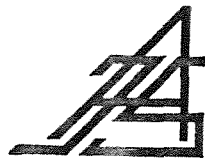


K. Müller

ARABIDOPSIS INFORMATION SERVICE

In collaboration with A. J. Müller, Gatersleben
G. P. Rédei, Columbia
J. Velemínský, Praha
Arranged by G. Röbbelen, Göttingen



No. 5
Prepared at the
Institut für Pflanzenbau und Pflanzenzüchtung
Universität Göttingen, Deutschland
March, 1968

CONTENTS

Preface

REDEI, G.P., and S.L. LI: Arabidopsis as a research tool in genetics	3
REDEI, G.P.: Arabidopsis for the classroom	5

A. Brief Notes

CROVELLO, T.J.: The value of numerical taxonomy for Arabidopsis research	7
HOLLAMBY, G.J.: The infeasibility of selecting simultaneously for growth rate and flowering time on Arabidopsis plants grown in nutrient agar	10
JONES, Mary E.: Variation in flowering time of natural populations of Arabidopsis thaliana (L.) HEYNH. with special reference to the breeding system	11
KARBE, Christine, and G.RÖBBELEN: Variation of plant size and other morphological characters in natural populations of Arabidopsis	13
CETL, I., and Jirina DOBROVOLINA: Further data on the developmental characters of natural populations of Arabidopsis thaliana (L.) HEYNH. from Western Moravia ..	15
EFFMERTOVA, Eva, and I.CETL: The behaviour of progenies derived from a "winter annual" natural population of Arabidopsis thaliana (L.) HEYNH.	16
DOBROVOLINA, Jirina: The heritability of characters "number of days to appearance of the flower primordia" and "number of rosette leaves" in a natural population of Arabidopsis thaliana (L.) HEYNH.	17
CORCOS, A.F.: Inhibition of seed germination in Arabidopsis thaliana by Actinomycin D	17
REINHOLZ, Erna: Germination of light requiring races of Arabidopsis thaliana in the dark after X-irradiation	18
SANKHLA, Daksha, and Narendra SANKHLA: Morphactin-cytokinin interaction in seed germination of Arabidopsis thaliana, En-2	19
SANKHLA, Daksha, and Narendra SANKHLA: Effect of helminthosporol and helminthosporic acid on seed germination and senescence of leaf discs of Arabidopsis thaliana, En-2	19
SANKHLA, Daksha, and Narendra SANKHLA: Reversal of abscisic acid-induced senescence of Arabidopsis leaf discs by cytokinins	20
SANKHLA, Daksha, and Narendra SANKHLA: Growth of Arabidopsis thaliana, En-2, in response to added morphactin	21
GINTER, T.N., and V.I.IVANOV: Chromosome numbers in Arabidopsis species	23
BERGER, Brigitte: Indirect measures of embryo development in interspecific crosses	23
BERGER, Brigitte: Relation of embryo to endosperm development after interspecific pollinations	24
REDEI, G.P.: Genetically determined differential recombination in the two sexes ..	25
BOUHARMONT, J., and J. Van Den HENDE: Inheritance of lethal chlorophyll mutants in tetraploid Arabidopsis thaliana	25
KASYANENKO, A.G., and Yu.S.NASYROV: Pigment concentration and photosynthetic activity of some chlorophyll mutants of Arabidopsis thaliana	26
ABEELS, M.: Existence of RNA-transmethylase in Arabidopsis	27
BERG, Beatrix I. Van Den, and W.J.FEENSTRA: Complementing alleles as a cause of apparent reversion of pyrimidineless mutants	27
LI, S.L., and G.P.REDEI: Temperature-sensitive, thiamine-requiring mutants in Arabidopsis	28
RÖBBELEN, G.: Functionally different, allelic mutants	29
HUSSEIN, H.A.S., and J.H. Van Der VEEN: Genotypic analysis of induced mutations for flowering time and leaf number in Arabidopsis thaliana	30
KUČERA, J.: Detection of induced late mutants in the early race Dijon	30
SAMUEL, J., and O.P.KAMRA: On radiostimulation of flowering in Arabidopsis thaliana	31
HIRONO, Y., and H.H.SMITH: Mutations induced with a base analog	32

(continued)

Acknowledgement

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

A.J.MÜLLER

G.RÖBBELEN

JACOBS, M.: Presumptive evidence for the extrachromosomal nature of laggard mutants induced by 5-bromodeoxyuridine	32
BONOTTO, S., M.JACOBS*, M.C.LOWI, S. Van PUYMBROECK, and R.KIRCHMANN: Study of the incorporation of 5-bromodeoxyuridine into the DNA of Arabidopsis by neutron activation analysis	33
JACOBS, M., and S.BONOTTO: Evaluation of the penetration and incorporation into DNA of tritiated thymidine in Arabidopsis seeds	34
JACOBS, M., and S.BONOTTO: Effects of actinomycin D on macromolecular synthesis and growth in Arabidopsis plants	34
AMANO, E.: Photoreactivation of UV-irradiated roots of Arabidopsis thaliana	35
REDEI, G.P.: A comparison of the somatic effects of X-rays and ethyl methane-sulfonate	36
BHANDARKAR, M.K., and B.K.GAUR: Lack of adaptation to Deuterium by Arabidopsis thaliana	37
ARNOLD, C.G., und D.CRUSE: Pflanzen mit väterlichen Eigenschaften nach Röntgenbestrahlung	39
CONTANT, R.B.: Changes in sensitivity of seeds to fast neutrons in the course of germination	39
CONTANT, R.B., and R.DANKERT: Effects of storage on dry irradiated Arabidopsis seeds	40
CONTANT, R.B., and R.DANKERT: Absence of a relation between early root growth and date of flowering in seed-irradiated Arabidopsis	41
CONTANT, R.B., and R.DANKERT: Comparison of effects on quantitative characters in the M ₁ of Arabidopsis after seed treatments with fast neutrons, gamma-rays and EMS	41
CONTANT, R.B., and R.DANKERT: Correlations within and between M ₁ and M ₂ , and genetic segregation, for various quantitative traits after neutron irradiation of Arabidopsis seeds	43
DANKERT, R., and R.B.CONTANT: In vitro germination of Arabidopsis pollen	45
DANKERT, R., and R.B.CONTANT: Male sterility induced by gamma-rays, fast neutrons and EMS	46
HIRONO, Y., H.H.SMITH, and J.LYMAN: Biological effectiveness of heavy ionizing particles relative to X-rays	47
FUJII, Taro: Killing efficiency of neutrons in Arabidopsis seeds	47
IVANOV, V.I., A.V.SANINA, and H.A.TIMOFEEFF-RESSOVSKY: Preliminary data on effects of various ionizing radiations on Arabidopsis	48
VEEN, J.H. Van der: Linear EMS-dose response for induced lethals, including maternally conditioned embryonic lethals	49
VEEN, J.H. Van der, and C. VAN HEEMERT: Decrease of genetic damage resulting from dry-storage of EMS-treated seeds	50
VELEMINSKY, J., V.POKORNY, and T.GICHNER: Absence of correlation between the frequency of mutations in M ₂ and some developmental characters in M ₁ generation ..	51
GICHNER, T., and J.VELEMINSKY: The influence of pH on the mutagenic activity of nitroso-compounds on Arabidopsis	52
VELEMINSKY, J., and T.GICHNER: Mutagenic activity of some nitrosamines	52
GICHNER, T., L.EHRENBERG, and C.A.WACHTMEISTER: The mutagenic activity of 8-hydroxyethyl methansulfonate (HOEMS), 8-methoxyethyl methansulfonate (MOEMS) and diethyl 1,3-propanedisulfonate (DEPD) on Arabidopsis	53
VELEMINSKY, J., and T.GICHNER: The mutagenic activity of N,N,N'-trimethylnitrosourea (TMNH) and p-tolylsulfonyl methyl nitrosamide (TSMN)	53
MÜLLER, A.J.: Genic male sterility in Arabidopsis	53
MÜLLER, A.J., und Ulrike HEIDECCKER: Lebensfähigkeit und letale fusca-Mutanten bei Arabidopsis thaliana	54
STEINITZ-SEARS, Lotti M., and Suzanne LEE-CHEN: Chromosome studies in Arabidopsis thaliana	55
KRANZ, A.R.: The fixation of radiation energy during the development in chlorophyll b deficient mutants of Arabidopsis	56

B. Techniques

PEDERSON, D.G., and D.F. MATZINGER: Techniques of artificial selection	58
LI, S.I., and G.P.REDEI: A simple technique for screening thiamine auxotrophs	58
ARNOLD, C.G., und D.CRUSE: Herstellung von Totalpräparaten zur Darstellung von Embryonen	59
BALKEMA, G.H.: Embryo test without seed loss	59
BONOTTO, S., and M.JACOBS: Further studies on Arabidopsis RNA	59

C. Bibliography

Comprehensive list until December 1967	61
In the press	68

C. Announcements

Meeting of Scientists interested in Arabidopsis Research	68
Change of Addresses	68
Material	68
Erratum	68

P R E F A C E

It is just 5 years, that on the occasion of the XIth International Congress of Genetics in Scheveningen an informal meeting was held, during which some cooperation of Arabidopsis research workers was suggested. It also appeared worthwhile to have new results and experiences in Arabidopsis research quickly circulated among the interested scientists. Thus this newsletter was proposed, of which the present 5 issues surely do not yet justify a jubilee edition. But the increasing interest in this "tachyplant" was followed by an increasing number of subscribers of the "Arabidopsis Information Service" and the first issues were soon out of print. The following articles are therefore intended to discuss the use of this plant from a more general point of view. A comprehensive list of literature was added, which will best be suited to place the existing information on Arabidopsis in the various fields of biology at everyone's easy disposal.

Arabidopsis as a research tool in genetics

G.F. RÉDEI and S.L. LI

Arabidopsis is not an economic plant. It is used only as a tool to study basic biological problems. Findings should be applicable to other plants, possibly to different organisms. A tool should be efficient and convenient, providing maximum information with minimal requirements for labor, facilities and expenditures. This plant has been in use for some time in several laboratories. Just knowing how Arabidopsis meets expectations and is regarded by fellow biologists is interesting to note. There are several reasons in making such an inquiry in this issue of the Arabidopsis Information Service.

Sixty years ago the first experimental paper on Arabidopsis appeared (LAIBACH, 1907). Twenty-five years ago the suitability of Arabidopsis for genetic and developmental research was demonstrated (LAIBACH, 1943). In 1967 the "father of Arabidopsis", Professor Dr. Friedrich LAIBACH, died leaving a heritage that he cultivated in the traditions of his teacher, Eduard STRASBURGER, the great founder of cytology.

For a long time Arabidopsis was a one man's research tool. This plant could not be meaningfully compared with Drosophila, maize or Escherichia coli as to the number of qualified investigators, or to the extent of facilities or funds involved in its use and development. Today the number of scientific reports dealing with this plant nears 300, an impressive record, hence outlining the major achievements within the framework of Arabidopsis Information Service is impossible.

In order to assess the present status and usefulness of Arabidopsis, a questionnaire was circulated among the members of the Department of Genetics of the University of Missouri to obtain their views on Arabidopsis as compared to nine other organisms ranging from man to viruses. Unfortunately many outstanding genetic organisms could not be included. Mouse, Ephestia, snapdragon, tomato, barley, rice, Oenothera, yeast, Paramecium, etc. have been omitted along with others equally useful tools. Enough burden was placed upon the cooperative volunteers by answering 140 questions conscientiously. The group includes former students or associates of MORGAN, BRIDGES, STURTEVANT, EAST, and STADLER, varying in rank from a graduate student to a member of the National Academy of Science. Persons polled had experience with all the organ-

Table: Total scores given by 9 faculty members and 2 graduate students indicating the potential usefulness of the named organisms for a particular field of research

Field of Genetic Research	ORGANISMS										Total Scores for Fields
	Homo	Droso- phila	Bombyx	Zea	Tri- ticum	Arabi- dopsis	Algae	Fungi	Bacteria	Viruses	
Formal	26	101	75	103	57	89	62	77	41	27	658
Cytogenetics	53	91	48	108	102	54	27	32	7	5	527
Polyploidy, aneuploidy	43	43	46	64	110	73	23	16	5	1	424
Gene evolution	46	78	48	63	88	70	37	46	40	32	548
Recombination	12	93	51	92	42	79	73	93	78	54	776
Mutation	19	78	45	73	56	81	73	93	95	84	697
Biochemical	50	55	44	54	45	75	84	105	103	61	676
Molecular	36	39	33	36	31	49	68	82	98	102	574
Developmental	42	73	73	62	60	83	38	36	19	13	499
Immunogenetics	75	45	44	24	25	19	35	35	58	49	409
Extranuclear	11	33	30	44	33	54	64	54	36	18	377
Population	77	97	51	42	50	76	47	58	65	52	615
Quantitative	43	65	53	87	82	86	36	37	33	25	547
Applied (Models)	23	59	50	58	50	64	20	26	21	25	396
Total scores for organisms	665	950	691	910	831	952	687	790	699	548	

isms listed in the questionnaire. Though only eleven answers were received, a good cross-section of the community of basic geneticists was represented, including both sexes and a wide variety of age groups. A maximum of 10 scores was given to the most suitable organisms for a particular type of research and zero marked the unsuitable. The combined scores are summarized in the table. Rating was anonymous. Homogeneity of the eleven responses was reasonably good. Total scores given to particular fields apparently reflects the subconscious appreciation of the participants for certain areas of research.

Evaluating the results proved interesting. Though Arabidopsis is relatively a new-

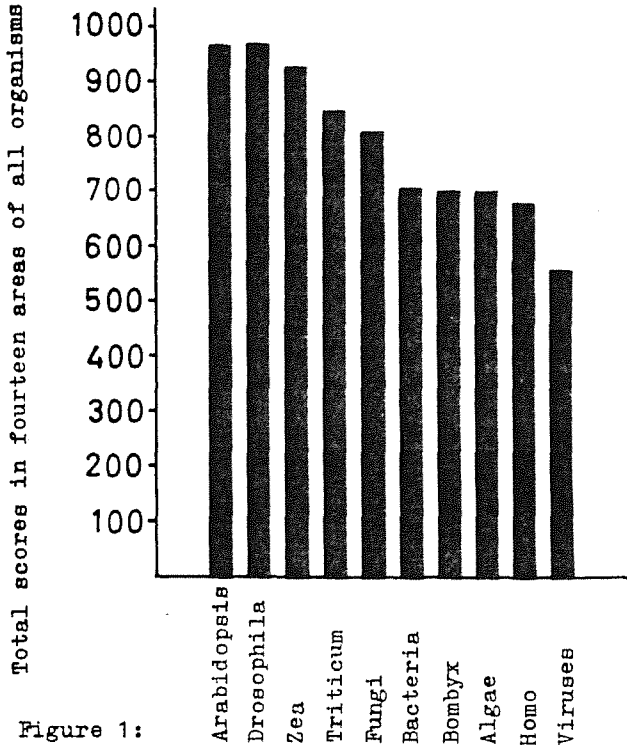


Figure 1:

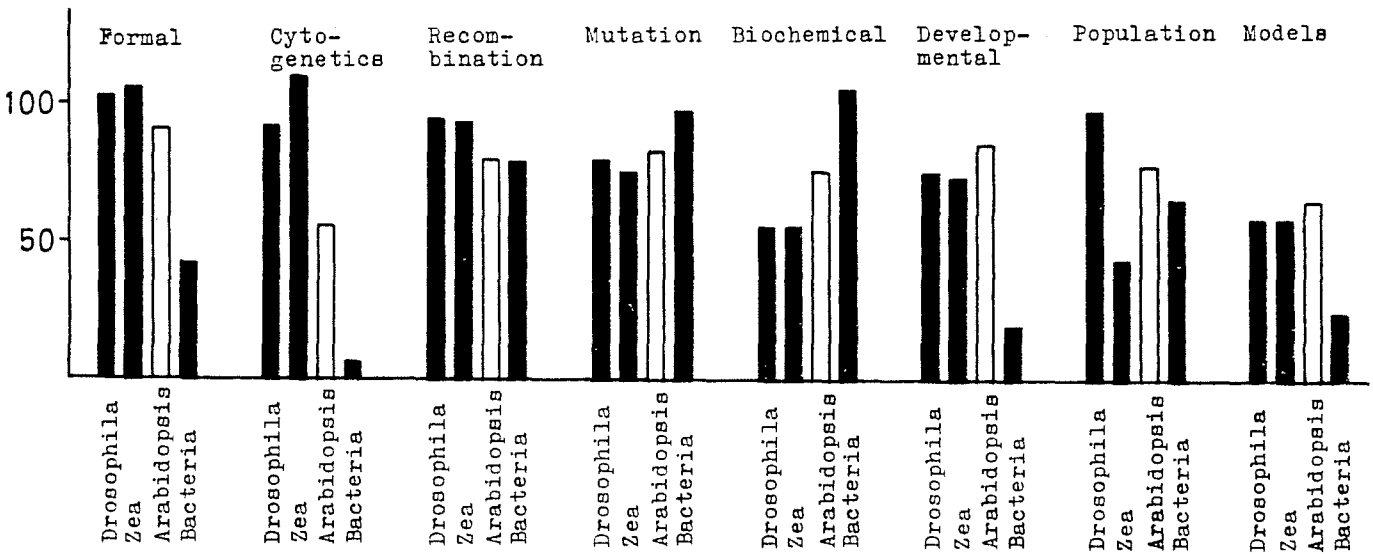
comer as a genetic tool, its overall suitability for genetic research was judged very favorably (Figure 1). This rating appears reasonable. There is little doubt that higher organisms are the most versatile tools of genetics; microorganisms (fungi, algae, and bacteria, and viruses) are not well-fitted to cytological approaches, as shown in the overall scores with the latter techniques counting high. The difficulties in genetic manipulations of higher mammals is reflected in the overall low rating of man as a genetic organism. In biochemical and molecular genetics microorganisms were rated well above higher organisms. But also in those two fields, Arabidopsis was definitely favored above all other multicellular organisms.

When Drosophila, maize, Arabidopsis, and bacteria are compared in the eight

Drosophila is 2, for maize 2, for bacteria 1 (Figure 2). In only one field is Arabidop-

in 2, the same index for

Figure 2: The scores of four representative organisms in selected areas of major importance



sis at the bottom, and this is perhaps one of the least active areas in genetics. - Other evaluations could be made, but the small samplings discourages this. In some areas the judgments were clearly biased by unknown factors. Bacteria should not have been rated as low for recombination studies and certainly not below Arabidopsis. Nonetheless the overall figures are not unrealistic.

LAIBACH in his prescient address at the Symposium on Arabidopsis Research in Göttingen, 1965, anticipated just what is presented in these statistics when he said: "...wenngleich...Viren und Bakterien sicherlich manches Grundsätzliche aufzeigen können, so können sie auf das Problem der Entwicklung und Phylogenie bei den Blütenpflanzen doch wohl schwerlich die letzte Antwort geben. Bei dem Versuch, diesem Ziel näher zu kommen, kann, so bin ich sicher, die kleine Arabidopsis thaliana Helfer, vielleicht sogar Schrittmacher sein".

Arabidopsis for the classroom

G.P. REDEI

At the University of Missouri Arabidopsis has been successfully used in courses on general genetics and experimental botany. During the past years numerous requests for seed to use Arabidopsis in classroom demonstrations were received. The outline of the following experiments for general biology or genetics laboratory courses may therefore be of some value to people interested in Arabidopsis as a teaching aid.

1. Mendelian segregation and linkage

Cross:	gi^2 is late even under continuous illumination taking about two months or more to get flowers; consequently, a large rosette is formed.
$ch^1 \times gi^2 pa$	pa dwarf, dark green // $gi^2 pa$ late, dark green and short stem ch^1 free of chlorophyll b, yellow green plants

Plants of this genotype should be planted at least at intervals 2-3 weeks apart and kept under 8 hrs daily illumination to meet the flowering time of the male partner.

The F_1 will be like the wild type, i.e., normal green and early. F_2 may be used for the demonstration of mendelian ratios and linkage in coupling and repulsion phase. Linkage intensity may be calculated in F_2 by the product method of FISHER and BALMUKAND (J. Genet. 20, 79). A few F_1 plants should be kept under short-day conditions in order to have available for F_2 several thousands of seeds if obtaining $ch^1 pa$ recombinants is also desired. Map distances: $gi-25-pa-8-ch$.

Crosses may be made with the aid of 5x watchmaker's magnifier using a pair of sharp forceps like Clay-Adams, Inc. (New York, N.Y. 10010) No. C-911 or Peer No. 46-206. Emasculation should be done before the flowers open, but could be omitted in this case since the female carries a recessive marker so that hybrids can be identified in F_1 .

Suggestions for successful culture: Store seed in open containers (like small vials) in a cool place. Germination is unimpaired for about 2 years. Plant seeds singly on the surface of fine, sterilized soil and never let them dry before germination. Good cultures can be secured in five inch diameter (ca. 12cm) short (Azalea) pots. Water by spraying with a fine nozzle, like the low volume "Water Fog", before germination or until early seedling stage. Later spray with the high volume nozzle of Fog-It Nozzle Col, P.O. Box 1752, Oakland, California 94604. A better method is placing the pots in a suitable aluminum or plastic-lined tray containing distilled water. - Good seed generally germinates within 3-4 days at 20-25°C, except in the first days after harvesting. Never transplant seedlings! Never provide more than 1200 foot candle light, if the plants are raised in growth chamber. Under 500 foot candle the growth is slow. Natural daylight and 100 foot candle incandescent continuous illumination during the night is necessary for the F_2 to be able to classify the late types. During the warm season protect plants from direct sunlight that may cause sunburn. Optimal temperature is around 25°C. Plants should be protected from insects by parathion or other suitable insecticides.

2. Biochemical mutants

Plants of the ch^1/ch^1 genotype are yellow green and free of chlorophyll b. Use any other suitable yellow green plants, and also pa/pa and wild plants, to demonstrate the difference between phenotype and genotype. The presence or absence of two chlorophylls can be demonstrated by paper- or thin-layer chromatography.

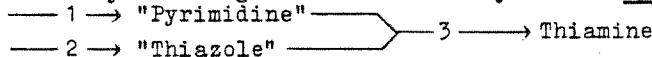
For paper chromatography cut paper strips (Whatman No. 1 or similar) not wider than 6-8 mm and ca. 20 cm long. Students will need two for each plant to be tested. Secure the same number of 150 x 16 mm or larger test tubes equipped with a tightly fitting cork. Add into each test tube ca. 1 ml mixture of petroleum ether: acetone (10:1.2 v/v). Also each student needs one small test tube (8 x 75 mm) for each genotype to be analyzed and half the number of glass rods rounded at both ends. Also necessary are a few small, fine tip capillary tubes that can be made in the lab with a Bunsen burner from suitable soft glass. A dropping

bottle equipped with a rubber bulb to keep abs. acetone is also needed. - Place one small or half leaf into the small test tube, and macerate it with 3-5 drops of acetone and apply a well-visible green spot about 2 cm from the end to each paper strip. The quantity of pigment on the two strips should be different. Too small amounts are not visible, while too large amounts may not separate satisfactorily. Place the strips into the larger test tube about 1/2 cm deep submerging the paper yet keeping the green out of the solvent. Carefully fix the strip with the cork in that position. Avoid any contact of the filter paper with the side of the tube. Perform all operations, including the development in dim light. - The separation is accomplished within 10-15 minutes and can be watched. At the solvent front, observe the orange-colored carotenes, next the xanthophylls, then a bluish-green spot of chlorophyll a immediately below the yellow green chlorophyll b is situated in all genotypes containing the ch^+ allele, while ch^1/ch^1 plants do not show anything at this place. Under a short-wave length ultraviolet lamp (Mineralight UVS-12, Ultraviolet Products, Inc., San Gabriel, California) chlorophyll displays a characteristic bright red color. This can be seen only in the absence of any other light source or in the darkroom.

Thin-layer chromatography gives superb separation in a shorter time. Apply a thin cellulose layer (MN 300, Macherey-Nagel & Co., Germany, or Brinkman, Westbury, N.Y., 11590) with the aid of an appropriate applicator on 2.5 x 10 cm glass plates. Mix the powder according to instructions of the label very quickly and dry the plates for 20 minutes at 90°C or over-night at room temperature. Apply extract and develop chromatogram in any suitable small container (larger vial, tightly sealed) with the mixture recommended above. An evaluation is possible within 5-10 minutes. - As an alternative, one may purchase Polygram Cel 300 precoated plastic sheets 20 x 20 cm (a package of 25 is \$22.00). Cut these into 60 strips (1 x 6.6 cm) and develop the chromatograms in 2 x 8 cm vials. Appropriately drilled wood blocks can hold the vials. Development time is about 2-3 minutes. A single experiment costs less than 2 cents. A dim light is not necessary as the operation is rapid.

3. Nutritional mutants

The genetic control of a biosynthetic pathway can be more easily demonstrated with Arabidopsis than with any microorganism. Thiamine synthesis in vivo follows the three main steps:



Mutant py is unable to make step No.1; mutant tz is blocked at step No.2, and mutant th has an absolute requirement for thiamine (block at step No.3). All three mutants are lethal unless thiamine or its appropriate moiety is supplied. On supplemented media all display normal growth. The requirement of py can be satisfied by 2-methyl-4-amino-5-aminomethyl pyrimidine HCl (obtainable from Nutritional Biochemicals, Cleveland, Ohio, 44128; \$1.00/g). Mutant tz requires 4-methyl-5-β-hydroxyethyl thiazole that is not a commercial product but can be conveniently prepared by autoclaving an appropriate solution of oxythiamine HCl (available from the above source: \$1.80/g). Any good commercial thiamine HCl can be used for mutant th.

Aseptic culture: Each student prepares the following mineral solution (mg/100ml distilled water): NH_4NO_3 40; $MgSO_4 \cdot 7 H_2O$ 20; $CaH_4(PO_4) \cdot H_2O$ 20; KH_2PO_4 20; K_2HPO_4 10; $FeC_6H_5O_7 \cdot 3 H_2O$ (ferric citrate) 0.5. Alternatively a larger batch may be prepared by the instructor, and 100 ml may be given to everyone in the class. Add to this enough good grade agar (ca. 1%) so that seeds will not submerge when applied to the surface. Sucrose or glucose content of the medium should be 2%. Divide the autoclaved batch into four equal portions (25 ml each). Add to batch No.1: 25 ml distilled water, No.2: 1 mg pyrimidine in 25 ml water, No.3: 0.01 mg oxythiamine in 25 ml water, No.4: 1 mg thiamine in 25 ml water. Mix and distribute each medium into 9 test tubes of 5 ml each; plug with cotton and autoclave at 15 lb pressure for 15 minutes. Sterilize simultaneously ca. 250 ml distilled water for each experiment. - After the agar is solidified, place approximately 50 seeds from each genotype into separate small cloth bags. Treat the seed with a 5% solution of calcium hypochlorite for 8 minutes. The disinfectant should be prepared at least one day ahead. Rinse the material carefully with at least five changes of cold sterile distilled water. Use a small inoculation chamber for planting. Precipitate microorganisms with a mist of 70% ethylalcohol. Place three seeds of each genotype on the surface of the agar in three tubes each of the four media. - Within two weeks the culture is ready for evaluation. The genotypes may carry code numbers so that the student can identify the requirement and can construct the pathway on the basis of his own observations. To avoid mechanical contamination of the seed, mutants on marked background py +;tz gl and th al can be used. A large quantity of seed of each mutant can be produced in pots by periodic spraying with dilute thiamine solution.

4. Genetic and physiological control of photoperiodic response

Segregation for late flowering can be seen from experiment 1. Differences in photoperiodic response may be well-demonstrated by planting in test tubes (as indicated in experiment 3) or on soil. Observe the germination of the following genotypes under continuous and 8 hour daily illumination, and record the order of the appearance of flower buds which is expected to be as follows:

	Illumination	
	Continuous	8 hours daily
wild type	1	2
<u>ld</u>	2	Not at all
<u>co</u>	3	1
<u>gi²</u>	4	3

The very striking effect of 5-bromodeoxycytidine, a DNA nucleoside analog, can also be demonstrated. - Prepare a mineral-sugar-agar medium as suggested in experiment 3. Make the following 5 variables, 10 to 20 test tubes each: (1) basal medium, (2) supplemented with 5-bromodeoxycytidine ($10^{-5}M$), (3) supplemented with 5-bromodeoxycytidine ($10^{-5}M$) plus deoxycytidine (the normal DNA nucleoside, $10^{-3}M$), (4) supplemented with deoxycytidine ($10^{-3}M$) alone, (5) supplemented with bromodeoxycytidine ($10^{-5}M$) + cytidine (the normal RNA nucleoside, $10^{-3}M$). The chemicals can be purchased in 100 mg quantities for a total cost of less than twenty dollars from any recognized supply house. - Place one seed of gi² genotype on the surface of the agar in each tube. Expose the culture to high intensity continuous illumination. Record the time of germination and the appearance of flower buds. Treatments (2) and (5) should flower within half the time of that required for the other treatments. In this system flowering is promoted by the suppression of DNA metabolism.

A. B R I E F N O T E S

The value of numerical taxonomy for Arabidopsis research

T.J. GROVELLO

(Department of Biology, University of Notre Dame, Indiana, USA)

This report has two purposes: (1) to summarize relationships among the strains of the LAIBACH collection of natural races based on comparative information available; and (2) to make nontaxonomists aware of the techniques of numerical taxonomy and its possible value to them. RÖBBELEN (1965) presented comparative data on 12 characters for 154 lines of 120 strains of Arabidopsis thaliana (L.) HEYNH. With his permission I proceeded to analyze the above data using the standard techniques of numerical taxonomy (SOKAL and SNEATH, 1963). Due to space limitations, the reader who is not a numerical taxonomist may find the following description of the particular technique used to be too brief. A complete treatment of the method and explanation of its terms may be found in the above book.

The data presented by RÖBBELEN may be represented as a character (12 rows) by line (154 columns) table. Call each line an OTU (Operational Taxonomic Unit - taxon to be classified). Each character was standardized to remove the weighting effect due to measurement of the different characters on different measurement scales. For example, the character leaf pubescence had four states while that of leaf width had only two states. The fact that different characters have different numbers of possible states would allow each character to affect subsequent estimates of similarity differently. It is this type of weighting that is removed here. The next step is to calculate the distance between each OTU in the context of a twelve dimensional space formed by the twelve characters. A phenogram, or tree diagram, is constructed from the calculated distance relations. SOKAL's distance coefficient was used and the OTU's were clustered into a phenogram by the Unweighted Pair Group Method, using averages. Computations required less than two minutes on the GE 625 computer at the University of Kansas. Total computing cost was less than \$10.

The results appear in Table 1 and Figure 1. Because of space limitations and for other reasons it seemed best to present the results as a "truncated phenogram", one that showed the relationships among groups of OTU's, with the members within each group listed in a table. Truncation was carried out at the distance value of 1.0. That is, OTU's clustering with each other at a distance of 1.0 or less were placed in the same group. Of course, the advantage of simplicity of presentation of the results is obtained only by a decrease in the accuracy of presentation of relationships among individual lines within a group. Normally, all OTU's (lines) would be shown separately in a phenogram. The agreement of the non-truncated phenogram with the original distance values is estimated by the cophenetic correlation coefficient, where zero suggests no agreement and one suggests perfect agreement. In this analysis the correlation was 0.776.

Twenty-seven clusters of lines (OTU's) of A. thaliana were apparent in the data. The study involves too many OTU's to allow an adequate summary of results in this short report. However, if one added the country of origin to each line in Table 1, several groups are found to consist mostly of lines from one country. But in general the results do not show a clearcut separation of strains according to their country of origin and of lines according to their strains. This is the result of several

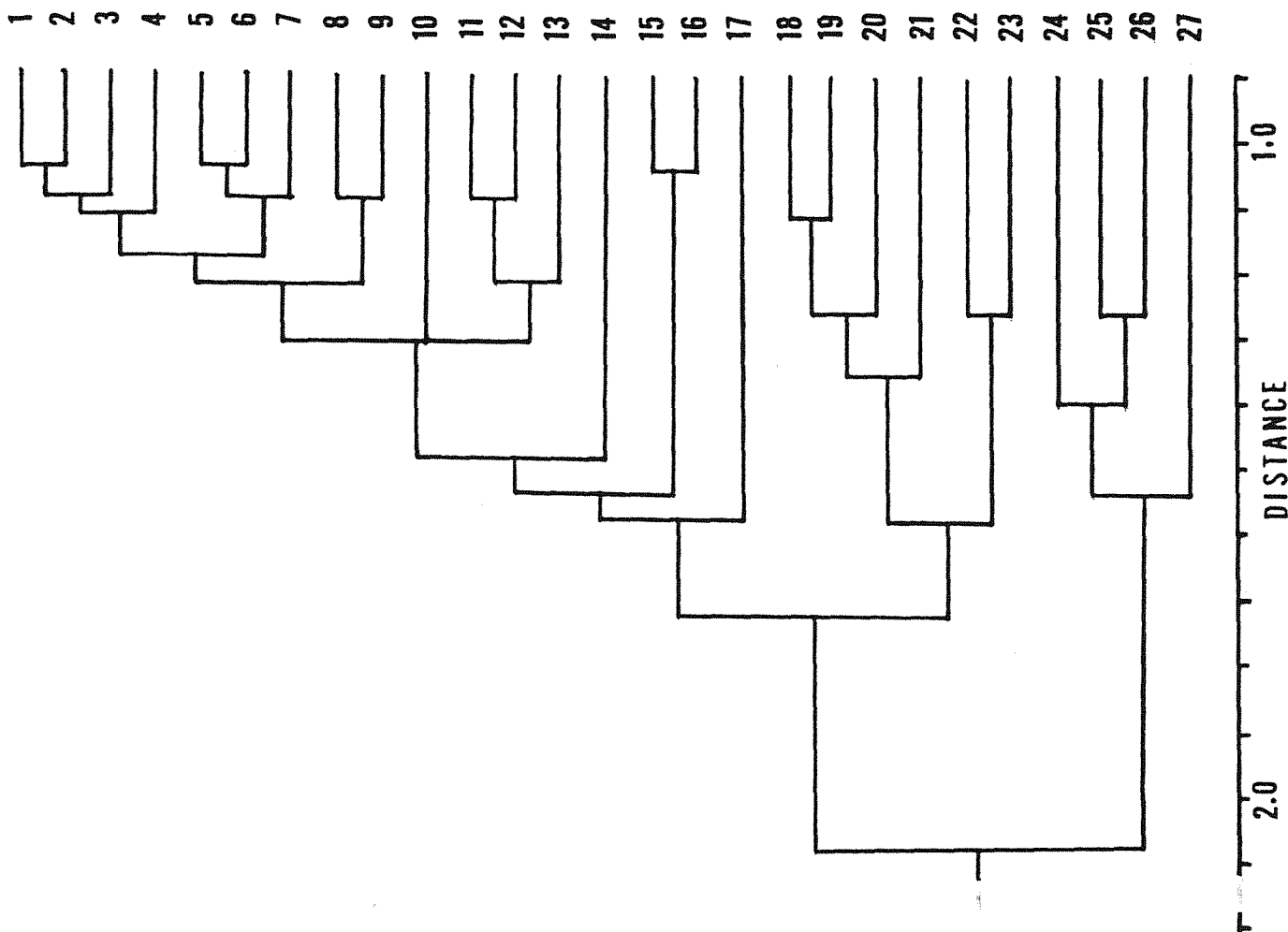


Figure 1: Truncated phenogram of the 154 lines of 120 strains of the LAIBACH Standard Collection of Natural Races

factors, most of which RÖBBELEN (1965, personal communication) has pointed out. These include low number of characters, relatively low sample size used to estimate each character in each line and the innate plasticity of the vegetative characters employed. For these reasons a truncated phenogram was used, because finer, more accurate resolution among the strains must await increased observations on a greater number of characters. But the present analysis still remains an objective summary of the relationships among lines on the basis of RÖBBELEN's data. This was its first purpose.

Turning to the second purpose, numerical taxonomy can aid the geneticist and physiologist by indicating efficiently the relationships (a) among lines within a strain and (b) between strains that he, or others are analyzing. For example, assume that we had obtained a more accurate estimate of phenetic relationships among the 120 strains, say one based on 100 characters. Investigator A reads that B, working with strain BIA observed 95% germination of seeds stored in a certain way for six months as well as several other interesting characters that were not included in the original 100. Investigator A works with several other strains and wants to know if his strains may be likely to possess the same characteristics. By reference to a phenogram depicting relations among all the strains, A can assess quickly how closely related phenetically strain BIA is to any of his strains. - Similarly, suppose A wishes to know why a certain two strains cluster together, or why a line of one strain clusters with other strains instead of its own. By examination of the data he can ascertain in which characters the two OTU's are similar and in which ones they differ, thus enhancing his understanding of their relationship.

Geneticists and evolutionists may be more interested in a multivariate character analysis instead of a strain analysis. One could use the same methods but instead of a tree diagram of strains, one would have groups of characters. The investigator would then be interested to generate hypotheses as to why certain characters cluster

Table 1: The lines (OTU's) in each group of the truncated phenogram

GROUP		LINES IN EACH GROUP										
No.												
1	AK-1	NW-2										
2	AN-3	BD-0	BE-0	BE-1	BLA-4	BS-2	BS-5	BSCH-0	BSCH-2	CO-2	DR-0	EI-2
	FR-2	GR-1	GR-2	GR-3	GR-4	GR-5	GR-6	HM-0	IS-1	KÄ-0	KL-0	KL-1
	LÖ-1	LÖ-2	MA-2	NA-1	NP-0	NW-1	NW-3	NW-4	PA-2	PF-0	PO-0	RSCH-4
	RU-0	TSU-0	TU-0	UK-2	VI-0	WÜ-0	ZÜ-0					
3	ST-0											
4	AN-1	BCH-1	BCH-4	BLA-6	BLA-10	CHI-0	EP-0	GD-1	HA-0	JE-0	LI-1	
	LI-5	LI-7	LI-12	NW-0	ROU-0	RSCH-0						
5	AN-2	DI-0	EL-0	GA-0	GÖ-0	GÖ-2	GÜ-0	HI-0	SG-1	SG-2		
6	CHI-1	CHI-2	CT-1	DA-0	EN-1	EN-2	EST-1	FE-1	FR-3	FR-4	GIE-0	IS-0
	LI-8	MT-0	PI-0	SI-0	UK-3							
7	IN-0	KN-0	LM-2	MA-0	STW-0	TU-1	WIL-3					
8	BCH-3	BLA-2	CO-3	EI-4	HS-0	KL-3	KL-4	RD-0	TA-0	UK-1	X-0	
9	BLA-1	BLA-3	BLA-5									
10	BL-1	BR-0	MRK-0	PO-1	WSO-OA	WSO-OB						
11	CO-1	PA-1	PA-3	PI-2								
12	PR-0											
13	LA-0											
14	EI-6	GU-1										
15	EST-0	LI-1	WIL-1									
16	WIL-2A	WIL-2B										
17	EI-5											
18	BS-1	ER-0	UK-4									
19	LA-1	LI-6A	LI-6B	PT-0								

GROUP No.	LINES IN EACH GROUP
20	MR-0
21	LI-10A LI-10B ÖST-0 TE-0
22	KL-2 LI-11
23	ØY-0
24	BU-0 LG-0 WA-1
25	KO-2
26	LU-1A LU-1B
27	KR-0

together. Is it due to pleiotropy, to an adaptive complex, etc.? Actually, the above method is relatively inaccurate. Better results can be obtained by related methods used in numerical taxonomy that do not result in a tree diagram, as, for example, principal components analysis (SEAL, 1964).

The above brief discussion cannot give justice to the value of taximetric techniques as an efficient heuristic procedure to summarize information and to serve as a context for generating biologically important hypotheses that can be tested by subsequent experiments. Its presentation here is chiefly to call attention to a technique of considerable promise. This does not mean that to use it a physiologist must become a numerical taxonomist. Rather it points to another direction in which cooperation among biologist of various disciplines could pay big dividends. Specifically, a continuing data bank of information on Arabidopsis strains could be set up and maintained along the lines used for the maintenance of strains at Göttingen. Revised reports that incorporate new information both about strain and character relationships could be issued periodically. Such a data bank for over 100 strains of Aedes aegypti is being established at Notre Dame to provide all investigators with as much information as possible about their strains. Perhaps a committee should be established to investigate the feasibility of this for Arabidopsis.

References:

RÖBBELEN, G.: Arabidopsis Inf. Serv. 2, 36-47 (1965)
 SEAL, H.L.: Multivariate Statistical Analysis for Biologists. J.Wiley, New York 1964
 SOKAL, R.R., and P.H.A. SNEATH: Principles of Numerical Taxonomy. W.H. Freeman, San Francisco 1963

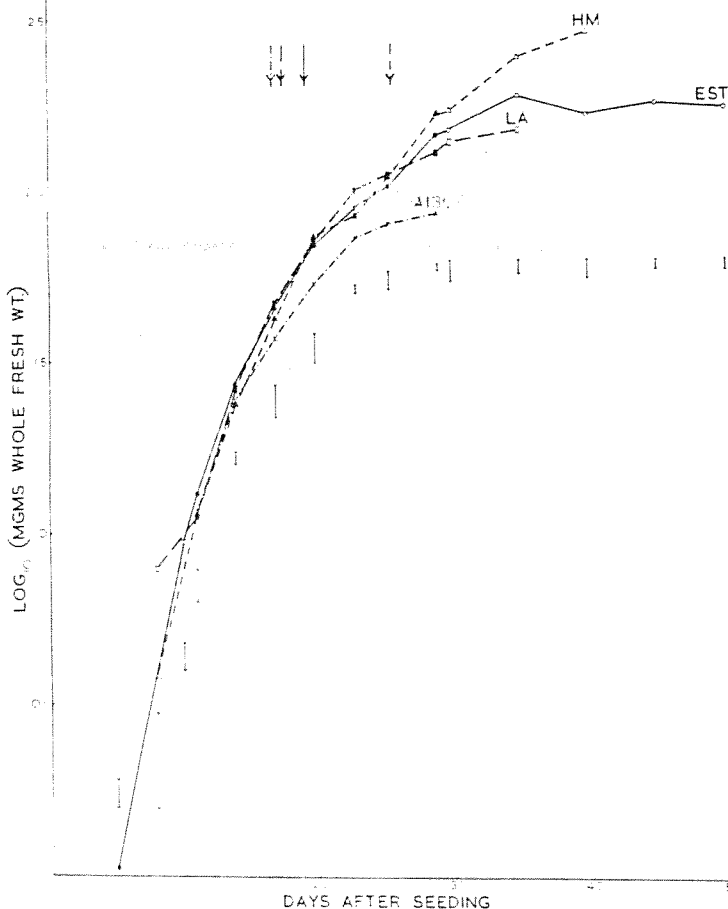
The infeasibility of selecting simultaneously for growth rate and flowering time on Arabidopsis plants grown in nutrient agar

G.J. HOLLAMBY

(Division of Plant Industry, C.S.I.R.O., Canberra. Present Address: Agricultural College, Roseworthy, South Australia)

Arabidopsis thaliana (L.) HEYNH. was used to investigate the efficiencies of different plant breeding methods when selecting simultaneously for two quantitative characters in self pollinated plants. Two such easily measured characteristics in Arabidopsis are flowering time and growth rate. Measuring flowering time and growth rate of a single plant (which is necessary in early generations where heterozygosity is high) rules out to take the standard measure of growth rate in the exponential phase, namely, log plant fresh weight at 11 days, as this is preflowering. BROCK (1967) selected for growth rate by weighing plants at 22 days, post flowering, and measured the response to selection by weighing plants at 11 days. He found that this selection technique for early growth rate was not efficient and warned against its use. I felt that a better technique would be to measure the plants' weights as they flowered rather than to measure them all at the one fixed time after flowering irrespective of their flowering times, in the hope that the growth rates estimated in this way would be an index of the growth rates during the exponential phase. An experiment to test the usefulness of this technique by constructing growth curves of several homozygous Arabidopsis lines of differing flowering times was performed.

Four lines of Arabidopsis were used: Landsberg/Warthe (La), Estland (Est), and Hannoversch-Münden (Hm), originally from the LAIBACH collection, and an early flowering mutant of Estland (A136) produced by Dr. BROCK, C.S.I.R.O. The mean numbers of days to flowering were: 16.84 (0.15)* for A136, 17.67 (0.12) for La, 19.39 (0.10) for Est, and 25.78 (0.24) for Hm. Plants were grown aseptically one per test tube (diameter 150mm x 16mm) in sterile nutrient agar at 25°C, in 1800 f.c. continuous light, and 65% rel. humid. using the technique described by LANGRIDGE (1957). At seeding there was a 7 ml aliquot of nutrient agar in each tube. Four plants from each of four replicates were sampled from each line at a harvest. Due to poor establishment in some lines there were fewer plants and hence fewer harvests from them.



* Standard errors

Figure 1: Growth curves for four lines of Arabidopsis grown under constant environment conditions in nutrient agar.

Arrows indicate flowering times. Flags below each harvest time indicate either L.S.D. 5%, or 95% fiducial limits. Open symbols in Hm, Est, and La represent data from plants sown on a different date to the rest of the experiment.

Figure 1 illustrates log whole plant fresh weight measurements. There were relatively large error variances in early harvests, probably due to uneven germination, and these may have masked differences between lines in these stages. The exponential growth phase ended soon after 14 days for all races. Final plant weight was ranked

according to flowering time, the latest flowering race being the heaviest, as one would expect. Growth rates estimated for each line by three different methods are shown in the following table. Method 3 is the technique that was hoped to be useful.

Table: Comparison of growth rates as measured by different methods:

Method 1: Log (Whole plant fresh weight at 11 days)/11;
 Method 2: Mean Log (Whole plant fresh wt at flowering)/Mean flowering time;
 Method 3: Mean (log whole plant fresh wt at flowering/flowering time).
 Figures in brackets are standard errors where calculable.

Race	Method 1	Method 2	Method 3
A136	0.09646 (0.00283)	0.09207	0.09122 (0.00390)
La	0.09556 (0.00283)	0.09634	0.09767 (0.00285)
Est	0.10153 (0.00283)	0.09229	0.08784 (0.00140)
Hm	0.09588 (0.00283)	0.07914	0.07821 (0.00113)

These data show that delaying the measurement of plant fresh weight until flowering and calculating the plant growth rate on this basis underestimates the growth rate during the exponential phase of the later flowering races. Estimates calculated the same way but based on log dry weights showed a similar tendency. These races may have genetically passed their period of exponential growth by flowering, or the decreased growth rate over the latter part of their vegetative stage may have been environmentally induced due to poor lighting of the rosette and a decreased root medium. The agar had dried to approximately one half its volume after 20 days pulling the still green parts of these later flowering races down into the wells in which the tubes sat. An analysis of the relative proportions of tops to roots and of root growth did not help to explain this underestimation of the exponential growth rate.

Whatever the cause, the conclusion must be drawn that under the environmental conditions described, taking the log fresh weight of a plant at flowering as an index of its growth rate while in the exponential phase is not valid. It therefore is not feasible to select simultaneously on the one plant for both growth rate and flowering time.

References:

- BROCK, R.D.: *Radiat.Bot.* 7, 193-203 (1967)
 LANGRIDGE, J.: *Austral.J.Biol.Sci.* 10, 243-52 (1957)

Grateful acknowledgement is made of the critical advice, facilities and assistance from the following C.S.I.R.O. personnel, Dr. R.D. BROCK, Mr. R. DUNSTONE, and Miss J. MUST.

Variation in flowering time of natural populations of *Arabidopsis thaliana* (L.) HEYNE. with special reference to the breeding system

Mary E. JONES

(Department of Genetics, The University, Birmingham 15, U.K.)

Investigations into the breeding system of British wild populations of *Arabidopsis* were initiated on samples of seed collected in 1965 from five randomly selected plants in each of the following six populations:

Population	Location	Habitat
1	Alcester, Warwickshire	Disused railway track
2	Broom, Warwickshire	" " "
3	Luddington, Warwickshire	" " "
4	Henley-in-Arden, Warwickshire	" " "
5	Cannon Hill Park, Birmingham	Weeds on cultivated land
6	Parks' Nurseries, Middlesex	" " " "

The plants were grown on a bench in the glasshouse (LAWRENCE, 1966), in a completely randomised block experiment. Measurements were taken of days to flowering from an arbitrary date. The experiment was terminated after 104 days, after which some plants still showed no signs of flowering; these are referred to as late forms.

The results of the analysis of variance showed that both populations and families within populations differed significantly. An indication of the differences between populations is given by the population means and variances:

Population	1	2	3	4	5	6
Mean	37.412	59.969	47.222	21.574	7.580	8.574
Variance	33.504	328.553	81.390	140.435	4.416	3.389

These fall into two distinct groups, populations 1, 2, 3 and 4 having high means and variances, and populations 5 and 6 with comparatively low means and variances. This shows good correlation with the origins of the populations, populations 5 and 6 being essentially weed populations which have probably been subject to strong directional selection for early flowering by gardeners.

The variation between families also shows marked heterogeneity (BARTLETT test for heterogeneity of variances $P < 0.001$) (Figure 1). This could be attributed to two factors. One is that the result is purely a scalar effect of the environment, family variance increasing with family mean. The other is that the large variances of some families are the result of segregation of the progeny of either a

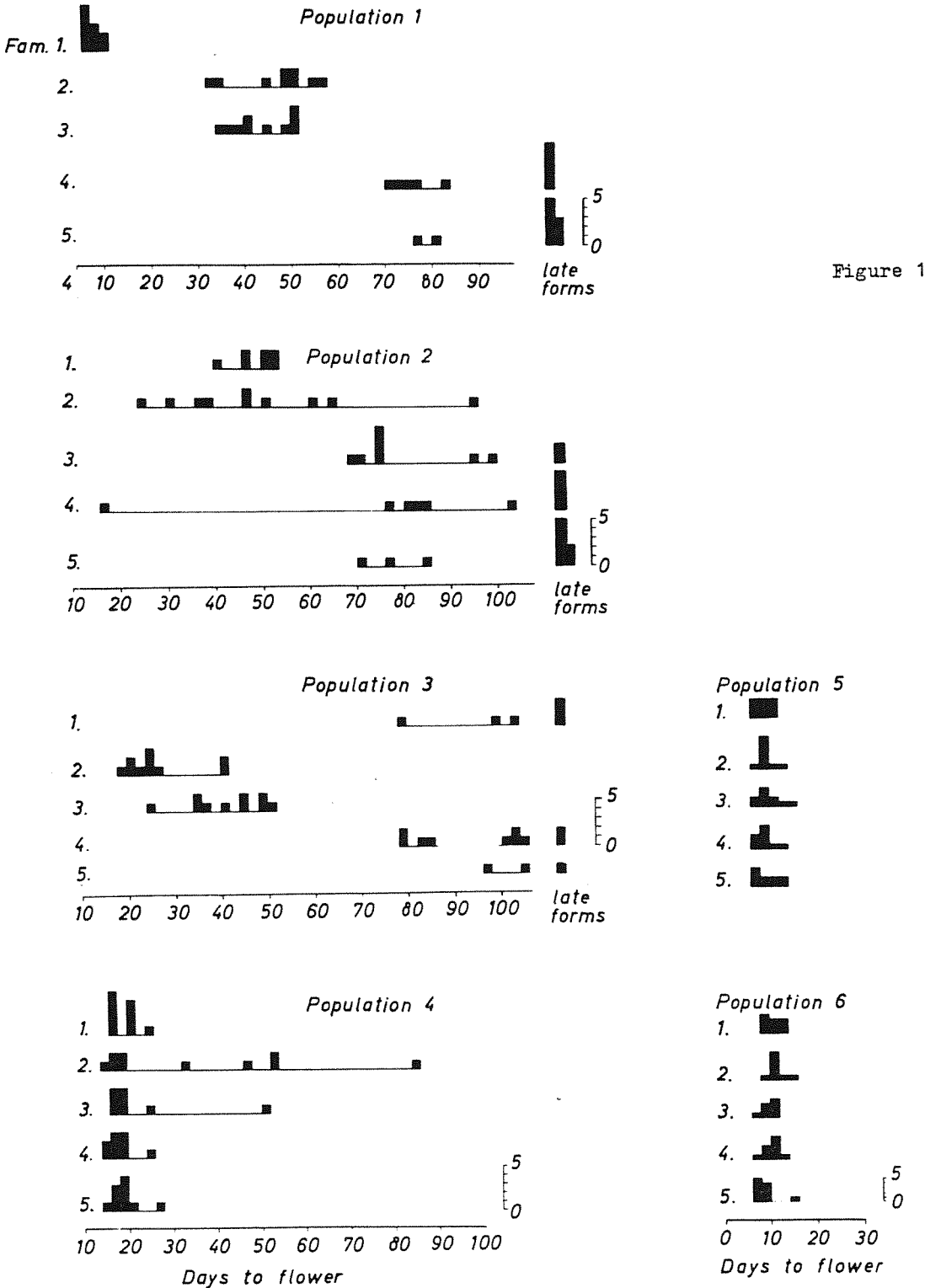


Figure 1

non-homozygous parent or a homozygote which had cross-pollinated with other heterozygous individuals. Thus small family variances would indicate that the parent was relatively homozygous and inbreeding.

A certain scalar effect of the environment would be expected especially in the very late families. However this cannot fully account for: (1) The enormity of some of the variances, plants from a single family flowering, for instance, over a period of up to 100 days; (2) The presence of families with similar means and widely differing variances. The distribution of variation in populations 5 and 6 would appear to indicate that the parent plants were relatively homozygous and inbreeding. If, however, cross-pollination did occur in such populations assuming all families are of this type, little segregation would be expected, and if this were so, and these families were segregating, this method of investigation would not be sensitive enough to detect it.

Further investigations were made on populations 5 and 6 on the progeny of the first selfed generation. Five individuals from each of the five first generation families in each population were selected at random and selfed. The selfed progeny were grown in a randomised block experiment and their flowering time measured. Each group of five families originating from a single wild individual will be referred to as a family group. - In the analysis of variance of this data both populations and families within groups within populations were significant. The item which is of interest is the between families within groups item. If there are significant differences between selfed families which have originated from one parent plant, then this is evidence that the progeny were segregating. Each family group was examined individually, the between family mean square being calculated for each group separately and tested against the appropriate summed within family mean square:

Population	Family group	Between M.S.	d.f.	Within M.S.	d.f.	V.R.	P
5	1	37.025	3	8.564	29	4.323	.01-001
	2	23.623	3	15.477	21	1.526	Not significant
	3	104.344	3	6.229	29	16.751	.001
	4	30.325	4	6.902	35	4.399	.01-001
	5	23.623	2	3.177	16	7.436	.01-001
6	1	5.065	4	4.664	39	1.086	Not significant
	2	7.671	4	2.686	42	2.856	.05-.01
	3	7.887	4	2.143	41	3.680	.05-.01
	4	12.600	4	2.578	44	4.888	.01-001
	5	9.125	4	2.395	43	3.810	.05-.01

The significance of the between family mean squares of all but two family groups shows that most of these selfed families have originated from segregating parents. This is positive evidence that the majority of plants sampled from these populations were at least partially heterozygous. Thus, despite the low family variances shown by these populations in the previous experiment, it does not follow that individuals undergo automatic self-pollination under natural conditions.

It is interesting to note that this type of situation maybe widespread in natural populations of *Arabidopsis*. Similar results to those found in the first experiment described i.e. large within family variation, have also been reported in German and Czechoslovakian populations by NAFF-ZINN (1964) and DOBROVOLNA (1966) respectively, and also more recently by RÖBBELEN (personal communication).

References:

- DOBROVOLNA, J.: Arabid. Inf. Serv. 4, 6-7 (1967)
 LAWRENCE, C.W.: Arabid. Inf. Serv. 3, 36 (1966)
 NAFF-ZINN, K.: Beitr. zur Phytologie, Festschr. H. WALTER. Pp. 39-49, 1964

Variation of plant size and other morphological characters in natural populations of *Arabidopsis*

Christine KARBE and G. RÖBBELEN

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

Arabidopsis is well known to be strictly autogamous under greenhouse conditions. Seed samples collected from wild populations, however, frequently produce variable progenies. In the LAIBACH collection (cf. RÖBBELEN, 1965) many of the "natural races" kept in the greenhouse for a long series of generations still appear heterogenous in various traits. Single plants often gave rise to different, but homogenous offsprings, while others "segregated" even after recurrent selection. The idea has been mentioned several times in the past (cf. the preceding paper of Mrs. JONES in this issue) that wild populations of *Arabidopsis* can be composed of different "pure lines" as well as of hybrids between them.

By the way of other projects an experiment was run to test this situation. In the spring of 1967 seeds were harvested from single plants within a small field of winter wheat in the south of Göttingen, near Holtensen. A random sample of 40 progenies of this population "H1-2" was grown in the greenhouse - each family, derived from a single plant, in a row of 8 pots. The diameter of the full grown rosette and the

number of days to flowering were determined. The data for the latter character are in perfect agreement with those of the preceding paper and, therefore, need not to be given here in detail.

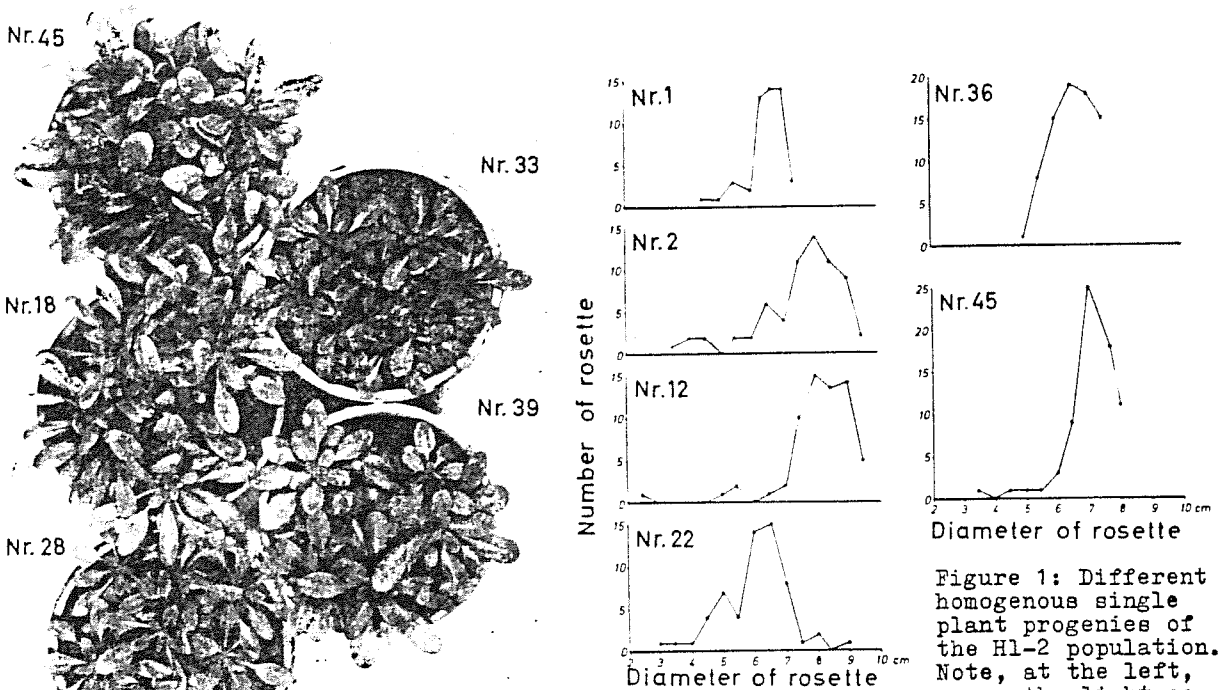


Figure 1: Different homogenous single plant progenies of the H1-2 population. Note, at the left, e.g., the light co-

lour and smooth margin of the leaves in Nr.18 or the broad leaves and early flowering in Nr. 45. (The plants were heavily sprayed with a fungicide.)

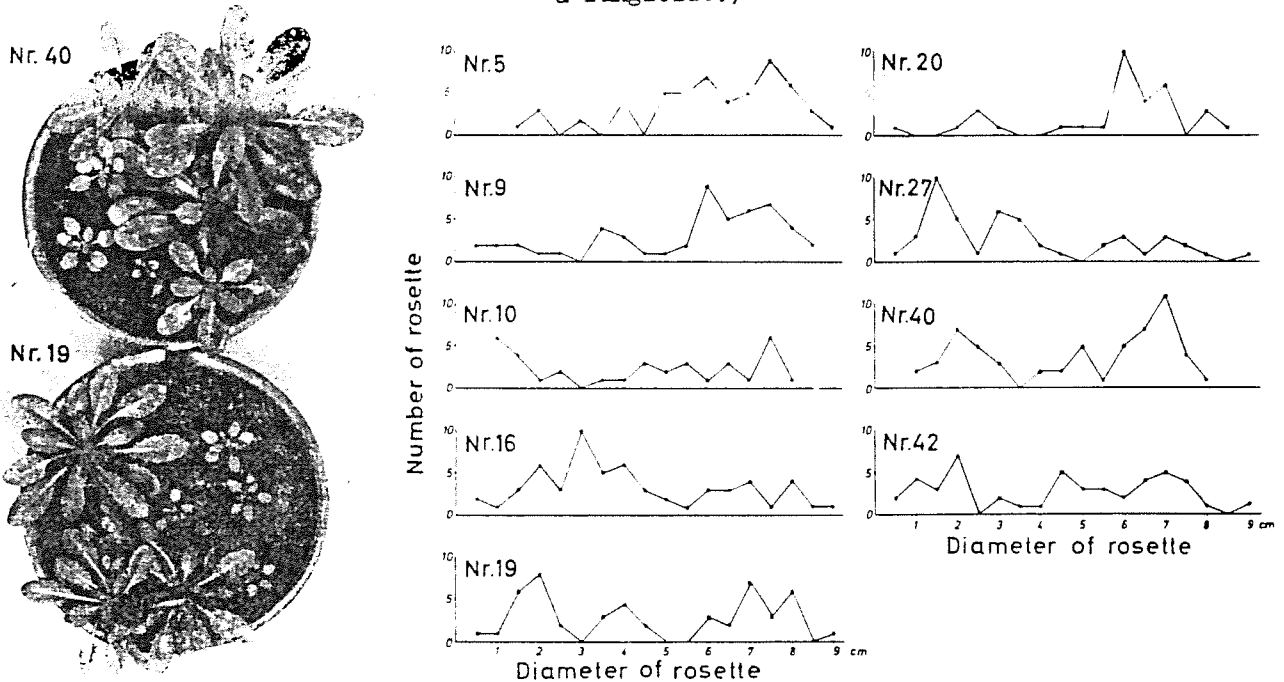


Figure 2: Single plant progenies of the H1-2 population "segregating" in rosette size

The morphological variation between and within the families, on the other side, illustrates the same facts at an additional level. 25 of the 40 families were phenotypically as uniform as any "pure line" can be. The graph in Figure 1 indicates the distribution of frequencies of the rosette diameter of 6 such homogenous families. The result was confirmed by an analysis of variance which, however, revealed significant differences between those families. Not only the rosette diameter, but also the number, shape, margin, or colour of the leaves differed characteristically (see photograph of Figure 1).

Figure 2 presents another group of 9 families out of this population of H1-2 in which the phenotypic criterions were highly variab. Some of those curves could easily be fitted into the scheme of simple mendelian segregations of a heterozygous mother plant. Seeds of single plants were harvested to investigate this possibility in a progeny test; but this experiment is at the moment not yet ready for evaluation. It appears very probable, however, that the chance for cross-pollination (or mutation?) under natural conditions is much higher in *Arabidopsis* than expected ever before. This is the more surprising as the stand in the tested population was very loose (in 1967!) with distances of many meters between the different plants or clusters of a few individuals. - The variability of the remaining 6 of the tested 40 families ranges in between the two groups mentioned. The rosette diameters within these families were more or less continuously scattered over a larger scale within these families. More complex analyses would thus be needed to trace this kind of variability back to its origin.

Reference:

RÖBBELEN, G.: *Arabid. Inf. Serv.* 2, 36-47 (1965)

Further data on the developmental characters of natural populations of *Arabidopsis thaliana* (L.) HEYNH. from Western Moravia

I. CETL and Jiřina DOBROVOLNÁ

(Department of Genetics, Purkyně University, Brno, Czechoslovakia)

The experiments with natural populations reported by CETL, DOBROVOLNÁ, and EFFMERTOVA (1965, 1967), CETL (1965), and EFFMERTOVA and CETL (1966) were continued in 1965 and 1966/67 under the same experimental conditions as in the previous experiments (soil culture of plants from unvernallized post-dormancy seeds at 25+3°C under continuous illumination, 1250 lux; duration of each experiment 42 days from full germination).

The results for the three periods expressed by the distributions (1) of the percentage of generative plants, (2) of the mean number of days to appearance of the flower primordia in the generative plant fraction, and (3) of its standard deviation, are presented in the Tables 1, 2, and 3. The characteristics (2) and (3) are not given in populations with <20 per cent generative plants. These data confirm that both among and within the studied populations a considerable variability in developmental characters is present.

The percentage of generative plants in single populations is quite different changing from 0 to 100. When using the earlier classification of populations (see CETL, 1965) about 20 per cent of all the 164 populations appear to be "winter annual", about 15 per cent "mixed" and about 65 per cent "summer annual":

Table 1: Per cent of generative plants

	0 - 10 - 20 - 30 - 40 - 50 - 60 - 70 - 80 - 90 - 100	S
1963/64	18 % 28,1	64 99,9
1965	11 % 24,4	45 99,8
1966/67	1 % 1,8	55 100,1
S	30 % 18,3	164 100,0

Among the populations there are also differences in the mean number of days to appearance of flower primordia in the fraction of the generative plants, the extreme values being 9,1 and 36,1 days:

Table 2: Mean number of days to appearance of flower primordia in the generative plant fraction

	7,5 - 10,0 - 12,5 - 15,0 - 17,5 - 20,0 - 22,5 - 25,0 - 27,5 - 30,0 - 32,5 - 35,0 - 37,5	S
1963/64	3 % 6,5	46 100,0
1965	2 % 5,9	34 100,0
1966/67	2 % 3,7	54 100,2
S	2 % 1,5	134 100,1

The standard deviations of the foregoing values indicating the variability within populations are also very different (minimum +0,42, maximum +12,85 days). Only in two "summer annual" populations the σ values are as low as in homozygous races (cf. DOBROVOLNÁ, 1967). Therefore, only these two populations can be regarded as homozygous for the studied character while the remaining 132 populations appear to be heterozygous:

Table 3: Standard deviation for the number of days to appearance of the flower primordia in the generative plant fraction

	0	1	2	3	4	5	6	7	8	9	10	11	12	13	S	
1963/64		1	3	6	13	10	7	5	1						46	
%		2,2	6,5	13,0	28,2	21,2	15,7	10,9	2,2						99,9	
1965		5	9	5	5	4		2	2	1			1		34	
%		14,7	26,5	14,7	14,7	11,8		5,9	5,9	2,9			2,9		100,0	
1966/67		2	14	15	11	4	3	3			1			1	54	
%		3,8	25,9	27,8	20,4	7,4	5,6	5,6			1,8			1,8	100,0	
S		2	20	27	22	22	17	10	7	3	2			1	1	134
%		1,5	14,9	20,2	16,4	16,4	12,7	7,5	5,2	2,2	1,5			0,7	0,7	100,0

The results show that the new experimental material confirms the conception suggested on the basis of the first experiments (CETL, 1965), that natural populations of *Arabidopsis* with respect to their developmental characters are genetically variable and internally complex entities.

References:

CETL, I.: In "Arabidopsis Research", Rept.Int.Symp. Göttingen. Pp. 46-52, 1965
 - , J. DOBROVOLNÁ, and E. EFFMERTOVA: Arabid.Inf.Serv. 2, 3 (1965)
 - , - , - : Arabid.Inf.Serv. 4, 9-10 (1967)
 DOBROVOLNÁ, J.: Arabid.Inf.Serv. 4, 6-7 (1967)

The behaviour of progenies derived from a "winter annual" natural population of *Arabidopsis thaliana* (L.) HEYNH.

Eva EFFMERTOVA and I. CETL

(Department of Genetics, Purkyně University, Brno, Czechoslovakia)

It has been shown that in natural populations of *A. thaliana* a large variability in developmental characters exists between populations and often also within them (NAPP-ZINN, 1964; CETL, 1965). The latter can be subdivided in the between-plant and within-plant component. The first step in such an investigation is to study the variability of both, between and within progenies obtained by natural selfing from single mother plants of a population. It has been found by DOBROVOLNÁ (1967) that in several populations of the "summer annual" type a high percentage of progenies showed a higher variation in two developmental characters than homozygous races and that, therefore, such progenies can be regarded as heterozygous for these characters. "Winter annual" populations have not yet been studied from this point of view.

A winter annual population, HV-3, was used in this experiment. If unvernalsed seeds were sown only about 7% of the plants were generative at 25+3°C in continuous illumination, 1250 lux, after 42 days from germination. About 6 weeks of vernalization were needed to have all of the plants generative in the given experimental conditions. Seeds of single plants were harvested at the locality and vernalized for 4 weeks at 2+1°C. From 293 harvested plants a representative sample of 120 progenies was sown without vernalization in the above experimental conditions, and 89 progenies with sufficient number of individuals were evaluated. Among these progenies there were 59 (66,3 %) fully vegetative, 24 (27,0 %) with both vegetative and generative plants, and 6 (6,7 %) with >90% generative plants. This result shows that in the original population more than 25% of the plants were segregating in vegetative ("winter annual") and generative ("summer annual") individuals, and that they can thus, be regarded as heterozygous for the genetic factors determining the winter annual vs. summer annual character.

It is surprising that the frequency of generative plants in the original sample (13 in 168; 7,7 %) coincides not only with the frequency of generative progenies (6 in 89; 6,7 %) but also with the frequency of generative plants in all vegetative and "segregating" progenies (94 in 1129; 8,3 %). The χ^2 -values were 0,39, $P = 0,50-0,70$, and 0,01, $P > 0,99$, respectively. This finding shows that the "summer annual" individuals must not represent an independent group within this "winter annual" population but that the same frequency of these individuals can be produced by means of segregation of phenotypically "winter annual" heterozygotes. - The two above facts, namely, the relatively high percentage of progenies segregating in "winter annual" and "summer annual" individuals, and the apparently constant frequency of "summer annual" individuals in such a "winter annual" population will be a matter of further studies.

References:

CETL, I.: In "Arabidopsis Research", Rep.Int.Symp. Göttingen. Pp. 46-52, 1965
 DOBROVOLNÁ, J.: Arabid.Inf.Serv. 4, 9-10 (1967)
 NAPP-ZINN, K.: Beitr. zur Phytologie, Festschr. H. WALTER. Pp. 39-49, 1964

The heritability of characters "number of days to appearance of the flower primordia" and "number of rosette leaves" in a natural population of *Arabidopsis thaliana* (L.) HEYNH.

Jiřina DOBROVOLNÁ

(Department of Genetics, Purkyně University, Brno, Czechoslovakia)

Phenotypical differences in developmental characters, e.g., in the number of days to flowering, observed between and within different natural populations of *A. thaliana* and also between and within different plants in a population are thought to be caused genetically (NAPP-ZINN, 1964; CETL, 1965). But yet no evidence has been brought forward for the correctness of this assumption. A possibility to test the extent to which these differences are of genetic nature, consists in the comparison of the values obtained for individual plants and their progenies. It is known that in an autogamous population the coefficient of heritability, h^2 , is represented by the offspring-parent regression coefficient, $h^2 = b_{OP}$. Thus, the h^2 value can be used to answer the above question.

In the present experiment with the natural population Strř which appears to be highly variable and is of "summer annual" type, the number of days to the appearance of flower primordia and the number of rosette leaves were studied in 147 mother plants and their progenies. The number of offspring was variable from 10 to 19 with a mean of 12.6 plants per progeny. Both, the mother plants and progenies were grown under controlled experimental conditions (25±3°C, continuous illumination of 1250 lux). - The b values were estimated (1) as an unweighted regression of progeny means on mothers, (2) as a regression of individual progeny data on repeated maternal ones, and (3) as a weighted regression of progeny means on mothers (KEMPTHORNE and TANDON, 1953; REEVE, 1955). The results are given in the following table:

Mode of estimation	Number of days to appearance of the flower primordia	Number of rosette leaves
	$b \pm s_b$	$b \pm s_b$
(1)	0.5232* ± 0.0577	0.4576* ± 0.0505
(2)	0.4659* ± 0.0197	0.4424* ± 0.0175
(3)	0.4993* ± 0.0265	0.3517* ± 0.0324

* Exceeds the 0.1% point

The regression coefficients for both characters are thus highly significant and show that in the number of days to appearance of the flower primordia about 50 % and in the number of rosette leaves about 40 % of variability is of genetic nature. In other words, in the two characters 50 and 40 % of the phenotypic variation is represented by the additive component of genetic variability. Therefore, at least a half of the phenotypical differences observed in the population studied can be ascribed to genes. - The experiments will be continued.

References:

- CETL, I.: In "Arabidopsis Research", Rep.Int.Symp. Göttingen. Pp. 46-52, 1965
 KEMPTHORNE, O., and O.B. TANDON: Biometrics 1, 90-100 (1953)
 NAPP-ZINN, K.: Beitr. zur Phytologie, Festschr. H. WALTER. Pp. 39-49, 1964
 REEVE, E.C.R.: Biometrics 3, 357-374 (1955)

Inhibition of seed germination in *Arabidopsis thaliana* by Actinomycin D

A.F. CORCOS

(Kedzie Laboratory, Department of Natural Science, Michigan State University, East Lansing, Mich., U.S.A.)

The antibiotic Actinomycin D is known to inhibit irreversibly and specifically DNA-dependent RNA synthesis in animal cells at a concentration of about 0.04 µg/ml. Recent studies have indicated that to obtain the same results with plant cells the concentration of Actinomycin D has to be increased to at least 10 µg/ml. LANGE and MOHR (1956) have shown that Actinomycin D at the concentration of 10 µg/ml blocks the synthesis of anthocyanin in *Sinapis alba* seedlings. McCALLA and ALLAN (1964) using Actinomycin D at a concentration of 30 µg/ml were able to inhibit the chlorophyll development of *Euglena viridis*.

Preliminary tests had indicated that concentrations of at least 15 µg/ml were necessary to prevent the germination of *Arabidopsis thaliana*. An experiment was carried out in a Sherer growth chamber at 22°C under 24 hr full illumination (2500 foot candle). Seeds of the variety Estland (graciously sent to the author by Dr. LANGRIDGE) were germinated on minimal medium (VELEMINSKY and GICHNER, 1964) in 200 mm test tubes (one seed per tube). On medium in which 20 µg of Actinomycin were added the seeds were unable to germinate. Only in 3 tubes out of 30 they were able to swell and a greenish cast appeared. Observation under the dissecting scope indicated that in those cases the radicle elongated to only one eighth of a centimeter before growth stopped; but the cotyledons had enlarged and turned light green.

This experiment which was repeated 4 times seems to indicate that it requires 20 µg/ml of Actinomycin D to prevent the germination of *A. thaliana*. Further experi-

ments are now being carried out to find the concentration of Actinomycin D at which the seedlings are unable to grow after they successfully germinated in minimal medium.

References:

LANGE, H., and H. MOHR: *Planta* 67, 107-121 (1965)
 McCALLA, D., and R. ALLAN: *Nature* 201, 504-505 (1964)
 VELEMÍNSKÝ, J., and T. GICHNER: *Arabid. Inf. Serv.* 1, 34 (1964)

The author is indebted to Dr. W.GALL of the MERCK Company for furnishing the Actinomycin D which was used in this experiment

Germination of light requiring races of *Arabidopsis thaliana* in the dark after X-irradiation

Erna REINHOLZ

(Max-Planck-Institute for Biophysics, Frankfurt/M., Germany)

Since KUGLER (1951) it is known, that the race Hm (Hann.Münden) of *A. thaliana* has obligate light requirement for germination. But it was demonstrated (REINHOLZ, 1967) that gibberellic acid can substitute for the light during germination: Unirradiated seeds and such irradiated in an air-dried state with doses between 100 and 800 kR fully germinated in a few days.

Table 1: Germination of X-irradiated seeds of *A. thaliana*, race Hm, in the dark at 20-22°C after 2 months

Dose in kR	Experiment A											Experiment B			
	0	100	200	400	600	800	1000	1500	2000	3000	0	1000	1500	2000	
Number of seeds tested	778	604	487	541	873	615	566	522	573	656	1234	1008	675	1422	
% germinated	0	0.3	0.2	0	0.7	1.3	8.8	33.9	88.4	0	0	4.4	94.8	92.5	

In the meantime new data were obtained according to which high doses of X-rays are also able to influence the light requirement for the germination of Hm. As can be seen from Table 1 X-ray doses as high as 1-2 MR are necessary to induce germination in the dark. This removal of the germination inhibition by X-irradiation, therefore, is different from the well known stimulation effects of small doses. The effectiveness of only very high doses in these experiments seems to suggest that the obligate light germination might be related to a destruction of certain molecules. It is easily conceivable that inhibiting substances, or their precursors, or enzymes catalyzing the formation of such substances, are destroyed by X-rays. Another hypothesis to explain the observed germination in the dark after X-irradiation would be that the X-rays injure the cellular membranes leading the diffusible inhibitors to a more rapid leaching out during the initial soaking phase.

The problem of the influence of X-rays on the obligate light requirement for germination has been investigated previously. Already 1947 REINHOLZ tried to replace the visible light by an X-ray treatment of presoaked seeds of *Arabidopsis*, but in vain. Also DEVI, PRASAD and RILEY (1964) found no effect on the light requirement of germination in any of the irradiated seeds of *A. thaliana*, race Estland. The present data explain, why the earlier investigations were not successful: The applied doses were too low and the observation period was too short; the effect is established not earlier than the fifth day of germination and only after doses on air-dried seeds as high as 1 MR.

Under such conditions the *Arabidopsis* race Estland (Est-1) out of LAIBACH's sortiment revealed a quite analogous germination behaviour as the race Hm, as shown in Table 2.

Table 2: Germination of race EST-1 in the dark after X-irradiation

Dose in MR	Experiment A				Experiment B			
	0	1	1.5	2	0	1	1.5	2
Number of seeds tested	662	665	717	752	715	552	508	468
% germinated	0	68.0	0.8	0	0	83.7	1.0	0

References:

DEVI, S.K., K.N. PRASAD, and E.F. RILEY: *Radiat. Bot.* 4, 347-353 (1964)
 KUGLER, I.: *Beitr. Biol. Pfl.* 28, 211-243 (1951)
 REINHOLZ, E.: *FIAT-Report* 1006, 19 (1947)
 - : *Arabid. Inf. Serv.* 4, 16-17 (1967)

Morphactin-cytokinin interaction in seed germination of Arabidopsis thaliana, En-2

Daksha SANKHLA* and Narendra SANKHLA*

(Institute of Botany, University of Frankfurt/M., Germany)

Morphactins (certain derivatives of fluorine-9-carboxylic acid) represent a new class of recently characterised growth regulators (SCHNEIDER, 1964). These compounds resemble other growth retardants (CCG, Phospon, B-995 etc.,) in their overall growth retarding activity. However, whereas the growth effects of the latter chemicals are frequently mediated through inhibition of gibberellin biosynthesis in the treated plants (NINNEMANN et al., 1964), morphactins have been shown to be ineffective in this respect (TOGNONI et al., 1967). Recently, we observed an apparent antagonism between morphactin and kinetin during the germination of lettuce seeds (SANKHLA and SANKHLA, 1967). Since generally the response to growth retardants is extremely specific and is known to differ in various cultivars of the same species (CATHEY, 1964), it was thought worthwhile to extend the above observation on another plant. Results relating to the effect of morphactin-butylester, alone and in combination with kinetin, 6-benzylaminopurine and zeatin, are presented in this report.

Seeds of *A. thaliana*, En-2, (harvested 1966) were germinated at 25°C in the presence of various concentrations of the above mentioned growth substances. During the germination period the seeds received a light intensity of about 1000 lux for 16 hrs a day. Details of the experimental procedure have already been reported previously (SANKHLA and SANKHLA, 1968).

Substance	Concentration in ppm	% Germination after hrs		
		48	72	96
Water	-	60	90	100
Morphactin	15	12	45	55
"	30	2	22	30
"	50	0	0	10
" + kinetin	50 + 2	0	30	55
" + "	50 + 10	0	35	65
" + 6-benzylaminopurine	50 + 1	10	60	90
" + "	50 + 2	15	70	98
" + zeatin	50 + 0.5	20	80	95
" + "	50 + 1	35	85	100

As in lettuce (SANKHLA and SANKHLA, 1967) morphactin proved to be a potent inhibitor of seed germination also in *Arabidopsis* (table). Not only this, but all the cytokinins tested (kinetin, 6-benzylaminopurine, and zeatin) successfully reversed the inhibitory effect of morphactin on seed germination. In order of effectiveness zeatin appeared to be the superior.

There are several evidences (see SANKHLA and SANKHLA, 1967, 1968) which suggest that, since cytokinins can effectively overcome the inhibitory effect of a variety of both synthetic as well as naturally occurring growth inhibitors and growth retardants, it might be possible that both, endogenous cytokinins and inhibitors interact in bringing about growth and senescence. However, it should be borne in mind that often plants are capable of 'adding' or 'subtracting' entirely unrelated effects and hence the visual perception of results might not necessarily represent a true and genuine interaction between the growth substances that are being tested. This view appears to hold true especially for the process of seed germination, since there is no way of measuring the steady state of this process as a whole.

References:

- CATHEY, H.M.: Ann.Rev.Plant Physiol. 15, 271-302 (1964)
 NINNEMANN, H., J.A.D. ZEEVAART, H. KENDE, and A. LANG.: Planta 61, 229-235 (1964)
 SANKHLA, N., and D. SANKHLA: Planta 76, 47-51 (1967)
 - , - : Z.Pflanzenphysiol., in press, 1968
 SCHNEIDER, G.: Naturwiss. 57, 416-417 (1964)
 TOGNONI, F., A.A.De HERTOOGH, and S.H. WITWER: Plant & Cell Physiol. 8, 231-239 (1967)

Our best thanks are due to: Dr. G. SHAW for his gift of zeatin; Prof. K. EGLE for facilities; Dr. G. SCHNEIDER (of Merck AG) for his help in obtaining morphactin; Merck, Sharp & Dohme Ltd., for their gift of 6-benzylaminopurine and Deutscher Akademischer Austauschdienst for financial support.

Effect of helminthosporol and helminthosporic acid on seed germination and senescence of leaf discs of Arabidopsis thaliana, En-2

Daksha SANKHLA and Narendra SANKHLA

(Institute of Botany, University of Frankfurt/M., Germany)

Recently, helminthosporol (H-ol) and helminthosporic acid (H-acid) have been shown to act very much like gibberellin (GA) in several plant systems (HASHIMOTO et al., 1967; HASHIMOTO and TAMURA, 1967a). These substances have also been claimed to be active in inducing germination of light sensitive seeds of tobacco in total darkness (HASHIMOTO and TAMURA, 1967b). In *Arabidopsis* gibberellic acid is well known for its promoting effect on the germination of seeds kept in darkness (KRIBBEEN, 1957; REINHOLZ, 1967).

* On leave of absence from Botany Department, University of Jodhpur, India

Recently, we observed that gibberellin can also effectively retard the senescence of excised leaf discs of *Arabidopsis* (SANKHLA and SANKHLA, 1968). It seemed, therefore, of interest to evaluate the comparative efficiency of GA, H-ol and H-acid in the process of seed germination and senescence of leaf discs of *Arabidopsis*.

Seeds of *Arabidopsis thaliana*, En-2 (obtained through the kindness of Dr. KRANZ of this institute), and the mature leaves (33 days old) obtained from the plants grown in the green-house served as the experimental material. In the table 'percentage senescence' was calculated on the basis of percentage of chlorophyll degradation during the experimental period in comparison to the control.

Substance	Concentration in ppm	% Germination after days			% Senescence after hrs		
		7	14	21	24	36	48
Water	-	0	0	2	45	65	80
Gibberellin	10	10	50	75			
"	50	25	95	100			
"	100	70	100	100			
Helminthosporol	10	0	0	2	5	45	70
"	50				5	50	65
"	100	0	0	1	40	85	95
Helminthosporic acid	10	0	0	1	5	50	60
"	50				30	75	90
"	100	0	0	2	5	55	60

Results relating to the effect of H-ol, H-acid and GA in the darkness at 25°C have been presented in the table. It is clear that while GA significantly increased the dark germination, both H-ol and H-acid remained completely ineffective in this respect. However, in their effect on leaf senescence both H-ol as well as H-acid did indicate some positive response, but the effect was clear only initially and at lower concentrations. Further, this effect varied from strain to strain and often differences in response were noticed when leaves of different ages were used for experimentation. In contrast, gibberellin always gave constant and reproducible results (SANKHLA and SANKHLA, 1968). Thus it would appear that both H-ol and H-acid probably have a restricted activity in comparison to GA, and in *Arabidopsis* these compounds do not appreciably indicate gibberellin-like properties.

References:

- HASHIMOTO, T., A. SAKURAI, and S. TAMURA: *Plant & Cell Physiol.* 8, 23-34 (1967)
 HASHIMOTO, T., and S. TAMURA: *Plant & Cell Physiol.* 8, 35-45 (1967a)
 - , - : *Plant & Cell Physiol.* 8, 197-200 (1967b)
 KRIBBEN, F.J.: *Naturwiss.* 44, 373 (1957)
 REINHOLZ, E.: *Arabid. Inf. Serv.* 4, 16-17 (1967)
 SANKHLA, N., and D. SANKHLA: *Biol. Plant.*, in press, 1968

Grateful thanks are due to: Professor S. TAMURA (Tokyo) for his kind gift of H-ol and H-acid; Prof. K. EGLE for facilities and Deutscher Akademischer Austauschdienst for financial support.

Reversal of abscisic acid-induced senescence of *Arabidopsis* leaf discs by cytokinins

Daksha SANKHLA and Narendra SANKHLA

(Institute of Botany, University of Frankfurt/M., Germany)

Zeatin - the only identified cytokinin from higher plants has recently been claimed to exhibit much stronger cytokinin-like properties than kinetin, 6-benzylaminopurine and certain other cytokinins (LETHAM, 1967). In contrast, abscisic acid - a naturally occurring abscission-accelerating, dormancy-inducing, senescence-enhancing and germination-inhibiting substance appears to possess properties that are apparently opposite to those shown by cytokinins (OKHUMA et al., 1963; CORNFORTH et al., 1966; SANKHLA and SANKHLA, 1968 a,b,c). Earlier we observed that kinetin can effectively overcome the senescence-accelerating effect of abscisic acid (SANKHLA and SANKHLA, 1968b). The present experiments were undertaken to evaluate the comparative potentialities of two other cytokinins, viz., zeatin and 6-benzylaminopurine, in reversing the effect of abscisic acid in leaf senescence of *Arabidopsis*.

Our observations indicated that mature rosette leaves of *Arabidopsis* can be used as an excellent experimental material in studies relating to senescence because in these leaves the disintegration of chlorophyll in the dark is very quick and it is very easy to grow the plants of desired age in the green-house. Therefore, the present experiments were performed with leaf discs punched from mature rosette leaves (33 days old) of *A. thaliana*, En-2. Lots of 20 leaf discs (8 mm in diameter) were placed on petri dishes containing filter paper moistened with 5 ml of distilled water or an equivalent amount of the solution of the growth substance to be tested. Then the petri dishes were kept in the dark for 48 hrs. During this period two observations were made on chlorophyll content of the senescing leaf discs: one after 24 hrs and the other after 48 hrs. Chlorophyll was determined by simply extracting the pigment of 15 leaf discs with 10 ml solution of 80 % acetone and measuring the optical density of this extract at 665 nm.

Table: Abscisin II - cytokinin interaction in chlorophyll degradation
(Initial optical density = 0.320)

Substance	Concentration in ppm	Optical density after hrs	
		24	48
Water	-	0.150	0.090
Abscisin II	2	0.085	0.030
6-Benzylaminopurine	0.5	0.285	0.195
"	1	0.295	0.225
Zeatin	0.5	0.300	0.245
"	1	0.310	0.295
Abscisin + 6-benzylaminopurine	2 + 0.5	0.255	0.235
" + "	2 + 1	0.275	0.255
" + zeatin	2 + 0.5	0.270	0.255
" + "	2 + 1	0.300	0.280

It is clear from the Table that whereas abscisin II considerably hastens leaf senescence both 6-benzylaminopurine and zeatin act as strong senescence-delaying factors in leaf discs of *Arabidopsis*. A comparison of these results with our earlier results (SANKHLA and SANKHLA, 1968b) indicates that both these chemicals are far more superior to kinetin in delaying the senescence of leaf discs of *Arabidopsis*. Also, it was noted with interest that both the cytokinins tested successfully suppressed the senescence-accelerating action of abscisin II. In this respect, zeatin appeared to be superior to 6-benzylaminopurine.

Experiments designed to explore the mechanism of kinetin action in retarding cellular senescence in *Xanthium* have clearly shown that senescence is directed through RNA synthesis that is DNA dependent (OSBORNE, 1967). It has also been observed that the decline in RNA synthesis during senescence is not the result of loss of total DNA, but rather reflects a failure of the DNA to provide an effective template for RNA synthesis. In some way, therefore, kinetin would appear to maintain this template (OSBORNE, 1967). On the other hand, abscisin II has been shown to reduce the incorporation of ¹⁴C-leucine into protein (OSBORNE, 1967). Our unpublished results indicate that abscisin II-induced inhibition of lettuce seedling growth could be greatly reversed by simultaneous addition of nucleic acid precursors. These results would probably suggest that one action of abscisin II might be exerted through nucleic acid metabolism. Incidentally it should also be mentioned that cytokinin - abscisin II antagonism has been observed also for the growth of calli obtained from the cotyledons of *Ipomea* and for seed germination of *Arabidopsis* (SANKHLA and SANKHLA, 1968c,d). It might be quite possible that the interaction between the endogenous cytokinins and abscisin II functions in bringing about growth and senescence.

References:

- LETHAM, D.S.: *Planta* 74, 228-242 (1967)
 OKHUMA, K., O.E. SMITH, J.L. LYON, and F.T. ADDICOTT: *Science* 142, 1592-1593 (1963)
 CORNFORTH, J.W., B.V. MILBORROW, and G. RYBACK: *Nature* 210, 627-628 (1966)
 SANKHLA, N., and D. SANKHLA: *Physiol. Plant.* 21, 190-195 (1968a)
 - , - : *Experientia*, in press, 1968b
 - , - : *Naturwissenschaften*, in press, 1968c
 - , - : *Z. Pflanzenphysiol.*, in press, 1968d
 OSBORNE, D.J.: *Symp. Soc. Expt. Biol.* 21, 305-321 (1967)

Grateful thanks are due to: Prof. K. EGGLE for facilities; Prof. J.W. CORNFORTH for donating a sample of abscisin II; Dr. G. SHAW for his gift of zeatin; Merck, Sharp & Dohme Ltd., for supplying 6-benzylaminopurine and Deutscher Akademischer Austauschdienst for financial support.

Growth of *Arabidopsis thaliana*, En-2, in response to added morphactin

Daksha SANKHLA and Narendra SANKHLA

(Institute of Botany, University of Frankfurt/M., Germany)

Recently certain derivatives of fluorine-9-carboxylic acid (morphactins), which lastingly influence growth and morphogenesis in higher plants, have been characterised as novel growth regulators (SCHNEIDER, 1964). Like other growth retardants (CATHEY, 1964) these chemicals can also effectively retard growth, inhibit germination, delay leaf senescence, and the foliage of the treated plants develop intense green color (SCHNEIDER, 1964; SANKHLA and SANKHLA, 1967; HARADA, 1967). Moreover, as in the case of other growth retardants exogenously applied gibberellin can also reverse some of the growth effects of morphactins (ZIEGLER et al., 1966). However, the apparent similarity between growth retardants and morphactins ceases at this point. Morphactins unlike growth retardants (NINNEMANN et al., 1964) do not affect gibberellin biosynthesis (TOGNONI et al., 1967; KRELLE and LIBBERT, 1967) and are known to disturb phototropism of shoots and geotropism of roots (KHAN, 1967). Recently, an apparent antagonism between morphactins and cytokinins has also been recorded (SANKHLA and SANKHLA, 1967). No detailed account is yet available on the effect of morphactins on growth and flowering of higher plants. In furthering our knowledge relating to the role of these chemicals in plant growth we observed that morphactins produce certain striking changes in the growth habit of the treated *Arabidopsis* plants; the observations are summarized in this report.

Plants of *A. thaliana*, race Eb-2, were raised in groups of 20 each in a greenhouse as described elsewhere (SANKHILA and SANKHILA, 1968). When the seedlings were 15 days old 20 ml solution of the desired concentration (ranging from 100 ppm to 500 ppm) of morphactin-butylester (obtained as a gift from Merck AG, Germany) was sprayed onto each pot. A single such treatment greatly inhibited the extension growth of the main shaft as well as of lateral branches; as a result the treated plants appeared much dwarfer in comparison to the controls. Not only this, morphactin treatment completely



Figure 1: Plants and inflorescences of *Arabidopsis* treated with morphactin. a (from left to right) Control, 100 ppm and 500 ppm. b Branches showing double flowers and fruits

released the dormancy of lateral branches (Figure 1a) and greatly stimulated the formation of 'secondary rosette leaves'. These leaves were initially dark green and extremely hairy, but soon developed an anthocyanin like pigment on the lower surface, got rolled upwards and simulated a spatula in their appearance. As the concentration of morphactin was increased (100 ppm to 500 ppm) the visible appearance of floral primordia in the treated plants was slightly delayed. Of special interest, however, was that the lateral branches of a few plants under these conditions frequently indicated various degrees of fasciations and/or produced - besides normal flowers - flowers that appeared to be almost double replicas of the normal ones (Figure 1b). Such flowers generally developed 8 sepals, 8 petals, 10-12 stamen and two bicarpellary laterally fused ovaries. Sometimes even 'triple flowers' were observed. It is not clear whether this increase in the number of floral parts was due to just splitting of the existing primordia or initials, or was the result of the union of two buds. Most of these flowers developed normally and matured into double or triple fruits that contained perfectly viable seeds. In contrast, in some of the flowers developing on 'late' lateral branches either the sepals and petals were dropped at an earlier stage or these organs as well as anthers did not develop at all and the flowers indicated a distinct tendency towards femaleness. In addition, following treatment with morphactin the senescence of the whole plant was greatly delayed.

The results of experiments designed to study morphactin-gibberellin interaction indicated that gibberellin effectively reversed the inhibition of extension growth caused by the former substance, but remained ineffective in reversing the main effects caused by morphactin, viz., release of dormancy of lateral buds, formation of secondary rosette leaves, induction of fasciation in lateral branches and abnormalities of floral parts. Thus these results appear to support the earlier postulate (TOGNONI et al., 1967; MANN et al., 1966) that gibberellin and morphactin act independently of each other. As a matter of fact our unpublished results relating to growth and flowering of *Nicotiana glauca* suggest that in their mode of action morphactins probably resemble more to synthetic substances like 2,4-D and TIBA. Recent results of TOGNONI et al. (1967) as well as KRELLE and LIBBERT (1967) also indicate that morphactins might affect auxin metabolism. The gross morphological effects observed in the present investigation are also not unlike those shown by 2,4-D and TIBA. However, until further experiments have been carried out no definite opinion can be put forth regarding the exact site of action of morphactins.

References:

- CARNEY, H.M.: *Ann. Rev. Plant Physiol.* 15, 271-302 (1964)
 HARADA, H.: *Naturwissenschaften* 54, 95 (1967)
 KHAN, A.A.: *Physiol. Plant.* 20, 306-313 (1967)
 KRELLE, B., and E. LIBBERT: *Planta* 76, 179-181 (1967)
 MANN, J.D., H. HILD, E. YUNG, and D. JOHNSON: *Plant Physiol.* 41, 1751-1752 (1966)
 NINKEMANN, H., J.A.D. SEBVAART, E. KENDE, and A. LANG: *Planta* 41, 229-235 (1964)
 SANKHILA, N., and D. SANKHILA: *Planta* 76, 47-51 (1967)
 - , - : in press, 1968
 SCHNEIDER, G.: *Naturwiss.* 57, 416-417 (1964)
 TOGNONI, P., A.A. De HERTOGH, and S.H. WITWER: *Plant & Cell Physiol.* 8, 231-239 (1967)
 ZIEGLER, H., D. KÖHLER u. B. STREITZ: *Z. Pflanzenphysiol.* 54, 118-124 (1966)

We are grateful to Prof. K. EGLE for providing facilities and Deutscher Akademischer Austauschdienst for financial support.

Chromosome numbers in Arabidopsis species

T.N. GINTER and V.I. IVANOV

(Institute of Medical Radiology, Obninsk, U.S.S.R.)

Three species of the genus Arabidopsis were studied cytologically: A. korshinskyi BOTSCH., obtained by the courtesy of S. YUNUSOV from the Botanical Institute in Dushanbe, A. griffithiana (BOISS.) BUSCH (A. pumila (STEPH.) BUSCH ?), and A. wallichii (J.D. HOOK.) BUSCH, both obtained by the courtesy of E.YA. RAZHIVINA from the Botanical Institute in Leningrad. Standard squash preparation technique was applied.

In all the species mitosis was studied in shoot tip cells and in young sepals. In some cases mitosis was also studied in leaf meristematic cells (A. korshinskyi) and in root tip cells (A. griffithiana). Besides, meiotic preparations of pollen mother cells and tetrads in A. korshinskyi and A. griffithiana were also examined. The following chromosome numbers were found:

<u>A. wallichii</u>	2n = 16,
<u>A. griffithiana</u>	2n = 32,
<u>A. korshinskyi</u>	2n = 48.

It is to be mentioned, however, that in some of the examined cells the chromosome numbers deviated from the above given ones by ± 2 .

Indirect measures of embryo development in interspecific crosses

Brigitte BERGER

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

According to MÜLLER (1963) the testa of the Arabidopsis seed colours brownish as soon as the pollen tube enters the embryo sac. This discoloration happens even without embryo development, but only after fertilization. Without fertilization the ovule collapses into a white mass of tissue. For an early determination of seed setting as well as for analyses of the basis of interspecific incompatibilities between species related to Arabidopsis this character proved to be of special interest. Silique elongation was included into the following study on indirect measures of seed development.

19 different species of the genera Arabidopsis, Cardaminopsis, Arabis, Cardamine, Sisymbrium and Turritis were combined in 194 different crosses (for details cf. BERGER, 1968). In most of the combinations the siliques started ripening under the prevailing culture conditions 3 weeks after pollination. At this time the colour of the ovules and the length of the siliques were determined and simultaneous paraffin preparations were examined.

The results demonstrated a close correlation between testa coloration and the stage of the embryogenesis. It was possible to distinguish the following 5 different types of seed development:

1. The ovule is collapsed to a white mass of tissue - the egg cell is unfertilized;
2. the testa is light brownish, and only in the region of the chalaza dark brown - tightly coiled pollen tubes may be observed in the embryosac of some ovules (3 weeks after pollination!); in other cases enlarged "zygotes" or proembryos with up to 4 cells are found in the paraffin sections;
3. not only the size of the ovule, but also its dark brown colour is similar to that of normal ripe seeds - the embryo, however, does not pass over the juvenile stage of a globular embryo;
4. the outward appearance equals to the foregoing stage 3; the ovule is definitely not collapsed - the included embryo is "heart-shaped" with the cotyledons clearly differentiated;
5. the seed is fully developed, its coat normal brown - the embryo reaches its final shape.

As another criterion for seed setting the final length of the silique was used. The figure shows the dependence of the silique length on the number of the fertilized ovules. It was interesting to note that the elongation of the silique - similar to seed coat coloration - was only induced by fertilization and independent of any further development of the embryos.

In addition of the results obtained in studies on pollen tube growth (BERGER, 1966; 1968) the compatibility between Arabidopsis and 18 related species was determined with the aid of these 3 criteria: ovule coloration, silique length, and final stage of embryo development.

References:

- BERGER, B.: Arabid. Inf. Serv. 3, 13-14 (1966)
- : Beitr. Biol. Pflanzen, in press, 1968
MÜLLER, A.: Biol. Zentralbl. 82, 133-163 (1963)

(Figure see Page 24)

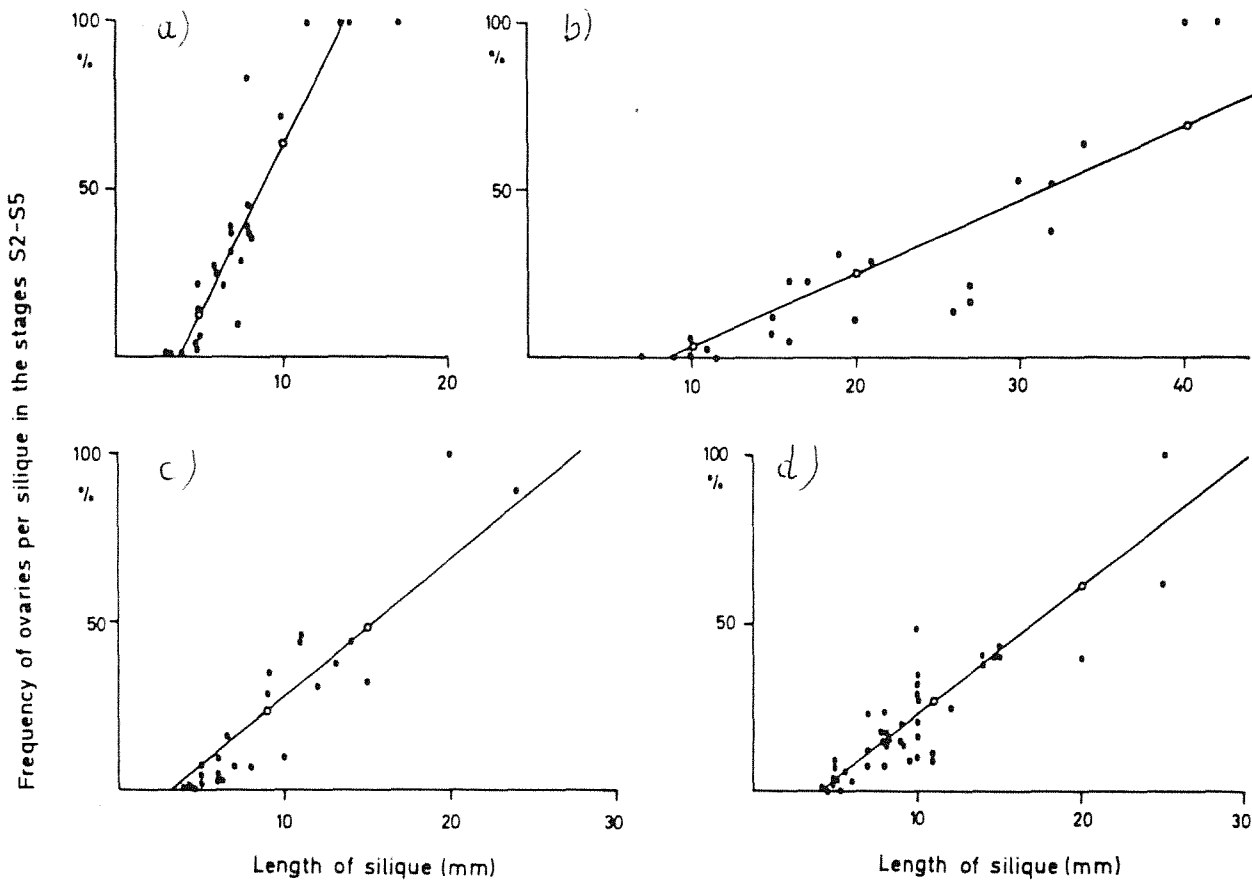


Figure: Correlation between length of the silique and seed setting after interspecific pollinations of the species Arabidopsis thaliana (a) Cardaminopsis arenosa (b) Arabidopsis pumila (c) and Cardaminopsis suecica (d)

Relation of embryo to endosperm development after interspecific pollinations

Brigitte BERGER

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

In order to clarify the developmental basis of the incompatibility between different species within the taxon Arabidopsis 194 crosses were analysed (BERGER, 1968). Fertilization was observed in 15% of these combinations, but only 3% of these cases yielded fertile seeds. The reason for this failure was investigated. The siliques were fixed every second day after pollination and paraffin sections were microscopically examined.

In intraspecific crosses the zygote was divided into two cells 2 days after pollination. 4 days later the apical part of the linear proembryo was differentiated into a globular embryo-"Anlage". Finally 12 days after pollination the embryo was full grown with the large hypocotyl and the flat cotyledons side by side. Until the sixth day after pollination the endosperm nuclei rapidly increased in number. Then the endosperm changed into the cellular state and was slowly reduced until its entire disappearance at about 12 days after pollination.

In interspecific combinations the same developmental processes were recorded, but with varying temporal delay. In no case morphologically abnormal embryos were found. The normal sequence of stages seemed to be blocked at different steps. Unexpectedly, however, the endosperm development was not retarded after interspecific pollinations, but followed the regular pattern. The maximum endosperm was observed 6 days after pollination, its breakdown at about 12 days. This means that the development of the endosperm and the embryo are independent from each other. In intraspecific combinations both are synchronized and the embryo enlarges at the expense of the endosperm. Hybrid embryos, however, are not full grown before the endosperm breaks down and their development, therefore, is blocked at about 12 days after pollination. In those combinations, e.g., Arabidopsis pumila x Arabidopsis thaliana or Arabidopsis pumila x Sisymbrium altissimum, in which the embryo reached more than 0,1 mm in length, cultures on 0,8% agar [minerals and 2% sucrose after BOUHARMONT (1961) + 0,2% yeast extract] were successful.

References:

- BERGER, B.: Beitr. Biol. Pflanzen, in press, 1968
BOUHARMONT, J.: Euphytica 10, 283-293 (1961)

Genetically determined differential recombination in the two sexes

G.P. REDEI

(Department of Genetics, University of Missouri, Columbia, Mo., U.S.A.)

The peculiar Gf factor in linkage group 2 decreases the recombination frequency in a chromosomal region of the female (REDEI, 1965). It has been observed furthermore that from 520 pollen grains tested, only 166 (31.9%) carried the gametophyte factor (Gf), while none of the functional eggs transmitted Gf. Tests on additional 1794 chromosomes of the male gametophyte demonstrated a 34.9% (611) transmission of Gf, while none of the additional 647 female second chromosomes were abnormal, thus agreeing with previous observations.

Table: Difference in recombination frequency in the two sporocytes in the presence of factor Gf. Constitution of the Gf carrier was Gf + py/+ hy +

	No. of chromosomes tested	Recombination frequency in region		
		<u>Gf - hy</u>	<u>Gf - py</u>	<u>hy - py</u>
Male	1749	21.7	26.5	6.5
Female	647	12.3	16.0	4.3
Reduction in the female (%)		43.3	39.6	33.8

In the present experiments recombination was studied in three regions with Gf + py/+ hy + plants. Results summarized in the table demonstrate that the crossing over reducing effect of Gf in the female is polarized. The reduction is greater in the immediate vicinity of the factor than in distal regions. The presence of Gf in the microsporocyte does not appear to have an influence on recombination.

Recently obtained other lines of evidence seem to indicate that of the three markers used, Gf is proximal to the centromere. Thus the sex differential recombination in Arabidopsis is similar to that observed by RHOADES in chromosome 5 of maize. That particular chromosome, however, did not display the peculiar behavior of the Gf carrier, i.e., the complete absence of female transmission. Both genes may be situated in the heterochromatin, since Arabidopsis chromosomes are also heterochromatic in the vicinity of the centromere.

References:

- REDEI, G.P.: Genetics 51, 857-872 (1965)
 RHOADES, M.M.: J. Amer. Soc. Agr. 33, 603-615 (1941)

Inheritance of lethal chlorophyll mutants in tetraploid Arabidopsis thaliana

J. BOUHARMONT and J. Van Den HENDE

(Institut Carnoy, Université Catholique, Louvain, Belgium)

Arabidopsis thaliana is a suitable material for the laborious study of tetrasomic inheritance because of its well known biological characteristics and the following three features as well: (a) the easy production of tetraploid strains which are highly fertile (BOUHARMONT, 1965), (b) an efficient induction of numerous mutations by the 1-methyl-3-nitro-1-nitrosoguanidine (MÜLLER and GICHNER, 1964) and (c) the possibility of using lethal mutations via the embryo-test of MÜLLER (1963).

Autotetraploid plants, heterozygous for chlorophyll mutations, have been obtained by two ways: (1) treatment of a tetraploid strain by MNG (1-methyl-3-nitro-1-nitrosoguanidine) and (2) chromosome doubling of a diploid offspring containing plants heterozygous for these mutations. The colchicine treatment was performed by placing a drop of a 0.1% solution on the apical meristem of seedlings. For the induction of mutations the seeds were presoaked in water (24 hrs) and treated in a solution of MNG (0.08 g/l; 24 hrs).

(ad 1) In tetraploid individuals the MNG treatment produces triplex heterozygotes. The M_2 -generation (observed at the embryo stage in the siliques of the M_1 -plants) contains duplex, triplex and quadruplex genotypes in a proportion of 1 : 2 : 1 (if chromosome segregation occurs). These three types of embryos are green and viable. In the M_3 -generation the d u p l e x plants segregate into quadruplex, triplex, duplex, simplex and nulliplex genotypes in a proportion of 1 : 8 : 16 : 8 : 1. It is necessary to observe a large number of seeds, since only the one nulliplex out of 36 individuals is homozygous recessive and phenotypically mutant. Moreover, only a maximum of 25% of the M_2 -plants is of the duplex type and the actual number of heterozygous M_2 -plants is always lower because of the chimeric nature of the M_1 -plants after mutagenic seed treatment. - The seeds collected on duplex M_2 -plants were sown. But it was only in the siliques of M_3 -plants (containing the M_4 embryos) that an adequate proportion of lethal mutants was recovered. Especially the s i m p l e x M_3 -plants (8 out of 36) theoretically give rise to 25% nulliplex lethal embryos. In some instances the number of white embryos corresponded to simplex or duplex segregation ratios, but in other cases they were lying between 1/4 and 1/36.

(ad 2) It is easier to produce heterozygous tetraploids by inducing the mutation at the diploid level. This method also allows to compare the transmission of a same

mutation at both chromosome levels. The seedlings, treated with colchicine, were derived from a heterozygous diploid plant, segregating about 25 % white embryos (the proportion, calculated from more than 9,000 embryos on several plants for two generations, was 24.3 %). The green plants of the colchicined population thus contained about 66 % heterozygotes, yielding duplex types after doubling of the chromosome number. Tetraploid plants were detected in the following generation by the number of the germination pores and the size of the pollen grains. Among these tetraploids, 3 plants were found with a rate of lethal embryos not significantly differing from 1/4 (simplex), 8 with a rate approaching 1/36 (duplex) and 11 without white seeds. The total percentages were 21.97 for the simplex and 3.39 for the duplex plants. These numbers did not deviate significantly from the expected figures (25.00 and 2.78 %).

In the following generation the offspring of two of the supposed simplex types was studied. In the first instance, 9 plants corresponded very well with a simplex segregation ratio (23.43 % lethal embryos) and 3 with a duplex type (2.83 %). The second offspring contained about the expected number of duplex plants (10 out of 18) with a normal proportion of lethals (3.10 %), but the rest of 8 plants, supposed to be simplex, gave a number of white embryos (19.03 %) significantly too low.

Further studies are necessary to explain several unexpected ratios and to compare the transmission of the mutations at several polyploidy levels. It will then be possible to estimate for some mutations the relative frequencies of chromosome- and chromatid-segregation. Some of the deviating segregation ratios may also be caused by a lower viability of those embryos containing higher doses of the recessive mutant alleles.

References:

- BOURHAMONT, J.: In "The use of induced mutations in plant breeding". Symp. IAEA/FAO, Rome 1965. Pp. 649-657
 MÜLLER, A.J.: Biol. Zentralbl. 82, 133-163 (1963)
 - , and T. GICHNER: Nature 201, 1149-1150 (1964)

Pigment concentration and photosynthetic activity of some chlorophyll mutants of *Arabidopsis thaliana*

A.G. KASYANENKO and Yu.S. NASYROV

(Institute of Plant Physiology and Biophysics, Tajik Academy of Sciences, Dushanbe, U.S.S.R.)

Chlorophyll mutants of *Arabidopsis thaliana* are suitable material for investigations on gene action. In the present study the following non-allelic recessive types were used: chlorina 80/2, dark green 203/13, light green 80/1, viridoalbina 40/3, green with yellow spots 801/6 (all of them induced by γ -irradiation, 10-80 kR, of seeds), the mutants V 156, V 44, V 120 and V 24/3 (kindly supplied by Prof. G. RÖBBELEN, Göttingen) and the mutant gl₁ (provided by Dr. K.V. KVITKO, Leningrad).

These chlorophyll mutants were grown during the autumn and winter season in a greenhouse with a light-period of 12-14 hrs and a temperature of 18-25°C. Leaves of rosettes before flowering were used for analysis. The pigment concentrations were determined after paper-chromatographic separation (SAPOSCHNIKOV, BRONSTEIN, and KRASOVSKAYA, 1955). The "potential intensity of photosynthesis" was studied with C¹⁴O₂ (ZALBENSKY, SEMIHATOVA, and VOZNESENSKY, 1955). This parameter signifies the intensity of photosynthesis in the leaf, when light, CO₂-concentration (1%), temperature and some other factors are optimal.

Table: Pigment concentration and potential intensity of photosynthesis in chlorophyll mutants of *Arabidopsis thaliana* (L.) HEYNH.

Genotype	Pigment μ /g fresh weight					total	mg CO ₂ per g dry weight per hr
	Chlorophyll a	Chlorophyll b	Carotene	Lutein	Violaxanthine		
Wild type (En-2)	526	382	28	54	30	1020	240 ± 10
Chlorina 80/2	436	185	23	32	24	700	230 ± 10
Grünlich gelb V 156	280	124	28	35	13	480	220 ± 15
Dark 203/13	811	384	68	89	37	1389	195 ± 6
Green with yellow spots 801/6	282	335	32	45	22	716	196 ± 10
Virescence 80/1	382	562	42	60	28	1074	197 ± 15
Grünlich V 120	384	182	17	29	13	625	192 ± 7
Grünlich gelb V 44	267	60	39	11	17	394	162 ± 24
Glucosa gl ₁	324	182	24	36	26	592	138 ± 5
Grünadrig V 24/3	266	149	24	39	16	494	134 ± 2
Viridoalbina 40/3	334	150	17	41	29	571	50 ± 5

The table demonstrates the complex effect of mutant genes on the photosynthetic apparatus. In some chlorophyll mutants the concentration of plastid pigments is decreased, in others it is increased. Simultaneously the morphology of the plastids is altered (cf. KASYANENKO, 1967; KASYANENKO and TIMOPHEEFF-RESSOVSKY, 1967). In consequence of these differences in pigment concentration and plastid morphology the photoabsorbing capacity of the leaves and thus their photosynthetic activity is changed (cf. KASYANENKO and GILLER, 1967).

Therefore the indices for the "potential photosynthesis" are different in the mutants. Just so the relative efficiency of the chlorophyll unit, i.e., the "assimilation number", is variable. Many of the chlorophyll mutants are characterized by a comparatively high "assimilation number". Only two mutants, darkgreen 203/13 and viridoalbina 40/3, have a low "assimilation number".

References:

- KASYANENKO, A.G.: In "Issledovaniya po fotosintezu". Dushanbe, 1967
- , and Y.E. GILLER: Genetica No. 6 (1967)
- , and N.B. TIMOFFEEFF-RESSOVSKY: MOIP, No. 3 (1967)
SAPOSCHNIKOV, D.I., I.A. BRONSTEIN, and T.A. KRASOVSKAYA: Biochimiya 203/3 (1955)
ZALENSKY, O.V., O.A. SEMIHATOVA, and V.L. VOZNESENSKY: In "Metody primeneniya C¹⁴ dlya izucheniya fotosintesa". AN SSSR, Moscow, 1955

Existence of RNA-transmethylase in Arabidopsis

M. ABEELS

(Laboratoire de Cytogénétique, Université Catholique, Louvain, Belgium)

The existence of the RNA-transmethylase enzyme in *A. thaliana* was tested by using sRNA extracted from a mutant of *E. coli*, strain K 12-58/161 F⁻, which synthesized RNA in the absence of methionine (BOREK and RYAN, 1958). The enzyme mediates the incorporation of methyl groups from S-adenosylmethionine-methyl C¹⁴ into the nucleotides of the sRNA. The enzyme activity was measured on the basis of the radioactivity appearing in the sRNA after incubation with the enzyme. The sRNA was then hydrolyzed and submitted to electrophoresis. The experiments demonstrated the existence of transmethylase in *A. thaliana*. It was also found, that the four main nucleotides from *E. coli* were methylated to a different extent, when submitted to the action of the transmethylase of *A. thaliana*.

Experimental procedure: The sRNA from *E. coli* was extracted following the method of MONIER et al. (1960) and KIRBY (1956); the purification was made by chromatography on DEAE-cellulose. The plants, 5 weeks old, were frozen and homogenized. The medium for the extraction of enzymes contained: 0.1 M Tris buffer, pH 7.2, with 16 mM MgCl₂, 20 mM potassium-metabisulfite and 0.45 mM sucrose (ANDERSON and ROWAN, 1966). The assay mixture for the methylation consisted of 1 μM MgCl₂, 2 μM 2-mercaptoethanol, 10 μM triethanolamin buffer, pH 8.8, 10 μM C¹⁴-CH₃-S-adenosyl-methionine, 100 μM nucleotides as methyl deficient sRNA, and sufficient enzyme to incorporate at least 1 μM of C¹⁴-CH₃ groups (HURWITZ et al., 1964). After incubation for 20 minutes at 38°C 5 μM sodium pyrophosphate, 0.05 ml of 0.5% bovine plasma albumin, and 0.2 ml of 7% HClO₄ were added and the insoluble material removed by centrifugation. The pellet was resuspended and washed twice again. One part from the washed pellet was dissolved with 1.5 ml of 0.2 M NH₄OH, decanted on metal planchets, dried and counted. The other part after hydrolysis of the sRNA was submitted to electrophoresis with pyridine acetate buffer, pH 3.6, at 2000 volts. Nucleotides were localized in the UV-light and the radioactivity counted.

References:

- ANDERSON, J.W., and K.S. ROWAN: Biochem.J. 101, 9 (1966)
BOREK, E., and A. RYAN: J. Bacteriol. 75, 72 (1958)
HURWITZ, J., M. GOLD, and M. ANDERS: J. Biol. Chem. 239, 3462-3474 (1964)
KIRBY, K.S.: Biochem.J. 64, 405 (1956)
MONIER, R., M.L. STEPHENSON, and P.C. ZAMECNIK: Biochim. Biophys. Acta 43, 1 (1960)

Complementing alleles as a cause of apparent reversion of pyrimidineless mutants

Beatrix I. Van Den BERG and W.J. FEENSTRA

(Department of Genetics, Agricultural University, Wageningen, and
Institute of Genetics, The University, Groningen, The Netherlands)

The studies on reversion of pyrimidineless mutants in *Arabidopsis* were continued (see the previous report: Van Den BERG et al., 1967), and the hypothesis was tested that growth of a revertant is due to interallelic complementation between the original mutant allele (py) and an allele that has undergone a further mutation (py^c). In this conception the latter allele when present homozygously would lead to a deficient phenotype. The roughly 50 % deficient plants found in M₃- and further generations of selfed revertants then should for equal parts be composed of py py and py^c py^c. When crossed to the original mutant (py py) the former should give a deficient, and the latter a (semi-) wild progeny. Crossing of non-deficient plants from the aforementioned segregating generations by the original mutant line should result in progenies, segregating deficient to non-deficient in a one to one ratio.

Five revertant lines were tested in this way, the results are given in the following table. In order to select the deficient plants from segregating families, seeds were sown on a mineral medium. After the phenotype of the parents had been established, growth of deficient plants was restored by supplementation of the medium with thiamine. This supplementation had to be carried out at an early moment in order to obtain well growing plants required for crossing purposes. Because of this a rather large number

of plants had not reached the developmental stage necessary for distinction between deficient and non-deficient, and hence could not be scored.

Revertant	Original mutant allele	Segregation in families tested			Progenies from					
					deficient x original mutant			non-deficient x original mutant		
		deficient	non-deficient	no score	deficient*	non-deficient*	nr. of progenies**	nr. of plants		
							deficient	non-deficient	no score	
V 43 R1	py-2 ¹	189	189	184	47	56	24	277	189	51
V 163 R1	py-1 ¹⁴	356	366	53	30	58	13	212	199	27
V 446 R1	py-2 ²	60	66	50	16	8	10	232	151	26
V 462 R1	py-1 ¹⁵	45	49	7	19	12	7	117	110	39
V 632 R1	py-1 ¹²	113	97	24	2	4	6	67	64	8

* All homogeneous.

** All showed segregation in deficient and non-deficient plants; the totals per revertant are given.

The data show that two types do occur among the deficient plants, although there are rather large deviations from the 1:1 ratio. However, differences in recovery from the starvation period between the genotypes may account for this. Some of the segregating progenies show an excess of deficient plants, which is difficult to explain at this moment. Because of their nature we would like to call the alleles arisen by a second mutation "complementing alleles", and refer to the homozygous deficient types as "complementants".

These data then may be taken as to support our hypothesis outlined above, although they do not exclude an alternative hypothesis of an external suppressor, acting only in heterozygous condition. However, results of crosses of complementants to pyrimidineless mutants other than the parent line do not support the latter possibility. As has been reported previously (FEENSTRA, 1964), mutant alleles of classes py-1 and py-2 show between-group complementation. E.g., V 462 and V 43, when crossed give a semi-wild F₁. Thus, if growth of revertants were due to the action of an external suppressor, one would expect that the complementant from V 462, in which then the unchanged py-1 allele would be present, would complement with V 43, which is py-2, and, in an analogous way, the complementant from V 43 would complement with V 462. Both F₁'s were made, but turned out to be deficient. This is indicative of changes in the py-1, resp. py-2 alleles themselves, or at least of changes in the py-region, if py-1 and py-2 would turn out to be two different closely linked cistrons.

In our hypothesis, growth of revertants and comparable types may be considered as an extreme case of heterozygote superiority, or monogenic heterosis. Compared with the parent allele, a complementing allele would differ from it in one site only, assuming that we are dealing with point mutations.

In microorganisms interallelic complementation is a common phenomenon, but is thought to involve alleles differing in two sites. The occurrence of complementation between alleles differing in one site is not excluded, but must be much more difficult to find, since complementation tests are necessary. In a diploid organism like Arabidopsis a complementing allele is selected for in the presence of its parent allele, and will show up immediately after mutagenic treatment. - The work is being continued.

References:

- Van Den BERG, B.I., J. HEYTING, and W.J. FEENSTRA: Arabid. Inf. Serv. 4, 46 (1967)
 FEENSTRA, W.J.: Arabid. Inf. Serv. 2, 24 (1965)

Temperature-sensitive, thiamine-requiring mutants in Arabidopsis

S.L. LI and G.P. RÉDEI

(Department of Genetics, University of Missouri, Columbia, Mo., U.S.A.)

LANGRIDGE (1955) isolated first one temperature-sensitive, thiamine-requiring mutant of Arabidopsis thaliana. Its phenotype is not distinguishable from that of the wild type at temperature above 27°C. At lower temperature (18-23°C) the mutant plants fail to grow normally and become characteristically chlorotic. - The majority of our thiamine mutants are obligate auxotrophs that live only with an exogenous supply of thiamine. A few can grow to variable extent without thiamine under certain ranges of temperature and are said to be temperature-sensitive.

Three types of pyrimidine mutants show different responses to temperature. One mutant type (L-2-2) obtained through X-ray induction on unmarked background is lethal at higher temperature (28°C), but at lower temperature (15°C) its growth approaches that of wild type after a lag period. Another mutant (L-13-412) produced by EMS on al (white seed) background shows just the opposite response to temperature, lethal at lower temperature yet normal at higher temperature. The third pyrimidine mutant

[(L-12-59), EMS induced on gl (hairless) background], is quite similar to the second type described above. This third is lethal at lower temperature, but sub-vital at higher temperature. All of these three types are allelic and belong to the same locus as several temperature insensitive pyrimidine-requiring mutants. These pyrimidine mutants, though allelic, have opposite responses to temperature indicating that they represent different genetic sites of the pyrimidine locus.

Table: Temperature-sensitive, thiamine-requiring mutants in Arabidopsis. The plants were grown on minimal agar media (2% glucose and minerals) in aseptic test tubes. The light intensity was about 450 foot candles. The experiment at higher temperature was terminated after 28 days, at lower temperature after 56 days. The fresh weight is the average of around 18 (16 - 20) plants.

Mutants	Requirement	Average fresh weight (mg)		15°C/28°C
		28°C	15°C	
wild type	none	37.39	73.69	1.97
L-2-2	pyrimidine	2.10	16.43	7.82
L-13-412	pyrimidine	23.29	1.20	0.05
L-12-59	pyrimidine	16.40	4.02	0.24
L-12-107	thiamine	29.40	11.36	0.39
1018-16	thiamine	40.10	9.16	0.23

Another thiamine mutant [(L-12-107) induced by EMS on gl (hairless) background] shows similar response to temperature as one of the pyrimidine mutants. This mutant is semi-lethal at lower temperature but grows almost like a wild type at higher temperature. This thiamine mutant is allelic to LANGRIDGE's thiamine mutant (1018-16).

Reference:
LANGRIDGE, J.: Nature 176, 260 (1955)

Functionally different, allelic im mutants

G. RÖBBELEN

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

The variegated mutant im (im¹) was obtained by RÉDEI (cf. 1965, 1967) after X-irradiation of presoaked seeds as a rare event. Just so in our mutation experiments with X-rays only a single im type (im^{2B}) within about 50,000 M₂ families was found. After treatment of seeds or pollen with alkylating agents (EMS, ethylene imine or ethylene oxide), however, 64 new im mutants were observed in 22,347 M₂ families, i.e., over 100 times more than after the use of radiations. No im mutant ever appeared spontaneously.

Of the total number of im mutants (some of which were sterile or otherwise impaired) 31 separate lines could be established. These can easily be grouped according to their light sensitivity (cf. RÖBBELEN, 1968) into at least three classes of phenotypes: the first class, including 23 mutants is characterized by the mutant im², the second with 6 types by im^{5C}, while the third with the prototype im²⁴⁵ contains the remaining 2 mutants. The table demonstrates that under the same illumination im^{5C} rosettes are almost white, while simultaneously grown im²⁴⁵ plants are rather green and im^{5C} individuals intermediate. Though all of our mutants originated from the same genetic line

Leaf colour of different im mutants under identical culture conditions

Mutant	% seedlings with colour of cotyledons				chlorophyll per g fresh-weight in full grown rosettes
	white	>50% white	>50% green	green	
<u>im</u> ¹	75.5	18.4	6.1	0	189.3
<u>im</u> ⁵²	89.0	7.7	3.3	0	162.8
<u>im</u> ⁵⁶	10.6	59.6	28.7	1.1	348.2
<u>im</u> ²⁴⁵	5.7	27.3	46.6	20.4	529.4

(En-2) and proved to be allelic without exception, it now needs to be tested, whether their functional differences are due to a change of different "mutons" within the same gene or to different extragenic modifiers.

Reference:
RÉDEI, G.P.: In "Arabidopsis Research", Rep.Int.Symp., Göttingen 1965. Pp. 119-127
- : Genetics 56, 431-443 (1967)
RÖBBELEN, G.: Planta, in press, 1968

Many thanks are due to Dr. G.P. RÉDEI, who generously supported these investigations in his laboratory during the tenure of a Visiting Professorship of the author at the University of Missouri, Columbia, Mo., U.S.A.

Genotypic analysis of induced mutations for flowering time and leaf number
in Arabidopsis thaliana

H.A.S. HUSSEIN* and J.H. Van Der VEEN

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

The very early variety "erecta", which is an X-ray mutant from Landsberg (by REDEI), flowers after 22-25 days with 6-7 rosette leaves. From this 'line C', an EMS mutant was derived by BHATIA and Van Der VEEN (1964), which flowers 5-6 days later and has 6-7 more rosette leaves. This 'line 51' has now been found to differ from 'line C' in a single partially recessive gene. After seed vernalization, 'line 51' (e_1e_1) closely resembles 'line C' (E_1E_1), which does not respond (HUSSEIN and Van Der VEEN, 1965). Both lines were subjected to large scale selection experiments after EMS- or X-ray treatments. No selections earlier than line C could be obtained. This is in accordance with BHATIA and Van Der VEEN (1964).

The following groups of mutants were obtained. All these mutants were identified as single gene mutants, but tests of linkage or allelism have been only done with his groups (A, B, etc.). It should be noted that only fully fertile and vigorous mutant lines were selected, and that these were only crossed after M_5 :

(A) Late mutants with large effect, i.e., flowering at least 10 days later and having at least 10 rosette leaves more than the parent of origin: These are 3 about completely recessive EMS-mutants (e_2e_2 from line C; e_4e_4 and e_6e_6 from line 51) and 1 EMS-mutant with an intermediate heterozygote (e_3e_3 , from line 51). A 6 x 6 F_2 -diallel (and backcrosses), including lines C and 51, showed that all mutant genes are at different loci and only E_3-e_3 and E_4-e_4 are rather closely linked. It is interesting to note that these latter two mutants show relatively little response to seed-vernalization, and show the same type of genotype-season interaction. All other mutants mentioned in this paper (except l_3l_3 ; see later), give complete or nearly complete response to vernalization.

(B) Late mutants with relatively small effect from line C: These are 3 partially or about completely recessive EMS-mutants (l_1l_1 , l_2l_2 , l_3l_3), and 3 partially dominant or nearly completely dominant X-ray mutants (X_1X_1 , X_2X_2 , X_3X_3). A 7 x 7 F_2 -diallel (including line 51) showed that among these E_1-e_1 (the C-51 locus) and X_2-x_2 are closely linked, and that L_3-l_3 is probably loosely linked to these two.

(C) Late mutants with relatively small effect from line 51: In this group we have 1 partially recessive EMS-mutant (e_7e_7) and 1 completely recessive X-ray mutant (e_7e_7). These two loci are probably closely linked, but no linkage to E_1-e_1 could be detected.

(D) Early mutants from line 51: None of these is as early as line C. These are 4 partially or almost completely recessive EMS-mutants (v_1v_1 , v_2v_2 , v_3v_3 , v_4v_4) and 2 partially recessive X-ray mutants (v_5v_5 , v_6v_6). A 7 x 7 F_2 -diallel (including line C) revealed that, of these loci, v_6-v_6 is either very closely linked to E_1-e_1 , or v_6 is a different allele of e_1 at the E_1 -locus.

(E) A second-cycle selection programme (after EMS- or X-ray treatment) was started with one late mutant from line C (viz., e_2e_2). Of the 2 early EMS-mutants obtained, one (R_1R_1) is dominant towards early and has a relatively small effect, the other (R_2R_2) shows an intermediate heterozygote and has a large effect (halfway between line C and e_2e_2). R_1-r_1 and R_2-r_2 are linked to each other but not linked to E_2-e_2 . It should be noted that R_1 is the only dominant EMS-mutation (which in this case is towards early).

One of the main objectives of this study was to identify single gene segregations and linkage relationships for a character which often appears to show typical quantitative inheritance. Further details can be found elsewhere (HUSSEIN, H.A.S., Ph.D. thesis, Wageningen, in preparation).

References:

- BHATIA, C.R., and J.H. Van Der VEEN: In: "The use of induced mutations in plant breeding". FAO/IAEA Symp., Rome 1964, 497-503
HUSSEIN, H.A.S., and J.H. Van Der VEEN: Arabid. Inf. Serv. 2, 6 (1965)

* On leave from the University of Cairo

Detection of induced late mutants in the early race Dijon

J. KUČERA

(Department of Genetics, Purkyně-University, Brno, Czechoslovakia)

Late mutants with vernalization requirement were induced in the early race Dijon by HUSSEIN and Van Der VEEN (1965). In our experiments M_2 -populations of this race were cultivated after treatment with N-methyl-N-nitrosourea (MN) and γ -irradiation at high temperature ($25\pm 3^\circ$) and under continuous illumination (1250 Lux). These conditions are highly selective for the detection of induced vernalization requirement.

9 to 13 days after germination all of the untreated control plants showed rosettes with 4 leaves and flower primordia. In M_2 -populations, however, many plants were found with a considerably longer vegetative phase and a higher number of rosette leaves. As the time until the appearance of flower primordia was strictly correlated with the number of rosette leaves, only the latter was evaluated.

After γ -irradiation 73 late M_2 -plants (= 7.1%) were registered in 44 out of 100 M_1 -progenies. After MU-treatment 124 out of 624 M_2 -plants (19.9%) were definitely later. The number of plants with one or more additional leaves is given in the following table:

Treatment	Total M_2 -plants	Additional leaves				Total late plants
		+1	+2	+3	+50	
γ -rays	1031	65 (6.3%)	6 (5.8%)	0	2 (0.2%)	73 (7.1%)
MU	624	88 (14.1%)	28 (4.5%)	8 (1.3%)	0	124 (19.9%)

All together a slight developmental delay - equal to the middle early natural races - was induced in 195 plants (11.8%), while 2 plants (0.1%) were drastically changed and similar to late races with vernalization requirement.

The progenies of the selected late M_2 -plants were investigated under the same culture conditions. Because of this cultivation technique, however, the number of seeds harvested in M_2 was comparatively low. Therefore, only 81 progenies with 6 or more plants could be evaluated in the M_3 -generation. In 13 progenies (16%) the recorded developmental delay of the M_2 -plant proved not to be genetically fixed; but in 68 progenies the changed phenotype reappeared. 58 progenies of the latter group segregated in respect to lateness, while 8 progenies (9.9%) were uniform. In the segregating group, besides the phenotype of the M_2 -plants, there were (a) plants identical with the control in 43 progenies (53.0%) and (b) plants with larger developmental delay than in M_2 in 7 progenies (8.6%), while (c) 8 progenies (9.9%) were highly variable including the original M_2 -types and later plants as well. (d) In 2 progenies (2.5%) all of the M_3 plants were later compared to the M_2 . - The correctness of the classification in M_3 was examined in a M_4 -generation cultivated under the same conditions. The high number of segregating progenies in the M_3 points towards the complexity of the genetic control of the number of rosette leaves and the possibility of various types of mutation in this typically developmental feature.

Reference:

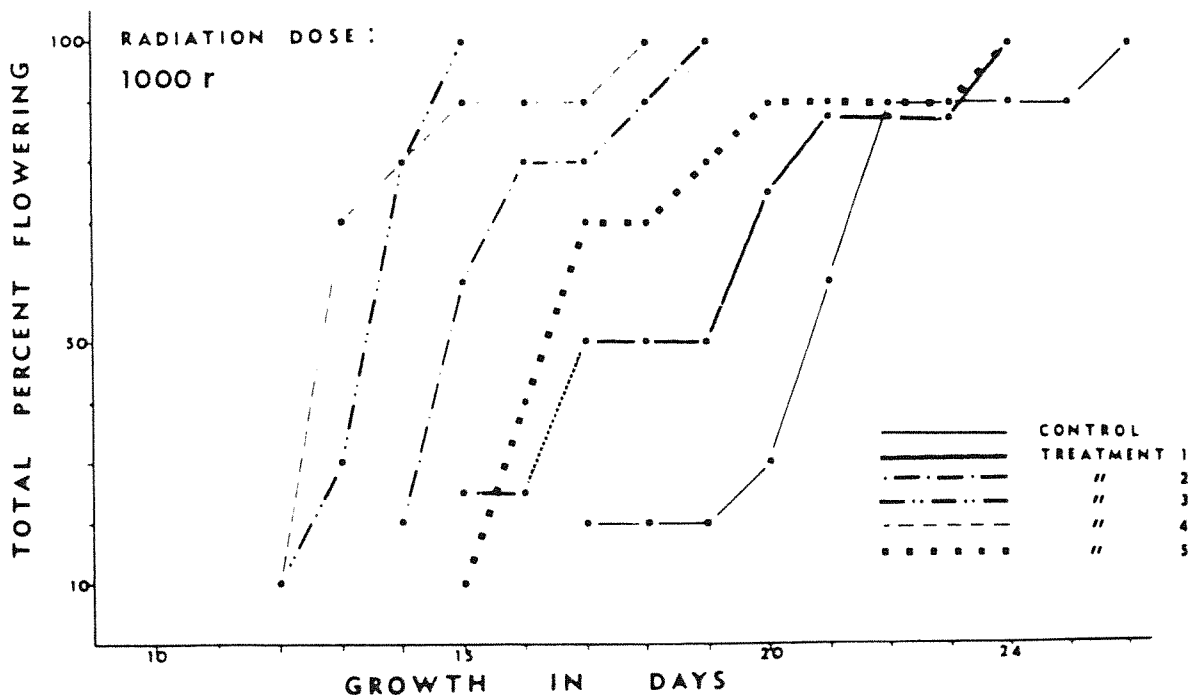
HUSSEIN, H.A.S., and J.H. Van Der VEEN: Arabid. Inf. Serv. 2, 6 (1965)

On radiostimulation of flowering in Arabidopsis thaliana

J. SAMUEL and C.F. KAMRA

(Laboratory of Radiation Biology, Dalhousie University, Halifax, N. S., Canada)

In an earlier report JEFFREY (1966) reported significant stimulation of flowering in Arabidopsis thaliana after incorporation of Sr^{90} and Cs^{137} . The stimulation was considered as a radiation effect since it was also induced by acute exposure of dry seeds to gamma rays.



In a preliminary experiment involving acute gamma irradiation of vegetative stages it has been found that not only flowering is stimulated but it seems to be of a higher magnitude than with dry seed irradiation. - Seeds were planted in test tubes on VELE-MINSKY's medium and grown under constant illumination of 40 W cool white fluorescent lamps at $25 \pm 1^\circ\text{C}$. The planted seeds were given a 4 day cold treatment at 4°C before transferring to the growth room. A dose of 1000 rads from a Cs ^{137} gamma beam was used and the material was irradiated at the same time on different days of growth:

(1) Dry seed irradiation on agar medium, (2) seeds irradiated on '0' day of growth, i.e., after the cold treatment, (3) young seedlings irradiated on the 4th day of growth, (4) seedlings irradiated on the 7th day of growth, (5) seedlings irradiated on the 9th day of growth. The growth and flowering of the treated plants was compared with that of controls and is presented in Figure 1.

There appears to be a pronounced stimulation in flowering after radiation exposure. Furthermore, the stimulation in flowering is apparently greater when seedlings are irradiated as compared to dry seed irradiation. In this experiment the most effective treatment was an irradiation of 4 day old seedlings. The total time required for 100% flowering in this treatment (3 days) also appears to be very short. - This preliminary experiment is being repeated at present. But positive radiostimulation of flowering seems to be of rather general occurrence in A. thaliana.

Reference:

JEFFREY, D.P.: Arabid. Inf. Serv. 3, 11 (1966). For details cf. M.Sc. thesis, Dalhousie University, Halifax, N.S., Canada, 1966

Mutations induced with a base analog

Y. HIRONO and H.H. SMITH

(Biology Department, Brookhaven National Laboratory, Upton, L.I., N.Y., U.S.A.)

An effort was made to induce mutations in Arabidopsis thaliana with a DNA base analog by growing plants in a medium (mineral nutrients + dextrose + agar) into which 5-bromodeoxy-cytidine was incorporated. Wild type seeds were sterilized with calcium hypochlorite, sown singly on the agar medium in a test tube, and the plants (M_1 -generation) were grown under well-controlled conditions in a growth chamber. Mature seeds were harvested from each plant, whose progeny (M_2 -generation) was then tested with about 50 seeds sown on soil in a pot kept in the greenhouse. For progeny tests of the next succeeding (M_2) generation, seeds harvested from plants grown in the pots were used.

Progeny tests have now been completed for about 1000 plants of the M_1 -generation. Among these M_2 -families, two segregated for albino lethal mutants and one for yellow-green leaf color mutants. The segregation ratios of wild type : mutant in each of these M_2 families were much greater than 3:1, thus indicating that the M_1 -plants were heterozygous and each parent contributed the mutation. The progeny tests in the M_2 generation showed that each of these mutants is controlled by a single mendelian recessive gene. More plant progenies are being tested and several additional families have recently been found which segregate for other mutants in the M_2 -generation. M_2 -progeny tests have not yet been completed for these families. - As controls about 1000 plants were grown in test tubes containing the agar medium without base analog added. These were progeny tested and no mutants were found.

Some slow-growing ("laggard") plants were observed to occur in both the M_2 -generation and corresponding control families, though their frequency was much higher in the former. Preliminary progeny tests indicate that the laggards were at least not due to single mutations. It seems likely that they developed from abnormal seeds which had been physiologically affected by the base analog or particular environmental condition under which the parental plant had been grown.

Research carried out at Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.

Presumptive evidence for the extrachromosomal nature of laggard mutants induced by 5-bromodeoxyuridine

M. JACOBS

(Laboratory of Genetics of Higher Plants, Free University of Brussels, Belgium)

In a previous note (Arabid. Inf. Serv. No. 4) a hypothesis of an extrachromosomal basis of 5-bromodeoxyuridine activity has been expressed, based on the high frequency and specificity of induced variants. For testing the validity of this hypothesis, reciprocal crosses between viable laggards obtained in a F_4 -progeny from plants treated by 5-bromodeoxyuridine and control plants have been made. Laggard plants chosen as parents are issued from families that include a high frequency of lethal and viable laggard seedlings.

The table shows that of the progenies out of 12 crosses between laggard ovules and control pollen, 11 gave laggard plants, lethal or viable. In progenies issued from the reciprocal cross, all plant phenotypes are normal. Arguments for the extranuclear heredity hypothesis are thus 3 in number: (1) differences observed between

reciprocal crosses, (2) specificity and high frequency of variants, (3) persistence of segregation in the progeny of laggard types. - The physical basis of the phenomenon is unknown.

Table: Phenotype distribution in the progenies of reciprocal crosses.

Laggard ♀ x normal ♂				Normal ♀ x laggard ♂			
No. of crossing	Viable laggard	Lethal laggard	Normal	No. of crossing	Viable laggard	Lethal laggard	Normal
1	1	1	9	1	-	-	4
2	-	2	6	2	-	-	9
3	-	3	3	3	-	-	11
4	-	1	11	4	-	-	12
5	4	3	18	5	-	-	18
6	4	-	6	6	-	-	3
7	-	-	2				
8	-	1	3				
9	2	2	4				
10	-	3	4				
11	-	1	6				
12	1	1	7				

Study of the incorporation of 5-bromodeoxyuridine into the DNA of Arabidopsis by neutron activation analysis

S. BONOTTO, M. JACOBS*, M.C. LONI, S. Van PUymbroeck and R. KIRCHMANN

(Department of Radiobiology, C.E.N., Mol, and

(*) Laboratory for Genetics of Higher Plants, Free University of Brussels, Belgium)

In a previous work (JACOBS and BONOTTO, 1967) we have studied the incorporation of labelled 5-bromodeoxyuridine (³H-BUDr) into the DNA of Arabidopsis, in different experimental conditions. It was found that a significant amount of radioactivity is incorporated by the plants when the base analogue is applied to the axils and even more when it is added to the culture medium. Moreover, the results obtained with aminopterin and FUDr, two inhibitors of pyrimidine synthesis, suggested that BUDr can replace thymidine even in the presence of a normal pool of thymidilate. The results reported by BROWN (1967) seem to be in accord with this hypothesis. In fact BUDr applied to the axils of the plants, suppressed temporarily the incorporation of ³H-thymidine, leading without any doubt to the formation of an BUDr-substituted DNA. This abnormal DNA may lead to the formation in the cells of abnormal proteins by the intermediary of modified m-RNA molecules (JACOBS, 1967) and consequently to the appearance of particular phenotypes, as chlorophyll mutants (JACOBS, 1964) or laggard plants (BROWN, BHATIA, and SMITH, 1965; JACOBS, 1967).

The possibility remains, however, that Arabidopsis, like bacteria, possesses some biochemical systems capable to restore the abnormal DNA, by excision of the analogue, or by removing the halogen from the analogue (HESLOT, 1966). In order to check this later possibility we have examined the DNA of Arabidopsis, Wil-2, for the presence of Br by neutron activation analysis, a technique which permits to measure extremely low amounts of the halogen (KIRCHMANN, 1960). Preliminary results obtained by this technique, have shown that the DNA extracted from plants cultivated in presence of BUDr, contains a significant amount of Br. The DNA of the controls does not contain the halogen. These results enable us to the following conclusion: The BUDr is incorporated into the DNA and remains unchanged in the macromolecule. We cannot exclude, however, that in some particular physiological conditions or for some races of Arabidopsis, a biochemical mechanism restores the abnormal DNA. In this case no mutants will be expected. - Detailed information about the extraction and the neutron activation analysis of Arabidopsis DNA will be reported in a further paper.

References:

- BROWN, J.A.M.: Arabid. Inf. Serv. 4, 30-31 (1967)
 - , C.R. BHATIA, and H.H. SMITH: In "Arabidopsis Research", Rep. Int. Symp. Göttingen. Pp. 171-183, 1965
 HESLOT, H.: Table ronde sur l'utilisation des mutations induites en horticulture, Gembloux, 1966, Pp. 6-13
 JACOBS, M.: Lejeunia 25, 1-7 (1964)
 - : Bull. Soc. Roy. Bot. Belgique 100, 259-281 (1967)
 - , and S. BONOTTO: Arabid. Inf. Serv. 4, 49-51 (1967)
 KIRCHMANN, R.: Ann. de Gembloux, 163-167, 2^e Trim. (1960)

We thank J.C. DELGOFFE for technical assistance. - This work has received assistance from the "Fonds National de la Recherche Fondamentale Collective".

Evaluation of the penetration and incorporation into DNA of tritiated thymidine in Arabidopsis seeds

M. JACOBS and S. BONOTTO

(Laboratory for Genetics of Higher Plants, Free University of Brussels, and Section of Phytobiology-Agronomy, Department of Radiobiology, C.E.N., Mol, Belgium)

There is great interest to know the quantitative evolution of the incorporation pattern of tritiated thymidine into the DNA of *Arabidopsis* meristems, among others to interpret sensitivity of embryo seeds to mutagens and to determine the length of the mitotic cycle and its main phases. Some preliminary results are here reported.

Seeds of *Arabidopsis*, Wil-2, were immersed in a solution of ^3H -thymidine (3 curies/mM; Radiochemical Centre, Amersham) at a concentration of 33 $\mu\text{C}/\text{ml}$ during increasing times up to 48 hrs and placed in an environment of continuous light at a temperature of $24 \pm 1.5^\circ\text{C}$. After washing, the extraction of acido-soluble fractions and acido-insoluble fractions has been operated and the amount of radioactivity measured in a scintillation spectrometer. The results are presented in the figure. These data indicate that after

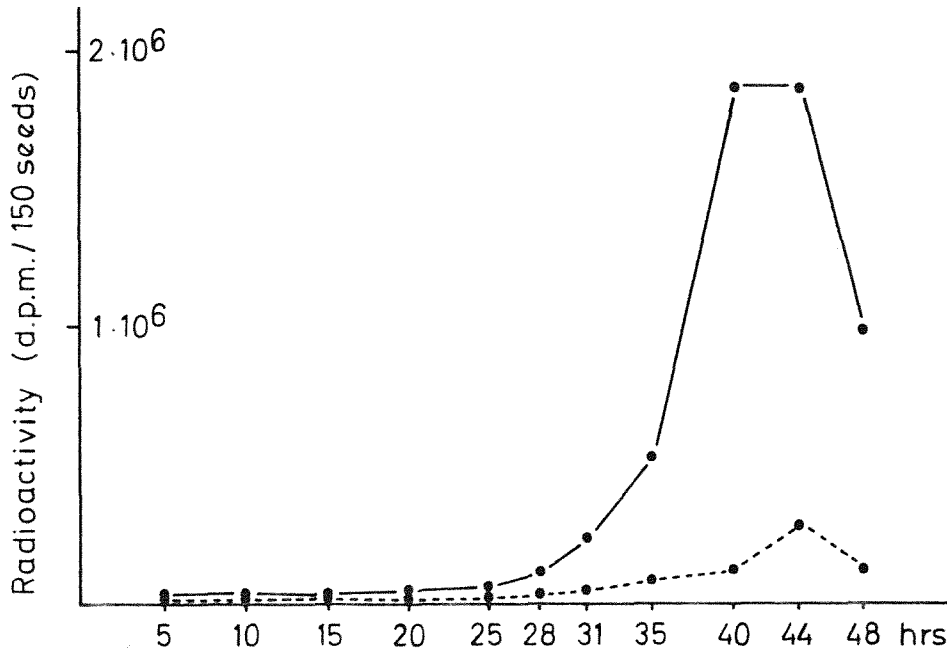


Figure: Kinetics of the penetration of ^3H -thymidine in acido-soluble fractions (—) and acido-insoluble fractions (---) of seeds.

soaking of seeds during 25 hrs, penetration and incorporation suddenly increase and reach a maximum \pm 40 hrs after sowing. The peak would represent the first wave of cells that go through the first metaphase. These results correspond approximately to those reported by RÖBBELEN (1965) who mentioned the beginning of DNA synthesis 30-35 hrs and the onset of mitosis later than 36 hrs after the soaking of the seeds.

Reference:

RÖBBELEN, G.: Beitr. Biol. Pflanzen 41, 323-35 (1965)

Effects of actinomycin D on macromolecular synthesis and growth in Arabidopsis plants

M. JACOBS and S. BONOTTO

(Laboratory for Genetics of Higher Plants, Free University of Brussels, and Section of Phytobiology-Agronomy, Department of Radiobiology, C.E.N., Mol, Belgium)

Actinomycin D, which is known as an inhibitor of DNA dependent RNA synthesis (HURWITZ et al., 1962) has proved to be a useful tool for the study of gene action (REICH and GOLDBERG, 1964). The morphological and biochemical alterations induced by this antibiotic on the embryonic development of amphibians and on the growth of *Acetabularia* have been studied by BRACHET and his collaborators (BRACHET, 1965). In higher plants, mitosis in root meristem of *Allium cepa* is blocked and differentiation of prevascular tissue disturbed in the presence of actinomycin (BAL and GROSS, 1963).

In *Arabidopsis* we have tested the influence of actinomycin D upon the incorporation of tritiated uridine (specific activity 760 $\mu\text{C}/\text{mM}$; Radiochemical Centre, Amersham) in seeds immersed in a solution which contained ^3H -uridine at a concentration of 20 $\mu\text{C}/\text{ml}$ in combination with two concentrations of actinomycin, 50 and 100 $\mu\text{g}/\text{ml}$ respectively. The results refer to the sum of radioactivity found at intervals of several hours in

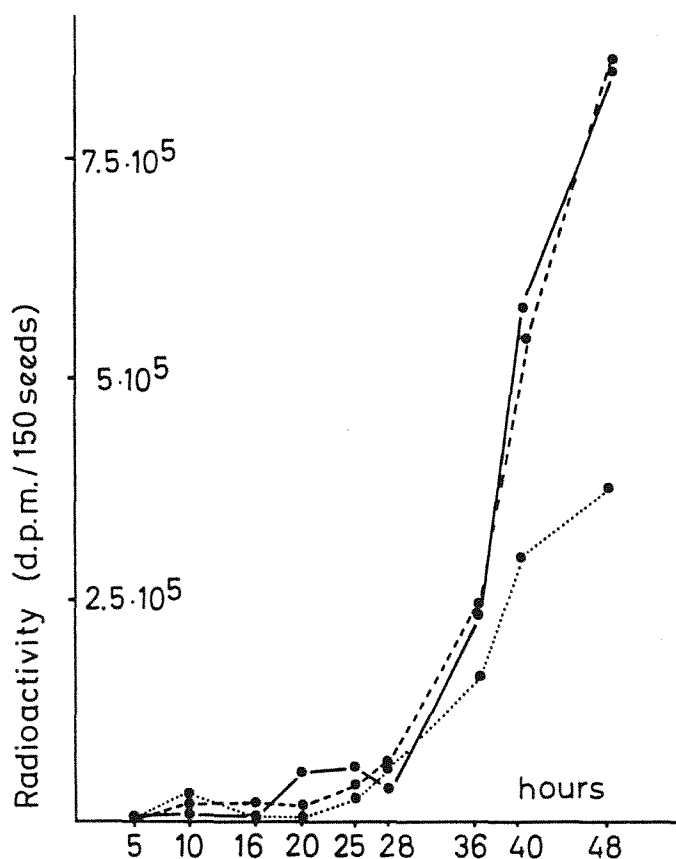


Figure 1: Kinetics of the ³H-uridine incorporation by seeds. — control, ---- 50 µg actinomycin/ml, 100 µg actinomycin/ml. Radioactivity: dpm/150 seeds.

References:

HURWITZ, J., et al.: Proc. Natl. Acad. Sci. U.S. 48, 1222 (1962)
 BAL, A., and P. GROSS: Science 139, 584-586 (1963)
 BRACHET, J.: L'année biologique 1-2, 21-48 (1965)
 REICH, E., and J.H. GOLDBERG: Prog. Nucl. Acid Research 3, 184-235 (1964)
 SCHMIDT, G., and S.T. THANNHAUSER: J. Biol. Chem. 161, 83-89 (1945)

We thank J.C. DELGOLFFE for his technical assistance.

Photoreactivation of UV-irradiated roots of Arabidopsis thaliana

E. AMANO

(National Institute of Genetics, Misima, Japan)

The recovery from irradiation with ultraviolet light (UV) by posttreatment with visible light was tested. Because of the weak penetrability of the UV it was intended to take advantage of the thin root of the *Arabidopsis* seedling for studying photoreactivation in a higher plant. Measurements were taken of the linear growth of the primary root after the UV irradiation.

The used seeds were a 1967 harvest of the strain "Estland" obtained from the Brookhaven National Laboratory, U.S.A. through Drs. A.H. SPARROW (BNL), K. YAMAKAWA (IRB) and T. FUJII (NIG). Seeds were surface-disinfected in 95% ethanol and 3% H₂O₂ (1:1) for 5 min at room temperature, rinsed twice with sterilized water, and sown on 0.8% or 1.0% agar in 9 cm Petri dishes. A pipette was found convenient, as suggested by VELEMINSKY and GICHNER in this newsletter, for placing the seeds on the agar media in lines. Then the dishes were stored in a refrigerator for 24 hrs at 3 - 5°C. After this cold treatment, the dishes were stacked together and placed on a support so that the agar plates were held vertically with seeds lined up horizontally, under the illumination of a 400W high pressure mercury fluorescent lamp

the acido-insoluble fractions obtained through a modified scheme of the procedure of SCHMIDT and THANNHAUSER (1945). The figure shows the expected incorporation into the acido-insoluble fractions. A depression of RNA synthesis appeared only for the highest concentration (100 µg/ml) of actinomycin, 25 hrs after sowing when the rate of incorporation suddenly increases in the case of the control seeds. It is also of interest to note that actinomycin inhibits not only the incorporation but also the penetration of the labelled riboside into the seeds.

Culture of *Arabidopsis* on mineral medium including actinomycin in a range of concentrations from 30 to 100 µg/ml shows in relation to these concentrations, a lot of perturbations, e.g., reduction of leaves surface, delay in development, partial bleaching of the leaves specially for the young ones and mortality. This inhibition of growth can be related to the decrease of RNA synthesis. Studies on the effects of this antibiotic on DNA and protein metabolism are now underway.

(3,000 lux) at 20 - 25°C. Several layers of glass (Petri dish walls etc.) were sufficient to filter out the harmful UV component of this light source. After germination the roots of the seedlings grew on the surface of the agar straight downward. When they were about 5mm long, they were irradiated with UV from four 4W germicidal lamps, at an intensity of 200 ergs/mm²/sec as measured by a Toshiba germicidal light meter. Luminescence from the germicidal lamps was light enough that no safety light was used. One group of the UV-irradiated plates remained in the dark room where the UV treatments were done. The other group was posttreated with visible light by continuing the culture under the first type of illumination. After these treatments, the dishes were again placed vertically, but with 90° rotated, so that the roots grew downward in a right angle to the parts grown before the treatment. The length of this second growth was measured through the bottom of the dish under a 10x binocular microscope with a micrometer.

Table: Photoreactivation of UV-irradiated primary roots of *Arabidopsis thaliana* seedlings

Experiment No.	Pretreatment	UV dose range tested (sec)	Posttreatment	Time of measurement (hrs after UV)	Photo- reactivation
1	light	10 - 600	dark 5 hrs	48	++
2	light	30 - 90	dark 5 hrs	20	+++
3	dark 1 hr	5 - 150	dark 6 hrs	24, 48	-*
4	dark	60	light 1-60 min**	48	****
5	dark	30	light 1-60 min**	20	+
6	light	30	light 1-60 min**	20	±, ****
	light	30	dark 5 hrs	20	± or +
7	light	10 - 180	dark 5 hrs****	26	± or -
			dark 1-3 hrs	46	****
8	light	30, 60	dark 1-3 hrs	24	++

* : The dark treatment suppressed root growth considerably; but the relative growth of the UV-irradiated roots to the non-irradiated ones was better than after light treatment.

** : After this short light period the samples were kept dark for 5 hrs.

*** : Considerable variation of root length was observed in the material.

**** : This posttreatment was followed by 1 hr light of the double intensity.

As shown in the table, in most of the experiments photoreactivation of root growth was detected, though the degree of the reactivation was not large. Some of the experiments were disturbed by the retardation of the root growth in consequence of the dark treatment. This interaction may be one of the reasons that the photoreactivation effect in our experiments was not as significant as in microorganisms (KELNER 1949). The use of root growth might be another and probably the main cause of the smallness of the detected photoreactivation. Less- or non-damaged cells can compensate the UV damage in terms of root length. This is the more likely as almost normal growth was regained after the temporary delay and the initial morphological reaction of UV irradiated roots. Therefore, cytological methods must be tried to confirm and further analyze photoreactivation in *Arabidopsis*.

Reference:

KELNER, A.: Proc. Nat. Acad. Sci. U.S. 35, 73-79 (1949)

A comparison of the somatic effects of X-rays and ethyl methanesulfonate

G.P. RÉDEI

(Department of Genetics, University of Missouri, Columbia, Mo., U.S.A.)

The effectiveness and efficiency of the two mutagens in title has been evaluated by various authors in different kinds of experiments. Some data obtained in our laboratory, by-products of some other studies, may be of interest because of the difference in the experimental technique.

F₂ seed of the cross *gl¹ ch¹ x gl² pa* (50% heterozygotes) was planted by the agar suspension technique at a density of 30 per 5 inch pots. The seed was either presoaked 24 hrs before X-irradiation or was treated directly overnight in an ethyl methanesulfonate solution of a given concentration. The number of seeds treated was about equal in both series. Within the ranges of these mutagenic treatments, germination was nearly identical. Survival and fertility were influenced by the environmental condition of the seasons. Because of the presence of the two alleles, *gl¹* and *gl²*, determining late flowering, the rosettes grew to a large size with excellent sectors developing. Many of sectors involved one or more whole leaves. Very small sectors were ignored because their classification is uncertain; distinguishing insect or fungus damage from genetic effects is not always possible. Sectors extending to at least a half leaf were cut off, and macerated in 2-3 drops of acetone in 8 x 75 mm test tubes with a glass rod. The crude extract was applied to thin layers of MN 300 cellulose powder on glass, and the pigments were separated in a petroleum-ether-acetone (10:1.2 v/v) mixture for 10 minutes. The presence or absence of chlorophyll b in the phenotypically *ch* sectors was easy to observe. In less clear cases the use of a Mineralight

USV-12 provided the required answer. Chlorophyll appears purple or greenish in the short wave length UV light, while chlorophyll b fluoresces bright red.

Details of the experimental results are represented in the table. The total number of sectors was larger after EMS treatments in comparison to that in the X-rayed series. The number of sectors varied greatly from experiment to experiment. The number of phenotypically ch sectors was larger in the EMS treated series. Chemical analysis demonstrated that with the exception of one experiment EMS did not produce more chlorophyll b deficient sectors than X-rays. Observations made in other experiments indicate, however, that similar EMS treatments produce more thiamine requiring mutants than X-rays.

Table: Classification of somatic sectors in gi^1ch^1/gi^2pa plants

Exp. No.	Dose	Population	No. of sectors								No. of <u>ch</u> estimated	
			total	white	non- <u>ch</u> -type yellow	phenotypically <u>ch</u>				total	% of population	
						total tested	chl-b free found	chl-b free %				
381	13.000 R 0.3% EMS	1317	18	2	0	16	16	9	56	9	0.68	
		1702	30	1	18	11	11	5	45	5	0.29	
386	13.000 R 0.5% EMS	1434	14	1	4	9	8	6	75	7	0.49	
		1056	185	39	120	46	19	6	32	15	1.42	
388	13.000 R 0.35% EMS	1122	14	0	4	10	8	8	100	10	0.89	
		1316	74	6	45	23	11	5	45	10	0.76	
394	13.000 R 0.24% EMS	1795	46	2	12	32	23	23	100	32	1.78	
		1327	56	1	33	12	12	2	17	2	0.15	
Total	X-ray EMS	5668	92	5	20	69	55	46			1.02	
		5401	345	47	216	92	53	18		32	0.59	

Somatic sectors in heterozygotes may appear as the result of mutation, deletion, recombination, non-disjunction, etc. Recessive mutants in the second generation of mutagen treated heterozygotes may appear only as a consequence of mutation or small deficiency. The frequency of ch sectors in the treated heterozygotes is at least five times higher than that of the combined frequency of alterations at the several thiamine loci and even much higher than the induced mutation rate of the ch locus itself as established from progeny analysis after similar treatments. Thus the vast majority of the ch leaf sectors were not produced by mutation or deletion of the wild allele at the ch locus. If this had been the case, EMS should have produced larger numbers of ch sectors than X-rays as observed in other experiments where thiamine mutants were isolated. (Note that much larger numbers of alterations can be identified as color sectors than the number of fertile inflorescence in chimeras bearing mutant sectors.) If X-ray is less efficient in producing mutations or small deficiencies than EMS, the equal or better efficiency of the first agent in the induction of the somatic ch sectors should be attributed to its effectiveness in causing recombination and non-disjunction, etc. Considering these data and the interpretation, the conclusion should be drawn that mutagenic efficiency should not be estimated on the basis of somatic sectoring.

Reference:

HIRONO, Y., and G.P. REDEI: Arabid. Inf. Serv. 1, 21 (1964)

Lack of adaptation to Deuterium by Arabidopsis thaliana

M.K. BHANDARKAR and B.K. GAUR

(Biology Division, Bhabha Atomic Research Centre, Trombay, Bombay, India)

Adaptation to higher D₂O concentrations by certain genotypes of bacteria, algae, yeast, molds, etc., is one of several interesting observations recently summarised by KATZ and CRESPI (1966). An improved capacity of the microorganisms to divide in higher D₂O concentrations than in lower ones was recognised by these authors as an expression of adaptation. The term 'adaptation' or 'habituation' in higher plants was first used by STEIN and FORRESTER (1963) for explaining the overcoming of low levels of tolerance to high concentrations of D₂O by pea roots. They expressed adaptation in terms of improved rate of growth due to stepwise rise of D₂O level in the growth medium. DOBBINS (1967) observed an initial decrease in the growth

rate of mesocotyl cells of *Zea mays* in 80% D₂O followed by a gradual rise in the growth rate with passage of time. Our studies (BHANDARKAR, 1967) on barley seedlings grown on 50% D₂O for 9 days also showed a decrease in D₂O-induced retardation of shoot-elongation rate with time. These observations illustrate the 'adaptation' of the tissue to D₂O.

An inhibition of growth in *Arabidopsis thaliana* by D₂O has been reported earlier (BHATTIA and SMITH 1966, BHANDARKAR and GAUR 1967). We have now investigated the possibility of adaptation to deuterium by this plant through stepwise increase in D₂O content in the media.

The culturing techniques, in the present study, were similar to those described earlier (BHANDARKAR and GAUR, 1967). The experiment consisted of six treatments as follows:

- D₁(0) = first generation of deuterium experiment: material grown on media with water.
 D₁(25) and D₁(37.5) = first generation of deuterium experiment: material grown on 25 and 37.5% D₂O respectively.
 D₂(0)* and D₂(25)* = second generation of deuterium experiment: material grown on H₂O and 25% D₂O respectively.
 D₂(1,25 + 1,37.5)* = second generation of deuterium experiment: material from D₁(25) grown on 37.5% D₂O.

*(Seeds obtained from D₁(0) and D₁(25) treatments were utilized in second generation).

Table 1: Days taken for the germination, growth and development of *A. thaliana* cultured on 0, 25 and 37.5% D₂O levels in the first and second generation.

Organogenetic development	D ₁ (0) (a)	D ₁ (25) (b)	D ₁ (37.5) (c)	D ₂ (0) (d)	D ₂ (25) (e)	D ₂ (1, 25 + 1,37.5) (f)
100% germination	4.0	5.0	7.0	7.0	7.0	13.0
<u>As observed in 50% of the population</u>						
1st leaf pair	2.0	3.0	5.0	2.7	6.8	10.0
2nd leaf pair	4.0	4.0	6.0	5.5	9.8	11.0
3rd leaf pair	5.0	5.0	8.0	9.3	12.0	17.0
4th leaf pair	8.0	9.0	13.0	12.3	13.5	20.4
5th leaf pair	10.0	13.0	14.5	16.4	19.7	27.3
Floral initial	13.0	17.0	20.0	18.0	27.8	32.0
1st open flower	20.0	22.0	23.0	25.7	32.0	39.0

The data in Table 1 represent the growth rates (as time taken to attain various growth and developmental stages) of plants grown in different deuteriated media and from seeds with and without any deuterium content. As reported earlier (BHANDARKAR and GAUR, 1967) postponement in the organogenetic development is observed when protracted seeds are grown in nutrient-agar containing 25% and 37.5% D₂O (columns a, b and c). The seeds used in D₁(0) and D₂(0) were obtained from soil and agar culture respectively. This may perhaps account for the relatively earlier growth of plants in D₁(0) than in D₂(0).

Table 2: D₂O-induced delay (days) in germination, growth and development of *A. thaliana* by differential deuteriation of seeds in D₁ generation (a, b, c, d, e and f are the same as in Table 1).

Organogenetic development	b-a (A)	c-a (B)	e-d (C)	f-d (D)	c-b (E)	f-e (F)
100% germination	1.0	3.0	0.0	6.0	2.0	6.0
<u>As observed in 50% of the population</u>						
1st leaf pair	1.0	3.0	4.1	7.3	2.0	3.2
2nd leaf pair	0.0	2.0	4.3	5.5	2.0	1.2
3rd leaf pair	0.0	3.0	2.7	7.7	3.0	5.0
4th leaf pair	1.0	5.0	1.2	8.1	4.0	6.9
5th leaf pair	3.0	4.5	3.3	10.9	1.5	7.6
Floral initial	4.0	7.0	9.2	14.0	3.0	4.2
1st open flower	2.0	3.0	6.3	13.3	1.0	7.0

Table 2 contains the data on the extent of delay due to various D₂O treatments. It can be seen from columns A and B that there is a greater delay in organogenesis (or decrease in growth rate) with increasing deuterium concentration in the culture media. The same is found to be true when partially deuteriated seeds, secured from D₁(25) treatment, are grown on 25% and 37.5% D₂O media (columns C and D). Taking into consideration their respective controls, plants from D₂(25) showed a more retarded growth rate than those from D₁(25) (columns A and C). Similarly, the delay in growth induced by an uniform rise of 12.5% D₂O appears to be greater in second generation than in the first (columns E and F). This is very significant yet contrary to expectation on the premise that prior culturing giving rise to partial deuteriation of seeds does impart adaptation in plants.

Taking improvement in growth rate leading to faster organogenetic development as a criterion of adaptation, our results show that under the experimental conditions followed here, Arabidopsis thaliana does not exhibit the phenomenon of adaptation to deuterium reported in some other higher plants.

References:

- BHANDARKAR, M.K.: M.Sc. thesis, Bombay Univ., Nov. 1967
- , and B.K. GAUR: Arabid. Inf. Serv. 4, 52-53 (1967)
BHATIA, C.R., and H.H. SMITH: Arabid. Inf. Serv. 3, 41 (1966)
DOBBINS, D.R.: Amer. J. Bot. 54, 640 (1967)
KATZ, J.J., and H.L. CRESPI: Science 151, 1187-1194 (1966)
STEIN, O.L., and G.M. FORRESTER: Planta 60, 349-359 (1963)

Pflanzen mit väterlichen Eigenschaften nach Röntgenbestrahlung

C.G. ARNOLD und D. CRUSE

Kastrierte Blüten von Arabidopsis thaliana wurden nach einer Röntgenbestrahlung mit unbehandeltem Pollen bestäubt. Die Befruchtung sowie die darauf folgende Embryoentwicklung wurde an 10 μ dicken Paraffinschnitten (mit Hämätin nach HANSEN gefärbt) verfolgt. Dabei fanden sich - neben Entwicklungsverzögerungen in der Embryoentwicklung - Fälle von dispermer Befruchtung der Eizelle sowie Embryonen, die Bezirke mit väterlichem und mit mütterlichem Kernmaterial nebeneinander enthielten. Dieses war in der frühen Embryoentwicklung aus der unterschiedlichen Kerngröße sowie der Lage der Kerne bzw. der Zellen zueinander zu folgern. Zellgruppen, welche die kleinen, offensichtlich männlichen Kerne enthielten, fanden sich in allen Teilen des Embryos und auch im Suspensor. Sofern solche Bezirke mit männlichen Kernen an der Bildung des Keimlings bzw. der Pflanze teilnehmen, können Pflanzen entstehen, die nur aus väterlichem Kernmaterial hervorgehen. - Um dieses zu prüfen, wurden kastrierte bestrahlte Blüten mit rezessivem Pollen bestäubt. Als 'marker' bot sich die Mutante co hy an (REDEI and HIRONO, 1964), die schon im Keimlingsstadium erkannt werden kann. Tatsächlich wurden in der F₁ einige Pflanzen erhalten, die mit dem Vater identisch waren und nach Selbstung konstant blieben.

Reference:

- REDEI, G.P., and Y. HIRONO: Arabid. Inf. Serv. 1, 9-10 (1964)

Changes in sensitivity of seeds to fast neutrons in the course of germination

R.B. CONTANT

(Institute for Atomic Sciences in Agriculture, Association Euratom - Ital, Wageningen, Netherlands)

Experiments are in progress to determine the changes in sensitivity to fast neutrons, of tomato and Arabidopsis seeds in the course of germination. A summary of preliminary results on the Arabidopsis experiment is presented below. The data will be further processed by electronic computer.

The first experiment included dry seeds and seeds imbibed at 27°C for 1/3, 1, 3 and 6 hours before irradiation; fast neutron exposures, in reactor 'BARN', at a dose rate of 2.5 krad/hr in water (+6% gamma contamination), ranged from 0 - 180 minutes with an extra treatment of 300 minutes for dry seeds. After a total incubation of 10 hours at 27°C (including the irradiation time), the seeds of all treatments were kept on agar at 2°C for 6 days, after which they were returned to 27°C and continuous light for germination. Subsequent treatment was as in the second experiment. - In the second experiment, seeds were laid out on agar in petri-dishes, imbibed for 6 days at 2°C and then incubated under continuous illumination at 27°C, for periods of 1/3, 3, 6, 12 and 24 hours respectively, after which they were immediately irradiated for periods ranging from 0 - 112 minutes. Temperature and light remained unchanged during irradiation and throughout germination, for a period of 7 days. The seedlings were then transplanted into soil and grown to maturity at 24 / 20°C (16 hour day / 8 hour night). - The first experiment dealt with changes in sensitivity during the period of water absorption. The second experiment was meant to study changes in neutron sensitivity after imbibition was complete. The 6-day treatment at 2°C was necessary in both cases to induce even germination, but constituted to some extent a complicating factor in the design of these trials.

First results: Experiment 1: For all criteria studied neutron sensitivity markedly increased with increasing pre-irradiation hydration time, mainly during the first 1/3 hour (table). For reduction in the percentage of ovules fertilised, sensitivity continued to increase slowly beyond six hours of hydration. With regard to the percentage of ovules fertilised, maximum sensitivity was reached after less than 3 hours of hydration (dose reduction factor approximately 3). Similarly, sensitivity to the induction of embryonic lethals rose sharply during the first 1/3 - 1 hour of hydration but not greatly thereafter.

Table : Exposures (minutes) producing an effect halfway between the control and the sublethal dose, for different criteria

Criterion	Experiment 1				Experiment 2
	Period of hydration at 27°C starting from dry seed (hours)				Average of all incubation treatments at 27°C, preceded by cold imbibition
	Dry	1/3-1	3	6	
20% reduction in number of ovules per silique	300	143	113	98	53
50% reduction of percentage of ovules fertilised	195	77	63	63	67
15% embryonic lethals	265	86	85	80	74

Experiment 2 : For almost all criteria analysed so far there was no consistent difference in sensitivity between the incubation treatments. The percentage of ovules fertilised (average of first 5 siliques on the main stem) was 64% in the un-irradiated control and decreased with dose to reach 17% at the 82 minutes exposure. The number of ovules per silique, expressed as a percentage of the control, was reduced to 72%, at the 82 minutes exposure. Flowering was slightly delayed at the higher exposures, but somewhat advanced at low and intermediate exposures, a fact which has not yet been adequately interpreted. The period from flowering to ripening of the siliques increased slightly but consistently with dose, leading to a maximum delay of 2/3 day at the sublethal exposure. - The frequency of embryonic lethals (cf. MÜLLER, 1963) was highly variable, though there was a high correlation with radiation exposure time; no significant differences were noted between the incubation treatments.

The data suggest that the main 'sensitivity' increase in germinating *Arabidopsis* seeds is associated with the actual uptake of water and only slightly with subsequent metabolic processes. Water uptake causes swelling of the seeds, an increase in their weight and a change in the elementary composition and the total amount of H and O atoms. A preliminary calculation permits the conclusion that the energy absorbed per seed, or per cell, from fast neutrons is more than doubled when imbibition is complete. This means that at least 70% of the so-called increase in sensitivity may be explained by the increase in energy absorbed. Further calculations allow a more accurate evaluation of true sensitisation.

Reference:
MÜLLER, A.J.: Biol. Zbl. 82, 133-163 (1963)

Effects of storage on dry irradiated *Arabidopsis* seeds

R.B. CONTANT and R. DANKERT

(Institute for Atomic Sciences in Agriculture, Association Euratom - Ital, Wageningen, Netherlands)

Fast neutron treated dry *Arabidopsis* seeds were stored at -20°C for periods of 0 - 11 hours (1 hour intervals) and 18 - 32 hours (1 or 2 hours intervals); gamma treated seeds were stored at the same temperature for periods of 24 - 34 hours (2 hour intervals) and 42 - 55 hours (1 hour intervals). For each dose of both kinds of radiation, the regression was calculated between storage time (x) and the number of hours needed to attain 50% germination (y), and between storage time and average root length 100 hours after germination (z). The results are tabulated below.

Type of radiation and dose	y after 24 hrs of storage	survival rate (%)	b_{xy}	confidence limits for b_{xy} (P = 0.05)	b_{xz}
Control	19.5	100	-0.007	ns*	+0.040 ns
Fast neutrons					
10 krad	20.0	61	-0.042	ns	-0.007 ns
17.5 krad	21.1	9	-0.007	ns	-0.008 ns
25 krad	22.3	0	-0.070	ns	-0.068 ns
Gamma rays					
50 krad	27.8	51	-0.161	-0.08 and -0.22	-0.12 ns
100 krad	31.0	21	-0.190	-0.11 and -0.23	-0.03 ns
150 krad	33.2	9	-0.228	-0.12 and -0.29	-0.12 ns

* ns = not significantly different from 0

Germination delay was considerably lower after neutron irradiation than following gamma irradiation, when doses are compared on the basis of equal survival rate. With fast neutrons no significant storage effect occurred within the range of storage times involved, at -20°C. With gamma-rays, radiation effects on germination were slightly but significantly reduced by storage (i.e. more rapid germination); although the regression coefficients did not differ significantly, there was a tendency towards a greater storage effect with increasing dose. This same trend was apparent after

neutron irradiation, though not very consistently. With regard to radiation induced root-length reduction there was, especially after gamma irradiation, a slight enhancement with storage (negative regression) but none of the effects were significant. These data are in agreement with the fact that neutron effects are less modified by secondary factors than the effects due to radiations of low linear energy transfer (LET), and with the fact that the modification of radiation effects is very slight at very low temperatures. One notes, however, that germination is slightly advanced whereas root-growth is slightly reduced; this suggests that processes of cell elongation (germination) may more readily recover than those involving cell division. This question requires more detailed study, in which also storage periods of 0 - 24 hours should be included in the case of gamma treated seeds.

Absence of a relation between early root growth and date of flowering in seed-irradiated Arabidopsis

R.B. CONTANT and R. DANKERT

(Institute for Atomic Sciences in Agriculture, Association Euratom - Ital, Wageningen, Netherlands)

In the experiment described by CONTANT and DANKERT (1968), root length was measured 100 hours after germination of each of the 3453 individuals studied; subsequently, the date of first anthesis was determined. These plants constituted a mixture of 120 F₁-families, derived from crosses between neutron irradiated M₁-individuals and control plants. The plants were classified into 'normal' and 'extremely thin' according to their root thickness at the time of measuring, and according to the degree of fertility ('normal', 'weak' or 'sterile') or heavily mutated appearance. The normal appearing plants represented approximately 90% of the total number. For each group of plants,

Table 1: Relation between root-length (x) and number of days to flowering (y)

Plant category	r _{xy} (corr.)	b _{xy} (regr.)
normal fertility / normal root	-0.19	-0.09
normal fertility / thin root	-0.14	-0.11
weak fertility / normal root	-0.17	-0.09
weak fertility / thin root	-0.16	-0.12
sterile / normal root	-0.24	-0.08
sterile / thin root	-0.25	-0.11
mutated / normal root	-0.18	-0.40*
mutated / thin root	-0.14	-0.33*

* significantly different from 0 at P = 0.05

the correlation and regression coefficient between rootlength and number of days to flowering is shown in the table. All regression coefficients were negative and very low; only those of the severely mutated plants differed significantly from 0 (P = 0.05). These results indicate only a very slight correspondence between reduced root growth in the very early seedling stage and delayed flowering.

The average root lengths of the different fertility groups did not differ significantly (21 - 24 mm). However, the roots of the heavily mutated plants were very significantly shorter (11 mm). The proportion of plantlets with extremely thin roots ranged from 0.8 to 1.3% in the three fertility classes while reaching 2.8% in the heavily mutated plants; none of the differences were significant.

Reference:

CONTANT, R.B., and R. DANKERT: Arabid. Inf. Serv. 5, 40-41 (1968)

Comparison of effects on quantitative characters in the M₁ of Arabidopsis after seed treatments with fast neutrons, gamma-rays and EMS

R.B. CONTANT and R. DANKERT

(Institute for Atomic Sciences in Agriculture, Association Euratom - Ital, Wageningen, Netherlands)

Dry seeds of *Arabidopsis thaliana*, race Li-2, moisture content 6.8%, 1000-seed weight 4.09 mg, were treated in air with: (a) 50, 100 and 150 krad of Cs ¹³⁷ gamma-rays (GR) at 23°C, at a rate of 200 krad/hr; (b) 10, 17.5 and 25 krad of fast neutrons (FN) at 21°C in reactor 'BARN', Wageningen, at a rate of 2.5 krad/hr plus a gamma-contamination of 154 rad/hr; (c) 0.01 N unbuffered EMS-solution, submersed (anaerobic), for a period of 24 hours at 25°C, followed by washing on a filter for 15 minutes. - Each treatment consisted of 18 x 25 seeds. There was a control of 10 x 25 seeds for treatments (a) and (b), and a separate control for treatment (c) consisting of 4 x 25 seeds treated according to the same procedure but with water instead of EMS. The method of culturing has been described by CONTANT (1966). During growth, various characteristics of the vegetative and the reproductive stage were studied of which the results are summarised in Table 1.

Table 1: Mean + S.D.* of: (1) number of hours incubation needed to attain 75% germination; (2) final percentage of germination; (3) percentage survival after 100 hours of germinated individuals; (4) root-length 100 hours after germination in mm; (5) number of leaves on main stem at beginning of anthesis; (6) length of longest leaf at anthesis in mm; (7) number of days from germination to first flowering

Treatment	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(n**)
Dry control	23.5	99	100	15.6±1.7	16.2±2.3	49.0± 4.1	35.0±3.2	138
FN 10 krad	24.7	98	61	3.3±0.4	16.6±4.6	38.0± 9.8	35.6±4.7	174
17.5 krad	25.0	97	9	1.9±0.2	14.3±2.6	34.1±15.6	45.2±7.0	21
25 krad	27.0	98	0	1.5±0.2	-	-	-	-
GR 50 krad	29.9	95	51	3.5±0.8	16.0±4.7	37.9±12.0	40.0±5.0	115
100 krad	32.9	97	21	2.6±0.6	14.6±5.6	31.9±15.0	43.0±5.8	24
150 krad	34.9	94	9	2.0±0.3	15.4±2.7	32.4±14.1	46.9±5.6	14
EMS control	16.9	98	97	9.9±1.5	15.8±2.6	48.5± 9.4	37.7±3.5	32
EMS 0.01 N.	21.2	98	85	9.0±1.7	14.6±2.7	41.9± 3.7	38.5±4.3	156

* each row-average was regarded as one observation (cf. MÜLLER, 1964)

** n = number of d.f. used for columns 5, 6 and 7

With regard to speed of germination (1), all differences except FN₁₀ / FN_{17.5} were significant (P = 0.05); for germination capacity (2), none were significant. The rapid germination of the EMS treatment and its control was due to prior imbibition for 24 hours; the fact that this advance in germination was only 6.6 hours (in the control) illustrates the blockage of the germination process by the anaerobic conditions of soaking. Germination delay was much greater following GR than after even a lethal dose of FN.

All differences in survival (3) between treatments and their control were significant; the EMS treatment was least affected. The survival curve for neutrons had a steeper slope than for gamma-rays; the LD₅₀'s were approximately 50 krad of GR and approximately 11.5 krad of FN.

Rootlength (4) was severely reduced even at the lowest radiation doses used. Except for the comparisons EMS-control / EMS, FN₁₀ / GR₅₀ and FN_{17.5} / GR₁₅₀, all differences were significant (P = 0.05). The EMS treatment was only affected by 10% compared to its control. Their relatively low values suggest a detrimental effect of prolonged submersion or an uncontrolled factor. The coefficient of variation was much greater in the EMS treatment and its control than in the dry control; it was hardly increased by FN but doubled after GR, which probably mainly reflects the large physiological variability usually resulting from GR.

A high correlation (r = 0.91) existed between survival and rootlength, which was due mainly to the correspondence between rootlengths less than 2 - 3 mm and the inability to survive. A more detailed analysis, on the FN₁₀ treatment only, confirmed this finding (Table 2). The 'critical' root-length, which is probably associated with complete inhibition of mitosis, seemed to be closer to 2 mm in the case of FN, and closer to 3 mm in the case of GR (Table 3). Or, for a given root-length, survival is slightly greater after FN treatment than after GR; this again supports the existence of more pronounced physiological effects after GR.

Table 2: Root-length and survival in the FN₁₀ treatment

Root-length category (mm)	1 - 2.5	2.5 - 3.5	3.5 - 4.5	4.5 - 6.5
Percentage survival	0	32	68	72

Table 3: Relation between root-length after 100 hours and survival

Treatment	% roots > 2 mm	% survival	% roots > 3 mm
Dry control	100	100	100
FN 10 krad	75	61	40
17.5 krad	10	9	0
25 krad	0	0	0
GR 50 krad	64	61	45
100 krad	40	21	17
150 krad	19	9	6
EMS control	100	97	100
EMS 0.01 N	100	85	100

The number of leaves at the beginning of anthesis (5) is a very insensitive criterion of radiation damage. Few differences were significant at P = 0.05 (control and FN₁₀/FN_{17.5}; FN₁₀/GR₁₀₀; GR₅₀/EMS). Even so, there was a tendency towards a reduction in average leaf number with dose. Except for some irregularities, variation coefficients increased with dose, more strongly in the case of GR than with FN.

For leaf-length (6), all treatments differed significantly from their control; the difference between the two controls was insignificant. The other significant differences were FN₁₀ / GR₁₀₀ or GR₁₅₀; FN_{17.5} / GR₅₀ (P = 0.05). Variation coefficients increased

very markedly with dose. The EMS treatment had a relatively high leaf-length and a low variation.

With regard to flowering date (7) all treatments differed significantly from their control except EMS and FN₁₀. Mean, standard deviation and variation coefficient all increased with dose (except GR₁₅₀); the EMS-control was 2.5 days later than the dry control. For a given survival rate, the delay seems to be somewhat greater with GR than with FN.

All sterile individuals were handcrossed with pollen from control plants, in order to study any genetic or plasmatic male sterility that might be involved. This is reported separately (DANKERT and CONTANT, 1968).

References:

- CONTANT, R.B.: Arabid. Inf. Serv. 3, 35-36 (1966)
 DANKERT, R., and R.B. CONTANT: Arabid. Inf. Serv. 5, 46-47 (1968)
 MÜLLER, A.J.: Kulturpflanze 12, 235-255 (1964)

Correlations within and between M₁ and M₂, and genetic segregation, for various quantitative traits after neutron irradiation of Arabidopsis seeds

R.B. CONTANT and R. DANKERT

(Institute for Atomic Sciences in Agriculture, Association Euratom - Ital, Wageningen, Netherlands)

In the course of a study on the sensitivity of *Arabidopsis* seeds, race Li-2, to fast neutrons the selfed progeny was grown of 125 visibly aberrant M₁ plants which differed significantly from the control in respect of (a) length of longest leaf at anthesis; (b) number of days to flowering and (c) number of days from flowering to harvesting. Twenty five of these M₁ plants, which were clearly chimeric, were represented by 2 - 4 M₂ seedlots each. For all 3 characters the frequency distributions of the selected M₁ plants were skewed towards the lower values, in contrast to the control; those of the original M₁ population were much less skewed or not at all.

Fifty seeds were sown per progeny whereas the control consisted of 1500 seeds. Methods of culturing were described earlier (CONTANT, 1966 a). Germination was recorded 6, 18, 24, 30, 42 and 54 hours after sowing. Root length was determined by taking photographs of the petridishes at 12 hour intervals; the negatives were projected on a smooth white wall on which the roots were measured with a curvimeter, which constitutes an improvement over the earlier method (CONTANT, 1966 b). Furthermore, the following parameters were recorded: (1) plant diameter at 1 - 1.5 week intervals; (2) number of leaves and length of the longest leaf at the B₆ stage of the first flowers (cf. MÜLLER, 1961); (3) the number of days to the B₆ stage of the first flowers; (4) segregation of morphological and colour aberrations. Fertility was only determined on the early flowering individuals.

M₂ segregation of non-germinating mutations: Thirty six M₂ lines (out of 150) segregated for germinating and non-germinating seeds, in frequencies ranging from 1/16 to over 1/2, and consistent with 1/4 in 29 cases. In 3 out of the 25 visible chimeras there was a significant difference in germination speed between the progenies of the two plant parts harvested.

M₂ segregation of visible mutations: Forty five progenies segregated for visible mutations in proportions ranging from less than 1/16 to over 1/2. Most did not differ significantly from a 3 : 1 ratio. Five types of phenotypic abnormalities occurred in several progenies, in approximately the same proportion: (a) tiny yellowish plantlet, dying after 3 - 4 weeks; in 6 M₂ lines, in proportions ranging from 0.05 - 0.10; (b) very compact tiny plantlet with darkgreen leaves; in 3 M₂ lines, in proportions ranging from 0.04 - 0.08; (c) darkgreen plant with small leaves, and short petioles; in 4 M₂ lines, in proportions ranging from 0.17 - 0.30; (d) plant with open growth habit and long petioles; in 2 M₂ lines, in proportions 0.06 and 0.08; (e) plant with mosaic leaves; in 3 M₂ families, in proportions ranging from 0.04 - 0.07 (another mosaic, occurring in part of a visibly chimeric M₁ plant, segregated 3 : 1). - The first categories are such drastic abnormalities that small phenotypic differences cannot be detected; however, the less severe mutations within categories (d) and (e) were in several quantitative respects almost identical (Table 1). Although the phenotypes suggest a certain degree of

Table 1: Mutant type	Code number	Mean leaf number	Mean length longest leaf (mm)	Mean number of days to flowering
(d)	37	24	31	56
	35 A	23	28	54
(e)	44	26	24	58
	109	24	24	58
	135	27	26	58

specificity in mutation induction, most of these mutations may be due to more or less unspecific deletions or other chromosomal disturbances, instead of gene mutations. Their genetics will be further studied.

Of the 52 visible mutants found, 8 had a greater leaf number and 5 a greater length of the longest leaf, whereas 6 mutants were earlier flowering than the control; 7 lines were retained for certain positive characteristics of their mutant segregants and examined in more detail (Table 2). - Mutant number 3 is of immediate interest because of its marker-gene which behaves as a single recessive, and its otherwise normal appearance and behaviour.

Table 2: Characteristics of 7 M₂ mutants

Mutant code number	Main feature(s)	Percentage occurrence in M ₂	Number of leaves			Leaf length			Number of days to flowering			Fertility relative to control Li-2
			(a)*	(b)	(c)*	(a)	(b)	(c)	(a)	(b)	(c)	
3	yellow green	0.20	31	30	35	52	50	54	44	45	46	normal
12	compact, distorted	0.05	30	29	30	33	43	8	54	48	39	15%
21	darkgreen, anthocyanin	0.25	31	31	16	45	42	14	44	49	45	30 - 40%
53	glossy, compact	0.21	30	29	30	42	45	41	46	49	41	normal
59	dark, small, narrow leaves	0.03	31	31	22	42	45	30	52	50	36	50%
56	small, very early	0.17	30	29	17	48	47	18	47	42	23	15%
84	small, very early	0.03	31	31	15	40	39	22	50	50	22	20%

* (a) control; (b) non-mutated M₂ plants; (c) mutant M₂ plants.

Except for number 53 all mutants had a very low fertility which offset the advantage of earliness. Mutant number 12 had a much more rapid succession of leaves (number of days to flowering / number of leaves) than the corresponding control; to a lesser extent this was also the case in no.'s 84, 56 and possibly 53. The inheritance of these phenomena will be studied. The search for similar mutants with full fertility will be continued.

Length of longest leaf: The variation coefficient (CV %) of the M₂ families was greater than of the control; only 3 out of 110 families had a significantly (F = 0.05) smaller CV % than the control. A comparison of leaf lengths, relative to the control, of the M₁ plants and their M₂ offspring revealed that selection for shorter leaves is not possible in the M₁ (X² for correspondence between M₁ and M₂ : 78.8; 3 d.f.). On the other hand, selection for longer leaves in the M₁ seems possible (X² = 0.11); as physiological disturbances only very rarely lead to leaf enlargement this result is not unexpected.

Number of days flowering: Also with regard to flowering date, most M₂ lines had a greater variation than the control; only 3 progenies out of 110 had a significantly smaller CV %. Several progenies significantly earlier than the control thus still contained significantly later individuals and vice versa. There appeared to be virtually no correspondence between days to flowering, relative to the respective controls (+ or - values), between the M₁ plants and their M₂ lines. Consequently, no selection for earliness or lateness would be possible in the M₁ generation. This shows that the differences in flowering date in the M₁ are of physiological origin. This is also demonstrated by the relationship between leaf length (x) and the number of days to flowering (y), for which the regression coefficients were: b_{xy} = -0.51 in the M₁; +0.08 in the M₂, and +0.11 in the control. This indicates a general weakness in the M₁, expressed as reduced leaf length combined with late flowering.

In the M₂ (72 lines and their control) no significant correlation was found between the number of days to flowering on the one hand and germination speed or length of the longest leaf on the other hand. As expected, there was only a slight and statistically insignificant correspondence between the variation coefficients of the M₂ relative to their control (CV% M₂ / CV% control) for leaf length and number of days to flowering, respectively. Although there was a marked regression between average root length 100 hours after germination and the number of days to flowering (b = 0.64), the correlation was too weak to allow selection for flowering date on the basis of the root-length measurements; furthermore, the control showed a very similar regression (b = 0.68).

Chimerism: Twenty five M₁ plants were visibly chimeric for fertility or for some morphological or colour change; these were partly dominant mutations or chromosome aberrations and partly plasmatic mutations. Of these plants, 2 - 4 parts were harvested separately. The offspring of 14 of these was tested for significant differences in respect of (a) speed of germination; (b) segregation of non-germinators; (c) root length after 100 hours; (d) leaf length, and (e) number of days to flowering.

Two M₁ plants appeared to be chimeric in respect of one of these quantitative traits, 3 M₁'s were chimeric for 2 traits, 3 M₁'s were chimeric for 3 traits and 1 M₁ was chimeric for 4 of these traits. In the two M₁ plants of which 4 parts were harvested, 3 parts proved to be of different idio-type. This illustrates that seed irradiation may induce significant chimerism for various quantitative characters.

References:
 CONTANT, R.B.: Arabid. Inf. Serv. 3, 35-36 (1966 a)
 - : Proc. 3rd Int. Congress Radiation Research, Cortina d'Ampezzo, Abstract No. 226, 58 (1966 b)
 MÜLLER, A.J.: Kulturpflanze 9, 364-395 (1961)

In vitro germination of Arabidopsis pollen

R. DANKERT and R.B. CONTANT

(Institute for Atomic Sciences in Agriculture, Association Euratom - Ital,
Wageningen, Netherlands)

Optimal conditions for the germination of Arabidopsis pollen are unknown. In order to approach this problem and furthermore to detect differences in germination and pollen tube growth between pollen from control plants and from certain mutants, the following experiment was carried out.

Anthers were taken from healthy Arabidopsis plants, grown in polystyrene dishes in a growth chamber under standard conditions (CONTANT, 1966), and squashed in Van Tieghem cells to make hanging drop preparations of a salt solution (BREWBAKER and KWACK, 1963) with a range of saccharose concentrations (10, 15, 17.5, 20, 25 or 30%) and with or without the addition of one detached and thoroughly squashed stigma (from the same plant) per preparation. Each treatment was done in duplicate. BREWBAKER's solution consists of: 300 ppm $\text{Ca}(\text{NO}_3)_2$, 200 ppm $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 100 ppm KNO_3 and 100 ppm H_2BO_3 . The preparations were incubated at 20°C in continuous light from Philips TL33RS fluorescent tubes (12000 lux), and inspected at 5-hour intervals.

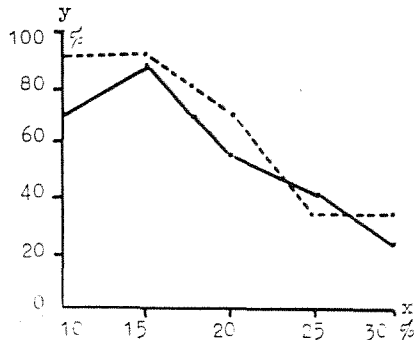


Figure 1: Saccharose concentration (x); percentage germinated pollen after 20 hours (y)

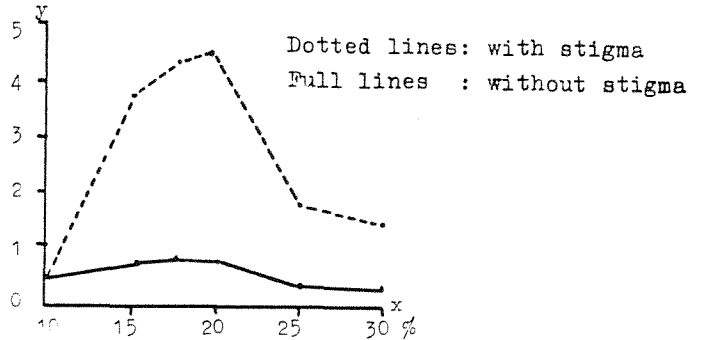


Figure 2: Saccharose concentration (x); average pollen tube length of germinated pollen in arbitrary units (y)

Figure 1 shows the germination percentage for each treatment after 20 hours of incubation. Germinated were considered to be all pollen grains in which plasma exudes from the germination pore(s), i.e. also the grains which burst at or shortly after germination. The low osmotic pressure of the 10 and 15% sugar solutions permitted rapid water uptake and germination but also led to a high percentage of bursting pollen. The addition of a squashed stigma to the medium induced more rapid pollen germination while also leading to a higher percentage of germinating pollen bursting. This effect caused by the stigma is probably associated with activity or concentration of enzymes; this is supported by the very marked effect on pollen tube growth, shown in Figure 2. The data demonstrate that for both pollen germination and pollen tube growth a saccharose concentration of 17.5 - 20% is optimal. More study is needed to establish whether further improvements can be made by changing the salt composition and/or total concentration, and whether this would affect the optimum sugar concentration.

The second part of the experiment, conducted at a sugar concentration of 20% with or without the addition of a stigma, aimed at a comparison of pollen tube growth from control plants and from 9 different mutants. All comparisons were made in duplicate, with independent control samples; in each preparation, 90 - 160 pollen tubes were measured. In the preparations without a stigma tube length after 20 hours ranged from 0.3 - 0.6 units; none of the differences were significant. In those with a stigma, growth had been very much faster (3.9 - 4.8 units), except for mutant no. 7 (1.4 units) which was also the only mutant significantly different from the control ($P < 0.001$). The variation between the 18 control samples is a measure of the reproducibility of the method: $x = 4.7 \pm 0.6$ units. It is suggested that mutant no.7 possesses a mutation which prohibits the reaction of its pollen to stimulatory substances from the squashed stigma. Also in pollinations in vivo such pollen grains may have a much slower tube growth, leading to certation of normal pollen were present. Experimentation with this mutant will be continued, using UV-fluorescence microscopy to study the growth of pollen tubes within the style.

References:

CONTANT, R.B.: Arabid. Inf. Serv. 3, 35-36 (1966)
BREWBAKER, J.L., and B.H. KWACK: Amer. J. Botany 50, 859-865 (1963)

Male sterility induced by gamma-rays, fast neutrons and EMS

R. DANKERT and R.B. CONTANT

(Institute for Atomic Sciences in Agriculture, Association Euratom - Ital, Wageningen, Netherlands)

Dry *Arabidopsis* seeds were treated with a range of doses of gamma-rays and fast neutrons, and with EMS. The M₁ plants were screened for male sterility; 113 plants or shoots without any normally developing siliques were retained for examination under the binocular and for crossing with unirradiated plants.

Microscopically, four types of male sterility were found: (a) thecae filled with pollen, but not dehiscent and ultimately shriveling (91 cases); (b) thecae of normal appearance but with only few pollen grains; shriveling at a later stage without dehiscence (20 cases); (c) thecae smaller, devoid of pollen and not dehiscent (1 case); (d) thecae shriveling at a very early stage (1 case). In all plants, the filaments and connective tissue of the anthers were normal.

As a preliminary to crossing studies the degree of successful emasculation was determined; 200 flowers on control plants were emasculated and isolated without pollination. Fourteen flowers (7%) produced a fertile silique. In these cases the emasculation was assumed to have failed; the confidence limits for this percentage were 0 and 12%, respectively (P = 0.05).

Male sterile M₁ plants were rather easily detected by absence of normal siliques, but also by the fact that a large proportion of the male sterile individuals possessed weak thin stems (50 - 80%, dependent on radiation dose); this weakness is probably chiefly the result rather than the cause of the sterility. The aberrant growth habit of the plants was itself not prohibitive to normal fruit development, as crossing with normal pollen led to normal siliques. There was no relation between sterility in the M₁ and the date of flowering. The length of the longest leaf at the beginning of anthesis was slightly greater in the male sterile plants than in the fertile plants; The differences were 1.6% in the neutron irradiated plants, 8.7% after gamma-rays and 7.5% after EMS; however, none of these differences were significant.

The occurrence of male sterility was verified by using anthers of the suspected plants for pollinating emasculated control flowers, 10 - 15 such pollinations were carried out per suspected plant; 97 plants were confirmed to be male sterile. There was only one doubtful case, yielding 2 siliques (more than the 12% allowed for errors in castration); it was discarded. The table shows that the incidence of male sterility increased with dose;

Table: Male sterility after irradiation and EMS

Treatment	Total number of plants	Number and percentage of male sterile plants throughout	Number of entirely fertile F ₁ families**	Number of F ₁ families** segregating for slight fertility or (sterility)
Control	170	0 0%	-	-
Fast neutrons				
10 krad*	174	43 24	41	1 + (1)
17.5 krad*	21	9 42	9	0
Gamma rays				
50 krad	115	25 21	24	(1)
100 krad	24	9 37	7	2
150 krad	14	5 35	5	0
EMS 0.01 N	156	6 4	6	0

* with 5% β -contamination

** cross of male sterile x normal

on the basis of comparable survival rate (lowest doses of both types of radiation approximately equivalent) there was no reliable difference between fast neutrons and gamma-rays; the EMS treatment produced a much lower percentage of male sterile plants.

Of each male sterile plant, 10 - 15 flowers were subsequently crossed with pollen from control plants; all M₁ plants proved to be fertile, indicating that no female sterility had occurred. Only two families (F₁) segregated for very slight fertility (table); one, from 10 krad of fast neutrons, segregated 16 fertile: 14 slightly fertile, while the other, from 50 krad of gamma-rays, segregated 9 : 10. Although several alternative explanations are possible and will have to be tested, these cases are tentatively thought to represent intermediary inheritance of a mutated gene for weak fertility or sterility, the slightly fertile plants being heterozygous; the sterility of the mother plants (M₁) may have been caused by additional physiological effects. Furthermore, 3 F₁ families segregated 3 fertile : 1 fully sterile; these may be cases of recessive mutations in which the M₁ plants have been selfed accidentally after unsuccessful emasculation. Alternatively, this segregation might have been caused by a chimeric situation for plasmatic male sterility and physiological or chromosomal male sterility in the flowering M₁ shoot. This will be further tested in the F₂. There were no fully sterile F₁ families, which shows that, except for the possibility just mentioned, no plasmatic male sterility had been induced; this agrees with J.H. van der VEEN (personal communication) who so far failed to find plasmatic

male sterility after EMS treatment. The selfed progenies of all F_1 families will be tested for segregation of infertility, to allow a distinction between genetic male sterility and physiological radiation effects in the M_1 ; in the first case, the offspring of 50% of the plants in each F_1 family will contain male sterile individuals, while in the latter case all progenies will be (almost) fully fertile on selfing. Finally, the selfed seed of several hundred fertile M_1 plants has also been harvested and will be screened for segregation of genetic male sterility.

Biological effectiveness of heavy ionizing particles relative to X-rays

Y. HIRONO, H.H. SMITH and J. LYMAN

(Biology Department, Brookhaven National Laboratory, Upton, N.Y.,
and Donner Laboratory, University of California, Berkeley, California, U.S.A.)

F_1 seeds of a genetic stock of *Arabidopsis thaliana* heterozygous for the yellow-green locus, Ch/ch , were used in all experiments on induced genetic change. The recessive gene, ch , gives a yellow-green color in the leaves when homozygous or hemizygous, and the presence of the dominant gene, Ch , produces full green color, so that the F_1 heterozygote has normal green leaves. Loss of the Ch allele (deletion) or change in its function (mutation) in heterozygotes gives a yellowish green phenotype in leaf cells in which the phenomenon occurs and in cell lineages of the altered genotypes. The frequency of F_1 plants, grown from irradiated seeds, that show yellow-green leaves (sectored or entire) was used as a measure of the frequency of radiation induced genetic change or damage.

Irradiation with heavy ionizing particles was carried out at the HILAC machine (University of California, Berkeley). The X-ray treatments were made with a machine operated at 250 kVp. Since the penetrating distances of the heavy ionizing particles are very short it was necessary to determine the precise internal geometry of the embryo and to position the seed consistently in order to be able to compute exact dosimetries and to make quantitative comparisons.

Experiments are in progress using He^4 , Li^7 , C^{12} , O^{16} , Ne^{20} and Ar^{40} -ions. Although final results with somatic mutations are not yet available it is clear that in comparison with X-rays the lowest RBE's are close to 10 and that those for He^4 , Li^7 , C^{12} and O^{16} are higher than for Ne^{20} and Ar^{40} -ions.

In separate experiments the effects of these same heavy ionizing particles on plant growth are being investigated, using both F_1 and wild type seeds. The plants are grown on a nutrient medium singly in test tubes and under well controlled environmental conditions (cf. HIRONO and SMITH, this volume). The dry weight of the plant is used as an indicator of growth and of radiation effects. The results with dry weight are consistent with those obtained from the mutation experiments described above in that the RBE's show the same general relationships.

Reference:

HIRONO, Y., and G.F. REDEI: Genetics 51, 519-526 (1965)

Research carried out at Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission and at Donner Laboratory.

Killing efficiency of neutrons in Arabidopsis seeds

Taro FUJII

(National Institute of Genetics, Misima, Japan)

The killing effects of gamma-rays and thermal neutrons on seeds soaked in water for 48 hours have been compared, and drastic effects of neutrons were observed (FUJII, 1967). The aim of the present experiment was to determine whether the damage of high LET radiations is modified by the water content of the treated material. For these studies irradiations with fast neutron were also included.

Dry and 24 hrs presoaked seeds were used. Thermal neutron exposures were done with the pneumatic tube which was provided in the reactor of the Kyoto University; the irradiation time was 10-150 sec with an out put of 50 kW; the total neutron flux, therefore, was 3.9×10^{12} in minimum and 7.1×10^{13} in maximum. The neutron beam of the pneumatic tube consisted of about 90 % thermal neutrons and 10 % fast neutrons, with a high gamma-ray contamination. Equal samples were also exposed to monoenergetic 14 MeV neutrons from the $T(d,n)$ reaction neutron generator in our institute. Its total neutron flux was 3.8×10^{11} - 1.8×10^{13} with the average intensity of 10^9 neutrons/sec; here the gamma-ray contamination was less than 10 %. Thus the neutron effect is expected to mask over the effect of the contaminating gamma-rays because of its high RBE.

The decrease of the survival rates after the neutron irradiation is shown in the table together with the results of the previous gamma-ray experiment, for comparison. In the experiment with thermal neutrons, severe killing was again observed: almost all plants died after a flux of 71.5×10^{12} neutrons on dry seeds and similar survival rates were observed after 35.6×10^{12} on wet seeds while 37.5 kR of gamma-rays alone had no effect on dry seeds and 16.5 kR might only slightly affect

wet seeds. Thus the killing effect of thermal neutrons is very high, and it seems to result from the high LET of the radiation modified by the water content of the material.

Gamma-rays*			Thermal neutrons			Fast neutrons		
Dosage (kR)	Dry seeds (%)	Wet seeds (%)	Total neutron flux** (x10 ¹²)	Dry seeds (%)	Wet seeds (%)	Total neutron flux*** (x10 ¹¹)	Dry seeds (%)	Wet seeds (%)
10	91.6	92.7	3.9 (2.5)	80.7	76.7	3.8	85.8	83.9
30	90.8	75.6	15.4 (7.5)	85.2	39.7	4.6	80.6	78.6
50	92.8	21.7	26.6 (12.5)	72.5	6.0	15.5	73.9	51.0
70	93.3	0.0	35.6 (16.5)	72.5	2.1	19.0	66.2	32.4
100	82.4	0.0	43.0 (22.5)	56.3	0.0	35.0	64.2	34.6
			53.1 (27.5)	40.9	0.0	176.0	1.1	0.0
			60.8 (32.5)	14.1	0.0			
			71.5 (37.5)	1.9	0.0			

* After FUJII (1967)

** Dosage was estimated from the activation of Au-foil. The figures in parentheses show the contaminating gamma-rays in kR.

*** Dosage was estimated from the S,P reaction.

But, the assessment of thermal neutrons' RBE is much complicated because of its multi-energetic condition and the abundance of contaminated gamma-rays. The survival rates in the neutron series are rather high compared to those of the gamma series, both in dry and wet seeds. Moreover, 48 hrs of soaking were used in the gamma-ray experiment while only 24 hrs in the neutron samples. This difference may also influence the intensity of cell or tissue killing.

Fast neutron irradiations of dry and wet seeds also showed an effect of the water content. The survival rates in wet seeds after 19 or 35x10¹¹ neutrons were about half of those in dry seeds. The fast neutron doses which the seeds received, were calculated from the first collision dose converted from the given tissue dose by using chemical seed analyses; the dosage in rad unit is reckoned from the multiplied total neutron flux by 5.98x10⁻⁹. From the calculations, 3.8 and 35.0x10¹⁰ neutrons are equivalent to 2.27 and 20.93 krad, respectively. When the results are compared with those after gamma-irradiation, the neutrons prove to be nearly 10 times more effective in dry and about 5 times in wet seeds at the LD₅₀ level. Modifications of radiation damage by the water content are known in 14 MeV neutron exposures. But nevertheless, the modification factor is too large in the present experiment, though the environmental modification of radiation effects is generally very small in high LET radiation. Further study is now underway.

Reference:

FUJII, G.: *Arabidopsis Serv.* 4, 59 (1967)

Preliminary data on effects of various ionizing radiations on Arabidopsis

V.I. IVANOV, A.V. SANINA, and H.A. TIMOFEEFF-RESSOVSKY

(Institute of Medical Radiology, Obninsk, U.S.S.R.)

The data presented here comprise a brief summary of results of experiments carried out in collaboration with Dr. H. ABEL and his colleagues from the Zentralinstitut für Kernforschung, Dresden. The results will be published in detail elsewhere.

Dry seeds of *A. thaliana* (L.) HEYNH., race Enkheim, about a year old, were irradiated with 200 kV X-rays (mean energy about 1 MeV, mean LET along the track = 1.6 keV/mcm), 6.3 MeV H ions (LET = 6.7 keV/mcm), and 25.1 MeV He ions (LET = 26 keV/mcm) at doses ranging from about 0.9 to about 40 krad. For H and He ions the energy values were calculated at the seed surface level, and LET for the matter of unit density.

Dose-response curves for root growth, survival, flowering date, and sterility grade were obtained. For root measurements the method elaborated by MÜLLER (1964) was applied, and it was found that the effectiveness of radiations increased with LET, although rather small RBE values were obtained (below 2). The other M₁ effects were studied only at doses ranging from about 0.9 to about 20 krad. In this dose range no statistically significant differences between the irradiated and control lots were observed. The flowering was delayed slightly with increasing doses, and no significant differences between dose-response curves for X, H, and He irradiation were found. However, the sequence of the curves was the same as that for the root growth. The same was true for sterility grade estimated as fraction of completely undeveloped embryos among all the embryos. It seems likely that in these two cases relatively small differences in RBE values were overlapped by rather high variability of the data. - At present screening for M₂ mutations is being in progress.

Reference:

MÜLLER, A.J.: *Kulturpflanze* 12, 237-255 (1964)

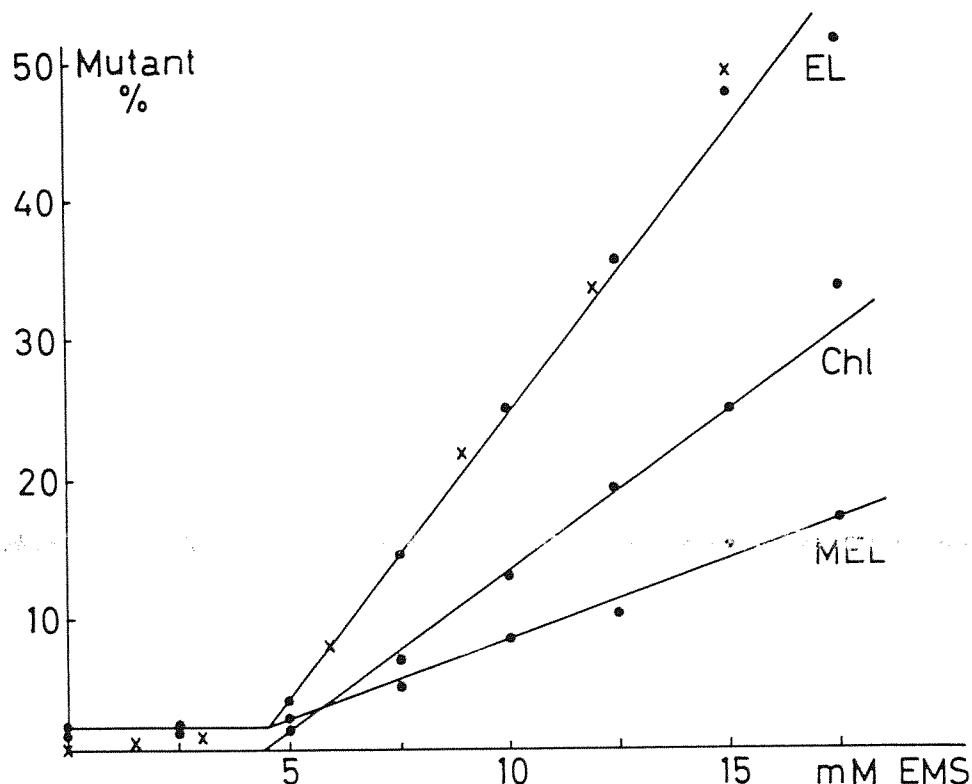
Linear EMS-dose response for induced lethals,
including maternally conditioned embryonic lethals

J.H. Van der VEEN

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

After 5 days on wet filterpaper at 20°C (to break dormancy) and redrying on filterpaper (24 hrs, 24°C), seeds of Landsberg-'erecta' were treated with 0.0, 2.5, 5.0, 7.5, 10.0, 12.5, 15.0 and 17.5 mM EMS (not-buffered, 24 hrs, 24°C, dark). After 5 min rinsing-off with tapwater, the seeds were put on agar, and the seedlings were treated as described earlier (Van der VEEN, 1965, 1967 a).

From a random sample of 100 M₁-plants per item, fruits No. 5 and 6 were used to apply MÜLLER's embryo-test, i.e., to score the fertility (% of ovules fertilized), embryonic lethals (% of seeds containing lethal embryos), and chlorophyll mutants (% among non-lethal embryos). In addition, flowers No. 5 and 6 (or 6 and 7) of a random sample of 40 M₁-plants per item were pollinated with pollen from the untreated control.



The results are given in the Figure. E.L. refers to embryonic lethals and Chl. to chlorophyll mutants, both after natural selfing, M.E.L. (i.e., maternally conditioned E.L.; see below) is used for embryonic lethals found after applying control pollen. The rare chlorophyll mutants found after crossing can be neglected.

It is seen that for each of the three categories (E.L., Chl., M.E.L.) the observations can be represented very well by a straight line, which originates at a point just left of the dose of 5 mM EMS. It should be noted that the level of this point coincides with the control level, i.e., 0% chlorophyll mutants, and 1.5% embryonic lethals (both, E.L. and M.E.L.). It can be added that the fertility after natural selfing (not given in the graph) drops linearly from precisely the same point left of 5 mM, from a level of 92% (which equals the control) to 15% at 17.5 mM EMS. It is attractive to consider a concentration just below 5 mM as a threshold, under which no EMS-effect becomes manifest.

MÜLLER (1966) concludes from his EMS-experiments (treatment with 2.5 mM up to 25.0 mM, 18 hrs (!), 22°C) that the frequency of both, embryonic lethals and chlorophyll mutants increases exponentially ($n = \pm 2$), like a two-hit curve does. His percentage for recessive lethals of all types (m_c in Exp. 1, Table 3, l.c.) are inserted into our graph with crosses (x). For ease of inspection his concentration scale (mM) was multiplied by 0.6, i.e., his dose of 25 mM is entered at our 15 mM, etc. Except for the three lower doses (including 0 mM), which are below our "threshold-dose",

his results can be adequately described by the same straight line. The notion of an exponential dose-response curve is mainly based on the three lower doses. This in turn is due to the fact that MÜLLER's mutant frequency goes down to virtually 0% (for 0 mM), in contrast to all our experiments which gave a minimum of 1-2%. MÜLLER's statement (p. 217, l.c.) that at lower doses a larger fraction of the alkylations might be repaired than a higher doses, seems not altogether irreconcilable with our concept of a "threshold-dose". - Nevertheless, the disturbing fact remains that GICHNER (1965), who also studied an EMS-dose range (18 hrs, 24°C, 6 hrs post-washing), found a dose-response curve for lethals of all types which is clearly exponential throughout the range.

A final point regards the non-recessive embryonic lethals (M.E.L.) found after pollinating with control (C) pollen. In a previous report (Van der VEEN, 1967b) the percentages of embryonic lethals after 10 mM (24 hrs, 24°C) were 1.1% for the Control, 1.7% for CxEMS, 7.1% (!) for EMSxC and 19.0% for EMS (natural selfing). The different hypotheses proposed for this reciprocal difference have now been tested. From this M₁-material 120 CxEMS-fruits and 120 EMSxC-fruits were harvested (one fruit per plant). In each M₂-family (from crossing) 2 plants were left to natural selfing and 2 plants were pollinated with C-pollen. The embryo-test was again applied on fruits No. 5 and 6. The array of genotypes is expected to be the same for CxEMS and EMSxC, except for those mutations which were subject to differential selection in male versus female gametes. Indeed, after natural selfing CxEMS and EMSxC segregated 7.5 and 5.1% chlorophyll mutants and 11.2 and 9.1% embryonic lethals respectively. Significant is that (CxEMS)xC and (EMSxC)xC produced resp. 5.9 and 4.8% embryonic lethals. The latter equality leads to the conclusion that the non-recessive embryonic lethals are mainly caused by maternal physiological effects (induced by the impaired genotype of the sporophyte). The reader can easily verify that the M₂-results exclude substantial contributions of (1) autonomous effects of the embryonic cytoplasm, (2) gene-dose effects in the triploid endosperm, and (3) dominant embryonic lethal mutations transmitted by the female gamete only.

References:

- GICHNER, T.: In "Arabidopsis Research". Rep.Int.Symp. Göttingen, 1965. Pp. 200-206
MÜLLER, A.J.: Züchter 36, 201-220 (1966)
VEEN, J.H. Van der: Arabid. Inf. Serv. 2, 31-32 (1965)
- : Arabid. Inf. Serv. 4, 63 (1967 a)
- : Arabid. Inf. Serv. 4, 45 (1967 b)

Decrease of genetic damage resulting from dry-storage of EMS-treated seeds

J.H. Van der VEEN and C. Van HEEBERT

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

MÜLLER (1967) treated presoaked seeds of the race Dijon with 50 mM EMS (3 hrs, 24°C), then submersed them in water (1 hr or 24 hrs), and finally dry-stored them (silicagel, 24°C) during various periods. After 1 hr water-storage, the genetic damage (M₁-sterility, embryonic lethals and chlorophyll mutants) gradually increased during the dry-storage, but after 24 hrs water-storage, the damage remained constant during the dry-storage from 0 hrs to 56 days. In contrast, a drastic decrease occurred in experiments of MÜLLER (1965, 1967) with MNU and other nitrosamides. This author argued in favour of reversion of premutational lesions during dry-storage, rather than inactivation of residual MNU or its reaction products. - Our EMS-results are at variance with MÜLLER's EMS-results, but resemble his MNU-results to some extent. In addition, we included dry-storage at -20°C.

After 5 days on wet filterpaper at 2°C (a routine procedure used to break dormancy) and 24 hrs redrying on filterpaper (24°C), one year old seeds of Landsberg-'erecta' were treated with 50 mM EMS (not-buffered, 5hrs, 24°C), followed by 5 min rinsing with tapwater. The posttreatments were: (1) Water-storage, either (W) or not (-). Seeds submersed in a large quantity of water (dark, 24 hrs, 24°C, no stirring or renewal). (2) Dry-storage, either (S) or not (-). Drying for 15 min on filterpaper (room temperature), followed by further drying and storage (exsiccator, silicagel), either at +24°C (S+24) or at -20°C (S-20), and during 1, 5 or 14 days (S+24/1, etc.). Prior to storage at -20°C a vacuum treatment was inserted (30 mm, 2°C, 45 min, silicagel). - The experiment was so timed that all items could be simultaneously sown on agar.

The results are shown in the following table, in which "Survival" means % of treated seeds giving flowering plants (soil, greenhouse), "Sterility" means % of non-fertilized ovulae, and the mutant frequencies are given as % of seeds containing lethal embryos (E.L.) and % of chlorophyll mutants among non-lethal embryos (Chl.; MÜLLER's embryo-test). Per each item the siliques No. 5 and 6 were scored on 35 M₁-plants.

Attention should be drawn to the following data: (1) No difference between (-,-) and (W,-). Outdiffusion of EMS on agar and under water at equal rates, or if not, a balance with differential change in sensitivity to residual EMS. This absence of difference is in agreement with MÜLLER (1966), who sowed in soil. - (2) No water-storage prior to dry-storage (-, S+24/1) gives an immediate sharp increase in genetic damage, as compared with (-,-). In MÜLLER's (1967) 1 hr water-storage item, this increase during dry-storage was gradual. - (3) Even after 24 hrs water-storage (W, S+24/1) the damage increases (though to a lesser extent), indicating residual EMS. Here, MÜLLER found no increase. -

(4) Both with- (W, S+24) and without (-, S+24) water-storage, all three types of damage decrease during dry-storage (1 to 14 days). MÜLLER found constancy resp. increase in his EMS-experiment. The decrease of the three types of damage, from (-, S+24/1) to (W, S+24/14) has relative proportions which can be precisely mimicked by a range of decreasing EMS-concentrations. -

Post-treatments	Survival	Sterility	Mutants	
			E.L.	Chl.
- , -	85	46	26	10
W, -	88	48	25	12
- , S+24/1	84	65	38	16 (?)
- , S+24/5	84	61	34	21
- , S+24/14	88	56	30	16
W, S+24/1	92	56	29	14
W, S+24/14	88	43	18	9
W, S-20/1	72	50	34	14
W, S-20/14	48	40	24	10

(5) Dry-storage at -20°C (W, S-20/1) shows a spectrum shift relative to +24°C (W, S+24/1), characterized by decreased sterility and increased number of embryonic lethals (the chlorophyll mutants remain constant). This shift arises within one day dry-storage and shows no further increase. It could be due to the inserted vacuum treatment. - (6) Most surprisingly, the decrease of all three types of

damage is of the same order of magnitude during dry-storage at -20°C and at 24°C. Repair of already induced lesions or prevention of damage to be induced (e.g., by residual EMS after sowing) thus resembles a physical process.

In extensive posttreatment experiments with barley seeds GAUL (1966) used a very high EMS-dose (290 mM, 3 hrs, 24°C), so that without postwashing (-,-) all seeds were killed, and no amount of washing could prevent 100% seed-killing when dry-stored for 14 days at 24°C. The only items which were left and which can be tentatively compared with our *Arabidopsis* experiment are 24 hrs washing (running tapwater, 24°C) and either directly sown in the field (W,-) or first dried (vacuum, 18 hrs, 40°C) and dry-stored at -20°C for 42 days (W, S-20/42). These treatments gave resp. 11.8 and 16.5 % M₁-seedset and resp. 21.4 and 21.0 % chlorophyll mutants. This trend is roughly similar to that found by us for (W,-) versus (W, S-20/14). - It is obvious that several points of divergence remain to be clarified. Further experiments are being planned.

References:

- GAUL, H., et al.: In "Mutations in plant breeding". Symp. IAEA/FAO, Vienna, 1966. Pp. 63-84. (See also Radiat. Bot. 6, 505-518, 1966; 7, 289-301, 1967)
 MÜLLER, A.J.: Biol. Zbl. 84, 759-762 (1965)
 - : Züchter 36, 201-220 (1966)
 - : Arabid. Inf. Serv. 4, 43-44 (1967)

Absence of correlation between the frequency of mutations in M₂ and some developmental characters in M₁ generation.

J. VELEMÍNSKÝ, V. POKORNÝ, and T. GICHNER

(Institute of Experimental Botany, Prague, Czechoslovakia)

In order to test the relations between some characters of the first (M₁) generation and the frequency of mutations in the second (M₂) generation after the mutagenic treatment, we combined the technique of CETL (1965, and pers. communic.) for the measurement of developmental characters and of MÜLLER (1963) for the detection of recessive embryonic and chlorophyll lethals. The seeds were treated with 0.2 mM and 0.3 mM methyl-nitrosourea for 24 hrs and sown in constant distances and depth in the soil. One part

Table 1

Treatment	Conditions	Number of plants	\bar{x}	$s_{\bar{x}}$	\bar{y}	$s_{\bar{y}}$	m_c
Control	growth chamber	169	41.2	0.20	12.5	0.21	0.07
	greenhouse	173	30.0	0.18	7.5	0.17	2.08
MNU, 0.2 mM	growth chamber	156	39.6	0.15	14.5	0.19	20.86
	greenhouse	177	28.8	0.09	10.6	0.16	21.65
MNU, 0.3 mM	growth chamber	170	40.1	0.16	12.9	0.18	34.33
	greenhouse	169	28.1	0.16	7.9	0.15	34.04

Table 2

	Concentration of MNU	Conditions	Number of plants	r
y:m _c	0.2 mM	growth chamber	148	-0.148
	0.3 mM	" "	160	0.024
	0.2 mM	greenhouse	129	-0.048
	0.3 mM	" "	132	0.005
x:m _c	0.2 mM	growth chamber	116	-0.041
	0.3 mM	" "	105	-0.086
	0.2 mM	greenhouse	121	-0.021
	0.3 mM	" "	123	0.069

r = coefficient of correlation

of plants was then cultivated during the whole life cycle in the greenhouse (in June and July) and the second part in a growth chamber with constant humidity (75 %) and temperature (18±1°C). In the first month the plants grew under an 8 hrs day, later under a long day (16 hrs). On each plant the number of leaves in the rosettes (y), the number of days between the emergence and the appearance of flower primordia (x) and the number of mutant seeds in all siliquae (m_c) were determined. Under greenhouse conditions the mean number of rosette leaves was significantly lower and the length of vegetative phase significantly shorter than under the conditions in the growth chamber. This did not, however, influence the frequency of recessive lethals (Table 1). We also did not find any correlation between the frequency of mutations and the number of rosette leaves (y:m_c) or number of days to the appearance of flower primordia (x:m_c) (Table 2).

References:

CETL, I.: Arabid. Inf. Serv. 2, 3-4 (1965)
MÜLLER, A.J.: Biol. Ztbl. 82, 133-163 (1963)

The influence of pH on the mutagenic activity of nitroso-compounds on Arabidopsis

T. GICHNER and J. VELEMÍNSKÝ

(Institute of Experimental Botany, Prague, Czechoslovakia)

The release of diazoalkanes is a condition for the activity of nitrosoamides and nitrosoamines. In the case of nitrosoamides, e.g., N-ethyl-N-nitro-N-nitrosoguanidine (ENG) and N-ethyl-N-nitrosoourea (ENH) the release of diazoalkanes is a result of a hydrolytic decomposition. Diazoalkanes are, however, very labile and if the decomposition takes place outside the seeds, they do not probably reach the

Table: The frequency of segregating M₁ siliquae

pH	2.4 mM ENG	1.4 mM ENH	12 mM MBNA	25 mM EVNA
3	-	94.1	55.0	84.1
4	66.2	85.1	-	-
5	54.8	85.9	53.1	61.0
6	64.8	81.8	-	-
7	12.3	1.3	22.1	72.1
8	-	-	-	-
9	-	-	32.2	65.5

mutable sites in the meristematic cells. The rate of hydrolysis depends on the temperature and on the pH of the solution. At pH 3 to pH 6 it is relatively slow, but at pH 7 and in alkaline solutions it steeply increases. For ENH at pH 7 the half-life is about 1.5 hr (see DRUCKREY et al., 1967) and as a consequence only a small amount of unhydro-

lyzed nitrosoamides is taken up in the seeds during the 24 hrs treatment. This can perhaps explain, why ENH and ENG are very active at pH 3 to pH 6 and why their activity is lowered at pH 7. - Nitrosoamines, e.g., methylbenzyl nitrosamine (MBNA) and ethylvinyl nitrosamine (EVNA) are very stable, even in alkaline solutions. Their decomposition takes only place in the seeds after the action of specific enzymes. They are mutagenic, therefore, regardless of the pH of the mutagenic solution, at least in the range of the pH-solutions tested.

References:

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

Mutagenic activity of some nitrosoamines

J. VELEMÍNSKÝ and T. GICHNER

(Institute of Experimental Botany, Prague, Czechoslovakia)

12 different nitrosoamines were applied on seeds of Arabidopsis, Dijon (24 hrs, 25°C) and their mutagenic activity was evaluated according to the frequency of siliquae segregating embryonic and chlorophyll recessive lethals = m_b (MÜLLER, 1963). Mutagenic active (table) were ethylvinyl nitrosamine (EVN) and further nitrosoamines, with at least one methyl group on the side chains and with at least one hydrogen atom bound on the alpha-C-atom. EVN is known as an unstable nitrosamine which is able to hydrolyse, whereas other active nitrosoamines are known as stable ones, decomposing only enzymatically (see DRUCKREY et al., 1967). It could be assumed that the seeds of Arabidopsis contain enzymes, specifically decomposing only nitrosamines with a methyl group.

Nitrosoamines	sublethal conc.	max. m _b	Nitrosoamines	sublethal conc.	max. m _b
dimethyl-	1.000 M	75.2	ethylvinyl-	0.100 M	73.0
methylethyl-	0.100 M	59.5	ethyl-terc.butyl-	0.125 M	1.3
methylbutyl-	0.075 M	18.6	dibutyl-	0.030 M	1.5
methyl-terc.butyl-	0.100 M	0.2	methylphenyl-	0.020 M	2.8
methylbenzyl-	0.020 M	81.6	methylpiperazine	1.000 M	0.5
diethyl-	0.225 M	1.2	nitrosomorpholine	1.000 M	0.6

References:

DRUCKREY, H., R. PREUSSMANN, S. IVANKOVIC, and D. SCHMÄHL: Z.f.Krebsforsch. 69, 103-201 (1967)
MÜLLER, A.J.: Biol. Ztbl. 82, 133-163 (1963)

The mutagenic activity of β -hydroxyethyl methanesulfonate (HOEMS), β -methoxyethyl methanesulfonate (MOEMS) and diethyl 1,3-propanedisulfonate (DEPD) on *Arabidopsis*

T. GICHNER, L. EHRENBERG, and C.A. WACHTMEISTER

(Institute of Experimental Botany, Prague, Czechoslovakia; - Institute of Biochemistry and Institute of Organic Chemistry, University, Stockholm, Sweden)

Monofunctional HOEMS and MOEMS as well as bifunctional DEPD induce high frequencies of embryonic and chlorophyll mutations. The high frequency of mutations induced by DEPD may be caused by the fact that this compound is not crosslinking but is able to provoke two independent ethylations. - The mutagenic efficiencies of these compounds, using mutation rate vs. sterility as a criterion, is approximately on the same level as that of EMS and much higher than after the X-ray treatment. - The mutagenic effectiveness, using molar concentration vs. mutation rate as a criterion, is for HOEMS and MOEMS much lower than for EMS and it is in line with their alkylation rates. The mutagenic effectiveness of DEPD is lower than the effectiveness of EMS, although the alkylation rates of DEPD are higher.

Abstract of a paper which will appear in "Hereditas"

The mutagenic activity of N,N,N' - trimethylnitrosoarea (TMNH) and p-tolylsulfonyl methyl nitrosamide (TSMN)

J. VELEMÍNSKÝ and T. GICHNER

(Institute of Experimental Botany, Prague, Czechoslovakia)

Two further nitrosoamides have proved to be very strong mutagens for *Arabidopsis thaliana*. Only the highest frequencies of induced mutations are given in the following table (treatment time 24 hrs, temperature 25°C). TMNH had no mutagenic activity

Treatment	Degree of sterility	Frequency of segregating M ₁ siliquae	on <i>Saccharomyces cerevisiae</i> (MARQUARDT et al., 1964); it was, however, strongly carcinogenic on rats (IVANKOVIC et al., 1965). TSMN was not mutagenic on <i>Saccharomyces cerevisiae</i> (MARQUARDT et al., 1964) and not carcinogenic on rats (DRUCKREY et al., 1961).
TMNH 75 mM	97.3	97.4	
TSMN 0.75 mM	54.0	82.4	

References:

- DRUCKREY, H., R. PREUSSMANN, D. SCHMÄHL, and M. MÜLLER: Naturwiss. 48, 165 (1961)
 IVANKOVIC, S., H. DRUCKREY, and R. PREUSSMANN: Z.f.Krebsforsch. 66, 541-548 (1965)
 MARQUARDT, H., F.K. ZIMMERMANN, and R. SCHWALER: Z.Vererbungslehre 95, 82-96 (1964)

Genic male sterility in *Arabidopsis*

A.J. MÜLLER

(Institut für Kulturpflanzenforschung, Gatersleben, Krs. Aschersleben, Germany)

A recessive mutant *ms* causing male sterility has been induced by X-ray treatment (12 kR on presoaked seeds) of "Dijon G". The M₂ family segregated into 12 fertile and 5 sterile plants. Pollination of the sterile plants with wild-type pollen resulted in 96.5% seed setting (250 ovules studied). 30 F₁ plants were grown to maturity; all proved to be fertile. The F₂ segregated into 22 fertile and 13 sterile plants. The result of the backcross "sterile x F₁" was 134 fertile: 146 sterile.

Table: Classification of anthers

Genotype	No. of pollen grains per anther							Total No. of anthers studied
	0	1-10	11-30	31-70	71-150	151-400	401-460	
<i>ms ms</i>	24	9	30	27	36	-	-	126
<i>ms ms+</i>	-	-	-	-	-	-	26	26
<i>ms+ms+</i>	-	-	-	-	-	-	24	24

Genotype	No. of normal pollen grains per anther							Total No. of anthers studied
	0	1-10	11-30	31-70	71-150	151-400	401-460	
<i>ms ms</i>	40	38	36	7	5	-	-	126
<i>ms ms+</i>	-	-	-	-	-	-	26	26
<i>ms+ms+</i>	-	-	-	-	-	-	24	24

The flowers of the *ms ms* plants contain 6 anthers of + reduced size, which open somewhat delayed. 126 anthers (2 per flower) from the basal flowers of 10 sterile plants were studied microscopically. As shown in the Table, the number of pollen grains per anther varied from zero to 148 (control: 431±12). Most of the pollen grains were abortive and of reduced size, but some were stainable with acetocarmine and, therefore, classified as normal. With respect to the mean number of abortive and normal pollen grains per anther, there were no significant differences between various *ms ms* plants. After natural selfing 835 seeds from about 280.000 ovules were harvested (seed setting: 0.3%). The genotype of these seeds was confirmed as *ms ms*. In a second

sowing performed in winter seed setting was only 0.005 % (7 seeds from about 150.000 ovules). Thus, it is difficult to propagate the mutant by natural selfing, although sterility is not complete.

Growth rate, vegetative characters and flower morphology are entirely normal. Only the terminal flowers of all inflorescences are regularly malformed. They consist of \pm effeminated organs. The margin of the sepals forms small white ovules and often a papillose stigma. The petals and stamens are partially aborted.

Lebensfähige und letale fusca-Mutanten bei Arabidopsis thaliana

A.J. MÜLLER und Ulrike HEIDECKER

(Institut für Kulturpflanzenforschung, Gatersleben, Krs. Aschersleben, Germany)

Unter der Bezeichnung fusca werden entsprechend der früher vorgeschlagenen Klassifizierung embryonaler Anomalien alle Mutanten zusammengefaßt, die sich durch das Auftreten von Anthozyan im Embryo des unreifen bzw. reifen Samens auszeichnen. Zwei durch Röntgenstrahlen induzierte rezessive Mutationen dieses Typs sind bereits kurz beschrieben worden (MÜLLER, 1963). Inzwischen konnte durch verschiedene chemische Mutagene eine große Zahl von fusca-Mutationen ausgelöst werden (vgl. MÜLLER, 1964, 1966). Über die relative Häufigkeit ihres Auftretens unterrichtet die folgende Tabelle,

M u t a g e n	Anzahl der Mutationen		
	<u>fusca</u>	Chlorophyll	<u>fusca</u> : Chlorophyll
N-Nitroso-N-methyl-N'-nitroguanidin	17	599	1 : 35,3
N-Nitroso-N-methylharnstoff	8	287	1 : 35,9
N-Nitroso-N-methylurethan	23	938	1 : 40,7
Äthylmethansulfonat	14	425	1 : 30,4
iso-Propylmethansulfonat	6	336	1 : 56,0
total	68	2585	1 : 38,0

in der unter Zusammenfassung der Ergebnisse verschiedener Versuchsserien (mit im einzelnen unterschiedlichen Mutationsfrequenzen) die Anzahl der rezessiven fusca-Mutationen der Anzahl der gleichzeitig induzierten rezessiven Chlorophyllmutationen gegenübergestellt ist (Chlorophyllmutanten = albina, xantha und chlorina, bestimmt nach der Farbe der Kotyledonen vor Beginn der Samenreife).

Die überwiegende Mehrzahl der bisher vorliegenden fusca-Mutanten läßt sich wie folgt charakterisieren: Die Mutanten-Embryonen unterscheiden sich vor Beginn der Samenreife hinsichtlich Größe und Gestalt nicht oder nur geringfügig vom Wildtyp (Dijon G). Die Kotyledonen weisen Chlorophyll auf, doch ist die Grünfärbung in den meisten Fällen von etwas geringerer Intensität als beim Wildtyp. Die Ausbildung des karminroten Farbstoffes beginnt frühestens im Stadium der Kotyledonenkrümmung und erreicht ihren Maximalwert im Verlauf der Samenreife. In manchen Fällen bestehen deutliche Unterschiede zwischen den einzelnen Mutantenstämmen. In manchen Fällen beschränkt sich die Farbstoffbildung weitgehend auf die Grenzzone zwischen Kotyledonen und Hypokotyl; in anderen Fällen sind außer dieser Zone (die dann besonders intensiv gefärbt erscheint) auch die Kotyledonen selbst und die sproßseitige Region des Hypokotyls karminrot gefärbt. Der Farbstoff weist die typischen Charakteristika eines Anthozyans auf: Er ist ausschließlich in den Vakuolen lokalisiert, wird unter dem Einfluß von NH_4OH (oder einer anderen Base) blaugrün und gibt mit FeCl_3 eine dunkelviolette Färbung (Phenolreaktion). Offensichtlich handelt es sich um das gleiche Anthozyan, das in Wildtyp-Keimpflanzen bei starker Belichtung bzw. Mineralstoffmangel gebildet wird und dort gleichfalls bevorzugt in der Grenzzone zwischen Kotyledonen und Hypokotyl auftritt. (In den Wildtyp-Embryonen entsteht unter unseren Kulturbedingungen kein Anthozyan.) Die Anthozyaneinlagerung bleibt bei der Samenreife erhalten, so daß eine klare Unterscheidung zwischen Mutante und Wildtyp in der Regel auch an vollreifen Samen möglich ist. Bei einem Teil der Mutantenstämme sind die Samen \pm geschrumpft, jedoch ist - wie das Vorkommen von fusca-Mutanten mit normal großen, ungeschrumpften Samen beweist - ein derartiges Defizit an Reservestoffen keineswegs obligate Begleiterscheinung der Anthozyaneinlagerung.

Die Keimung erfolgt meist etwas verzögert. Bei der Mehrzahl der bisher geprüften Mutantenstämme verlieren die Keimlinge das Anthozyan nicht und sterben auf Erde oder Minimalmedium vor der Ausbildung der Primärblätter. Bei anderen Mutantenstämmen bilden sich aus den Keimlingen unter Verlust des Anthozyans lebensfähige, grüne Pflanzen, die meist in ihrem Wachstum gehemmt sind. Die Fertilität ist in der Regel gut.

Auf eine fusca-Mutante, die auch wegen ihrer Eignung als genetische Marke Interesse beansprucht, sei im folgenden näher eingegangen:

fusca (315): Intensive Rotfärbung der Kotyledonen. Die reifen Samen sind stark geschrumpft. Das der Schrumpfung zugrunde liegende Defizit an Reservestoffen (Fett!) beschränkt sich auf die Kotyledonen, während das Hypokotyl fest und rund erscheint. Voll ausgereifte Samen konnten bisher noch nicht zum Keimen gebracht werden. Im Gelbreife-Stadium befindliche und daher noch nicht maximal geschrumpfte Samen keimen dagegen 100%ig ohne Keimruhe. (Aus den gleichen Schoten stammende Wildtyp-Samen keimten erst nach 2-5 Wochen.) Das Anthozyan wird innerhalb von 3-5 Tagen nach Keimbeginn vollständig zurückgebildet. Die entstehenden Pflanzen haben einen völlig normalen Habitus und unterscheiden sich in Größe und Blühalter nicht signifikant vom Wildtyp. Die Fertilität der Pollen- und Samenanlagen ist normal. F_2 -Spaltung = 341 wild : 107 fusca.

Die Anthozyanbildung stellt ein relativ unspezifisches Indiz für solche Stoffwechselanomalien dar, die zu einem Überschuß an Pyruvat bzw. Acetyl-CoA führen. Bei Mutantensstämmen mit geschrumpften Samen ist offensichtlich die Synthese von Reservestoffen gehemmt. Der normale Ablauf der postembryonalen Entwicklung von fusca (315) zeigt, daß in diesem Fall ein ausschließlich zur Bildung von Speicherfett notwendiger Reaktionsschritt blockiert ist. - Genaue genetische und biochemische Untersuchungen an fusca-Mutanten wurden begonnen.

S u m m a r y : Recessive mutants with anthocyanin in the embryos have been isolated after various mutagenic treatments. Most strains are lethal in the seedling stage, but some are viable and fertile.

Literatur:

- MÜLLER, A.J.: Biol.Zbl. 82, 133-163 (1963)
- : Züchter 34, 102-120 (1964)
- : Züchter 36, 201-220 (1966)

Chromosome studies in Arabidopsis thaliana

Lotti M. STEINITZ-SEARS and Suzanne LEE-CHEN

(Genetics Department, University of Missouri, Columbia, Missouri, U.S.A.)

On the basis of occasional good pachytene to diplotene figures, it was determined that trisomic II involves the main nucleolar chromosome (figures 1 a-d). It was reported earlier (CHEN and SEARS 1967) that II carries REDEI's linkage group 4. The weakest trisomic, which had been lost, was reobtained among the offspring of 3n material. It is shown in figures 1 e-h. The linkage group of this chromosome is not yet known.*

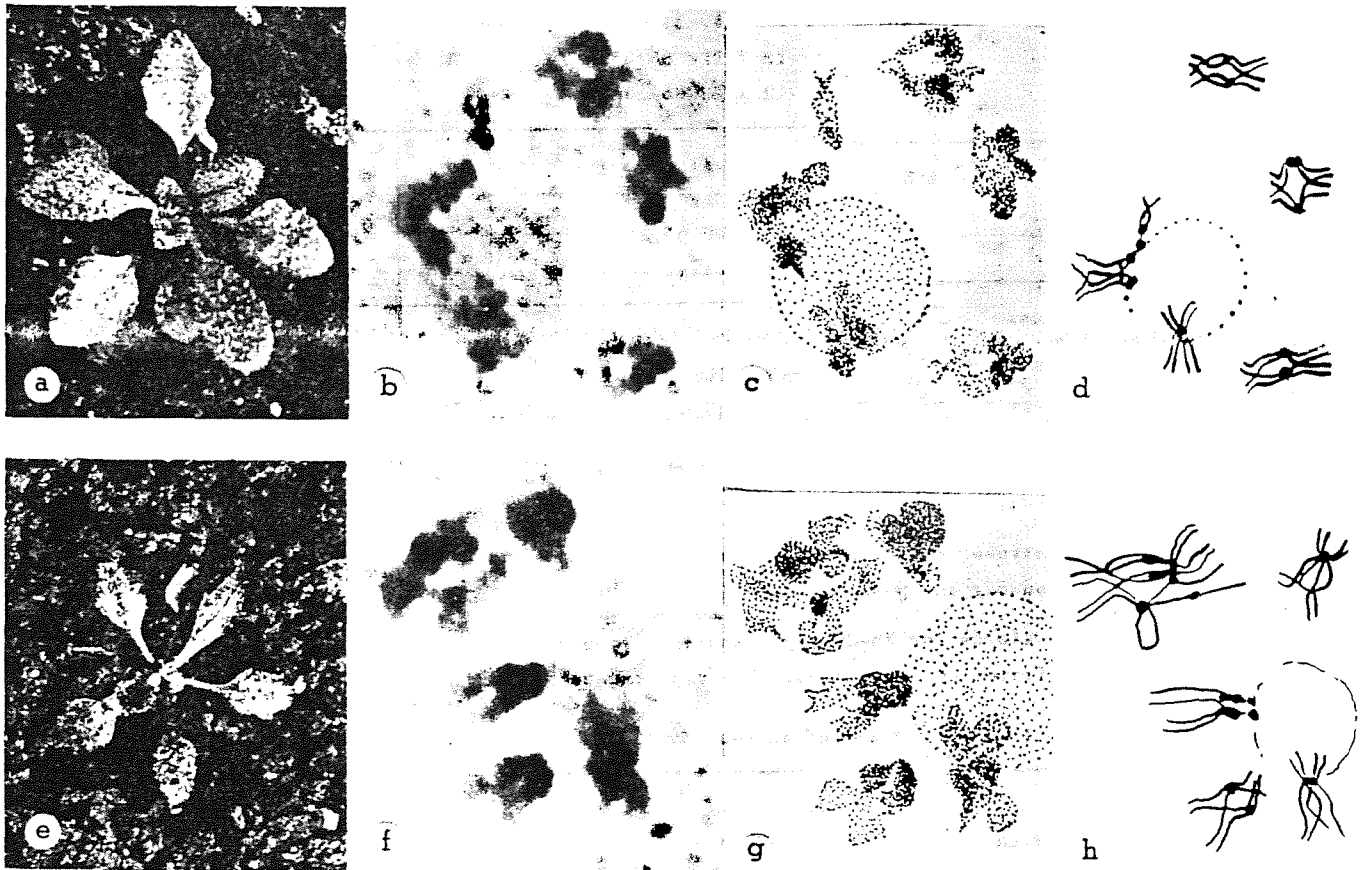


Figure 1: Trisomic plants (a and e), photomicrographs of diplotene nuclei (b and f), drawings of the same diplotene cells (c and g), and diagrammatic interpretation of the same cells (d and h).

Reference:

LEE-CHEN, Suzanne, and Lotti M. STEINITZ-SEARS: Can.J.Genet.Cytol. 9, 381-384 (1967)

* With these two types the set of 5 trisomics in Arabidopsis is complete. The Editor.

The fixation of radiation energy during the development in chlorophyll b deficient mutants of Arabidopsis

A.R. KRANZ

(Institute of Botany, University of Frankfurt-M., Germany)

The still unknown function of the chlorophyll b has been discussed in the new research work about the two sensibilization systems for the light reactions I and II of the photosynthesis (ARNON et al., 1964; WITT, 1966). But certain chlorophyll b

Table 1: Experimental conditions in growth chamber III ('66-10)

Arabidopsis thaliana	ch ⁺ = En-2 ch ¹ ch ² ch ³ = V 81
substrate	8 p. loam 2 p. peat 1 p. sand
temperature	light 16 h 25 ± 2 °C dark 8 h 15 ± 2 °C
humidity	light 16 h 50 ± 5 % dark 8 h 80 ± 5 %
radiation	normal 32.1 cal · cm ⁻² · d ⁻¹ yellow 24.1 cal · cm ⁻² · d ⁻¹ red 20.2 cal · cm ⁻² · d ⁻¹

Table 2: Spectral radiation energy (μwatts · cm⁻²) distribution in the plant level ('66-10, 13./14.3.67). Spectral light meter after ROBERTSON and HOLMES (1965)

wavelength -- millimicrons (interference filter no.)	342 1	437 2	535 3	642 4	742 5
normal 12 x TLF 65/55 12 x WU 65/25	< 0.1	4.35	4.00	4.70	1.12
yellow 12 x TLF 65/55 12 x WU 65/25 RH-plexiglass 300	< 0.1	0.43	4.10	4.80	1.12
12 x TLF 65/55 12 x WU 65/25 RH-plexiglass 501	< 0.1	0.25	0.01	4.40	1.12

Table 3: Ranges of the genotypes, radiation qualities and ontogenetic stages in running time, energy and pigment value.

Ref.: parenthesis stands for En-2 only. Sample 1 = young plant, sample 2 = rosette phase, sample 3 = flowering time, sample 4 = ripening husks.

	Genotype (mutant)	Radiation quality (filter)	Ontogenetic stages (samples)
Running time: p.d.	ch ⁺ < ch ² < ch ¹ < ch ³	(red) » yellow > normal	1 < 2 < 3 < 4
Energy value: Cal/pl. Cal/dm ² Cal/g	ch ¹ ≈ ch ⁺ > ch ³ ≈ ch ² ch ¹ ≈ ch ⁺ > ch ² > ch ³ ch ⁺ ≈ ch ¹ > ch ² > ch ³	(red) » normal > yellow (red) » yellow > normal (red) ≈ yellow < normal	2 < 3 << 4 2 << 3 < 4 2 < 3 << 4
Pigment value: Chl.a./dm ² Chl.b./dm ² Carot./dm ²	ch ⁺ » ch ¹ ≈ ch ² > ch ³ ch ⁺ » ch ² ≈ ch ³ > ch ¹ ch ⁺ » ch ¹ > ch ² > ch ³	(red) < yellow < normal (red) ≈ (yellow) < normal (red) < yellow < normal	1 < 2 < 3 ≈ 4 1 < 2 << 3 » 4 1 < 2 > 3 < 4

deficient mutants of Chlorella have evidentially the ability of normal photosynthesis (METZNER et al., 1965; WILD and EGLER, 1967), although there is a deficiency of starch production at least in some cases of Pisum (MÜLLER, 1964).

Starting from these results we have been interested in the problem of the radiation energy fixation during the ontogenetic dry matter production in three quantitative chlorophyll b deficient mutants of Arabidopsis. We are grateful to Profs. REDEI and RÖBBELEN for seeds of the genetic material we have used.

Our experiments were carried out under constant climatic conditions (see table 1) in the three radiation ranges produced with plexiglass filters: normal (~300-800 nm) and red (~600-800 nm). This results in a gradual elimination of the radiation energy within the region of the leaf pigment absorption, whereas at the phytochrome action peaks (660 and 730 nm) the energy is not changed (see table 2). Thus the phytochrome system can function normally (HOCK and MOHR, 1965; RUDOLPH, 1965).

Here the gained results shall be presented briefly in a range comparison statistically tested by SPEARMAN's range correlation. As shown in table 3 all the analyzed characters - the running time of development, the energy values of the dry matter, and the leaf pigments - increase with progressive ontogenesis. During the ripening of the husks and the flowering only chlorophyll b and carotenoid contents decrease. The various radiation qualities cause the expected decrease of the developmental time from red to yellow and to normal, while the energy values are increasing except for the calories per gram of dry weight and dm² of leaf area. The pigment values rise as expected with increasing normalization of the radiation quality but there is no essential difference in chlorophyll b content under red or yellow light. The three monogenic mutants of the ch-locus show the known range order in the chlorophyll b content: ch⁺ > ch² > ch¹ (RÖBBELEN and VELEMINSKY, 1965). But there is another order of the mutants in the six remaining characters. The values of calories per gram, chlorophyll a and carotenoids indicate the order ch⁺ > ch¹ > ch² > ch³. The other characters deviate from this order.

In accordance to HIRONO and REDEI (1963) we have detected an equal amount of assimilation starch in the leaf parenchym chloroplasts of the mutant ch¹ which shows after thin layer chromatographic separation under the UV-lamp absolutely no chlorophyll b in all ontogenic stages. The apparent radiation energy used as defined by the author (KRANZ, 1968) runs up to ~1 p.c. like in other annual higher plants and this character is positively correlated with the values of the chlorophyll a and the carotenoids.

Summarizing, we may state that there is no proved genetic and ontogenetic correlation between chlorophyll b and the fixation of the radiation energy in the dry matter. Whenever the analyzed genotypes would only differ in their alleles within the ch-locus (LEE-CHEN and STEINITZ-SEARS, 1967) they would cause a specifically pleiotropic action on the remaining characters studied in this paper. Then we could postulate there is on one hand the main effect of the ch-locus on the chlorophyll a -- and the carotenoid synthesis, the calorie value p.g. and the time of development and on the other hand the secondary effects on the chlorophyll b content and the other calorie values. The interaction of the phytochrome system with the studied reactions of characters may be eliminated on account of the arranged radiation conditions.

References:

- ARNON, D.J., et al.: Proc.nat.Acad.Sci.(Wash.) 51, 1274-1282 (1964)
HIRONO, Y., and G.P. REDEI: Nature (Lond.) 197, 1324-1325 (1963)
HOCK, B., and H. MOHR: Planta (Berl.) 65, 1-16 (1965)
KRANZ, A.R.: Arabid.Inf.Serv. 4, 20-21 (1967)
- : Angew.Bot.(Berl.) 41, in press (1968)
LEE-CHEN, S., and L.M. STEINITZ-SEARS: Canad.J.Genet.Cytol. 9, 381-384 (1967)
METZNER, H., et al.: Planta (Berl.) 65, 186-194 (1965)
MÜLLER, F.: Planta (Berl.) 63, 65-82 (1964)
ROBERTSON, G.W., and R.M. HOLMES: Ecology 44, 419-423 (1963)
RUDOLPH, E.: Planta (Berl.) 66, 75-94 (1965)
VELEMINSKY, J., and G. RÖBBELEN: In "Arabidopsis Research", Rep.Int.Symp.Göttingen. Pp. 131-137, 1965
WILD, A., and K. EGLER: Biol.Zbl. (Leipz.) in press (1967)
WITT, H.T.: Umschau (Frankf./M.) 66, 589-596 (1966)

This work has been supported by a grant from Deutsche Forschungsgemeinschaft.

B. TECHNIQUES

Techniques of artificial selection

D.G. PEDERSON and D.F. MATZINGER

(Department of Genetics, North Carolina State University, Raleigh, N.C., U.S.A.)

A mass selection study is being carried out on 14-day fresh-weight in several artificially constructed populations. The plants are grown in a controlled environment under aseptic conditions (LANGRIDGE, 1957), and after being weighed they are stored prior to selection of the best plants. The storage trays are commercially-made holders for "Dispo" assay trays, each with a sheet of "Plexiglass" laid across the top. A number of 8 mm holes are bored in each cover so that one tray corresponds to one of the wooden blocks used to hold test tubes. In use, the trays are filled with water and plants are placed one to a hole, in which position they will remain viable for 24 hours or more. If it is known that some plants will not be included in the selected group, circles are drawn around the holes corresponding to those plants. The time saved may be important, since one person can weigh and store only about 80 plants per hour. - The plants are potted, initially on agar blocks, by a technique similar to that of Van Der VEEN (1966) giving an ultimate survival rate of close to 100 per cent. An overall generation time of 3 months has been maintained in the study involving cross-fertilization of selected plants.

References:

LANGRIDGE, J.: Aust.J.Biol.Sci. 10, 243-252 (1957)
Van Der VEEN, J.E.: Arabid.Inf.Serv. 2, 31 (1965)

A simple technique for screening thiamine auxotrophs

S.L. LI and G.P. RÉDEI

(Department of Genetics, University of Missouri, Columbia, Mo., U.S.A.)

Several years ago a program to isolate nutritional mutants was started in our laboratory. Initially the progenies of single seeds exposed to mutagen were handled individually. By this method in approximately six thousand progenies of X-ray treated seeds 5 thiamine auxotrophs could be obtained. Similarly handled populations of over 2,000 plants obtained after ethylmethane sulfonate treatments of the seed yielded 6 thiamine deficient mutants. Obviously this method is too laborious for the isolation of a larger number of mutations at specific loci.

The following modifications have since been introduced. Instead of placing with forceps only five mutagen treated seeds per five-inch diameter pots, 50 seeds were planted to the same. The treated seeds were suspended in 0.12% agar in a concentration of 2.5 seeds per ml. This free-flowing liquid was supplied dropwise to the surface of the soil or distributed as a very fine continuous flow from a separatory funnel. 20,000 or more seeds can be planted in good distribution within an hour with some experience. The germination and growth of a material planted in this manner is entirely satisfactory. In one experiment 47,415 seed bearing plants were obtained from 88,235 seeds treated with 12,000 R X-rays after 24 hours presoaking. Another similar seed population exposed to 0.24% ethyl methane sulfonate produced 48% survivors.

The plants grown in the same pot were harvested in bulk by threshing the seed on a sheet of paper. Approximately 300-400 seeds from each bulk progeny were then broadcast on the surface of soil in five-inch pots again. The mutants were scored several times during the first three weeks after germination. Since the great majority of the thiamine auxotrophs display a normal or nearly normal transmission, the statistical probability assures us of about 80% recovery of all the thiamine mutants induced. Most of those that remain undetected are not very useful for genetic studies because of the poor penetrance. This new isolation procedure required only about 1/10 the space and labor relative to the old technique.

The nutritional mutants are first selected in the second generation after the treatment in soil cultures. A "complete medium" containing vitamins, amino acids, nucleic acid precursors is applied to the non-growing or poorly developing seedlings after germination. The exact requirement, if any, is verified later in aseptic agar cultures.

To be sure that all new isolates are really new occurrences rather than mechanical seed contaminations from previous experiments, genetically marked different stocks of Columbia wild type background were used for each experiment.

So far 62 independent thiamine auxotrophs have been analyzed in our laboratory. From these 40 (64.5%) require only the pyrimidine moiety; 9 (14.5%) respond to "vitamin thiazole"; 13 (21%) grow only with intact thiamine.

References:

FEENSTRA, W.J.: Genetica 35, 259 (1964)
RÉDEI, G.P.: Genetica 45, 1007 (1960)
- : Genetica 47, 979 (1962)
- : Amer.J.Bot. 52, 834 (1965)

Herstellung von Totalpräparaten zur Darstellung von Embryonen

C.G. ARNOLD und D. CRUSE

Fruchtknoten bzw. Schoten von Arabidopsis thaliana werden ohne vorherige Fixierung in Karminessigsäure eingelegt, der auf 200 ml je 1 ml einer 10%igen FeCl₃ Lösung zugesetzt wurde. Die Dauer der Einwirkung schwankt je nach dem Entwicklungsstadium der



Ovules with embryos
stained in carmine

Fruchtknoten zwischen 2 und 7 Wochen; bei längerer Aufbewahrung in der Lösung werden die Objekte zu stark mazeriert. Nach dieser Behandlung mit Karminessigsäure + Fe bringt man die Objekte auf einen Objektträger in einen Tropfen Karminessigsäure ohne Fe. Die Klappen der Schoten werden vorsichtig abpräpariert, die Plazenten vom Septum getrennt und beide Hälften etwas auseinandergezogen. Darauf wird das Präparat mit einem Deckglas bedeckt und einige Male vorsichtig durch eine kleine Flamme gezogen, ohne es dabei zu kochen. Mikroskopische Kontrolle! Auch ist jede Druckeinwirkung auf das Deckglas unbedingt zu vermeiden. Die Präparate sind höchstens einen Tag haltbar. -
E r g e b n i s : Die dunkelrot gefärbten Embryonen sind durch die junge Samenschale hindurch deutlich zu erkennen (siehe Abb.).

Embryo test without seed loss

G.H. BALKEMA

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

For MÜLLER's embryo test seeds are scored for mutations 2 or 3 days before the seedcoat turns brown. I found that seeds of unopened siliques of this age germinate in about 14 days when the siliques are placed on agar (with a trace of KNO₃, in light, 24°C). The seedlings force open the siliques and can then be scored for mutations. - Green seeds, e.g. seeds from the embryo test, sown straight on the agar (KNO₃, light, 24°C) give over 95% germination. Green seed germinates after 11-12 days, whereas brown (almost ripe) seed takes 15-16 days. Cold treatment (3 days, 3°C, dark) after sowing r e t a r d e d germination in both cases. - Seeds sown in this way after the embryo test can give additional information on chlorophyll mutations scored at the seedling stage, and can be used to raise M₂ plants for further studies.

Further studies on Arabidopsis RNA

S. BONOTTO and M. JACOBS

(Section of Phytobiology-Agronomy, Department of Radiobiology, C.E.N., Mol, and Laboratory for Genetics of Higher Plants, Free University of Brussels, Belgium)

In No.4 of this newsletter, we have described a method of the extraction of Arabidopsis RNA (BONOTTO and JACOBS, 1967). This method has been further improved. We know from the work of RÉDEI (1967) that Arabidopsis possesses enzymes capable to digest RNA: a neutral ribonuclease, an acidic ribonuclease and a phosphodiesterase. It is therefore necessary to protect the RNA during extraction as good as possible against the action of these enzymes.

Although the utilization of naphthalene disulfonate (NDS) and of sodium dodecylsulfate (SDS) has previously provided good results, we have tried polyvinyl sulfate (PVS) as another inhibitor of ribonuclease. It was reported that this polyanion, introduced by FELLING and WILEY (1959), protected very well the RNA extracted from the endosperm of castor bean seeds at pH 5.0 (COCUCCI and STURANI, 1966). The RNA preparations which we have obtained from Arabidopsis seedlings with a mixture of NDS (1 mg/ml), PVS (100 µg/ml) and SDS (1%) seem quite satisfactory. Besides the utilisation of PVS, we have introduced in our method the following modifications:

After precipitation from the aqueous phase by the addition of 0.1 vol of 10% NaCl and 2.5 vol of absolute alcohol, the RNA, collected by centrifugation, is dissolved at 0°C in a small volume of 0.01 M NaCl, containing 100 µg/ml of PVS. The RNA solution, adjusted to a NaCl final concentration of 2 M with a solution of NaCl 4 M, containing 100 µg/ml of PVS is then allowed to stand 24 hrs at 4°C. In these conditions the ribosomal RNA (r-RNA) precipitates, while the soluble RNA (s-RNA) remains in solution. The r-RNA is collected by centrifugation and washed with cold 2 M NaCl in order to eliminate the contaminant s-RNA. The s-RNA remained in the supernatant is precipitated by the addition of 2.5 vol of cold absolute alcohol and collected by centrifugation. - The r-RNA and s-RNA are then dissolved in 0.3 ml of acetate buffer, layered on the top of 4.6 ml of 5-20% sucrose gradients, containing 50 µg/ml PVS, and centrifuged 15-16 hrs at 22.000 rev/min or 5 hrs at 37.500 rev/min in the SW 39 rotor of the Spinco ultracentrifuge. Two drop fractions, collected by puncturing the bottoms of the tubes, are diluted with 0.9 ml of distilled water and the absorbance at 260 mµ measured in the Cary spectrophotometer.

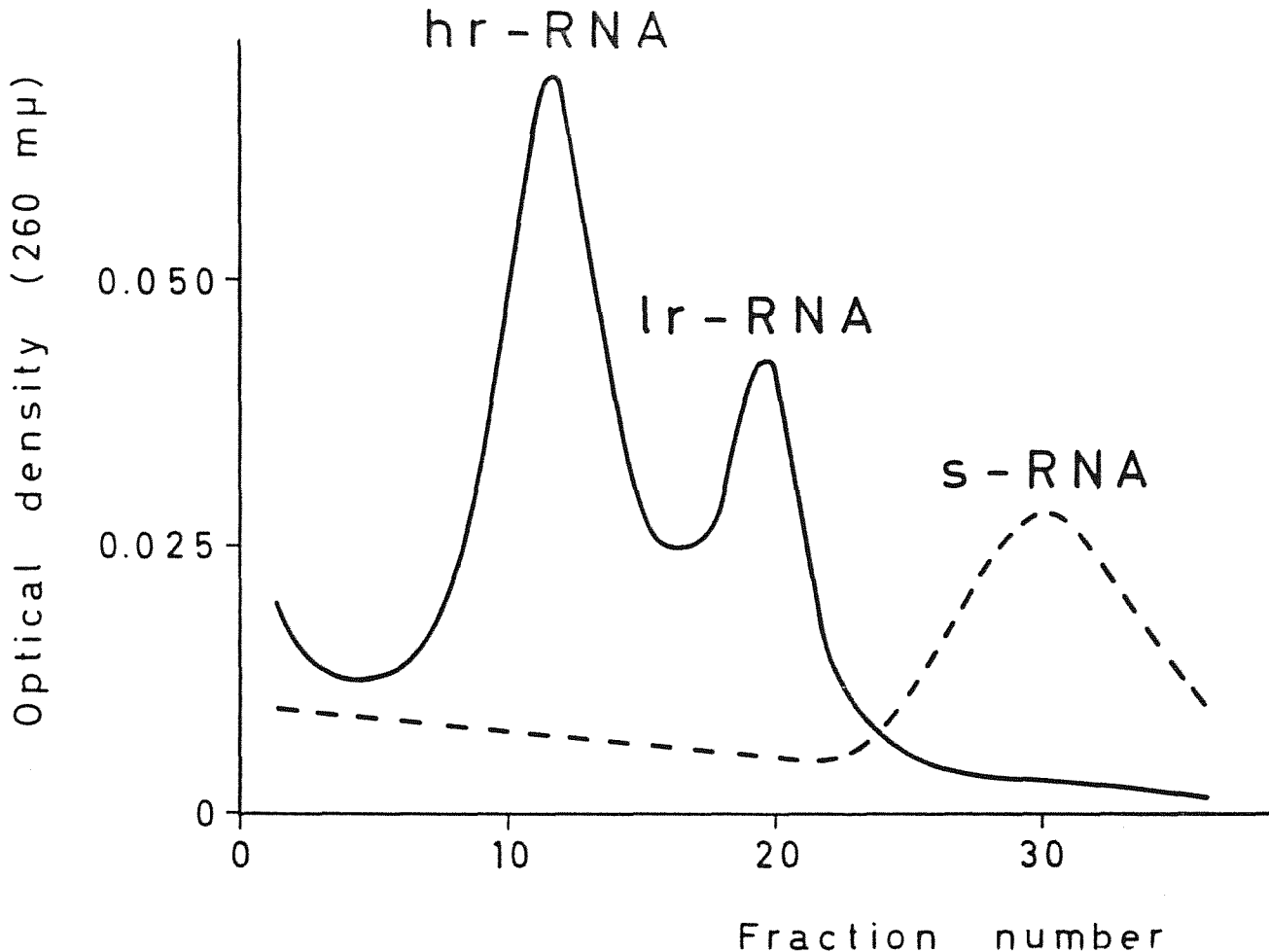


Figure: Sedimentation pattern of r-RNA and s-RNA extracted from 1 month old Arabidopsis plants. hr-RNA = heavy ribosomal RNA, lr-RNA = light ribosomal RNA, s-RNA = soluble RNA.

The figure shows the centrifugation pattern of r-RNA and s-RNA extracted from 1 month old Arabidopsis plants. It appears from the figure that our method allows a good separation of r-RNA from s-RNA, without any significant degradation. Moreover, a deoxyribonuclease treatment of the RNA preparation before ultracentrifugation in order to eliminate contaminant DNA, seems to be unnecessary. Finally, the presence of PVS in the sucrose gradients, utilized also by MURPHY and LOVETT (1966) for the RNA of Blastocladiella emersonii, is advantageous with respect to NDS because it does not absorb at 260 mμ. The RNA of the whole cells and of cellular organelles (chloroplasts and mitochondria) is now being studied.

References:

- BONOTTO, S., and M. JACOBS: Arabid. Inf. Serv. 4, 65-67 (1967)
- COCUCCI, S., and E. STURANI: Ital. J. Biochem. 15, 273-278 (1966)
- FELLING, J., and C.E. WILEY: Arch. Biochem. Biophys. 85, 313-316 (1959)
- MURPHY, M.N., and J.S. LOVETT: Developmental Biology 14, 68-95 (1966)
- REDEI, G.P.: Arabid. Inf. Serv. 4, 33 (1967)

We thank Mrs. Eliane BONNIJNS-Van GELDER for the technical assistance during a part of this work. This work was carried out with help of the "Fonds National de la Recherche Fondamentale Collective".

C. B I B L I O G R A P H Y

(Comprehensive list of titles on Arabidopsis published until December 1967, including those already cited in Arabid. Inf. Serv. Nos. 1-4)

- "Arabidopsis Research", Report of an International Symposium held at the University of Göttingen, Germany, April 21-24, 1965. Edit. Gerhard RÖBBELEN. Pp. 210 (with 30 original papers). Institut für Pflanzenbau und Pflanzenzüchtung, Universität Göttingen, July 1965
- ARNOLD, C.G., and D(oris) CRUSE: Eine 8-chromosomige Mutante von Arabidopsis thaliana. Flora 155, 474-476 (1965)
- , - : Die Wirkung von Thalidomid auf die Embryoentwicklung von Arabidopsis thaliana. Z.Pflanzenphysiol. 56, 292-294 (1967)
- BARENDSE, G.W.M.: Vernalisatie van Arabidopsis thaliana. In "Tuinbouwkundig Onderzoek, Jaarsverslag 1963". Ed.: Ministry of Agriculture, The Hague, Abstr., p. 281
- BARTHELMUSS, I(ise): Merkmalskorrelationen und Selektion bei Arabidopsis thaliana (L.) HEYNH. Z.Pflanzenzücht. 52, 273-332 (1964)
- BHATIA, C.R.: Discussion to paper of S.K.DEVI, K.N.PRASAD, and E.F.RILEY (in Radiat. Bot. 4, 347, 1964). Radiat.Bot. 6, 83-84, 376 (1966)
- : Increased mutagenic effect of ethyl methanesulfonate when dissolved in dimethylsulfoxide. Mutat.Res. 4, 375-376 (1967)
- , and J.H. Van der VEEN: Two-way selection for EMS-induced micromutations in Arabidopsis thaliana (L.) HEYNH. In "The Use of Induced Mutations in Plant Breeding". Rep.Meeting FAO/IAEA, Rome 1964, pp. 497-503. Pergamon, Oxford, 1965
- , and K.R.NARAYANAN: Genetic effects of ethyl methanesulfonate in combination with copper and zinc ions on Arabidopsis thaliana. Genetics 52, 577-581 (1965)
- BÖCHER, T.W., and K.LARSEN: Experimental and cytological studies on plant species. IV. Further studies in short-lived herbs. Dansk Vid.Selsk.Biol.Medd. 10, 1-24 (1958)
- BONOTTO, S.: A modification of MÜLLER's experimental device for obtaining a growth of the primary roots of Arabidopsis thaliana (L.) HEYNH. under controlled conditions. Experientia 22, 481 (1966)
- , and M.JACOBS: Effect of gamma radiation on the uptake of ³H-EMS by seeds of Arabidopsis thaliana (L.) HEYNH. 3rd Int.Congr.Radiat.Res., Cortina, Book of Abstr., p. 40, 1966
- , - , and L.LEDOUX: Activation de l'incorporation de la thymidine-H³ dans le DNA d'Arabidopsis thaliana (L.) HEYNH. provoquée par les radiations gamma. Arch.Int. Phys.Biochem. 74, 311-312 (1966)
- , - , G.MILIC, et L.LEDOUX: Effet des radiations gamma sur la pénétration du méthane sulfonate d'éthyl tritié dans les graines d'Arabidopsis thaliana (L.) HEYNH. Bull.Soc.Roy.Bot.Belgique 100, 163-171 (1967)
- BOTSCHANTZEV, V.P.: De cruciferis notae criticae, II. (Russ.). Bot.Materialy Gerbariia Bot.Inst. 18, 104-108 (1957)
- : De cruciferis notae criticae, III. (Russ.). Bot.Materialy Gerbariia Bot.Inst. 19, 105-108 (1959)
- BOUHARMONT, J.: Action des rayons X sur une série autopolyploide d'Arabidopsis. Rep. Meeting FAO/IAEA, "The Use of Induced Mutations in Plant Breeding". Pp. 649-657, Rome 1964
- BROCK, D.R.: Induced quantitative variation in Arabidopsis thaliana (Abstract). In "Induced Mutations and their Utilization". Erwin-BAUR-Gedächtnis-Vorlesungen IV. Abh.dtsch.Akad.Wiss.Berlin, Kl.Med., No. 2, pp. 263-267. Akademie Verlag, Berlin 1967
- BRODFÜHRER, U(rsula): Der Einfluß einer abgestuften Dosierung von ultravioletter Sonnenstrahlung auf das Wachstum der Pflanzen. Planta 45, 1-56 (1955)
- : The effect of temperature on the reaction of plants to ultraviolet radiation. Carnegie Inst.Wash.Yearbook 56, 288-291 (1957)
- BRODFÜHRER-FRANZGROTE, U(rsula): Der Einfluß des roten und blauen Spektralbereiches auf ultraviolettt bestrahlte Pflanzen. Unpubl. 1956. Cit. in W.RUHLAND: Handb.d. Pflanzenphysiol. XVI, 532-554, Springer, Berlin 1961
- : The influence of temperature on the reaction of plants to ultraviolet radiation. Unpubl. 1958. Cit. in W.RUHLAND: Handb.d.Pflanzenphysiol. XVI, 532-554, 1961
- BRONCKERS, F.: Variations polliniques dans une série d'autopolyploides artificiels d'Arabidopsis thaliana (L.) HEYNH. Pollen et Spores 5, 233-238 (1963)
- BROWN, J.A.M.: Effects of thymidine analogues on the apical meristem of Arabidopsis thaliana (L.) HEYNH. Plant Physiol. Suppl. 37, 22-23 (1962)
- : Effect of thymidine analogues on reproductive morphogenesis in Arabidopsis thaliana (L.) HEYNH. Nature (Lond.) 196, 51-53 (1962)
- : A temperature sensitive late flowering mutant of Arabidopsis. Genetics 50, 237-238 (1964)
- , and H.H.SMITH: Incorporation and effects of thymidine analogues in gametophytic tissue of Arabidopsis thaliana. Mutation Res. 1, 45-53 (1964)
- , J.P.MIKSCHE, and H.H.SMITH: An analysis of H³-thymidine distribution throughout the vegetative meristem of Arabidopsis thaliana (L.) HEYNH. Radiat.Bot. 4, 107-113 (1964)
- BUIATTI, M., and C.LORENZONI: Effetto dell'irradiazione con raggi X di semi di Arabidopsis thaliana (L.) HEYNH. Caryologia 16, 101-110 (1963)

- CLAUSS, H., and W. BAUH: Über die Blütenbildung von *Hyoscyamus niger* und *Arabidopsis thaliana* in 72-Stunden-Zyklen. Z. Bot. 44, 437-454 (1956)
- DALY, K.: Effect of temperature on survival of gamma-irradiated *Arabidopsis* seed. Radiat. Res. 12, 430 (1960)
- : The induction of quantitative variability by gamma radiation in *Arabidopsis thaliana*. Genetics 45, 983 (1960)
 - : The effect of fast neutrons on quantitative variability in *Arabidopsis thaliana*. Genetics 46, 861 (1961)
- DEVI, S.K., K.N. PRASAD, and E.F. RILEY: Survival and dark germination of X-irradiated *Arabidopsis thaliana*, race Estland. Radiat. Bot. 4, 347-353 (1964)
- DIERKS, W.: Untersuchungen zum Heterosisproblem. Z. Pflanzenzücht. 40, 67-102 (1958)
- FEENSTRA, W.J.: Isolation of nutritional mutants in *Arabidopsis thaliana*. Genetica 35, 259-269 (1966)
- FRANK, H.: Über den Stickstoffverlust bei alternden Pflanzen. Planta 44, 313-340 (1954)
- FREYN, J.: Über einige kritische Arabis-Arten. Oesterr. Bot. Zeitschr. 39, 101-108, 128-134, 167-171 (1889)
- FUJII, T.: Radiation effects on *Arabidopsis thaliana*. I. Comparative efficiencies of γ -rays, fission and 14 MeV neutrons in somatic mutation. Japan. J. Genet. 32, 91-101 (1964)
- , M. IKENAGA, and J.T. LYMAN: Radiation effects on *Arabidopsis thaliana*. II. Killing and mutagenic efficiencies of heavy ionizing particles. Radiat. Bot. 6, 297-306 (1966)
 - , - : Killing and mutagenic efficiencies of heavy ionizing particles in *Arabidopsis thaliana*. Nature (Lond.) 213, 175-176 (1967)
- GICHNER, T.: Relation of some M₁ characters to the frequency of M₂ mutants in *Arabidopsis thaliana*. Biol. Plant. (Praha) 8, 209-212 (1966)
- , and J. VELEMINSKY: Induction of chlorophyll chimeras by X-rays and ethyl-methanesulphonate (EMS) in different heterozygous strains of *Arabidopsis thaliana*. Proc. Symp. "Induction of Mutations and the Mutation Process". Praha, 27-29. Sept. 1963, pp. 54-56, 1965
 - , - : The influence of EDTA on the mutagenic activity of ethyl methanesulphonate (EMS) in *Arabidopsis thaliana*. Biol. Plant. (Praha) 7, 162-164 (1965)
 - , - : Mutagenic action of N-nitroso-N-methylurea and N-nitroso-N-ethylurea. (Czech). Genetika a šlechtění (Praha) 1, 67-84 (1965)
 - , - : Preliminary tests for the detection of suitable concentrations of chemomutagens in plants. (Czech with Engl. Summary) Genetika a šlechtění (Praha) 2, 81-86 (1966)
 - , - : The mutagenic activity of 1-alkyl-1-nitrosoureas and 1-alkyl-3-nitro-1-nitrosoguanidine. Mutation Res. 4, 207-212 (1967)
- GOMEZ-CAMPO, C., and L. DELGADO: Radioreistance in crucifers. Radiat. Bot. 4, 479-483 (1964)
- GREGORY, F.G., and G.G. HUSSEY: Photoperiodic responses of *Arabidopsis thaliana*. Proc. Linn. Soc. London 164, 137-139 (1953)
- GRIFFING, B., and J. LANGRIDGE: Phenotypic stability of growth in the self-fertilized species *Arabidopsis thaliana*. In "Statistical Genetics and Plant Breeding". Nat. Acad. Sci., Bot. Res. Council, Publ. No. 222, pp. 768-334, Washington 1962
- MACCIUSI, B. (Barbara), and E. REICHERT: Restitutionserscheinungen an pflanzlichen Meristemen nach Röntgenbestrahlung. I. Die Genese strahleninduzierter Sproßgabelungen. Planta 60, 289-306 (1963)
- HÄRER, L. (uise): Die Vererbung des Blühalters früher und später sommereinjähriger Rassen von *Arabidopsis thaliana* (L.) HEYNH. Pflanzl. Rep. 1090, 1-27 (1947); Beitr. Biol. Pflanzen 28, 1-35 (1951)
- HILL, J.: Conditioning in *Arabidopsis thaliana*. Heredity 16, 513-515 (1961)
- HIRONO, Y.: A genetic method for the localization of chromosome defects. Genetics 50, 255 (1964)
- : Mitotic exchange of linked markers in *Arabidopsis*. Thesis, pp. 92 + XXXII, Univ. of Missouri (1964)
 - , and G.P. REDEI: Somatic recombination in *Arabidopsis*. Genetics Today, Proc. XI. Internat. Congr. Genet., The Hague, Sept. 1963, Vol. I, p. 15, 1963
 - , - : Multiple allelic control of chlorophyll b level in *Arabidopsis thaliana*. Nature (Lond.) 197, 1324-1325 (1963)
 - , - : Induced premeiotic exchange of linked markers in the angiosperm *Arabidopsis*. Genetics 51, 519-526 (1965)
 - , - : Acceleration of flowering of the long-day plant *Arabidopsis* by 8-azaadenine. Planta 68, 88-93 (1966). Cf. Erratum: Planta 70, 206 (1966)
 - , - : Early flowering in *Arabidopsis* induced by DNA base analogs. Planta 71, 107-112 (1966)
- HOFFMANN, P.: Zur Physiologie der Photosynthese bei höheren Pflanzen. Botan. Studien, Heft 18, 152 S., VEB Gustav Fischer Verlag, Jena 1968
- , and R. IRMLER: Vitamin K₁-Gehalt und Photosynthese bei höheren Pflanzen. Ber. dtsch. bot. Ges. 72, 71-86 (1966)
- HUSSEY, G.: Experiments with two long-day-plants designed to test BÜNNING's theory of photoperiodism. Physiol. Plantarum (Kopenh.) 7, 253-260 (1954)
- HYLANDER, N.: *Cardaminopsis suecica* (FR.) HILL., a northern amphidiploid species. Bull. Jard. bot. Bruxelles 27, 591-605 (1957)
- VANOV, V.I., A.G. KASYANENKO, A.V. SARINA, and R.A. TIMOFEEFF-RESSOVSKY: Studies in radiation genetics of *Arabidopsis thaliana* (L.) HEYNH. I. Brief description of the experimental plant and some methods of its cultivation, crossing and estimation of variability. (Russ. with Engl. Summary). Genetika Akad. Nauk SSSR 2 (8), 55-70 (1966)

- IVANOV, V.I., A.V.SANINA, and H.A.TIMOFEEF-RESSOVSKY: Studies in the radiation genetics of *Arabidopsis thaliana* (L.) HEYNH. II. Survival and fertility of G₁ plants after irradiation of dormant seeds. (Russ.with Engl.Summary). *Genetika Akad. Nauk SSSR* 3 (5) 16-23 (1967)
- JACOBS, M.: Test de l'activité mutagénique de la 5-bromodeoxyuridine et de la 5-iododeoxyuridine chez une plante supérieure *Arabidopsis thaliana* (L.) HEYNH. cultivée "in vitro". *Lejeunia* 25, 1-7 (1964)
- : Etude comparative des fréquences et types de mutations induites par le méthane sulfonate d'éthyle et le méthane sulfonate de méthyle chez *Arabidopsis thaliana* (L.) HEYNH. *Bull.Soc.Roy.Bot. Belgique* 98, 43-66 (1964)
 - : Isolement de mutants biochimiques chez *Arabidopsis thaliana*. Méthodes et résultats préliminaires. *Bull.Acad.Roy. Belgique, Classe des Sciences 5^{ème} sér.*, 51, 725-747 (1965)
 - : Effets induits chez *Arabidopsis thaliana* (L.) HEYNH. par un analogue de la thymidine incorporé dans le milieu de culture. *Bull.Soc.Roy.Bot. Belgique* 100, 259-281 (1967)
- JAFRI, S.M.H.: Some cruciferae of W.Pakistan, Afganistan and N.W.Himalaya. *Notes Roy.Bot.Garden, Edinburgh* 22 (2), 95-119 (about 1960)
- JARETZKI, R.: Untersuchungen über Chromosomen und Phylogenie bei einigen Cruciferen. *Jahrb.wiss.Bot.* 68, 1-45 (1928)
- : Beziehungen zwischen Chromosomenzahl und Systematik bei den Cruciferen. *Jahrb.wiss.Bot.* 76, 485-527 (1932)
- KASYANENKO, A.G., and Yu.E.GHILLER: Some characteristic features of the photosynthetic apparatus of chlorophyll mutants of *Arabidopsis thaliana* (L.) HEYNH. (Russ.with Engl.Summary). *Genetika Akad. Nauk SSSR* 3 (6), 42-49 (1967)
- KRIBBEN, F.J.: *Arabidopsis thaliana* (L.) HEYNH., eine botanische Drosophila. *Naturwiss.Rundsch.* 17, 139-145 (1964)
- : Die Abkürzung der Samenruhe bei *Arabidopsis* durch Gibberellinsäure. *Naturwiss.* 44, 313 (1957)
- KUČERA, J.: Induction of developmental mutations by N-methyl-N-nitrosourea in *Arabidopsis thaliana*. In "Mechanism of Mutation and Inducing Factors". *Proc.Symp. Prague, Aug. 1965*, pp. 313-316. *Academia, Prague 1966*
- KUGLER, I(da): Untersuchungen über das Keimverhalten einiger Rassen von *Arabidopsis thaliana* (L.) HEYNH. - Ein Beitrag zum Problem der Lichtkeimung. *Fiat Rep.* 1094, 1-23 (1947); *Beitr.Biol.Pflanzen* 28, 211-243 (1951)
- KVITKO, K.V.: Aseptic culture of *Arabidopsis thaliana* (L.) HEYNH. and prospects of utilization of these methods in botanical investigations. (Russ.). *Vestn.Leningrad Univ., Ser.Biol.* No. 15/3, 47-56 (1960)
- , and A.MÜLLER: *Arabidopsis thaliana* (L.) HEYNH., a new object for genetic investigations. (Russ.). *Issledovaniya po genetike (Leningrad)* 1, 79-91 (1961)
- LAIBACH, F.: Zur Frage nach der Individualität der Chromosomen im Pflanzenreich. *Beih.Bot.Cbl., 1.Abt.,* 22, 191-210 (1907)
- : Die Ursachen der Blütenbildung und das Blühhormon. *Natur u. Volk* 70, 55-65 (1940)
 - : *Arabidopsis thaliana* (L.) HEYNH. als Objekt für genetische und entwicklungsphysiologische Untersuchungen. *Bot.Arch.* 44, 439-455 (1943)
 - : Zur Ätiologie der Blütenbildung. *Naturwiss.* 31, 246 (1943)
 - : Zur Blütenbildung bei Lang- und Kurztagpflanzen. *Ber.dtsch.bot.Ges.* 62, 27-32 (1949)
 - : Über sommer- und winterannuelle Rassen von *Arabidopsis thaliana* (L.) HEYNH. - Ein Beitrag zur Ätiologie der Blütenbildung. *Beitr.Biol.Pflanzen* 28, 173-216 (1951)
 - : Über die Brechung der Samenruhe bei *Arabidopsis thaliana* (L.) HEYNH. *Naturwiss.* 43, 164 (1956)
 - : Über den Artbastard *Arabidopsis suecica* (FR.) NORRL. x *A. thaliana* (L.) HEYNH. und die Beziehungen zwischen den Gattungen *Arabidopsis* HEYNH. und *Cardaminopsis* (C.A.MEYER) HAY. *Planta* 51, 148-166 (1958)
 - , and F.J.KRIBBEN: Apikaldominanz und Blühreife. *Beitr.Biol.Pflanzen* 30, 127-158 (1953)
 - , and A(dele) ZENKER: Zur Kälteeinflussung der Blütenbildung bei Langtagpflanzen. *Planta* 43, 250-252 (1954)
- LANGRIDGE, J.: Biochemical mutations in the crucifer *Arabidopsis thaliana* (L.) HEYNH. *Nature (Lond.)* 176, 260-261 (1955)
- : Effect of day-length and gibberellic acid on the flowering of *Arabidopsis*. *Nature (Lond.)* 180, 36-37 (1957)
 - : The aseptic culture of *Arabidopsis thaliana* (L.) HEYNH. *Austral.J.biol.Sci.* 10, 243-252 (1957)
 - : A hypothesis of developmental selection exemplified by lethal and semilethal mutants of *Arabidopsis*. *Austral.J.biol.Sci.* 11, 58-68 (1958)
 - : An osmotic mutant of *Arabidopsis thaliana*. *Austral.J.biol.Sci.* 11, 457-470 (1958)
 - : A genetic and molecular basis for heterosis in *Arabidopsis* and *Drosophila*. *Amer.Naturalist* 96, 5-27 (1962)
 - : The genetic basis of climatic response. In "Environmental Control of Plant Growth". *Symp.Canberra, Aug. 25-31, 1962*. Pp. 367-379. *Academic Press, London 1963*
 - : Temperature-sensitive vitamine requiring mutants of *Arabidopsis thaliana*. *Austral.J.biol.Sci.* 18, 311-321 (1965)
 - , and B.GRIFFING: A study of high temperature lesions in *Arabidopsis thaliana*. *Austral.J.biol.Sci.* 12, 117-135 (1959)
- LEE-CHEN, S(uzanne), and L(otti) M.STEINITZ-SEARS: The location of linkage groups in *Arabidopsis thaliana*. *Can.J.Genet.Cytol.* 9, 381-384 (1964)
- LI, S.-L., and G.P.REDEI: Nutritional mutants in a higher plant. *Transact.Missouri Acad.Sci.* 1, 78 (1967)

- LI, S.-L., G.F. REDEI, and C.S. GOWANS: A phylogenetic comparison of mutation spectra. *Molec. Gen. Genetics* 100, 77-83 (1967)
- LÖVE, A.: *Hylandra* - a new genus of Cruciferae. *Svensk Bot. Tidskr.* 55, 211-217 (1961)
- , and D(oris) LÖVE: Chromosome numbers of central and northwest european plant species. *Opera Botanica (Soc. Bot. Lundensi)* 5, 1-581 (1961)
- MANTON, I(rene): Introduction to general cytology of the Cruciferae. *Ann. Bot.* 46, 509-556 (1932)
- McKELVIE, A.D.: The uses of radiation in plant breeding and investigations into the induction of mutations. *Genet. agrar. (Pavia)* 14, 197-205 (1961)
- : A list of mutant genes in *Arabidopsis thaliana* (L.) HEYNH. *Radiat. Bot.* 1, 233-241 (1962)
- : Differential response to mutagens in *Arabidopsis thaliana*. *Nature (Lond.)* 195, 409-410 (1962)
- : Studies in the induction of mutations in *Arabidopsis thaliana* (L.) HEYNH. *Radiat. Bot.* 3, 105-123 (1963)
- X MEIJER, J.: The spectral dependence of flowering and elongation. *Acta bot. Neerland.* 8, 189-246 (1959)
- MELCHERS, G.: In "Symp. on Biochem. of Morphogenesis". *Proc. IV. Int. Congr. Biochem.*, Vol. VI, p. 140. Vienna 1958
- X MICHNIEWICZ, M., and A. KAMIENSKA: Flower formation induced by kinetin and vitamin E treatment in long-day plant (*Arabidopsis thaliana*) grown in short day. *Naturwiss.* 52, 623 (1965)
- MIKSCH, J.P., and J.A.M. BROWN: Development of the vegetative and floral meristem of *Arabidopsis thaliana* under controlled conditions of growth. *Amer. J. Bot.* 50, 616-617 (1963)
- X - , - : Development of vegetative and floral meristems of *Arabidopsis thaliana*. *Amer. J. Bot.* 52, 533-537 (1965)
- MISRA, R.C.: Contribution to the embryology of *Arabidopsis thaliana* (GAY and MOUN.) *Agra Univ. J. Res., Sci.*, 11, 191-199 (1962)
- MÜLLER, A.J.: Zur Charakterisierung der Blüten und Infloreszenzen von *Arabidopsis thaliana* (L.) HEYNH. *Kulturpflanze* 9, 364-393 (1961)
- : Mutationen mit embryonaler Manifestation bei *Arabidopsis thaliana*. *Naturwiss.* 48, 579 (1961)
- : Embryonentest zum Nachweis rezessiver Letalfaktoren bei *Arabidopsis thaliana*. *Biol. Zbl.* 82, 133-163 (1963)
- : Keimwurzeltest zur Bewertung des somatischen Strahlenschadens bei *Arabidopsis thaliana*. *Kulturpflanze* 12, 237-255 (1964)
- : Mutationsauslösung durch Nitrosomethylharnstoff bei *Arabidopsis thaliana*. *Züchter* 34, 102-120 (1964)
- : The chimerical structure of M₁-plants and its bearing on the determination of mutation frequencies in *Arabidopsis thaliana*. *Proc. Symp. "Induction of Mutations and the Mutation Process"*, Praha, 27.-29. Sept. 1963, pp. 46-52, 1965
- : Über den Zeitpunkt der Mutationsauslösung nach Einwirkung von N-nitroso-N-methylharnstoff auf quellende Samen von *Arabidopsis thaliana*. *Mutagenesis Res.* 2, 426-437 (1965)
- : Durch Röntgenbestrahlung des Pollens von *Arabidopsis thaliana* induzierte diplophasische und haplophasische Letalmutationen. *Kulturpflanze* 13, 163-171 (1965)
- : Beeinflussung der radiomimetischen Wirksamkeit von Nitrosamiden durch Stoffwechsellinhibitoren. *Naturwiss.* 52, 213-214 (1965)
- : Reparation chemisch induzierter prämutativer Läsionen durch Rücktrocknung der behandelten Samen? *Biol. Zbl.* 84, 759-762 (1965)
- : Die Induktion von rezessiven Letalmutationen durch Äthylmethansulfonat bei *Arabidopsis thaliana*. I. Dosis-Effekt-Beziehungen und deren Beeinflussung durch die Behandlungsbedingungen. *Züchter* 36, 201-220 (1966)
- : Genetic analysis of sterility induced by highly efficient mutagens in *Arabidopsis thaliana*. In "Induced Mutations and their Utilization". Erwin BAUR-Gedächtnis-Vorlesungen IV. *Abh. dtsh. Akad. Wiss. Berlin, Kl. Med.*, No. 2, pp. 89-97. Akademie Verlag, Berlin 1967
- : Die Induktion von rezessiven Letalmutationen durch Äthylmethansulfonat bei *Arabidopsis thaliana*. II. Sensibilitätsänderungen in quellenden und keimenden Samen. *Biol. Zbl., Suppl.*, 86, 89-106 (1967)
- , and T. GICHNER: Mutagenic activity of 1-methyl-3-nitro-1-nitrosoguanidine on *Arabidopsis thaliana*. *Nature (Lond.)* 201, 1149-1150 (1964)
- NAPP-ZINN, K.: Die Vernalisation. *Kosmos* 49, 65-67 (1953)
- : Thermostabile und thermolabile Zwischenstadien im Vernalisationsprozess. *Ber. dtsh. bot. Ges.* 66, 362-367 (1953)
- : Vernalisation und Blattbildung. *VIII^e Congr. intern. Bot.*, Rapp. et Comm., 11/12, 287-289 (1954)
- : Vergleichende Atmungsmessungen an Sommer- und Winterannuellen. Untersuchungen an Caryopsen und Embryonen von *Secale cereale* und an Samen von *Arabidopsis thaliana*. *Z. Naturforschg.* 9b, 216-229 (1954)
- : Genetische Grundlagen des Kaltebedürfnisses bei *Arabidopsis thaliana* (L.) HEYNH. *Naturwiss.* 42, 650 (1955)
- : Spontanes Auftreten von Ketylvarianten bei *Arabidopsis thaliana* (L.) HEYNH. *Ber. dtsh. bot. Ges.* 68, 369-373 (1955)
- : Zur Frage nach der Übertragbarkeit des durch die Vernalisation bewirkten Blühimpulses. *Ber. dtsh. bot. Ges.* 69, 193-198 (1956)
- : Untersuchungen über den Aufbau der Infloreszenz bei *Arabidopsis thaliana*. *Beitr. Biol. Pflanzen* 34, 113-128 (1957)

- NAPP-ZINN, K.: Die Abhängigkeit des Vernalisationseffektes bei *Arabidopsis thaliana* vom Quellungsgrad der Samen und vom Lichtgenuß der Pflanzen nach der Kältebehandlung. *Flora* 144, 403-419 (1957)
- : Untersuchungen zur Genetik des Kältebedürfnisses bei *Arabidopsis thaliana*. *Z. indukt. Abst. u. Vererb. lehre* 88, 253-285 (1957)
 - : Die Abhängigkeit des Vernalisationseffektes bei *Arabidopsis thaliana* von der Dauer der Vorquellung der Samen sowie vom Alter der Pflanzen bei Beginn der Vernalisation. *Z. Bot.* 45, 379-394 (1957)
 - : Weitere Untersuchungen über die Beziehungen zwischen Atmungsintensität und Blühalter. *Planta* 48, 683-695 (1957)
 - : Untersuchungen über das Vernalisationsverhalten einer winterannuellen Rasse von *Arabidopsis thaliana*. *Planta* 50, 177-210 (1957)
 - : Über den Einfluß von KH_2PO_4 auf die Vernalisation. *Z. Bot.* 46, 506-515 (1958)
 - : Vernalization, light, and age with special regard to *Arabidopsis thaliana*. *Proc. IX. intern. bot. Congr.* 2, 278 (1959)
 - : Vernalisation, Licht und Alter bei *Arabidopsis thaliana* (L.) HEYNH. I. Mitteilung. Licht und Dunkelheit während Kälte- und Wärmebehandlung. *Planta* 54, 409-444 (1960)
 - : Vernalisation, Licht und Alter bei *Arabidopsis thaliana* (L.) HEYNH. II. Mitteilung. Die Rolle der vor und nach der Kältebehandlung herrschenden Lichtintensität. *Planta* 54, 445-452 (1960)
 - : Grundlagen der Blütenbildung. *Math. u. Naturwiss. Unterr.* 13, 154-158 (1960)
 - : Über die Bedeutung genetischer Untersuchungen an kältebedürftigen Pflanzen für die Aufklärung von Vernalisationserscheinungen. *Züchter* 31, 128-135 (1961)
 - : Vernalization, light, and age with special regard to *Arabidopsis thaliana* (L.) HEYNH. *Recent Adv. Bot.* 2, 1206-1207 (1961)
 - : Über die genetischen Grundlagen des Vernalisationsbedürfnisses bei *Arabidopsis thaliana*. I. Mitt. Die Zahl der beteiligten Faktoren. *Z. Vererbungslehre* 93, 154-163 (1962)
 - : Künstlich induziertes Vernalisationsbedürfnis bei sommerannuellen Pflanzen. *Naturwiss.* 49, 473-474 (1962)
 - : Zur Genetik der Wuchsformen. *Beitr. Biol. Pfl.* 38, 161-177 (1963)
 - : Über den Einfluß von Genen und Gibberellinen auf die Blütenbildung von *Arabidopsis thaliana*. *Ber. dtsh. bot. Ges.* 76, 77-89 (1963)
 - : Über die Beziehungen zwischen Vernalisation, Keimung und Atmung. Untersuchungen an *Arabidopsis thaliana* (L.) HEYNH. *Z. Bot.* 51, 317-339 (1963)
 - : Über genetische und entwicklungsphysiologische Grundlagen jahreszeitlicher Aspekte von Pflanzengesellschaften. In "Beiträge zur Phytologie". Festschrift H. WALTER. Edit. K. KREEB, pp. 33-49. Ulmer, Stuttgart 1964
 - : Antimetabolites in vernalization. *Symp. Differentiation of Apical Meristems and Some Problems of Ecological Regulation of Development of Plants. Praha-Nitra.* Abstr. pp. 54-56, 1964
 - : Zur Frage nach der Abhängigkeit der Pflanzenentwicklung vom Samenalter. *Ber. dtsh. bot. Ges.* 77, 235-242 (1964)
 - : Physiologische Aspekte der Blütenbildung. *Scientia* 100, 1965
 - : Aspects physiologiques de la mise à fleurs. *Scientia* 100, Suppl. 1965
 - : Zur Physiologie der Verzweigung krautiger Pflanzen. *Ber. dtsh. bot. Ges.* 80, 218-226 (1967)
- NIEMANN, E.G.: Wirkungen eines künstlichen ^{90}Sr -Fallout auf Pflanzen. I. Aufnahme und Verteilung des ^{90}Sr . *Atompraxis* 7, 370-375 (1961)
- : Wirkungen eines künstlichen ^{90}Sr -Fallout auf Pflanzen. II. Strahlenbiologische Wirkungen. *Atompraxis* 8, 51-56 (1962)
- NIETHAMMER, A. (n. l. e.): Der Einfluß von den Reizchemikalien auf die Samenkeimung. *II. Mitt. Jahrb. wiss. Bot.* 67, 223-241 (1926)
- NOVIKOV, G.N.: The morphogenetic effect produced by NaCl on *Arabidopsis thaliana* (L.) HEYNH. and *A. pumila* (STEPH.) N. BUSCH. (Russ.). *Acta Inst. bot. Acad. Sci. URSS*, Ser. IV., Fasc. 3, 279-292 (1937)
- OVERBECK, H.J.: Versuche zur Mutationsauslösung durch Trypaflavin an *Lepidium sativum* und *Arabidopsis thaliana* (1. Mitteilung). *Wiss. Zeitschr. Univ. Greifswald*, Jahrg. II, Math. nat. Reihe Nr. 5, 327-344 (1952/53)
- POSTLETHWAIT, S.N., and N.J. ENOCHS: Tachyplants - suited to instruction and research. *Plant Sci. Bull.* 13 (2), 1-5 (1967)
- RAJEWSKY, B.: The limits of the target theory of the biological action of radiation. *Brit. J. Radiol. NS* 25, 550-552 (1953)
- RATCLIFFE, D.: Adaptation to habitat in a group of annual plants. *J. Ecol.* 49, 187-203 (1961)
- REDEI, G.P.: Genetic control of 2,5-dimethyl-4-aminopyrimidine requirement in *Arabidopsis thaliana*. *Genetics* 45, 1007 (1960)
- : Genetic block of "vitamin thiazole" synthesis in *Arabidopsis*. *Genetics* 47, 979 (1962)
 - : Single locus heterosis. *Z. Vererbungslehre* 93, 164-170 (1962)
 - : Supervital mutants of *Arabidopsis*. *Genetics* 47, 443-460 (1962)
 - : Somatic instability caused by a cysteine-sensitive gene in *Arabidopsis*. *Science* 139, 767-769 (1963)
 - : A genetic oscillatory mechanism in *Arabidopsis*. *Genetics Today*, Proc. XI. Internat. Congr. Genet., The Hague, Sept. 1963, Vol. I, p. 41, 1963
 - : Megaspore selection in *Arabidopsis thaliana*. *Genetics* 50, 278-279 (1964)
 - : Non-mendelian megagametogenesis in *Arabidopsis*. *Genetics* 51, 857-872 (1965)
 - : Genetic blocks of thiamine synthesis in the angiosperm *Arabidopsis*. *Amer. J. Bot.* 52, 834-841 (1965)

- RÉDEI, G.F.: A defective genetic regulation of ribonuclease synthesis in *Arabidopsis*. *Genetics* 54, 356 (1966)
- : X-ray induced phenotypic reversions in *Arabidopsis*. *Radiat.Bot.* 7, 401-407 (1967)
 - : Biochemical aspects of a genetically determined variegation in *Arabidopsis*. *Genetics* 56, 431-443 (1967)
 - : Suppression of a genetic variegation by 6-azapyrimidines. *J.Hered.* 58, 229-235 (1967)
 - : Genetic estimate of cellular autarky. *Experientia* 23, 584 (1967)
 - , and L(otti) K. STEINITZ-SEARS: X-ray mutants with high selective advantage in *Arabidopsis*. *Genetics* 46, 892-893 (1961)
- REINHOLZ, E(rna): Röntgenmutationen bei *Arabidopsis thaliana* (L.) HEYNH. *Naturwiss.* 34, 26-28 (1947)
- : X-ray mutations in *Arabidopsis thaliana* (L.) HEYNH. and their significance for plant breeding and the theory of evolution. *Fiat Report* 1006, 1-70 (1947)
 - : Beiträge zur indirekten Strahlenwirkung. I. Röntgenbestrahlung biologischer Objekte in fester Phase. *Strahlentherapie* 95, 131-147 (1954)
 - : Weitere Untersuchungen zur Induktion von Keimblattveränderungen durch Röntgenstrahlen. *Experientia* 10, 486-488 (1954)
 - : Beeinflussung der Morphogenese embryonaler Organe durch ionisierende Strahlungen. I. Keimlingsanomalien durch Röntgenbestrahlung von *Arabidopsis thaliana*-Embryonen in verschiedenen Entwicklungsstadien. *Strahlentherapie* 109, 537-553 (1959)
 - : Biologische Strahleneinwirkung bei tiefen Temperaturen. *Max-Planck-Institut für Biophysik*, 1937-1962. Pp. 88-99
 - , und K.AURAND: Untersuchungen über die Wirksamkeit von Strahlenschutzsubstanzen bei Pflanzen. *Strahlentherapie* 94, 646-656 (1954)
- RIJVEN, A.H.G.C.: Glutamine and asparagine as nitrogen sources for the growth of plant embryos in vitro: A comparative study of 12 species. *Austral.J.biol.Sci.* 9, 511-527 (1956)
- RÖBBELEN, G.: Über die Protochlorophyllreduktion in einer Mutante von *Arabidopsis thaliana* (L.) HEYNH. *Planta* 47, 532-546 (1956)
- : Untersuchungen an strahleninduzierten Blattfarbmutanten von *Arabidopsis thaliana* (L.) HEYNH. *Z.indukt.Abst.u.Vererbungslehre* 88, 189-252 (1957)
 - : Über Heterophyllie bei *Arabidopsis thaliana* (L.) HEYNH. *Ber.dtsch.bot.Ges.* 70, 39-44 (1957)
 - : Eine Blattfarbmutante ohne Chlorophyll b von *Arabidopsis thaliana* (L.) HEYNH. *Naturwiss.* 44, 288-289 (1957)
 - : Instabile Genmanifestation nach Röntgenbestrahlung von *Arabidopsis thaliana* (L.) HEYNH. *Z.Naturforsch.* 13b, 14-16 (1958)
 - : Untersuchungen über die Entwicklung der submikroskopischen Chloroplastenstruktur in Blattfarbmutanten von *Arabidopsis thaliana*. *Z.Vererbungslehre* 90, 503-506 (1959)
 - : Über die unterschiedliche genetische Reaktion von *Arabidopsis thaliana* auf eine Röntgenbestrahlung in verschiedenen Entwicklungsstadien. *Ber.dtsch.bot.Ges.* 73, (41)-(42) (1960)
 - : Über die Wirkung von 6-Äthylmethansulfonat auf die Röntgeninduzierte Mutationen bei *Arabidopsis thaliana*. *Z.Vererbungslehre* 93, 25-34 (1962)
 - : Über Unterschiede in den genetischen Folgen einer Röntgenbestrahlung verschiedenartiger Pflanzenzellen - Untersuchungen an *Arabidopsis thaliana* (L.) HEYNH. *Z.Vererbungslehre* 93, 127-153 (1962)
 - : Zur Mutagenität von Urethanen. *Z.Vererbungslehre* 93, 256-263 (1962)
 - : Wirkungsvergleich zwischen Äthylmethansulfonat und Röntgenstrahlen im Mutationsversuch mit *Arabidopsis thaliana*. *Naturwiss.* 49, 65 (1962)
 - : The effects of two endogenous factors on artificial mutagenesis in *Arabidopsis thaliana*. *Proc.Symp. "Induction of Mutations and the Mutation Process"*, Praha, 27.-29.Sept. 1963, pp. 42-45, 1965
 - : Extranuclear inheritance of induced plastid mutations in *Arabidopsis thaliana*. *Genetics Today, Proc.XI.Intern.Congr.Genet.*, The Hague, Sept. 1963, Vol.I, p. 205-206, 1963
 - : Beiträge zur Mutabilität des Plastoms. *Ber.dtsch.bot.Ges.* 76, 416 (1964)
 - : Wirkung von Äthylmethansulfonat nach verschieden langer Vorquellung der Samen im Mutationsversuch mit *Arabidopsis thaliana* (L.) HEYNH. *Beitr.Biol.Pflanzen* 41, 323-335 (1965)
 - : EMS efficiency in seeds and gametes of *Arabidopsis thaliana*. In "Mechanism of Mutation and Inducing Factors". *Proc.Symp.Prague*, Aug. 1965. Pp. 309-312. *Academia, Prague* 1966
 - : Genic and plastomic control of plastid fine structure. *Genetics* 54, 357 (1966)
 - : Chloroplastendifferenzierung nach geninduzierter Plastommutation bei *Arabidopsis thaliana* (L.) HEYNH. *Z.Pflanzenphysiol.* 55, 387-403 (1966)
 - : Gestörte Thylakoidbildung in Chloroplasten einer xantha-Mutante von *Arabidopsis thaliana* (L.) HEYNE. *Planta* 69, 1-26 (1966)
 - , und W.WEHRMEYER: Gestörte Granabildung in Chloroplasten einer chlorina-Mutante von *Arabidopsis thaliana* (L.) HEYNE. *Planta* 65, 105-128 (1965)
- SARKAR, S.: Über einige Gibberellin-Wirkungen. *Colloque Intern.sur le Photothermo-periodism*. P. 189-190 (Abstr.), Parma 1957
- : Role of gibberellic acid on the flowering of *Arabidopsis thaliana*. *Symp.on Plant Metabolism*, University of Allahabad, January 1958
 - : Versuche zur Physiologie der Vernalisation. *Biol.Zbl.* 77, 1-49 (1958)
- SCHOLZ, H.: Nomenklatorische und systematische Studien an *Cardaminopsis arenosa* (L.) HAYEK. *Willdenowia* 2, 137-149 (1952)
- : *Hylandra suecica* (*Cardaminopsis suecica*) im Herbar ROMAN SCHULZ. *Willdenowia* 3, 325-328 (1962)
- SCHWANITZ, F.: Mutationsauslösung. *Kerntechnik* 8, 500-505 (1966)

- SEYFFERT, W.: Untersuchungen über die Vererbung quantitativer Charaktere an *Arabidopsis thaliana* (L.) HEYNH. Z.Pflanzenzüchtg. 42, 356-401 (1960)
- SHEN-MILLER, J., and W.R.SHARP: An improved medium for rapid initiation of *Arabidopsis* tissue culture from seed. Bull.Torrey bot. Club 93, 68-69 (1966)
- SHROPSHIRE, W.jr., W.H.KLEIN, and V.B.ELSTAD: Action spectra of photomorphogenic induction and photoinactivation of germination in *Arabidopsis thaliana*. Plant a.Cell Physiol. 2, 63-69 (1961)
- SMITH, H.H.: Experiments utilizing labeled nucleic acid precursors and analogues in plant cell research. Genetics Today, Proc.XI.Intern.Congr.Genet., The Hague, Sept. 1963, Vol.I, p. 85-86, 1963
- SOSNA, M.: The origin of radiomutants of the chlorine type in *Arabidopsis thaliana* (L.) HEYNH. Biol.Plant. (Praha) 4, 255-259 (1962)
- STEINITZ-SEARS, L(otti) M.: Chromosome studies in *Arabidopsis*. Amer.J.Bot. 49, 663-664 (1962)
- : Chromosome studies in *Arabidopsis thaliana*. Genetics 48, 483-490 (1963)
- ŠVACHULOVÁ, J.: Content of total chlorophyll and free amino acids of the chlorina mutants of *Arabidopsis thaliana* in artificial glucose nutrition. Biol.Plant. (Praha) 9, 34-40 (1967)
- TAUBÖCK, K.: Über physiologische Rassen mit verschiedenem Boranspruch. Bot.Arch. 44, 52-69 (1943)
- TITOVA, F.N.: Search for a plant *Drosophila*. (Russ.). Sovietskaya Botanika No. 2, 61-67 (1935)
- VANDENDRIES, R.: Contribution à l'histoire du développement des crucifères. La Cellule 25, 412-459 (1909)
- VAUGHAN, J.G.: Structure of the angiosperm apex. Nature (Lond.) 169, 458-459 (1952)
- : The morphology and growth of the vegetative and reproductive apices of *Arabidopsis thaliana* (L.) HEYNH., *Capsella bursa pastoris* (L.) MEDIC. and *Anagallis arvensis* L. J.Linnean Soc., London, Bot. 55, 279-301 (1955)
- , and F.R.JONES: Structure of the angiosperm inflorescence apex. Nature (Lond.) 171, 751-752 (1953)
- VEEN, J.H. van der: Door EMS (ethyl methansulfonaat) geïnduceerde moederlijke effecten bij *Arabidopsis thaliana*. Genen en Phaenen 11, 39-41 (1966)
- : EMS induced non-recessive embryonic lethals in *Arabidopsis thaliana*. (Abstr.) Genetica 38, 61-62 (1967)
- VELEMINSKÝ, J., and T.GICHNER: Cytological and genetic effects of the insecticide Systox on *Vicia faba* L. and *Arabidopsis thaliana* (L.) HEYNH. Biol.Plant. (Praha) 5, 41-52 (1963)
- , - : The effect of X-rays on dry and on soaked seeds of *Arabidopsis thaliana* (L.) HEYNH. (Czech with Engl.a.Germ.Summary). Genetika a šlechtění (Praha) 2, 45-54 (1965)
- , - : Action of X-rays on seeds of *Arabidopsis thaliana*. (Czech with Engl.Summary). Genetika a šlechtění (Praha) 2, 45-54 (1965)
- , and G.RÖBBELEN: Beziehungen zwischen Chlorophyllgehalt und Chloroplastenstruktur in einer chlorina-Mutante von *Arabidopsis thaliana* (L.) HEYNH. Planta 68, 15-35 (1966)
- , T.GICHNER, and V.POKORNY: The effect of inhibitors on the mutagenic activity of N-methyl-N-nitrosourea and N-ethyl-N-nitrosourea in *Arabidopsis thaliana*. Biol.Plant. (Praha) 7, 325-329 (1965)
- , - , - : The action of 1-alkyl-1-nitrosoureas and 1-alkyl-3-nitrosoguanidines on the M₂ generation of barley and *Arabidopsis*. Biol.Plant.(Praha) 9, 249-262 (1967)
- , J.ŠVACHULOVÁ, and T.GICHNER: Free amino acids in the chlorophyll mutants of *Arabidopsis thaliana* and *Hordeum vulgare*. Proc.Symp. "Induction of Mutations and the Mutation Process". Praha, 27.-29.Sept. 1963, pp. 57-61, 1965
- VIVIAND-MOREL, M.: Exemple de nanisme chez un pied d'*Arabis thaliana*. Ann.Soc.bot., Lyon 6, No. 2, 98-99 (1877/78)
- WEHRMEYER, E., and G.RÖBBELEN: Räumliche Aspekte zur Membranschichtung in den Chloroplasten einer *Arabidopsis*-Mutante unter Auswertung von Serienschnitten. III.Mitt. Über Membranbildungsprozesse im Chloroplasten. Planta 64, 312-329 (1965)
- WEBERLING, F.: Morphologische und entwicklungsgeschichtliche Untersuchungen über die Ausbildung des Unterblattes bei dikotylen Gewächsen, unter besonderer Berücksichtigung der Ohrchenbildungen an Compositen- und Cruciferenblättern sowie der Verbreitung rudimentärer Stipeln bei den Cruciferen. Beitr.Biol.Pflanzen 32, 27-105 (1955)
- WINGE, Ö.: Contributions to the knowledge of chromosome numbers in plants. La Cellule 35, 305-324 (1925)
- WRICKE, G.: Ein Fall von Superdominanz bei einer experimentell hergestellten Autotetraploiden von *Arabidopsis thaliana*. Z.ind.Abst.u.Vererb.lehre 87, 47-64 (1955)
- YOKOYAMA, K., and W.H.JONES: Tissue cultures of *Arabidopsis thaliana*. (Abstr.). Plant Physiol. 40, p. LXXVII (1965)
- ZANDSTRA, K.J., en D.M. de VRIES: Het ontwikkelingsrhythme van een akkeronkruid-gemeenschap. (With Engl.Summary). Project 218. Versl.Centr.Inst.Landbouwk.Onderz. 34-39, 1951
- ZENKER, A(dele) K.: Jarowisationsuntersuchungen an sommerannuellen *Arabidopsis*-Rassen. Beitr.Biol.Pflanzen 32, 135-170 (1955)

(In the press)

- BERGER, B(rigitte): Entwicklungsgeschichtliche und chromosomale Ursachen der verschiedenen Kreuzungsverträglichkeit zwischen Arten des Verwandtschaftskreises Arabidopsis. Dissertation Landw.Fak.Univ. Göttingen. 55 Pages. January 1968. To be publ. in: Beitr.Biol.Pflanzen
- BHATIA, C.R., and H.H.SMITH: Adaptation and growth response of Arabidopsis thaliana to deuterium. Planta
- BOELPAEPE, R.De : Etude des mutations léthales récessives induites par la MNG chez Arabidopsis thaliana (L.) HEYNH. Agricultura (Louvain)
- BOUHARMONT, J.: Evolution of the chromosome numbers in Arabidopsis polyploids. Chromosomes Today
- GICHNER, T., L.EHRENBERG, and C.A.WACHTMEISTER: The mutagenic activity of beta hydroxyethyl methanesulfonate, beta methoxyethyl methanesulfonate and diethyl, 1,3-propane disulfonate. Hereditas
- HABERER, G., K.EIHHORN, H.ABEL, and V.IVANOV: Strahlenbiophysikalische Untersuchungen an Arabidopsis thaliana. Teil 1. Physikalische Grundlagen. (Russ.). Studia biophysika.
- KRANZ, A.R.: Endogene und exogene Beeinflussung der apparenten Strahlungsenergienutzung annueller Pflanzen. Angew.Bot.(Berl.)
- FEDERSON, D.G.: Environmental stress, heterozygote advantage and genotype - environment interaction in Arabidopsis. Heredity
- RÖBBELN, G.: Genbedingte Rotlicht-Empfindlichkeit der Chloroplastendifferenzierung bei Arabidopsis. Planta
- SANKHLA, N., and D.SANKHLA: Interaction between growth regulators and (\pm)-abscisin II in seed germination. Z.Pflanzenphysiol.
- , - : Abscisin II-kinetin interaction in leaf senescence. Experientia
- ŠVACHULOVA, J., and J.VELEMINSKÝ: N-metabolism and chlorophyll content in chlorophyll mutant supplied by sucrose. Studia Biophysica
- VELEMINSKÝ, J., V.POKORNY, and T.GICHNER: The effect of visible light on mutagenic activity of methylnitrosourea and methylnitro-nitrosoguanidine. Biol. Plantarum

D. A N N O U N C E M E N T S

Meeting of Scientists interested in Arabidopsis Research

During the 12th International Congress of Genetics, held in Tokyo, Japan, during August 19-28, 1968 an informal meeting is scheduled to give the possibility for discussion of Arabidopsis Research. Dr. Y.HIRONO will be kind enough to act as an organizer. For details, please, pay attention to the announcement at the boards of the

Change of Addresses

BHATIA, C.R., Modular Laboratories, Biology Division, Bhabha Atomic Research Centre, Trombay, Bombay 74, India

FEENSTRA, W.J., Genetisch Instituut, Rijksuniversiteit, Groningen, Rijkstraatweg 76, Nederland

Material

X-ray induced single gene recessive "miniature" (mit) mutant from REDEI's very early race Landsberg-"erecta": small, roundish, yellowish-green rosette leaves in a fine regular pattern; flowers only 2 days later, perfect fertility, unimpaired growth vigour, and retaining all profits of 'erecta' as an experimental plant. Worthwhile trying for test tube cultures, etc. Available from: Dr. H.A.S. HUSSEIN, Dept of Genetics, University of Agriculture, Wageningen, Netherlands.

Erratum

In Arabid.Inf.Serv. No. 4, p. 50, table 1 read AIS/AS instead of AS/AIS