ARABIDOPSIS
INFORMATION SERVICE

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## CONTENTS

**Preface**

RÉDEI, G.P., and S.L. LI: Arabidopsis as a research tool in genetics .......... 3

RÉDEI, G.P.: Arabidopsis for the classroom ........................................ 5

**A. Brief Notes**

CROVELLO, T.J.: The value of numerical taxonomy for Arabidopsis research ...... 7

HOLLANDY, 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.) HEYN. with special reference to the breeding system .......... 11

KAME, Christine, and G. RÖBHELEN: Variation of plant size and other morphological characters in natural populations of Arabidopsis ................ 13

CETL, I., and Jirina DOROVOLNA: Further data on the developmental characters of natural populations of Arabidopsis thaliana (L.) HEYN. from Western Moravia. 15

EFERMERTOVA, Eva, and I. CETL: The behaviour of progenies derived from a "winter annual" natural population of Arabidopsis thaliana (L.) HEYN. ........ 16

DOROVOLNA, 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.) HEYN. .......................... 17

COROS, A.J.: 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, Dakeha, and Narendra SANKHLA: Mophactin-cytokinbin interaction in seed germination of Arabidopsis thaliana, En-2 .......................... 19

SANKHLA, Dakeha, and Narendra SANKHLA: Effect of helminthosporol and helminthosporic acid on seed germination and senescence of leaf discs of Arabidopsis thaliana, En-2 .......................... 19

SANKHLA, Dakeha, and Narendra SANKHLA: Reversal of abscinbin II-induced senescence of Arabidopsis leaf discs by cytokinin .......................... 20

SANKHLA, Dakeha, and Narendra SANKHLA: Growth of Arabidopsis thaliana, En-2, in response to added morphactin .................................. 21

GINTER, T.N., and V.I. IVATOY: 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

RÉDEI, G.P.: Genetically determined differential recombination in the two sexes ........ 25

BOUHARNONT, J., and J. Van Den HEENE: Inheritance of lethal chlorophyll mutants in tetraploid Arabidopsis thaliana ............................. 25


BERG, Beatrice I. Van Den, and W.J. FEENSTRA: Complementing alleles as a cause of apparent reversal of pyrimidineless mutants ...................... 27

LI, S.L., and G.P. RÉDEI: Temperature-sensitive, thiamine-requiring mutants in Arabidopsis ......................................................... 28

RÖBHELEN, G.: Functionally different, allelic im mutants ....................... 29

HUSSERL, E.A.S., and J.H. Van Der Veen: Genotypic analysis of induced mutations for flowering time and leaf number in Arabidopsis thaliana ............. 30

KUKU, J.: Detection of induced late mutants in the early race Dijon ............. 30

SAMUEL, J., and O.P. KAME: On radiostimulation of flowering in Arabidopsis thaliana .......... 31

HIRONO, Y., and H.H. SMITH: Mutations induced with a base analog ................ 32

(continued)

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A.J. MÜLLER

G. RÖBHELEN
JACOBS, M.: Presumptive evidence for the extrachromosomal nature of laggard mutants induced by 5-bromodeoxyuridine

BONOTTO, S., M. JACOBS*, M. C. LONI, S. Van PUYMBROECK, and R. KIRCHMANN: Study of the incorporation of 5-bromodeoxyuridine into the DNA of Arabidopsis by neutron activation analysis

JACOBS, M., and S. BONOTTO: Evaluation of the penetration and incorporation into DNA of 5-bromodeoxyuridine in Arabidopsis seeds

JACOBS, M., and S. BONOTTO: Effects of actinomycin D on macromolecular synthesis and growth in Arabidopsis plants

AMANO, E.: Photoreactivation of UV-irradiated roots of Arabidopsis thaliana

REDDE, G.P.: A comparison of the somatic effects of X-rays and ethyl methane-sulfonate

BHADARAKAR, M.K., and B. K. GAUR: Lack of adaptation to Deuterium by Arabidopsis thaliana

ARNDT, C.G., and D. GRAU: Pflanzen mit wasserlichen Eigenschaften nach Röntgenbestrahlung

CONTANT, R.B.: Changes in sensitivity of seeds to fast neutrons in the course of germination

CONTANT, R.B., and R. DANKERT: Effects of storage on dry irradiated Arabidopsis seeds

CONTANT, R., and R.D. DANKERT: Absence of a relation between early root growth and date of flowering in seed-irradiated Arabidopsis

CONTANT, R.B., and R. DANKERT: Comparison of effects on quantitative characters in the M1 of Arabidopsis after seed treatments with fast neutrons, gamma-rays and EMS

CONTANT, R.B., and R. DANKERT: Correlations within and between M1 and M2, and genetic segregation, for various quantitative traits after neutron irradiation of Arabidopsis seeds

DANKERT, R., and R.B. CONTANT: Male sterility induced by gamma-rays, fast neutrons and EMS

HIRONO, Y., H. H. SMITH, and J. LYMAN: Biological effectiveness of heavy ionizing particles relative to X-rays

PULI, Taro: Killing efficiency of neutrons in Arabidopsis seeds

IVANOV, V.I., A. V. SAKINA, and H. A. TIMOFEEFF-RESSOVSKY: Preliminary data on effects of various ionizing radiations on Arabidopsis

VEEN, J. H. van der: Linear EMS-dose response for induced lethals, including maternally conditioned embryonic lethals

VEEN, J. H. van der, and C. Van HEEMERT: Decrease of genetic damage resulting from dry-storage of EMS-treated seeds

VELENKINSKY, J., V. POKORSKI, and T. GICHNER: Absence of correlation between the frequency of mutations in M1 and some developmental characters in M2 generation

GICHNER, T., and J. VELENKINSKY: The influence of pH on the mutagenic activity of nitroso-compounds on Arabidopsis

GICHNER, J., and T. GICHNER: Mutagenic activity of some nitroso-compounds

GICHNER, T., L. HERRNBERG, and C. A. VONKAIMESCH: The mutagenic activity of 8-hydroxyethyl methansulfonate (HOEMS), 8-methoxyethyl methansulfonate (MOEMS) and diethyl 1,3-propanediulfonate (DEPED) on Arabidopsis

VELENKINSKY, J., and T. GICHNER: The mutagenic activity of N,N,N',N'-trimethylnitrosourea (TMNO) and p-tolylsulfonfyl methyl nitrosamide (TMNO)

MÜLLER, A.J.: Killing and sterility in Arabidopsis

MÜLLER, A.J., und Ulrike REDECKER: Lebensfähige und letale fusca-Mutanten bei Arabidopsis thaliana

STEINITZ-SCHERZ, Lotti W., and Suzanne LEE-CHEN: Chromosome studies in Arabidopsis thaliana

KRANT, A.E.: The fixation of radiation energy during the development in chlorophyll-deficient mutants of Arabidopsis

E. Techniques

PEDERSON, D.G., and D.F. NATZINGER: Techniques of artificial selection

LI, S.J., and G.P. REDEI: A simple technique for screening thiamine auxotrophs

AMEND, C.U., und D. GÜSE: Herstellung von Totalpräparaten zur Darstellung von EMS

BALKEMA, G.H.: Embryo test without seed loss

BONOTTO, S., and M. JACOBS: Further studies on Arabidopsis RNA

C. Bibliography

Comprehensive list until December 1967

In the press

C. Announcements

Meeting of Scientists interested in Arabidopsis Research

Change of Addresses

Material

Erratum
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. RÖDEL 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, 1940. 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, Ephedra, 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 organisms.

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<th>Field of Genetic Research</th>
<th>Homo</th>
<th>Drosophila</th>
<th>Bombyx</th>
<th>Zea</th>
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</table>

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.

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 newcomer 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

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Figure 1: Total scores in fourteen areas of all organisms

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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.

LAIICH in his prescient address at the Symposium on Arabidopsis Research in Göttingen, 1965, anticipated just what is presented in these statistics when he said: "...wenn gleich...Virus und Bakterien sicherlich manches Grundlegendes aufzeigen können, so könnten sich auf das Problem der Entwicklung und Phylogenie bei den Blüttenpflanzen doch wohl schmerzlich 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. RÉDEI

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:*

\[ g^2 \times p \]

\[ g^1 \times p \]

Plants of this genotype should be planted at least at intervals 2-3 weeks apart and kept under 8 hr 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 \( g^1 \) \( p \) recombinants is also desired. Map distances: \( g^1 - 25-2: g^2 - 8-0. \)

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. 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 pots, spraying with a low volume "Water Fog", before germination or until early seedling stage. Later spray with the high volume nozzle of Fogg-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 inadequate 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 \( p/p \) 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 (40-60°C) and 70% ethanol. Add 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 of 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. The separation of pigments, including the development in dim light, is readily performed 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 b situated in all genotypes containing the chl allele, while chl/chl 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 b 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 (NH3 500, 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 these in a large scale. 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:

1. "Pyrimidine"
2. "Thiazole"
3. Thiamine

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-G-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: $2/lb). Any good commercial thiamine HCl can be used for mutant th.

Aseptic culture: Each student prepares the following mineral solution (mg/100 ml distilled water): NH4NO3 40; MgSO4·7 H2O 20; CaH2(PO4)2·H2O 20; K2HPO4 10; FeC6H507·3 H2O (ferricitrate) 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 the culture is seeded. Surfase or glucose content of the medium should be 2%. Divide the agar into four portions of equal amounts (25 ml each). Add the autoclaved batch 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 requirements and can construct the pathway on the basis of his own observations. To aid in the identification of the seed mutants on marked background py ± tz ± th 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 by 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:

- py
- tz
- th
Illumination
Continuous 8 hours daily

<table>
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<th>1</th>
<th>2</th>
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<td>wild type</td>
<td>1</td>
<td>2</td>
<td></td>
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<tr>
<td>ld</td>
<td>2</td>
<td>Not at all</td>
<td></td>
<td></td>
</tr>
<tr>
<td>g2</td>
<td>3</td>
<td></td>
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<td>g12</td>
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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^{-5}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 g12 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.

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A. BRIEF NOTES
The value of numerical taxonomy for Arabidopsis research
T.J. CROVELLO
(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. RÖBERLEIN (1965) presented comparative data on 12 characters for 154 lines of 120 strains of Arabidopsis thaliana (L.) HENYH. 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 RÖBERLEIN 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 oophenetio 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 or 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 ROBBEIEN (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 resolu-
tion among the strains must await increased observations on a greater number of
characters. But the present analysis still remains an objective summary of the rela-
tionships among lines on the basis of ROBBEIENs data. This was its first purpose.

Turning to the second purpose, numerical taxonomy can aid the geneticist and phy-
siologist 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 BLA
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 phenetical-
ly strain BLA 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.

Genetists 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

<table>
<thead>
<tr>
<th>GROUP No.</th>
<th>LINES IN EACH GROUP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AK-1, NW-2</td>
</tr>
<tr>
<td>2</td>
<td>AN-3, BD-0, BE-0, BE-1, BLA-4, BS-2, BS-5, BSCH-0, BSCH-2, C0-2, DR-0, EI-2</td>
</tr>
<tr>
<td>3</td>
<td>LU-1, LU-2, MA-2, MA-1, NE-0, NW-1, NW-3, NW-4, PA-2, FT-0, PO-0, BSCH-4</td>
</tr>
<tr>
<td>4</td>
<td>SRO-0</td>
</tr>
<tr>
<td>5</td>
<td>AN-1, BCH-1, BCH-4, BLA-6, BLA-10, CHI-0, EP-0, GD-0, HA-0, JE-0, LI-1</td>
</tr>
<tr>
<td>6</td>
<td>LI-5, LI-7, LI-12, NW-0, ROU-0, BSCH-0</td>
</tr>
<tr>
<td>7</td>
<td>AN-2, DI-0, EL-0, GA-0, GÖ-2, GU-0, HI-0, SG-1, SG-2</td>
</tr>
<tr>
<td>8</td>
<td>CHI-1, CHI-2, CT-1, DA-0, ER-1, ER-2, ET-1, FR-1, FR-3, FR-4, GIE-0, IS-0</td>
</tr>
<tr>
<td>9</td>
<td>LI-8, MT-0, PI-0, SI-0, UK-3</td>
</tr>
<tr>
<td>10</td>
<td>IN-0, KA-0, LK-2, MA-0, STW-0, TU-1, WI-3</td>
</tr>
<tr>
<td>11</td>
<td>BCH-1, BCH-4, BLA-2, CO-3, EI-4, HS-0, KL-3, KL-4, RD-0, TA-0, UK-1, X-0</td>
</tr>
</tbody>
</table>

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 biologist must become a numerical taxonomist. Rather it points to another direction in which cooperation among biologists of various disciplines could pay big dividends. Specifically, a continuing database 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 about strain and character relationships could be issued periodically. Such a database 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:
The infeasibility of selecting simultaneously for growth rate and flowering time on Arabidopsis plants grown in nutrient agar

G.J. HOLIAMBY

(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 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 16m=) 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 equivalent 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

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* Standard errors

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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:

<table>
<thead>
<tr>
<th>Race</th>
<th>Method 1</th>
<th>Method 2</th>
<th>Method 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>A156</td>
<td>0.09646</td>
<td>0.09207</td>
<td>0.09122</td>
</tr>
<tr>
<td>Ls</td>
<td>0.09556</td>
<td>0.09634</td>
<td>0.09767</td>
</tr>
<tr>
<td>Est</td>
<td>0.10153</td>
<td>0.09229</td>
<td>0.08784</td>
</tr>
<tr>
<td>Hm</td>
<td>0.09588</td>
<td>0.07914</td>
<td>0.07624</td>
</tr>
</tbody>
</table>

Figures in brackets are standard errors where calculable.

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 in 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:

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 1: Alcester, Warwickshire
- Population 2: Broom, Warwickshire
- Population 3: Luddington, Warwickshire
- Population 4: Henley-in-Arden, Warwickshire
- Population 5: Cannon Hill Park, Birmingham
- Population 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:

<table>
<thead>
<tr>
<th>Population</th>
<th>Mean</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>37.412</td>
<td>33.504</td>
</tr>
<tr>
<td>2</td>
<td>59.969</td>
<td>328.553</td>
</tr>
<tr>
<td>3</td>
<td>47.222</td>
<td>81.390</td>
</tr>
<tr>
<td>4</td>
<td>21.574</td>
<td>140.435</td>
</tr>
<tr>
<td>5</td>
<td>7.580</td>
<td>4.416</td>
</tr>
<tr>
<td>6</td>
<td>8.574</td>
<td>3.389</td>
</tr>
</tbody>
</table>

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 (BARTLET 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
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 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:

<table>
<thead>
<tr>
<th>Population</th>
<th>Family group</th>
<th>Between W.S. d.f.</th>
<th>Within W.S. d.f.</th>
<th>V.R.</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>1</td>
<td>37.025</td>
<td>5.564</td>
<td>4.323</td>
<td>.01-001</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>25.623</td>
<td>15.477</td>
<td>1.526</td>
<td>.001</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>104.344</td>
<td>6.229</td>
<td>16.751</td>
<td>.001</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>30.325</td>
<td>6.902</td>
<td>4.399</td>
<td>.01-001</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>23.623</td>
<td>3.177</td>
<td>4.496</td>
<td>.001</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>5.905</td>
<td>4.664</td>
<td>1.866</td>
<td>.05-01</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>7.671</td>
<td>2.666</td>
<td>2.856</td>
<td>.05-01</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>7.887</td>
<td>2.143</td>
<td>3.680</td>
<td>.05-01</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>12.900</td>
<td>2.578</td>
<td>4.888</td>
<td>.01-001</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>9.125</td>
<td>2.395</td>
<td>3.510</td>
<td>.05-01</td>
</tr>
</tbody>
</table>

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 NAPP-ZINN (1964) and DOBRZENKA (1966) respectively, and also more recently by RÜBELEN (personal communication).

References:

DOBROVTINA, J.: Arabid. Inf. Serv. 4, 6-7 (1967)

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

Christine KARR and G. RÜBELEN

(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. HOEBELEN, 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 off-springs, while others "segredated" 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 "H1-2w" was grown in the greenhouse - each family, derived from a single plant, in a row of 6 pots. The diameter of the full grown rossette 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 colour 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 Hl-2 in which the phenotypic criterions were highly variable. Some of these 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:

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 EFFERTOVA (1965, 1967), CETL (1965), and EFFERTOVA 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 unvernalized 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>
<thead>
<tr>
<th>Year</th>
<th>0 - 10</th>
<th>10 - 20</th>
<th>20 - 30</th>
<th>30 - 40</th>
<th>40 - 50</th>
<th>50 - 60</th>
<th>60 - 70</th>
<th>70 - 80</th>
<th>80 - 90</th>
<th>90 - 100</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>1963/64</td>
<td>18</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>12</td>
<td>20</td>
<td>64</td>
<td>28,1</td>
<td></td>
</tr>
<tr>
<td>1965</td>
<td>4,3</td>
<td>6,2</td>
<td>3,1</td>
<td>3,1</td>
<td>4,7</td>
<td>18,8</td>
<td>31,2</td>
<td>99,9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1966/67</td>
<td>11</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>6</td>
<td>22</td>
<td>45</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1966/67</td>
<td>1</td>
<td>5,5</td>
<td>3,6</td>
<td>1,8</td>
<td>1,6</td>
<td>85,4</td>
<td>100,1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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>
<thead>
<tr>
<th>Year</th>
<th>7,5 - 10,0</th>
<th>10,0 - 12,5</th>
<th>12,5 - 15,0</th>
<th>15,0 - 17,5</th>
<th>17,5 - 20,0</th>
<th>20,0 - 22,5</th>
<th>22,5 - 25,0</th>
<th>25,0 - 27,5</th>
<th>27,5 - 30,0</th>
<th>30,0 - 32,5</th>
<th>32,5 - 35,0</th>
<th>35,0 - 37,5</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>1963/64</td>
<td>3</td>
<td>11</td>
<td>9</td>
<td>8</td>
<td>7</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>46</td>
<td>2,2</td>
<td>100,0</td>
<td>46</td>
<td></td>
</tr>
<tr>
<td>1965</td>
<td>6,5</td>
<td>23,9</td>
<td>19,6</td>
<td>17,4</td>
<td>15,2</td>
<td>13,0</td>
<td>2,2</td>
<td>2,2</td>
<td>100,0</td>
<td>34</td>
<td>100,0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1966/67</td>
<td>5,9</td>
<td>17,7</td>
<td>38,2</td>
<td>20,6</td>
<td>8,8</td>
<td>2,9</td>
<td>5,9</td>
<td>1</td>
<td>54</td>
<td>1,9</td>
<td>100,2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>1,5</td>
<td>6,7</td>
<td>17,9</td>
<td>23,1</td>
<td>17,9</td>
<td>11,2</td>
<td>7,5</td>
<td>7,5</td>
<td>4,5</td>
<td>0,7</td>
<td>1,5</td>
<td>100,1</td>
<td></td>
</tr>
</tbody>
</table>

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 s 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

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>1963/64</td>
<td>1.4</td>
<td>3.6</td>
<td>6.6</td>
<td>13.0</td>
<td>20.2</td>
<td>21.2</td>
<td>15.7</td>
<td>10.9</td>
<td>10.5</td>
<td>5.1</td>
<td>2.2</td>
<td>1.8</td>
<td>1.8</td>
<td>1.8</td>
<td>1.8</td>
</tr>
<tr>
<td>1965</td>
<td>1.4</td>
<td>3.6</td>
<td>6.6</td>
<td>13.0</td>
<td>20.2</td>
<td>21.2</td>
<td>15.7</td>
<td>10.9</td>
<td>10.5</td>
<td>5.1</td>
<td>2.2</td>
<td>1.8</td>
<td>1.8</td>
<td>1.8</td>
<td>1.8</td>
</tr>
<tr>
<td>1966/67</td>
<td>1.4</td>
<td>3.6</td>
<td>6.6</td>
<td>13.0</td>
<td>20.2</td>
<td>21.2</td>
<td>15.7</td>
<td>10.9</td>
<td>10.5</td>
<td>5.1</td>
<td>2.2</td>
<td>1.8</td>
<td>1.8</td>
<td>1.8</td>
<td>1.8</td>
</tr>
</tbody>
</table>

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:
DOBROVOLNA, J. and E. EFFMERTOVA: Arabid.Inf.Serv. 2, 3 (1965)
DOBROVOLNA, J.: Arabid.Inf.Serv. 1, 6-7 (1967)

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

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 (NAFF-ZINN, 1964; CETL, 1965). The latter can be subdivided into 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 DOBROVOLNA (1967) that in several populations of the "summer annual" type a high percentage of progenies showed a higher variation in two developmental characters than homoyzymous 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 unvernalised seeds were sown only about 75% 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 166; 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 χ²-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:
DOBROVOLNA, J.: Arabid.Inf.Serv. 1, 9-10 (1967)
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.) HEYNE.

Jiříma DOBOROVNÁ

(Department of Genetics, Purkyňův 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 Arabidopsis 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, $h = \frac{b_{op}}{s_b}$. Thus, the $h^2$ value can be used to answer the above question.

In the present experiment with the natural population Stř which appears to be highly variable and in 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:

<table>
<thead>
<tr>
<th>Mode of estimation</th>
<th>Number of days to appearance of the flower primordia</th>
<th>Number of rosette leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$b \pm s_b$</td>
<td>$b \pm s_b$</td>
</tr>
<tr>
<td>(1)</td>
<td>0.5252 ± 0.0577</td>
<td>0.4576 ± 0.0505</td>
</tr>
<tr>
<td>(2)</td>
<td>0.4659 ± 0.0197</td>
<td>0.4424 ± 0.0175</td>
</tr>
<tr>
<td>(3)</td>
<td>0.4993 ± 0.0265</td>
<td>0.3517 ± 0.0324</td>
</tr>
</tbody>
</table>

* 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:

KEMPThORNE, O., and O.R. TANDON: Biometrics 1, 90-100 (1953)
REEVE, E.C.R.: Biometrics 1, 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. LANG 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 2200 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 (VLEMRUSKY 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)
VELEMISZKY, J., and T. GICHER: 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

<table>
<thead>
<tr>
<th>Dose in kR</th>
<th>0</th>
<th>100</th>
<th>200</th>
<th>400</th>
<th>600</th>
<th>800</th>
<th>1000</th>
<th>1500</th>
<th>2000</th>
<th>3000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of</td>
<td>778</td>
<td>604</td>
<td>541</td>
<td>873</td>
<td>615</td>
<td>566</td>
<td>522</td>
<td>573</td>
<td>656</td>
<td>1234</td>
</tr>
<tr>
<td>% germinated</td>
<td>0</td>
<td>0.3</td>
<td>0.2</td>
<td>0.7</td>
<td>1.3</td>
<td>6.8</td>
<td>33.9</td>
<td>68.4</td>
<td>94.8</td>
<td>92.5</td>
</tr>
</tbody>
</table>

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 their formation, 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 difusible 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, a shown in Table 2.

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

<table>
<thead>
<tr>
<th>Dose in MR</th>
<th>0</th>
<th>1</th>
<th>1.5</th>
<th>2</th>
<th>0</th>
<th>1</th>
<th>1.5</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of</td>
<td>662</td>
<td>665</td>
<td>717</td>
<td>752</td>
<td>715</td>
<td>552</td>
<td>508</td>
<td>468</td>
</tr>
<tr>
<td>% germinated</td>
<td>0</td>
<td>68.0</td>
<td>0.8</td>
<td>0</td>
<td>83.7</td>
<td>1.0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

References:
REINHOLZ, E.: PIAT-Report 107, 12 (1947)
- : Arabid.Inf.Serv. 4, 10-17 (1967)
Morphactin-cytokinin interaction in seed germination of Arabidopsis thaliana, En-2
Daksha SANXELA* 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 (CCC, Phoepon, 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 (WINNEMANN 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 (GATHEY, 1964), it was thought worthwhile to extend the above observation to 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).

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:
WINNEMANN, H., J.A.D. ZEYKANT, R. EINKE, and A. LANG.: Planta 61, 229-235 (1964)
SANKHLA, N., and D. SANKHLA: Planta 76, 47-51 (1967)
SCHNEIDER, G.: Naturwiss. 51, 416-417 (1964)

Our best thanks are due to: Dr. G. SHAW for his gift of zeatin; Prof. K. BOLE for facilities; Dr. G. SCHNEIDER (of Merck AG) for his help in obtaining morphactin; Kerkow, 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 SANXELA and Narendra SANKHLA
(Institute of Botany, University of Frankfurt/M., Germany)

Recently, helminthosporol (H-o1) 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 TANTRA, 1967a). These substances have also been claimed to be active in inducing germination of light sensitive seeds of tobacco in total darkness (HASHIMOTO and TANTRA, 1967b). In Arabidopsis gibberellin acid is well known for its promoting effect on the germination of seeds kept in darkness (KRIBEN, 1957; REINHOLD, 1967).
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.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Concentration in ppm</th>
<th>% Germination after days 7</th>
<th>% Senescence after hrs 24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>-</td>
<td>0</td>
<td>45</td>
</tr>
<tr>
<td>Gibberellin</td>
<td>10</td>
<td>10</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>25</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>70</td>
<td>100</td>
</tr>
<tr>
<td>Helminthosporol</td>
<td>10</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Helminthosporic acid</td>
<td>10</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

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 gave a consistent and reproducible result (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)
KRIEBEN, F.J.: Naturwiss. 44, 373 (1957)
REINHOLZ, E.: Arabid.Inf.Serv. 4, 16-17 (1967)

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

Reversal of abscisin II-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 (CEKUMA et al., 1965; CORNFORTH et al., 1966; SANKHLA and SANKHLA, 1966 a, b, c). Earlier we observed that kinetin can effectively overcome the senescence-accelerating effect of abscisin II (SANKHLA and SANKHLA, 1966b). The present experiments were undertaken to evaluate the comparative poten-tialities of two other cytokinins, viz., zeatin and 6-benzylaminopurine, in reversing the effect of abscisin II 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

<table>
<thead>
<tr>
<th>Substance</th>
<th>Concentration in ppm</th>
<th>Optical density after hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>-</td>
<td>0.150</td>
</tr>
<tr>
<td>Abscisin II</td>
<td>2</td>
<td>0.085</td>
</tr>
<tr>
<td>6-Benzylaminopurine</td>
<td>0.5</td>
<td>0.285</td>
</tr>
<tr>
<td>Zeatin</td>
<td>0.5</td>
<td>0.295</td>
</tr>
<tr>
<td>Abscisin II + 6-benzylaminopurine</td>
<td>2 + 0.5</td>
<td>0.275</td>
</tr>
<tr>
<td>&quot; + zeatin</td>
<td>2 + 1</td>
<td>0.255</td>
</tr>
<tr>
<td>&quot; + &quot;</td>
<td>2 + 1</td>
<td>0.300</td>
</tr>
</tbody>
</table>

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 cytokinines 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, 1966c,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.: Plants 74, 228-242 (1967)
SANKHLA, N., and D. SANKHLA: Physiol. Plant. 21, 190-195 (1966a)
- - : Experientia, in press, 1966b
- - : Naturwissenschaften, in press, 1968c
- - : Z.Pflanzenphysiol., in press, 1968d

Grateful thanks are due to: Prof. L. BOLE for facilities; Prof. J.W. CORNFORTH for donating a sample of abscisin II; Dr. G. SHAW for his gift of zeatin; Merek, 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; HARA, 1967). Moreover, 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. Morphactin unlike growth retardants (SCHNEIDER et al., 1964) do not affect gibberellin biosynthesis (TOSCHI et al., 1967; BESLIS and LIBBERT, 1967) and are known to disturb phototropism of shoots and geotropism of roots (KHAH, 1967). Recently, an apparent antagonism between morphactins and cytokinines has also been recorded (SANKHLA and SANKHLA, 1967). So 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 compounds 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.
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 lateral branches, and abnormalities of floral parts. Thus these results appear to support the earlier postulate (Tognoni et al., 1967; Mark 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 paniculata 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 Libeert (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 our no definite opinion can be put forth regarding the exact site of action of morphactins.

References:

Hartmann, B.: Naturwissenschaften 54, 25 (1967)

to be published in press

We are grateful to Prof. E. Bolz 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. SUNUSOV from the Botanical Institute in Dushanbe, A. griffithiana (BOISS.) BUSCH (A. pumila (STEPH.) BUSCH ?), and A. wallichii (J.D. HOOE.) 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:

- A. wallichii
  \(2n = 16\)
- A. griffithiana
  \(2n = 32\)
- A. korshinskyi
  \(2n = 48\)

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

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 embryo sac 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 nearly 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:


(Figure see Page 24)
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 synchronised 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 thaliana x Arabidopsis thaliana or Arabidopsis thaliana x Sisymbrium altissimum, in which the embryo reached more than 0.7 mm in length, cultures on 0.5% agar (pH 6.5) and 2% sucrose after BOURJOMONT (1961) + 0.2% yeast extract were successful.

References:
Genetically determined differential recombination in the two sexes

G.F. 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/+ by +

<table>
<thead>
<tr>
<th>No. of chromosomes tested</th>
<th>Recombination frequency in region</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gf - hv</td>
</tr>
<tr>
<td></td>
<td>Gf - py</td>
</tr>
<tr>
<td>Male</td>
<td>1749</td>
</tr>
<tr>
<td></td>
<td>21.7</td>
</tr>
<tr>
<td></td>
<td>26.5</td>
</tr>
<tr>
<td></td>
<td>6.5</td>
</tr>
<tr>
<td>Female</td>
<td>647</td>
</tr>
<tr>
<td></td>
<td>12.9</td>
</tr>
<tr>
<td></td>
<td>16.0</td>
</tr>
<tr>
<td></td>
<td>4.3</td>
</tr>
<tr>
<td>Reduction in the female (%)</td>
<td>43.3</td>
</tr>
<tr>
<td></td>
<td>39.6</td>
</tr>
<tr>
<td></td>
<td>33.8</td>
</tr>
</tbody>
</table>

In the present experiments recombination was studied in three regions with Gf + py/+ by + 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 centromeres.

References:
REDEI, G.P.: Genetics 51, 857-872 (1965)

Inheritance of lethal chlorophyll mutants in tetraploid Arabidopsis thaliana

J. BOUGHORMAT and J. Van Den HEDE

(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 (BOUGHORMAT, 1965), (b) an efficient induction of numerous mutations by the 1-methyl-3-nitro-1-nitrosoguanidine (MÜLLER and GICHER, 1964) and (c) the possibility of using lethal mutations via the embryo-test of MÜLLER (1965).

Autotetraploid plants, heterozygous for chlorophyll mutations, have been obtained by two ways: (1) treatment of a tetraploid strain by NNG (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 NNG (C5K 0.1; 24 hrs).

(ad 1) In tetraploid individuals the NNG treatment produces triplex heterozygotes. The E2-generation (observed at the embryo stage in the siliques of the E1-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 E1-generation, duplex, simplex and triplex genotypes are in proportion of 1 : 2 : 36 : 8 : 1. It is necessary to observe a large number of seeds, since only the one nulliplex out of 36 individuals is homogenic recessive and phenotypically mutant. Moreover, only a maximum of 25 % of the E2-plants is of the duplex type and the actual number of heterozygous E2-plants is always lower because of the chimeric nature of the E1-plants after mutagenic seed treatment. - The seeds collected on duplex E2-plants were sown. But it was only in the siliques of E3-plants (containing the E2 embryos) that an adequate proportion of lethal mutants was recovered. Especially the simplex and triplex E2-plants (6 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
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 24.5%). The green plants of the colchicine 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), 6 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 (2.63%). The second offspring contained about the expected number of duplex plants (10 out of 18) with a normal proportion of lethals (3.10%). At the rest of 6 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:
- , and T. GÖHNKE: Nature 210, 1149-1150 (1964)

Pigment concentration and photosynthetic activity of some chlorophyll mutants of Arabidopsis thaliana
A.G. EKSTRÖM and Yu.S. EKSTRÖM
(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 60/2, dark green 203/13, light green 60/1, viridoalbina 40/3, green with yellow spots 601/6 (all of them induced by γ-irradiation, 10-50 kR, of seeds), the mutants V 156, V 44, V 120 and V 24/3 (kindly supplied by Prof. G. ROBBLELEN, ttinmer), and the mutant γ (provided by Dr. E.V. PETZKO, Leninras).

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 (SAPOSCHIKOV, BRONSTEIN, and Krasovskaya, 1955). The "potential intensity of photosynthesis" was studied with C14O2 (ZALENIEV, SEKIRATOV, and VESSENBERG, 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.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Pigment concentration</th>
<th>Potential intensity of photosynthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chlorophyll</td>
<td>Carotene</td>
</tr>
<tr>
<td>Wild type (En-2)</td>
<td>526</td>
<td>362</td>
</tr>
<tr>
<td>Chlorina 60/2</td>
<td>436</td>
<td>185</td>
</tr>
<tr>
<td>Grünlich gelb V 156</td>
<td>280</td>
<td>124</td>
</tr>
<tr>
<td>Dark 203/13</td>
<td>811</td>
<td>364</td>
</tr>
<tr>
<td>Green with yellow spots 601/6</td>
<td>262</td>
<td>335</td>
</tr>
<tr>
<td>Virencence 60/1</td>
<td>582</td>
<td>362</td>
</tr>
<tr>
<td>Grünlich V 120</td>
<td>354</td>
<td>152</td>
</tr>
<tr>
<td>Grünlich gelb V 44</td>
<td>527</td>
<td>160</td>
</tr>
<tr>
<td>Glucosa 41</td>
<td>722</td>
<td>182</td>
</tr>
<tr>
<td>Grünealbiga V 24/7</td>
<td>266</td>
<td>129</td>
</tr>
<tr>
<td>Viridoalbina 40/3</td>
<td>334</td>
<td>120</td>
</tr>
</tbody>
</table>

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. EASTHREN, 1967; EKSTRÖM and TILLOFFELT-ROSSOVSKY, 1967). In consequence of these differences in pigment concentration and plastid morphology the photosynthetic activity of the leaves and thus their photosynthetic activity is changed (cf. EKSTRÖM and KÜLLER, 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 40/5, have a low "assimilation number".

References:

Existence of RNA-transmethylase in Arabidopsis
M. ABEELS
(Laboratoire de Cytogenétique, 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 P-, 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 BORIS 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 10 mM MgCl2, 2 mM 2-mercaptoethanol, 10 mM triethanolamin buffer, pH 8.5, 10 mM C14-CH3-S-adenosylmethionine, 100 mM nucleotides as methyl deficient sRNA, and sufficient enzyme to incorporate at least 1 nCi of C14-CH3 groups (HURWITZ et al., 1964). After incubation for 20 minutes at 38°C 5 μl sodium pyrophosphate, 0.05 ml of 0.5% bovine plasma albumin, and 0.2 ml of 7% H2O2 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 Na2H2O, deanted 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:

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 (pyc). In this conception the latter allele when present homogenously would lead to a deficient phenotype. The roughly 50 % deficient plants found in W= - and further generations of selfed revertants then should for equal parts be composed of py py and py py pyc. 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 progeny, 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

<table>
<thead>
<tr>
<th>Revertant</th>
<th>Original mutant allele</th>
<th>Segregation in families tested</th>
<th>Progenies from deficient x original mutant</th>
<th>non-deficient x original mutant</th>
</tr>
</thead>
<tbody>
<tr>
<td>V 43 R1</td>
<td>py-2-1</td>
<td>189 189 184</td>
<td>47 56</td>
<td>24</td>
</tr>
<tr>
<td>V 163 R1</td>
<td>py-1-14</td>
<td>356 366 53</td>
<td>30 58</td>
<td>13</td>
</tr>
<tr>
<td>V 466 R1</td>
<td>py-2-15</td>
<td>60 66 76</td>
<td>16 8</td>
<td>10</td>
</tr>
<tr>
<td>V 462 R1</td>
<td>py-1-4</td>
<td>45 49 7</td>
<td>19 12</td>
<td>7</td>
</tr>
<tr>
<td>V 652 R1</td>
<td>py-1-12</td>
<td>113 97 24</td>
<td>2 4</td>
<td>6</td>
</tr>
</tbody>
</table>

* 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 pyrimidinless 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 is 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:

Temperature-sensitive, thiamine-requiring mutants in Arabidopsis

S.L. Li and G.P. Edebi

(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 high 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.

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

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:

Functionally different, allelic im mutants

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

The variegated mutant im (im^1) was obtained by RÖDEI (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^2) 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 pheno-types: the first class, including 23 mutants is characterized by the mutant im^3^, the second with 6 types by im^1^, while the third with the prototype im^4^ containing the remaining 2 mutants. The table demonstrates that under the same illumination im^2^ rosettes are almost white, while simultaneously grown im^4^ plants are rather green and im^5^ individuals intermediate. Though all of our mutants originated from the same genetic line.

Leaf colour of different im mutants under identical culture conditions

<table>
<thead>
<tr>
<th>Mutant</th>
<th>% seedlings with colour of cotyledons</th>
<th>Chlorophyll per g fresh weight in full grown rosettes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>white</td>
<td>&gt;50% white</td>
</tr>
<tr>
<td>im^1</td>
<td>75.5</td>
<td>18.4</td>
</tr>
<tr>
<td>im^2</td>
<td>89.0</td>
<td>7.7</td>
</tr>
<tr>
<td>im^3</td>
<td>10.6</td>
<td>59.6</td>
</tr>
<tr>
<td>im^4</td>
<td>5.7</td>
<td>27.3</td>
</tr>
</tbody>
</table>

(Zn-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 different extragenic modifiers.

Reference:

Many thanks are due to Dr. G.P. RÖDEI, 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

(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,e) closely resembles 'line C' (E,E), 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 within 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 E5:

(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 (e2e2 from line C; e4e4 and e6e6 from line 51) and 1 EMS-mutant with an intermediate heterozygote (e3e3, from line 51). A 6 x 6 F2-di-allelle (and backcrosses), including lines C and 51, showed that all mutant genes are at different loci and only E2-e2 and E4-e4 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 1313; 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 (1111, 1212, 1313), and 3 partially dominant or nearly completely dominant X-ray mutants (X1X1, X2X2, X3X3). A 7 x 7 F2-di-allelle (including line 51) showed that among these E1-e1 (the C-51 locus) and X2-x2 are closely linked, and that L3-l3 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 (e9e9) and 1 completely recessive X-ray mutant (e7e7). These two loci are probably closely linked, but no linkage to E3-e3 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 (v1v1, v2v2, v3v3, v4v4) and 2 partially recessive X-ray mutants (v5v5, v6v6). A 7 x 7 F2-di-allelle (including line C) revealed that, of these loci, V6-v6 is either very closely linked to E3-e3, or v6 is closely linked to 'e' at the E3 locus.

(E) A second-cycle selection programme (after EMS- or X-ray treatment) was started with one late mutant from line C (viz., e9e9). Of the 2 early EMS-mutants obtained, one (R1R1) is dominant towards early and has a relatively small effect, the other (R2R2) shows an intermediate heterozygote and has a large effect (halfway between line C and e9e9). R1-R1 and R2-P2 are linked to each other but not linked to E3-e3. It should be noted that R1 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:


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. KUCERA

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

Late mutants with vernalization requirement were induced in the early race Dijon by HUSSEIN and Van Der VEEN (1966). In our experiments M2-populations of this race were cultivated after treatment with N-methyl-N-nitrosourea (MN) and j-irradiation at high temperature (25°C) 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 M2-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 $\gamma$-irradiation 73 late M2-plants (= 7.1%) were registered in 44 out of 100 M1-progenies. After MU-treatment 124 out of 624 M2-plants (19.9%) were definitely later. The number of plants with one or more additional leaves is given in the following table:

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total M2-plants</th>
<th>Additional leaves</th>
<th>Total late plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\gamma$-rays</td>
<td>1031</td>
<td>+1 65 (6.3%)</td>
<td>+2 6 (5.8%)</td>
</tr>
<tr>
<td>MU</td>
<td>624</td>
<td>+1 88 (14.1%)</td>
<td>+2 28 (4.5%)</td>
</tr>
</tbody>
</table>

All together a slight developmental delay - equal to the middle early natural races - was induced in 195 plants (11.6%), while 2 plants (0.1%) were drastically changed and similar to late races with vernalization requirement.

The progenies of the selected late M2-plants were investigated under the same culture conditions. Because of this cultivation technique, however, the number of seeds harvested in M2 was comparatively low. Therefore, only 81 progenies with 6 or more plants could be evaluated in the M3-generation. In 13 progenies (16.1) the recorded developmental delay of the M2-plant proved not to be genetically fixed; but in 68 progenies the changed phenotype reappeared. 56 progenies of the latter group segregated in respect to lateness, while 8 progenies (9.9%) were uniform. In the segregating group, beside the phenotype of the M2-plants, there were (a) plants identical with the control in 43 progenies (53.0%) and (b) plants with larger developmental delay than in M2 in 7 progenies (8.6%), while (c) 8 progenies (9.9%) were highly variable including the original M2-types and later plants as well. (d) In 2 progenies (2.5%) all of the M3 plants were later compared to the M2. - The correctness of the classification in M3 was examined in a M4-generation cultivated under the same conditions. The high number of segregating progenies in the M3 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.P. LAKRA

(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-KHN' medium and grown under constant illumination of 40 W cool white fluorescent lamps at 25 ± 1°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 Cs¹³⁷ 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:

Mutations induced with a base analog

Y. HIRONO and H.H. SMITH

(Plant Breeding 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-bromo-deoxy-cytidine was incorporated. Wild type seeds were sterilized with calcium hypochlorite, sown singly on the agar medium in a test tube, and the plants (M₁-generation) were grown under well-controlled conditions in a growth chamber. Mature seeds were harvested from each plant, whose progeny (M₂-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 (M₃) generation, seeds harvested from plants grown in the pots were used.

Progeny tests have now been completed for about 1000 plants of the M₁-generation. Among these M₂-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 M₂ families were much greater than 3:1, thus indicating that the M₂-plants were true-breeding homozygous parents derived from the M₁. The segregation ratios of the M₃-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 M₂-generation. M₃-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 M₂-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 is 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

K. 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 M₄-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 crosses, all seedlings were normal. Arguments for the extranuclear heredity hypothesis are thus 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.

<table>
<thead>
<tr>
<th>No. of crossing</th>
<th>Viable laggard</th>
<th>Lethal laggard</th>
<th>Normal laggard</th>
<th>Viable normal</th>
<th>Lethal normal</th>
<th>Normal normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>9</td>
<td>1</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>2</td>
<td>6</td>
<td>-</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
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<td>1</td>
<td>1</td>
<td>11</td>
<td>4</td>
<td>-</td>
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<td>18</td>
<td>5</td>
<td>-</td>
<td>6</td>
</tr>
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<td>6</td>
<td>-</td>
<td>2</td>
<td>6</td>
<td>6</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>-</td>
<td>6</td>
<td>6</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>2</td>
<td>4</td>
<td>-</td>
<td>4</td>
<td>11</td>
</tr>
<tr>
<td>9</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>4</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>10</td>
<td>3</td>
<td>-</td>
<td>4</td>
<td>6</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>11</td>
<td>-</td>
<td>1</td>
<td>6</td>
<td>-</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>12</td>
<td>1</td>
<td>-</td>
<td>7</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
</tbody>
</table>

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 r-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, NIL-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:
HESLOT, H.: Table ronde sur l'utilisation des mutations induites en horticulture, Gembloux, 1966, Fs 6-13
JACOBS, M.: Lejeunia 22, 1-7 (1964)

We thank J.C. DELGOFPE 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 triated thymidine in Arabidopsis seeds

H. 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 triated 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, W1-2, were immersed in a solution of 3H-thymidine (3 curies/mM; Radiochemical Centre, Amersham) at a concentration of 33 \mu c/m1 during increasing times up to 48 hrs and placed in an environment of continuous light at a temperature of 24 ± 1.5°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 soaking of seeds during 25 hrs, penetration and incorporation suddenly increase and reach a maximum 1 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 Roßbeelen (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:

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

H. 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 meristems 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 triated uridine (specific activity 760 \mu c/mM; Radiochemical Centre, Amersham) in seeds immersed in a solution which contained 3H-uridine at a concentration of 20 \mu c/ml in combination with two concentrations of actinomycin, 50 and 100 \mu c/ml respectively. The results refer to the sum of radioactivity found at intervals of several hours in...
the acido-insoluble fractions obtained through a modified scheme of the procedure of SCHMIDT and THANNBAUSER (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.

References:
HAL, A., and F. GROSS: Science 130, 584-586 (1960)
BRACHET, J.: L'année biologique 1-2, 21-48 (1965)
REICH, E., and J.H. GOLDBERG: Prog. Nucl. Acid Research 1, 184-235 (1964)

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

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

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. YAMAZAWA (NIG), and T. FUJII (BNL). 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 0.9% or 1.0% agar in 9 cm Petri dishes. A pipette was found convenient, as suggested by VELEMINSKY and GIOHNER 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 attacked 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.
Several layers of Esse et...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. Then 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. Illumination for 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

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Pretreatment</th>
<th>UV dose range tested (sec)</th>
<th>Posttreatment</th>
<th>Time of measurement (hrs after UV)</th>
<th>Photoreactivation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>light</td>
<td>10 - 600</td>
<td>dark 5 hrs</td>
<td>48</td>
<td>++</td>
</tr>
<tr>
<td>2</td>
<td>light</td>
<td>30 - 60</td>
<td>dark 5 hrs</td>
<td>20</td>
<td>+++</td>
</tr>
<tr>
<td>3</td>
<td>dark 1 hr</td>
<td>9 - 150</td>
<td>light 1-60 min**</td>
<td>24, 48</td>
<td>*</td>
</tr>
<tr>
<td>4</td>
<td>dark</td>
<td>60</td>
<td>light 1-60 min**</td>
<td>48</td>
<td>+++</td>
</tr>
<tr>
<td>5</td>
<td>light</td>
<td>30</td>
<td>light 1-60 min**</td>
<td>20</td>
<td>++</td>
</tr>
<tr>
<td>6</td>
<td>light</td>
<td>30</td>
<td>light 1-60 min**</td>
<td>20</td>
<td>++</td>
</tr>
<tr>
<td>7</td>
<td>light</td>
<td>10 - 180</td>
<td>dark 5 hrs</td>
<td>46</td>
<td>***</td>
</tr>
<tr>
<td>8</td>
<td>light</td>
<td>90, 60</td>
<td>dark 1-3 hrs</td>
<td>24</td>
<td>++</td>
</tr>
</tbody>
</table>

*: 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. But the more likely reason for 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:

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

G.P. REDEI

(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.

P₂ seed of the cross ch₁ ch₂ X ch₃ ch₄ (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, ch₁ and ch₄, 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 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-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 mineral light...
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 eh 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 g1\*ch\*/g2\*pa plants

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Dose</th>
<th>Population</th>
<th>No. of sectors</th>
<th>phenotypically eh sectors</th>
<th>No. of eh estimated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>total</td>
<td>white</td>
<td>non-ch-type yellow</td>
</tr>
<tr>
<td>381</td>
<td>13,000 R</td>
<td>1317</td>
<td>18</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0.3% EMS</td>
<td>1702</td>
<td>30</td>
<td>1</td>
<td>18</td>
</tr>
<tr>
<td>386</td>
<td>13,000 R</td>
<td>1434</td>
<td>14</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>0.5% EMS</td>
<td>1056</td>
<td>185</td>
<td>39</td>
<td>120</td>
</tr>
<tr>
<td>386</td>
<td>13,000 R</td>
<td>1122</td>
<td>14</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>0.35% EMS</td>
<td>1516</td>
<td>74</td>
<td>6</td>
<td>45</td>
</tr>
<tr>
<td>394</td>
<td>13,000 R</td>
<td>1795</td>
<td>46</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>0.24% EMS</td>
<td>1327</td>
<td>56</td>
<td>1</td>
<td>33</td>
</tr>
<tr>
<td>Total</td>
<td>X-ray</td>
<td>5668</td>
<td>92</td>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>EMS</td>
<td>5401</td>
<td>1347</td>
<td>47</td>
<td>216</td>
</tr>
</tbody>
</table>
rate of meristem cells of Zea mays in 80% D2O followed by a gradual rise in the growth rate with passage of time. The studies (BHANDALKAR, 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 (BHATIA and SMITH, 1966, BHANDALKAR 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 (BHANDALKAR 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.

<table>
<thead>
<tr>
<th>Organogenetic development</th>
<th>D1(0)</th>
<th>D1(25)</th>
<th>D1(37.5)</th>
<th>D2(0)</th>
<th>D2(25)</th>
<th>D2(1, 25 + 1, 37.5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100% germination</td>
<td>4.0</td>
<td>5.0</td>
<td>7.0</td>
<td>7.0</td>
<td>7.0</td>
<td>13.0</td>
</tr>
<tr>
<td>As observed in 50% of the population</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st leaf pair</td>
<td>2.0</td>
<td>3.0</td>
<td>5.0</td>
<td>2.7</td>
<td>6.8</td>
<td>10.0</td>
</tr>
<tr>
<td>2nd leaf pair</td>
<td>4.0</td>
<td>4.0</td>
<td>6.0</td>
<td>5.5</td>
<td>9.8</td>
<td>11.0</td>
</tr>
<tr>
<td>3rd leaf pair</td>
<td>5.0</td>
<td>5.0</td>
<td>8.0</td>
<td>9.3</td>
<td>12.0</td>
<td>17.0</td>
</tr>
<tr>
<td>4th leaf pair</td>
<td>8.0</td>
<td>9.0</td>
<td>13.0</td>
<td>12.3</td>
<td>13.5</td>
<td>20.4</td>
</tr>
<tr>
<td>5th leaf pair</td>
<td>10.0</td>
<td>13.0</td>
<td>14.5</td>
<td>16.4</td>
<td>19.7</td>
<td>27.3</td>
</tr>
<tr>
<td>Floral initial</td>
<td>12.0</td>
<td>17.0</td>
<td>20.0</td>
<td>18.0</td>
<td>27.8</td>
<td>32.0</td>
</tr>
<tr>
<td>1st open flower</td>
<td>20.0</td>
<td>22.0</td>
<td>23.0</td>
<td>25.7</td>
<td>32.0</td>
<td>39.0</td>
</tr>
</tbody>
</table>

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 (BHANDALKAR and SMITH, 1967) retardment in the organogenetic development is observed when pro-ferile seeds are grown in nutrient-solution containing 60% and 37.5% D2O (column b, c, d and e). 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).

<table>
<thead>
<tr>
<th>Organogenetic development</th>
<th>b-a</th>
<th>c-a</th>
<th>e-d</th>
<th>f-d</th>
<th>c-b</th>
<th>f-e</th>
</tr>
</thead>
<tbody>
<tr>
<td>100% germination</td>
<td>1.0</td>
<td>3.0</td>
<td>0.0</td>
<td>6.0</td>
<td>2.0</td>
<td>6.0</td>
</tr>
<tr>
<td>As observed in 50% of the population</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st leaf pair</td>
<td>1.0</td>
<td>3.0</td>
<td>4.1</td>
<td>7.3</td>
<td>2.0</td>
<td>3.2</td>
</tr>
<tr>
<td>2nd leaf pair</td>
<td>3.0</td>
<td>4.0</td>
<td>4.3</td>
<td>5.5</td>
<td>2.0</td>
<td>1.2</td>
</tr>
<tr>
<td>3rd leaf pair</td>
<td>0.0</td>
<td>3.0</td>
<td>2.7</td>
<td>7.7</td>
<td>3.0</td>
<td>5.0</td>
</tr>
<tr>
<td>4th leaf pair</td>
<td>1.0</td>
<td>5.0</td>
<td>1.2</td>
<td>8.1</td>
<td>4.0</td>
<td>6.9</td>
</tr>
<tr>
<td>5th leaf pair</td>
<td>3.0</td>
<td>4.5</td>
<td>3.3</td>
<td>10.9</td>
<td>1.5</td>
<td>7.6</td>
</tr>
<tr>
<td>Floral initial</td>
<td>4.0</td>
<td>7.0</td>
<td>9.2</td>
<td>14.0</td>
<td>3.0</td>
<td>4.2</td>
</tr>
<tr>
<td>1st open flower</td>
<td>2.0</td>
<td>5.0</td>
<td>6.5</td>
<td>13.3</td>
<td>1.0</td>
<td>7.0</td>
</tr>
</tbody>
</table>

Table 2 contains the data on the extent of delay due to various D2O treatments. It can be seen from column k and l 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) (column 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 (column e and f). This is very significant yet contrary to expectation on the precise 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:

Changes in sensitivity of seeds to fast neutrons in the course of germination
R.B. CONTANT
(Institute for Atomic Sciences in Agriculture, Association Euratom - Itál, 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 f i r s t e x p e r i m e n t 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, the seeds 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, 1, 3, 10, 12 and 24 hours, respectively, after which they were immediately irradiated for periods ranging from 0 - 12 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 20°C was necessary in both cases to induce even germination, but constituted to some extent a complicating factor in the design of these trials.

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 0.5 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

<table>
<thead>
<tr>
<th>Criterium</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Period of hydration at 27°C starting from dry seed (hours)</td>
<td>Average of all incubation treatments at 27°C preceded by cold imbibition</td>
</tr>
<tr>
<td></td>
<td>Dry 1/3-1 3 6</td>
<td></td>
</tr>
<tr>
<td>20% reduction in number of ovules per silique</td>
<td>300 143 113 98</td>
<td>53</td>
</tr>
<tr>
<td>50% reduction of percentage of ovules fertilised</td>
<td>195 77 63 63</td>
<td>67</td>
</tr>
<tr>
<td>15% embryonic lethals</td>
<td>265 86 65 80</td>
<td>74</td>
</tr>
</tbody>
</table>

**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 unirradiated control and decreased with dose to reach 17% at the 82 minutes exposure. The number of siliques 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:

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Effects of gamma and fast neutron irradiation on germination of Arabidopsis thaliana
R.B. CONTANT and R. BANKERT
(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 - 53 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.

<table>
<thead>
<tr>
<th>Type of radiation and dose</th>
<th>y after 24 hrs of storage</th>
<th>b_{xy}</th>
<th>confidence limits</th>
<th>b_{xz}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>19.5 100 -0.007</td>
<td>ns*</td>
<td>+0.040 ns</td>
<td></td>
</tr>
<tr>
<td>Fast neutrons</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 krad</td>
<td>20.0 61 -0.042</td>
<td>ns</td>
<td>-0.007 ns</td>
<td></td>
</tr>
<tr>
<td>17.5 krad</td>
<td>21.1 5 -0.007</td>
<td>ns</td>
<td>-0.006 ns</td>
<td></td>
</tr>
<tr>
<td>25 krad</td>
<td>22.3 0 -0.070</td>
<td>ns</td>
<td>-0.066 ns</td>
<td></td>
</tr>
<tr>
<td>Gamma rays</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 krad</td>
<td>27.8 51 -0.161</td>
<td>-0.08 and -0.22</td>
<td>-0.12 ns</td>
<td></td>
</tr>
<tr>
<td>100 krad</td>
<td>31.0 21 -0.190</td>
<td>-0.11 and -0.23</td>
<td>-0.03 ns</td>
<td></td>
</tr>
<tr>
<td>150 krad</td>
<td>33.8 9 -0.228</td>
<td>-0.12 and -0.29</td>
<td>-0.12 ns</td>
<td></td>
</tr>
</tbody>
</table>

* 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. 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, the correlation and regression coefficient between root-length 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:

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, submers (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 each treatment (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% germina-
tion; (2) final percentage of germination; (3) percentage survival after
100 hours of germinated individuals; (4) root-length 100 hours after germina-
tion 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 ger-
mination to first flowering

<table>
<thead>
<tr>
<th>Treatment</th>
<th>(1)</th>
<th>(2)</th>
<th>(3)</th>
<th>(4)</th>
<th>(5)</th>
<th>(6)</th>
<th>(7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry control</td>
<td>23.5</td>
<td>99</td>
<td>100</td>
<td>15.6+1.7</td>
<td>16.2+2.3</td>
<td>49.0+4.1</td>
<td>35.0+3.2</td>
</tr>
<tr>
<td>FN 10 krad</td>
<td>24.7</td>
<td>98</td>
<td>61</td>
<td>3.5+0.4</td>
<td>16.6+4.6</td>
<td>36.0+9.8</td>
<td>35.6+4.7</td>
</tr>
<tr>
<td>17.5 krad</td>
<td>25.0</td>
<td>97</td>
<td>9</td>
<td>1.5+0.2</td>
<td>14.3+2.6</td>
<td>34.1+15.6</td>
<td>45.2+7.0</td>
</tr>
<tr>
<td>25 krad</td>
<td>27.0</td>
<td>98</td>
<td>0</td>
<td>1.5+0.2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>GR 50 krad</td>
<td>29.9</td>
<td>95</td>
<td>51</td>
<td>3.5+0.8</td>
<td>16.0+4.7</td>
<td>37.9+12.0</td>
<td>40.0+4.5</td>
</tr>
<tr>
<td>100 krad</td>
<td>32.9</td>
<td>97</td>
<td>21</td>
<td>2.0+0.6</td>
<td>14.6+5.6</td>
<td>31.9+15.0</td>
<td>40.0+5.8</td>
</tr>
<tr>
<td>150 krad</td>
<td>34.9</td>
<td>94</td>
<td>9</td>
<td>2.0+0.3</td>
<td>15.4+2.7</td>
<td>32.4+14.1</td>
<td>46.9+5.6</td>
</tr>
<tr>
<td>EMS control</td>
<td>16.9</td>
<td>98</td>
<td>97</td>
<td>0.6+1.5</td>
<td>15.8+2.6</td>
<td>46.5+9.4</td>
<td>37.7+3.5</td>
</tr>
<tr>
<td>EMS 0.01 N</td>
<td>21.2</td>
<td>98</td>
<td>85</td>
<td>0.6+1.7</td>
<td>14.6+2.7</td>
<td>41.5+3.7</td>
<td>36.7+3.3</td>
</tr>
</tbody>
</table>

* 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 NF10 / NF17.5 were
significant (P = 0.05); for germination capacity (2), none were significant. The rapid
generation of the EMS treatment and its control was due to prior imbibition for 24
hours; this fact that this advance in germination was only
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 signifi-
cant; the EMS treatment was least affected. The survival curve for neutrons had a
steep 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 FN17.5 / 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
approximately to 2 3 mm and the
unability 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 FM treatment than after GR; this again supports the existence of
more pronounced physiological effects after GR.

A high correlation (r = 0.97) existed between survival and rootlength, which was
also justified by the correspondence between rootlengths less than 2 - 3 mm and the
unability 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 FM 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

<table>
<thead>
<tr>
<th>Rootlength category (mm)</th>
<th>1 - 2.5</th>
<th>2.5 - 3.5</th>
<th>3.5 - 4.5</th>
<th>4.5 - 6.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>percentage survival</td>
<td>0</td>
<td>32</td>
<td>68</td>
<td>72</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>&lt; roots &gt; 2 mm</th>
<th>% survival</th>
<th>% roots &gt; 3 mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry control</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>FN 10 krad</td>
<td>75</td>
<td>61</td>
<td>40</td>
</tr>
<tr>
<td>17.5 krad</td>
<td>10</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>25 krad</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>GR 50 krad</td>
<td>64</td>
<td>61</td>
<td>45</td>
</tr>
<tr>
<td>100 krad</td>
<td>40</td>
<td>47</td>
<td>37</td>
</tr>
<tr>
<td>150 krad</td>
<td>19</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>EMS control</td>
<td>100</td>
<td>97</td>
<td>100</td>
</tr>
<tr>
<td>EMS 0.01 N</td>
<td>100</td>
<td>85</td>
<td>100</td>
</tr>
</tbody>
</table>

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/FN17.5;
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 (7), all treatments differed significantly from their control; the
difference between the two controls was insignificant. The other significant differences
were FN10 / GR100 or GR150; FN17.5 / 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 2.6 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, 1966).

References:
MÜLLER, A.J.: Kulturpflanze 12, 235-236 (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 Buraton - 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 families 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 petriplates 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. LÆRMAN, 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 specificity in mutation induction, most of these mutations may be due to more or less unspecified deletions or other chromosomal disturbances, instead of gene mutations. Their genetics will be further studied.

Table 1: Mutant type | Code number | Mean leaf number | Mean leaf length (mm) | Mean number of days to flowering
(a) 37 | 24 | 31 | 56
35 A | 23 | 26 | 54
(e) 44 | 26 | 24 | 58
109 | 24 | 24 | 58
155 | 27 | 26 | 58

specification in mutation induction, most of these mutations may be due to more or less unspecified 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 segregates 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>
<thead>
<tr>
<th>Mutant code number</th>
<th>Main feature(s)</th>
<th>Percentage occurrence in M2</th>
<th>Number of leaves (a)</th>
<th>Leaf length (a)</th>
<th>Number of days to flowering (b)</th>
<th>Fertility relative to control Li-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>yellow green</td>
<td>0.20</td>
<td>31 30 35</td>
<td>52 50 54</td>
<td>44 45 46</td>
<td>normal</td>
</tr>
<tr>
<td>12</td>
<td>compact, distorted</td>
<td>0.05</td>
<td>30 29 30</td>
<td>33 43 8</td>
<td>54 48 39</td>
<td>15%</td>
</tr>
<tr>
<td>21</td>
<td>darkgreen, anthocyanin</td>
<td>0.25</td>
<td>31 31 16</td>
<td>45 42 14</td>
<td>44 49 45</td>
<td>-90%</td>
</tr>
<tr>
<td>53</td>
<td>glossy, compact</td>
<td>0.21</td>
<td>30 29 30</td>
<td>42 45 41</td>
<td>46 49 41</td>
<td>normal</td>
</tr>
<tr>
<td>59</td>
<td>dark, small, narrow leaves</td>
<td>0.03</td>
<td>31 31 22</td>
<td>42 45 30</td>
<td>52 50 36</td>
<td>50%</td>
</tr>
<tr>
<td>56</td>
<td>small, very early</td>
<td>0.17</td>
<td>30 29 17</td>
<td>48 47 18</td>
<td>47 42 23</td>
<td>15%</td>
</tr>
<tr>
<td>84</td>
<td>small, very early</td>
<td>0.03</td>
<td>31 31 15</td>
<td>40 39 22</td>
<td>50 50 22</td>
<td>20%</td>
</tr>
</tbody>
</table>

* (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, (X 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 length, these phenomena will be studied. The search for similar mutants with full fertility will be continued.

Number of days to 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 significant earliness and 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 = -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 root on the other hand. As expected, there was only a slight and statistically insignificant correlation between plant height and number of days to flowering (b = 0.34), the correlation was too weak to allow selection for flowering date on the basis of the root-length measurements; furthermore, the control showed a similar regression (b = 0.39).

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 idiotype. This illustrates that seed irradiation may induce significant chimerism for various quantitative characters.

References:
- : Proc. 3rd Int. Congress Radiation Research, Cortina d'Ampezzo, Abstract No. 226, 58 (1966 b)
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 KNACK, 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 Ca(NO₃)₂, 200 ppm MgSO₄.7H₂O, 100 ppm KNO₃ and 100 ppm H₃B₃O₄. 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 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.

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

R. BANKERT and N.B. CONTANT

(Institute for Atomic Sciences in Agriculture, Association Buraton - Itai, Wageningen, Netherlands)

Dry Arabidopsis seeds were treated with a range of doses of gamma-rays and fast neutrons, and with EMS. The 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 siliques. 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 of male 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 1% 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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total number of plants</th>
<th>Number and percentage of male sterile plants</th>
<th>Number of entirely fertile families</th>
<th>Number of F1 families segregating for slight fertility or (sterility)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>170</td>
<td>0</td>
<td>0 %</td>
<td>-</td>
</tr>
<tr>
<td>Fast neutrons</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 krad*</td>
<td>174</td>
<td>43</td>
<td>24 %</td>
<td>41</td>
</tr>
<tr>
<td>17.5 krad*</td>
<td>21</td>
<td>9</td>
<td>42 %</td>
<td>9</td>
</tr>
<tr>
<td>Gamma rays</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 krad</td>
<td>115</td>
<td>25</td>
<td>21 %</td>
<td>24</td>
</tr>
<tr>
<td>100 krad</td>
<td>24</td>
<td>9</td>
<td>37 %</td>
<td>7</td>
</tr>
<tr>
<td>150 krad</td>
<td>14</td>
<td>5</td>
<td>35 %</td>
<td>5</td>
</tr>
<tr>
<td>EMS 0.01 N</td>
<td>156</td>
<td>6</td>
<td>4 %</td>
<td>6</td>
</tr>
</tbody>
</table>

* with 5% j-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 (F2) 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 F2 families segregated 5 fertile : 1 fully sterile; these may indicate wide deviations 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 F1 families will be
tested for segregation of infertility, to allow a distinction between genetic male
sterility and physiological radiation effects in the F1; in the first case, the off-
spring of 50% of the plants in each F1 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 N1 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. LIKAN

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

F1 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
hemiizygous, and the presence of the dominant gene, Ch, produces full green color, so
that the F1 heterozygote has normal green leaves. Loss of the Ch allele (deletion)
or change in its function (mutation) in heterozygotes gives a yellowish green pheno-
type in leaf cells in which the phenomenon occurs and in cell lineages of the altered
genotypes. The frequency of F1 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 He4, Li7, O12, O16, Ne20 and Ar40-ions. Although
final results with somatic mutations are not yet available it is clear that in com-
parison with X-rays the lowest RBE's are close to 10 and that those for He4, Li7,
O12 and O16 are higher than for Ne20 and Ar40-ions.

In separate experiments the effects of these same heavy ionizing particles on plant
growth are being investigated, using both F1 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 con-
sistent with those obtained from the mutation experiments described above in that the
RBE's show the same general relationships.

Reference:

HIRONO, Y., and G.I. REDLIN: Genetics 51, 519-526 (1965)

Research carried out at Brookhaven National Laboratory under the auspices of the
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**Killing efficiency of neutrons in Arabidopsis seeds**

Taro FUJII

(National Institute of Genetics, Hisama, 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 x 1012 in minimum and 7.1 x 1013 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 (D,n) reaction neutron generator in our institute. Its total
neutron flux was 3.8 x 1011-1.8 x 1012 with the average intensity of 109 neutrons/sec;
here the gamma-ray contamination was less than 10 %. Thus the neutron effect is ex-
pected 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 7.5 x 1012 neutrons on dry seeds and
similar survival rates were observed after 35.6 x 1012 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
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.

<table>
<thead>
<tr>
<th>Gamma-rays*</th>
<th>Total neutron * Flux*</th>
<th>Fast neutrons * Flux**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dosage (kR)</td>
<td>Total neutron (x10^12)</td>
<td>Total neutron (x10^11)</td>
</tr>
<tr>
<td>Dry seeds</td>
<td>Wet seeds</td>
<td>Dry seeds</td>
</tr>
<tr>
<td>10</td>
<td>91.6</td>
<td>92.7</td>
</tr>
<tr>
<td>30</td>
<td>50.8</td>
<td>75.6</td>
</tr>
<tr>
<td>50</td>
<td>92.8</td>
<td>21.7</td>
</tr>
<tr>
<td>70</td>
<td>63.3</td>
<td>0.0</td>
</tr>
<tr>
<td>100</td>
<td>82.4</td>
<td>0.0</td>
</tr>
</tbody>
</table>

* 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. 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.

Preliminary data on effects of various ionizing radiations on Arabidopsis

V.I. IVANOV, A.V. SANINA, and R.A. TIMOFIEFF-RESOVSKY
(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 2°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 (1), 22°C) that the frequency of both, embryonic lethals and chlorophyll mutants increases exponentially (n = 1), like a two-hit curve does. His percentage for recessive lethals of all types (E⁻ 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", we
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. The in turn, due to the soil used, the genetic damage goes down to virtually 0 (for 0 mM), in contrast to all our experiments which gave a minimum of 1-2%. MILLER stated (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) also studied an EMS-dose range (16 hrs, 24°C, 6 hrs post-washing), found a dose-response curve for lethals of all types which is clearly exponential through the range.

A final point regards the non-recessive embryonic lethals (N.E.L.) found after pollinating with control (C) pollen. In a previous report (Van der VEEN, 1967b) the percentage of embryonic lethals after 10 mM (24 hrs, 24°C) were 1.4% for the Control, 1.7% for CxEMS, 7.4% (1) for EMSx0 and 19.0% for EKS (natural selfing). The different hypotheses proposed for this reciprocal difference have now been tested. From this M1-material 120 CxEMS-fruits and 120 EMSx0-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 embry-test was again applied on fruits No. 5 and 6. The array of genotypes is expected to be the same for CxEMS and EMSx0, except for those mutations which are subject to different selection in male versus female gametes. Indeed, after natural selfing CxEMS and EMSx0 segregated 7.5 and 5.1% chlorophyll mutants and 11.2 and 9.1% embryonic lethals respectively. Significant is that (CxEms)xEMSx0 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 N.E.L-result (excl. C-pollen) occur in experiments of MILLER with MNU and other nitrosamides. This author argued in favour of reversion of premutational lesions during dry-storage, rather than inactivation of residual EMS or its reaction products. - Our EMS-results are in agreement with MILLEs 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 +2°C (S+24) or at -2°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 item 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 ovules, and the mutant frequencies are given as % of seeds containing lethal embryos (E.L.) and % of chlorophyll mutants among non-lethal embryos (Chl.; MILLER's embry-test). For each item the silicagel No. 5 and 6 were scored on 35 M1-plants.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Survival</th>
<th>Sterility</th>
<th>Lethal frequency</th>
<th>Chlorophyll mutants</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-,-)</td>
<td>95.5%</td>
<td>0.16%</td>
<td>2.3%</td>
<td>2.3%</td>
</tr>
<tr>
<td>(W,-)</td>
<td>95.5%</td>
<td>0.16%</td>
<td>2.3%</td>
<td>2.3%</td>
</tr>
<tr>
<td>(W,S+24)</td>
<td>95.5%</td>
<td>0.16%</td>
<td>2.3%</td>
<td>2.3%</td>
</tr>
<tr>
<td>(W,S-20)</td>
<td>95.5%</td>
<td>0.16%</td>
<td>2.3%</td>
<td>2.3%</td>
</tr>
<tr>
<td>(W,S+24/1)</td>
<td>95.5%</td>
<td>0.16%</td>
<td>2.3%</td>
<td>2.3%</td>
</tr>
</tbody>
</table>

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 MILLER (1966), who sowed in soil. - (2) No water-storage prior to dry-storage (-,S+24) gives an immediate increase in genetic damage, as compared with (-,-). In MILLER (1967) 1 hr water-storage ites, 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, MILLER found no increase. -

References:
MILLER, A.J.: alchter 16, 201-220 (1966)
- - Arabid.Inf.Serv. 4, 65 (1967 a)
- - Arabid.Inf.Serv. 4, 45 (1967 b)
(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 Post- Survival Sterility Mutants (-, 5+24/1) to (W, 5+24/14) has treatments E/L Ch1. relative proportions which can be precisely mimicked by a range of decreasing EMS-concentrations. - (5) Dry-storage at -20°C (W, S-20/1), 84 61 34 21 characterized by 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:
- : 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. FOKORNÝ, and T. ŠIČNER
(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 ČERL (1965) and pers. commun. 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 $\bar{x}$ $s_{\bar{x}}$ $\bar{y}$ $s_{\bar{y}}$ $r$

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

$r$ = coefficient of correlation

Table 2: Concentration of MNU Conditions Number of plants $r$

<table>
<thead>
<tr>
<th>Concentration of MNU</th>
<th>Conditions</th>
<th>Number of plants</th>
<th>$r$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2 mM</td>
<td>growth chamber</td>
<td>148</td>
<td>-0.148</td>
</tr>
<tr>
<td>0.3 mM</td>
<td>growth chamber</td>
<td>160</td>
<td>0.024</td>
</tr>
<tr>
<td>0.2 mM</td>
<td>greenhouse</td>
<td>129</td>
<td>-0.048</td>
</tr>
<tr>
<td>0.3 mM</td>
<td>greenhouse</td>
<td>132</td>
<td>0.005</td>
</tr>
<tr>
<td>0.2 mM</td>
<td>growth chamber</td>
<td>116</td>
<td>-0.041</td>
</tr>
<tr>
<td>0.3 mM</td>
<td>&quot;</td>
<td>105</td>
<td>-0.066</td>
</tr>
<tr>
<td>0.2 mM</td>
<td>greenhouse</td>
<td>121</td>
<td>-0.021</td>
</tr>
<tr>
<td>0.3 mM</td>
<td>&quot;</td>
<td>123</td>
<td>0.069</td>
</tr>
</tbody>
</table>

$r$ = 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°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) 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) or number of days to the appearance of flower primordia (x:m) (Table 2).

References:
CETL, I.: Arabid.Inf.Serv. 2, 3-4 (1965)

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-nitrosourea (ENU) 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 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 ENN 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 unhydrolyzed nitrosoamines is taken up in the seeds during the 24 hrs treatment. This can perhaps explain, why ENN 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:

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 = mb (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 hydrolyze, 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 nitrosoamines with a methyl group.

Table: Nitrosoamines

<table>
<thead>
<tr>
<th>Nitrosoamines</th>
<th>sublethal conc.</th>
<th>max. mb</th>
<th>Nitrosoamines</th>
<th>sublethal conc.</th>
<th>max. mb</th>
</tr>
</thead>
<tbody>
<tr>
<td>dimethyl-</td>
<td>1.000 M</td>
<td>75.2</td>
<td>ethylvinyl-</td>
<td>0.100 M</td>
<td>73.0</td>
</tr>
<tr>
<td>methylethyl-</td>
<td>0.100 M</td>
<td>59.5</td>
<td>ethyl-terc.butyl-</td>
<td>0.125 M</td>
<td>13.1</td>
</tr>
<tr>
<td>methylbutyl-</td>
<td>0.075 M</td>
<td>18.6</td>
<td>dibutyl-</td>
<td>0.050 M</td>
<td>1.5</td>
</tr>
<tr>
<td>methyl-terc.butyl-</td>
<td>0.100 M</td>
<td>0.2</td>
<td>methylphenyl-</td>
<td>0.020 M</td>
<td>2.6</td>
</tr>
<tr>
<td>methylbenzyl-</td>
<td>0.020 M</td>
<td>61.6</td>
<td>methylpiperazine</td>
<td>1.000 M</td>
<td>0.5</td>
</tr>
<tr>
<td>diethyl-</td>
<td>0.225 M</td>
<td>1.2</td>
<td>nitrosomorpholine</td>
<td>1.000 M</td>
<td>0.6</td>
</tr>
</tbody>
</table>

References:
The mutagenic activity of \( \beta \)-hydroxyethyl methansulfonate (HOEMS), \( \beta \)-methoxyethyl methansulfonate (MOEMS) and diethyl 1,-propanedisulfonate (DEPD) on Arabidopsis

T. GICHNER, L. EHHENBERG, 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'[tris(phenylmethylnitrosourea (TMNH) and p-tolylsulfonyl methylnitrosamide (TSMN)

J. VELEMINSKY 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 on Saccharomyces cerevisiae (MARQUARDT et al., 1964); it was, however, strongly carcinogenic on rats (IVANKOVIC et al., 1965). TSMN was not mutagenic on Saccharomyces cerevisiae (MARQUARDT et al., 1964) and not carcinogenic on rats (DRUCKREY et al., 1961).

References:

MARQUARDT, H., F.X. ZIELINSKY, and R. SCHMIDT: Z.Vererbungsl. 65, 82-96 (1964)

Genic male sterility in Arabidopsis

A.J. MÜLLER

(Institut für Kulturpflanzenforschung, Gatersleben, Mrs. 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

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. of pollen grains per anther</th>
<th>Total No. of anthers studied</th>
</tr>
</thead>
<tbody>
<tr>
<td>ms ms</td>
<td>0-10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>11-30</td>
<td></td>
</tr>
<tr>
<td></td>
<td>31-70</td>
<td></td>
</tr>
<tr>
<td></td>
<td>71-150</td>
<td></td>
</tr>
<tr>
<td></td>
<td>151-400</td>
<td></td>
</tr>
<tr>
<td></td>
<td>401-460</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. of normal pollen grains per anther</th>
<th>Total No. of anthers studied</th>
</tr>
</thead>
<tbody>
<tr>
<td>ms ms</td>
<td>0-10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>11-30</td>
<td></td>
</tr>
<tr>
<td></td>
<td>31-70</td>
<td></td>
</tr>
<tr>
<td></td>
<td>71-150</td>
<td></td>
</tr>
<tr>
<td></td>
<td>151-400</td>
<td></td>
</tr>
<tr>
<td></td>
<td>401-460</td>
<td></td>
</tr>
</tbody>
</table>

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 146 (control: 43±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 825 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
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 4 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 HEIDEMEYER

(Institut für Kulturpflanzenforschung, Gatersleben, Kre. Aschaffenburg, Germany)


<table>
<thead>
<tr>
<th>Mutagen</th>
<th>Anzahl der Mutationen</th>
<th>fusca</th>
<th>Chlorophyll</th>
<th>fusca : Chlorophyll</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-Nitroso-N-methyl-N'-nitroguanidin</td>
<td>17</td>
<td>599</td>
<td>1 : 35,3</td>
<td></td>
</tr>
<tr>
<td>N-Nitroso-N-methylurethan</td>
<td>8</td>
<td>267</td>
<td>1 : 39,9</td>
<td></td>
</tr>
<tr>
<td>N-Nitroso-N-methylmethanesulphonat</td>
<td>23</td>
<td>459</td>
<td>1 : 46,7</td>
<td></td>
</tr>
<tr>
<td>iso-Propylmethanesulphonat</td>
<td>14</td>
<td>425</td>
<td>1 : 30,4</td>
<td></td>
</tr>
<tr>
<td>total</td>
<td>68</td>
<td>2585</td>
<td>1 : 38,0</td>
<td></td>
</tr>
</tbody>
</table>

in der unter Zusammensetzung der Ergebnisse verschiedener Versuchsreihen (mit einzelnen unterschiedlichen Mutationsfrequenzen) die Anzahl der recessiven fusca-Mutationen der Anzahl der gleichzeitig induzierten recessiven Chlorophyllmutationen gegenübergestellt ist (Chlorophyllmutanten = alba, xantha und chlorina, bestimmt nach der Farbe der Kotyledonen vor Beginn der Samenreife).


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

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

Summary: 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:
- Züchter 34, 102-120 (1964)
- Züchter 38, 201-220 (1966)

Chromosome studies in Arabidopsis thaliana
Lotti M. STEINITZ-SEARS and Suzanne LEE-CHEN
(Plant 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 re-
ported earlier (CHEN and SEARS 1967) that II carries REDÉI'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), photomicrographes of diplotene nuclei (b and f),
drawings of the same diplotene cells (c and g), and diagrammatic interpre-
tation of the same cells (d and h).

Reference:

* 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 sensibilisation 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

<table>
<thead>
<tr>
<th>Arabidopsis thaliana</th>
<th>ch^+ = En-2</th>
<th>ch^1</th>
<th>ch^2</th>
<th>ch^3 = V 81</th>
</tr>
</thead>
<tbody>
<tr>
<td>substrate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>loam</td>
<td>8 p.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>peat</td>
<td>2 p.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sand</td>
<td>1 p.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>light</td>
<td>16 h</td>
<td>25 ± 2 °C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>dark</td>
<td>8 h</td>
<td>15 ± 2 °C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>temperature</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>humidity</td>
<td>16 h</td>
<td>50 ± 5 %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>light</td>
<td>16 h</td>
<td>50 ± 5 %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>dark</td>
<td>8 h</td>
<td>50 ± 5 %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>radiation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>normal</td>
<td>12 x TIP 65/55</td>
<td>&lt;0.1</td>
<td>4.35</td>
<td>4.00</td>
</tr>
<tr>
<td>yellow</td>
<td>12 x WU 65/25</td>
<td>&lt;0.1</td>
<td>0.43</td>
<td>4.10</td>
</tr>
<tr>
<td>red</td>
<td>12 x TIP 65/55</td>
<td>&lt;0.1</td>
<td>0.25</td>
<td>0.01</td>
</tr>
<tr>
<td>normal</td>
<td>RH-plexiglass 301</td>
<td>&lt;0.1</td>
<td>0.25</td>
<td>0.01</td>
</tr>
<tr>
<td>yellow</td>
<td>RH-plexiglass 501</td>
<td>&lt;0.1</td>
<td>0.25</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Table 2: Spectral radiation energy (watt/cm^2) distribution in the plant level ('66-10, 13./14.3.67).

<table>
<thead>
<tr>
<th>Wavelength (millimicrons)</th>
<th>342</th>
<th>437</th>
<th>575</th>
<th>642</th>
<th>742</th>
</tr>
</thead>
<tbody>
<tr>
<td>interference filter no.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Genotype (mutant)</th>
<th>Radiation quality (filter)</th>
<th>Ontogenetic stages (samples)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ch^+ &lt; ch^2 &lt; ch^1 &lt; ch^3</td>
<td>(red) &gt; yellow &gt; normal</td>
<td>1 &lt; 2 &lt; 3 &lt; 4</td>
</tr>
<tr>
<td>Cal pl.</td>
<td>ch^1 = ch^2 &gt; ch^1 &gt; ch^3</td>
<td>(red) &gt; normal &gt; yellow</td>
</tr>
<tr>
<td>Cal/dm^2</td>
<td>ch^1 = ch^2 &gt; ch^1 &gt; ch^3</td>
<td>(red) &gt; yellow &gt; normal</td>
</tr>
<tr>
<td>Cal/g</td>
<td>ch^1 = ch^2 &gt; ch^1 &gt; ch^3</td>
<td>(red) &gt; yellow &lt; normal</td>
</tr>
</tbody>
</table>

Pigment value:

<table>
<thead>
<tr>
<th>Chl a/dm^2</th>
<th>ch^+ &gt; ch^2 &gt; ch^1</th>
<th>(red) &lt; yellow &lt; normal</th>
<th>1 &lt; 2 &lt; 3 &lt; 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chl b/dm^2</td>
<td>ch^+ &gt; ch^2 &gt; ch^1</td>
<td>(red) = (yellow) &lt; normal</td>
<td>1 &lt; 2 &lt; 3 &lt; 4</td>
</tr>
<tr>
<td>Carot./dm^2</td>
<td>ch^+ &gt; ch^2 &gt; ch^1</td>
<td>(red) &lt; yellow &lt; normal</td>
<td>1 &lt; 2 &lt; 3 &lt; 4</td>
</tr>
</tbody>
</table>
deficient mutants of Chlorella have evidentially the ability of normal photosynthesis (METZNER et al., 1965; WILL and EGLE, 1967), although there is a deficiency of starch production at least in some cases of Pituin (KÜLLER, 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 ROBBELEN 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 KÖHR, 1965; RUDOLPH, 1965).

Here the gained results shall be presented briefly in a range comparison statistically tested by SIEARMAN’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. 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: \( \text{ch}^b > \text{ch}^a > \text{ch}^3 > \text{ch}^4 \) (ROBBELEN 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 \( \text{ch}^b > \text{ch}^1 > \text{ch}^a > \text{ch}^4 \). The other characters deviate from this order.

In accordance to HIROKO and REDEI (1965) we have detected an equal amount of assimilation starch in the leaf parenchym chloroplasts of the mutant \( \text{ch}^1 \) 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 (KHANZ, 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-SIEARS, 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 b content 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.

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This work has been supported by a grant from Deutsche Forschungsgemeinschaft.
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 beet plant. The storage trays are commercially-made holders for “Densol” 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 in the bottom, 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 (1965) 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:
Van Der VEEN, J.E.: Arabid.Inf.Serv. 2, 31 (1965)

A simple technique for screening thiamine auxotrophs

S.L. LI and G.F. REDDIE

(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 been introduced. Instead of planting 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 0.245 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 500-400 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 (54.5%) require only the pyrimidine moiety; 9 (14.5%) respond to “vitamin thiamine”; 13 (21%) grow only with intact thiamine.

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REDDIE, G.F.: Genetics 45, 1007 (1965)
- : Genetics 47, 979 (1962)
- : Amer.J.Bot. 52, 834 (1965)
Herstellung von Totalpräparaten zur Darstellung von Embryonen

C.G. ARNOLD und D. CRUSE


Embryo test without seed loss

G.H. BAIKEMA

(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 14-12 days, whereas brown (almost ripe) seed takes 15-16 days. Cold treatment (5 days, 30°C, dark) after sowing retards 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 REDDI (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 naphtalene 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 utilization of PFS, 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 contaminating 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 0.5 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 μμ measured in the Cary spectrophotometer.
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 Elastocladilla emersonii, is advantageous with respect to NDS because it does not absorb at 260 nm. The RNA of the whole cells and of cellular organelles (chloroplasts and mitochondria) is now being studied.

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We thank Mrs. Eliane BONNIENS-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".
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Naturwiss. 52, 623 (1965)


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- : Die Induktion von reessiven Letalmutationen durch Äthylethansulfonat bei Arabidopsis. II. Sensibilitätsänderungen in quellenden und keimenden Sämen. Z. Naturforch. 26b, 216-229 (1971)

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D. ANNOUNCEMENTS

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 dis-
cussion of Arabidopsis Research. Dr. Y.MIZOGU will be kind enough to act as an or-
ganizer. For details, please, pay attention to the announcement at the boards of the

Change of Addresses

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Trombay, Bombay 74, India

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

Material

X-ray induced single gene recessive "miniature" (mit) mutant from RÉDEI's very 
early race Landeberg-"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 : read A12/A5 instead of A5/A12