Kranz

ARABIDOPSIS INFORMATION SERVICE

In collaboration with A. Corcos, East Lansing
W. J. Feenstra, Groningen
G. P. Rédei, Columbia
G. Röbbelen, Göttingen
Arranged by A. R. Kranz, Frankfurt/Main

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A R A B I D O P S I S

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

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Acknowledgement

In presenting this first issue at Frankfurt/Main it is a pleasure to acknowledge my gratitude to Professor G. RÖBBELEN, the former editor of AIS, for his generous support in handing over the edition of this newsletter and the LAIBACH-collection of seed populations to me. The authors of the back volumes would like to express their thanks to G.RÖBBELEN who has initiated with enthusiasm this international forum of Arabidopsis research ten years ago and excellently organized this form of cooperation round the world for one decade.

A.R. Kranz

B. Techniques

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I would like to express my gratitude to my wife for her assistance in reading the manuscripts and also to Mrs. Brigitte KIRCHHEIM for the editing and typing of this volume of AIS.

A.R. Kranz

A. BRIEF NOTES

Complications with an "arginine mutant"

A. CORCOS

(Kedzie Laboratory, Department of Natural Science,
Michigan State University, East Lansing, Michigan, USA)

received January 1974

In the last issue of A I S (CORCOS 1973), I reported the isolation of an arginine mutant. This report was premature, since further work soon demonstrated that it was a "leaky" mutant whose growth was repressed by glucose. In other words, it grows normally on glucose-free media, but as the concentration of glucose is increased, its growth is decreased (Table 1). Other sugars were tested. Sucrose enhanced in a similar manner the growth of both the normal and the mutant strain (Table 2). Maltose and cellobiose, on other hand, seemingly depressed the growth of the mutant.

Table 1. Repression by glucose of the "arginine" mutant 241 after 4 weeks. Dry weight of the arginine mutant (15 plants) in presence of dextrose after 4 weeks at different sugar concentrations.

Sugar Concentration	Dry Weight in mgs
1. No dextrose	41.5
2. 1g/liter	26.5
3. 5g/liter	22.3
4. 10g/liter	9.0
5. 15g/liter	9.0
6. 20g/liter	4.4

Table 2. Dry weight in mgs of 5 normal and 5 mutant plants after 4 weeks on media without sugar and with sugars.

Media	Normal	Mutant 241
1. No sugar	21.5	22.1 (102.8)*
2. Maltose	21.1	17.3 (81.9)
3. Cellobiose	19.0	16.0 (84.2)
4. Glucose	22.6	11.3 (50.0)
5. Sucrose	34.1	34.5 (101.7)

* These numbers in parentheses represent dry weight of mutant 241 compared to the normal plant in p.c.

Only a few examples of glucose-effect are known in higher plants. In *Arabidopsis thaliana*, LI and RÊDEI (1968) have reported that glucose and maltose are inhibitors of a set of mutants in the thiamine pathway. The point that it is the mutant, not the normal plant, which is inhibited by glucose should be stressed, since it might lead to a genetic understanding of the glucose-effect.

Because of the nature of this mutant and its importance in research, as attested by the demand for seeds and tissue, we are attempting to get a non-leaky type as well as a haploid strain.

References:
CORCOS, A.: Arabidopsis Inf.Serv.10, 32 (1973)
LI, S.L. and G.P. RÊDEI: Plant Physiol. 44, 225-229 (1968)

Gene analysis of seed germination induced by phytochrome(P_{fr}) and/or gibberellic acid(GA_3) in Arabidopsis thaliana.

A.R.KRANZ

(Section Biology, Botany, University Frankfurt/Main, GFR)

received January 1974

The inheritance of seed germination has been studied only in a few cases and it is impossible to conclude how many and what kinds of genes are involved (BORRIS and GÜNTHER 1967). From the early work of KUGLER (1951) we know that the seed germination of certain Arabidopsis populations is light dependent and may be controlled by a major recessive factor, but maternal effects may also occur. Recent results of SHROPSHIRE and coworkers (SHROPSHIRE et al.1961, 1971) and our group (KRANZ et al. 1971, 1972) have shown that the phytochrome mechanism is involved in this process and that the promoting effect of the activated phytochrome (P_{fr}) can be substituted by gibberellic acid (GA_3). In those studies it was most important that homo- and heteroallelic ch mutants of Arabidopsis differed significantly; but at this time the sole responsibility of the ch genes for these reactions was questionable.

The wildtype and two heteroallelic genotypes (ch^1ch_+/ch^1ch_+, ch^3ch_3/ch^+ch_3) grown under greenhouse conditions have been crossed. The seeds (F_3) of the F_2 plants, which were classified later for their genotype by the leaf colour of the F_3 plants, were tested in treatments with H_2O and 10^{-3}M GA_3 solution in the dark (D) or 5 minutes with far-red (FR) plus 5 minutes red (R) radiation. The interfering effect of paper filters (REHWALDT 1967) has been eliminated by membrane filters (Sartorius-Membranfilter SM 11306). The temperature was 25± 1°C and the germinated seeds were evaluated after 12 days.

Table 1 gives the data obtained, the mean frequency and its standard deviation of the germinated seeds in the parents and the selected F_3 lines of the diallel crosses. Altogether the columns show that the known promoting effect of FR and/or GA_3 is confirmed for each genotype studied. In the D- and H_2O-treatment the non-segregating F_3 lines (parental types) equal to the parents concerned, but in the other treatments some deviations occur especially when the second gene (ch.)is involved. The seed germination of lines derived from the heterozygous F_2 plants resembles the wildtype generally. Further in the first treatment (D, H_2O) the values for the chlorophyll b defect of the F_2 mother plant and the germination of its F_3 seeds show a significantly negative correlation (SPEARMAN's range correlation coefficient $\varrho = -.839$ with $\alpha >> .99$).

Table 1: Mean (p.c.) and standard deviation of the number of seeds germinated in the parents and F_3-generation of the diallel cross.

material	F_2-generation		treatment			
			D		5'FR + 5'R	
parents rsp. crosses	phenotype	genotype	H_2O	10^{-3}M GA_3	H_2O	10^{-3}M GA_3
P ♂	green	ch^+ch_+/ch^+ch_+	0.0±0.00	93.1±10.52	74.0± 1.00	88.6± 6.35
P ♀	light-green	ch^1ch_+/ch^1ch_+	3.1±3.15	100.0± 0.00	100.0± 0.00	93.0± 2.00
	yellow	ch^+ch_3/ch^+ch_3	0.0±0.00	92.5± 3.50	8.8 ± 4.60	100.0± 0.00
ch^1ch_+/ch^1ch_+	green	ch^+ch_+/ch^+ch_+	0.4±0.83	97.3± 4.62	93.6± 5.47	95.7± 4.63
x	green	ch^+ch_+/ch^1ch_+	6.9±7.72	100.0± 0.00	88.3±12.25	89.9±10.38
ch^+ch_+/ch^+ch_+	light-green	ch^1ch_+/ch^1ch_+	28.7±3.30	90.5± 0.50	79.0± 6.00	89.2± 4.90
ch^+ch_3/ch^+ch_3	green	ch^+ch_+/ch^+ch_+	0.8±0.83	93.9± 6.69	27.5± 7.26	97.9± 2.61
x	green	ch^+ch_+/ch^+ch_3	0.4±0.95	92.7± 4.23	20.4± 8.84	96.7± 3.11
ch^+ch_+/ch^+ch_+	yellow	ch^+ch_3/ch^+ch_3	0.0±0.00	55.0±14.00	5.0± 5.00	60.5±0.50
ch^1ch_+/ch^1ch_+ x ch^+ch_3/ch^+ch_3	green	ch^+ch_+/ch^+ch_+	7.9±3.07	92.5± 0.45	80.0± 7.82	100.0± 0.00
	green	ch^+ch_+/ch^+ch_3	7.3±5.20	84.6 °/.	62.4± 0.60	99.0± 1.00
	green	ch^+ch_+/ch^1ch_3	3.4±2.20	93.2± 3.71	45.4±15.19	95.3± 2.04
	light-green	ch^1ch_+/ch^1ch_3	13.1±7.67	97.9± 2.90	51.6±11.47	98.2± 3.03
	light-green	ch^1ch_+/ch^1ch_+	0.0±0.00	28.6 °/.	93.7 °/.	38.7± 4.55
	yellow	ch^+ch_3/ch^+ch_3 ch^+ch_3/ch^1ch_3	3.4±3.16	87.5±11.02	82.5±13.81	88.5±13.02

From this result it can be concluded that the genes concerned control both characters, the chlorophyll (Cb defect) and the seed germination (P_{fr} and/or GA_3 reaction), but that the genetic and physiological interactions of the two genes are complex. RÖBBELEN (1972) has shown that in crosses involving the mutant V 81 (=$\underline{ch^+ch_3}/\underline{ch^+ch_3}$) the digenic interactions were complex too and the ratios of segregation are modified by deficiencies of the recessives; further misclassification could not be ruled out This was also true for our experiments and it may be one reason of the deviations mentioned above. Another reason may be that the sensitivity of light germinating in seeds for photoinduction by red energy has been changed differently in the genotype during storage (SHROPSHIRE et al.1970).

In summary the data of this study have yielded that each ch gene controls the P_{fr} and/or GA_3 dependent seed germination, but in the recessive state both genes reveal deviation of partial complementation depending on the photoinduction of the seeds.

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RÖBBELEN, G.: Arabidopsis Inf.Serv.9, 21-25 (1972)
SHROPSHIRE, W., Jr., KLEIN, W.H.and ELSTAD, V.B.: Plant and Cell Physiol.2, 63-69 (1961)

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Is Hylandra an amphidiploid of Arabidopsis and Cardaminopsis arenosa?

G.P. RÉDEI

(Department of Agronomy, University of Missouri, Columbia, Mo.,USA)

received February 1974

HYLANDER (1957) suggested first that Arabidopsis suecica (FR.)NORRL. is an amphiploid of Cardaminopsis arenosa (L.)HAYEK and Arabidopsis thaliana (L.)HEYNH. Subsequently LÖVE (1961) considered the hypothesis so plausible that he renamed the plant as Hylandra suecica (FR.)LÖVE. MĚSÍČEK (1967) questioned the validity of this hypothesis concerning the evolution of Hylandra because his attempt to hybridize C. arenosa with Arabidopsis failed just as an earlier attempt of LAIBACH (1958) was unsuccessful. MĚSÍČEK, however, succeeded in producing a steril hybrid of C.petraea (n=16) x Arabidopsis. In our laboratory we produced viable seed by crossing C.arenosa (2n=32) with Arabidopsis (2n=20). The majority of the seed developed into crippled seedling with distorted narrow leaves, a few however appeared as vigorous plants resembling more C.arenosa than Hylandra. Actually none of the traits characteristic for Hylandra could be revealed in the F_1. The fertility in F_1 was fairly good. The F_2 generation segregated into a few different types, including some with narrow leaves. The C.arenosa x Arabidopsis hybrid could be easily crossed with Hylandra. This F_1 also displayed fairly good fertility and it was uniform and intermediate type without any clear Hylandra traits. The F_2 of the triple hybrid segregated in manner similar to the C.arenosa x Arabidopsis F_2. The Hylandra x (C.arenosa x Arabidopsis) F_1 was morphologically different from the Hylandra x C.arenosa or Hylandra x Arabidopsis (2n=10) hybrids. C.petraea was successfully crossed with a dominant of Arabidopsis. The only vigorous survivor was steril (2n=13) and expressed the dominant yellow-green trait of the Arabidopsis male parent. The evidence at hand does not lend much support to the HYLANDER-LÖVE hypothesis concerning the evolution of Hylandra. Thus the origin of Hylandra requires more penetrating experimental studies before firm conclusions can be drawn.

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HYLANDER, N.: Bull.Jard.Bot.Bruxelles 27, 253 (1957)
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Defective regulation of the de novo pyrimidine pathway in the im^1 mutant.

G.P. RÉDEI and S.C. CHUNG

(Department of Agronomy, University of Missouri, Columbia, Mo.,USA)

received February 1974

We have reported earlier (CHUNG and RÉDEI 1973) that the level of orotidylic acid decarboxylase (4. 1. 1. 23) level is elevated in the im^1 mutant. Subsequent experiments showed an increased activity also of the preceeding enzyme, orotidylic acid pyrophosphorylase (2.4.2.10).

Table 1. Activities of three enzymes on protein basis.

Genotype	Medium	Decarboxylase	Pyrophosphorylase	Phosphatase
wild	basal	20.8	14.1	21.1
	azauracil	53.4	6.9	20.9
im^1	basal	42.7	21.2	21.4
	azauracil	76.5	8.5	21.1

Though azauracil increased the level of decarboxylase even further, the activity of pyrophosphorylase was substantially reduced on the analog medium. Acid phosphatase was used as a control enzyme. This reduction created an effective bottleneck in the pyrimidine pathway. A slight reduction in total RNA was detectable in the mutant compared to the wild type control. In 6-azauracil-grown plants there was a higher pyrimidine: purine ratio in the RNA. Though 6-azauracil-C^{14} was incorporated into the RNA in a small yet detectable amount, the major consequences of azauracil feeding could be found in the inhibition and suppression of the pyrimidine pathway. 6-azauracil-C^{14} was readily converted into 6-azauridylic acid in the plant. In the plants grown on azauracil media, orotic acid-C^{14} was converted to nucleic acids with an efficiency reduced to 1/7-1/8 of that found in its absence. Plants grown without azauracil readily metabolized orotic acid-C^{14} into orotidine-5-monophosphate and uridine-5-monophosphate. While those plants which were raised on azauracil media, accumulated in their cell the orotic acid and synthesised 1/5-1/7 amounts of orotidine-5-phosphate and barely detectable amounts of uridine-5-phosphate. These analytical findings are consistant with observed activities of the decarboxylase and pyrophosphorylase. Though the synthesis of decarboxylase increased on azauracil media, the activity of this enzyme was inhibited. The synthesis of pyrophosphorylase was reduced on azauracil media while its activity was apparently not affected.

Hereditary orotic aciduria in man is caused by a defective regulation of the same two enzymes. Curiously, there are several similarities between the consequences of a regulatory mutation in the two different organisms (CHUNG and RÉDEI 1974).

References:

CHUNG, S.C.and G.P. REDEI: Arabidopsis Inf.Serv.10, 6-7 (1973)
- - : Biochem.Genet. (in press)

The role of light in photoperiodic induction.

G.P. RÉDEI and G.ACEDO

(Department of Agronomy, University of Missouri, Columbia, Mo., USA)

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Arabidopsis is a facultative long-day plant. It does not have a critical day-length yet under continuous illuminations flower differentiation proceeds much faster than under natural daily light periods (LAIBACH 1951). Several induced mutants show differences in their photoperiodic response (RÉDEI 1962) and for some 5-bromodeoxyuridine, an antimetabolite, may be substituted for long light cycles to accelerate flowering (BROWN 1972, HIRONO and RÉDEI 1966).

On soil or on agar medium Arabidopsis does not grow in total darkness even if a carbohydrate source is provided aseptically through the roots. In submerged culture (RÉDEI 1972) using 5-10 ml medium per seedling, in the presence of 0.1-2% glucose or sucrose, prolonged growth can be maintained in the total absence of light. The seed is imbibed in the dark for 12-18 hrs, then exposed to day light for 1 hr, fol-

lowed by total darkness for the rest of the experiment, under such conditions the plants develop flower buds, flowers and fruits sometimes with macroscopically well visible seed initials. The time required for flowering in the dark is somewhat longer than in continuous light. If flower initiation is measured, however, by the number of leaves formed before the differentiation of flower primordia, very little difference can be seen between flowering in continuous light and dark culture. In total darkness thus flowering takes place early, while under short days flower formation is much delayed whether measured by time or number of vegetative leaves. In darkness the wildtype and the late flowering mutants flower with little difference. Under 8 hours daily light regimes, two of the mutants fail to flower in 120 or more days while they form flower buds within a month in the dark.

On the basis of these experiments, the conclusion appears obvious that in the long-day plant Arabidopsis, light is not required for flowering and shorter daily periods of illumination can effectively prevent this process of differentiation.

References:
BROWN, J.A.M.: Amer.J.Bot.59, 228 (1972)
HIRONO, Y.and G.P.RÉDEI: Planta 71, 107 (1966)
LAIBACH, F.: Beitr.Biol.Pfl.28, 173 (1951)

Effect of 6-azauracil feeding on plastid differentiation in the im^1 mutant,

G.P. RÉDEI and S.B. PURAD

(Department of Agronomy, University of Missouri, Columbia, Mo., USA)

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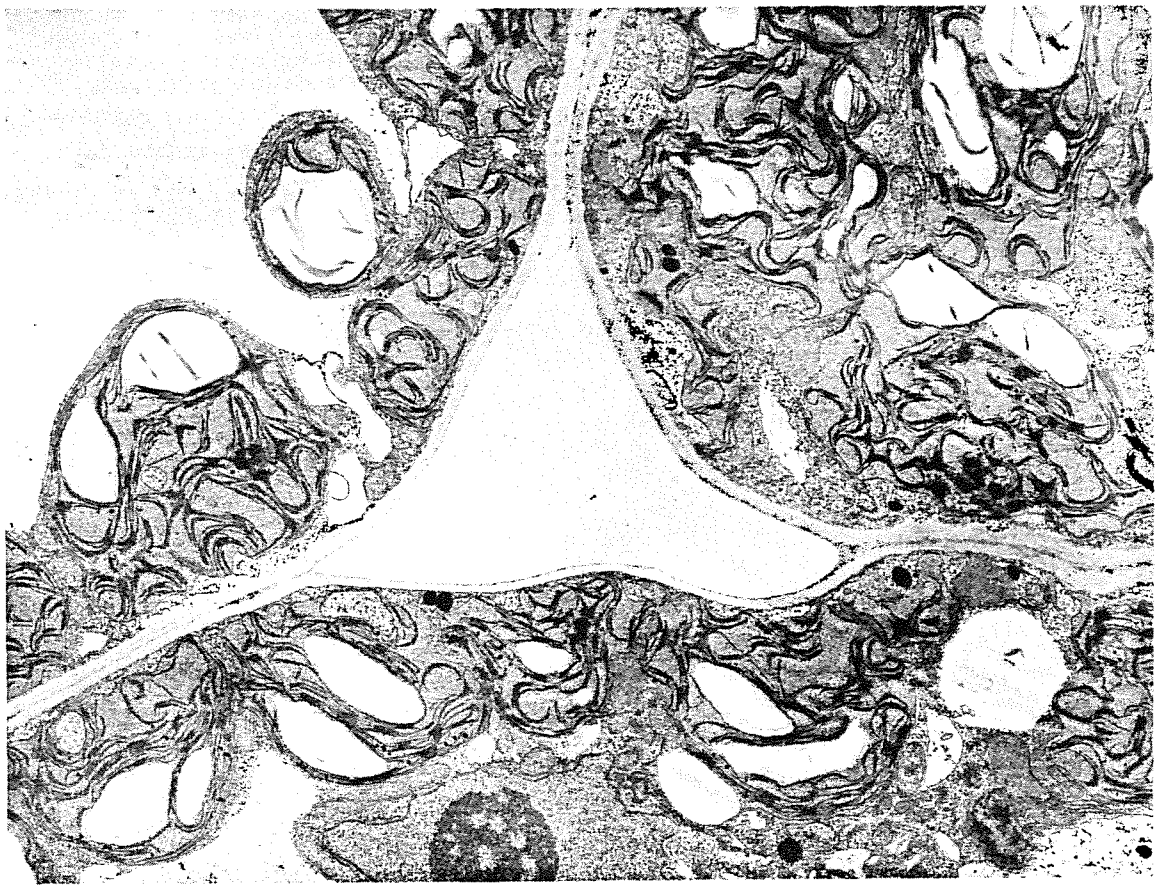
The im mutant fail to differentiate chloroplasts in the majority of their cells under high light intensity continuous illumination (RÉDEI 1965 1967, RÖBBELEN 1968). Chloroplast differentiation significantly improves, when measured by pigment production, if the plants are grown in $1\text{-}2\times10^{-5}$M 6-azauracil media.

Electronmicroscopic study of the cells revealed that at lower concentrations of the analog a more or less normal chloroplast differentiation is restored, while at high concentrations the lamellae in the plastids display an unusual curved shape. The size of these plastids is often larger than normal. As the large number of good size starch granules indicate, these chloroplasts are capable of converting sugar into starch and can perform other functions apparently well (Figure 1).

Figure 1: Palisade cells of the im mutant grown in vitro on 1.6×10^{-5}M 6-azauracil medium



This effect of 6-azauracil is conspicuous in the mutant but it is much reduced in the wild type.

References:
RÉDEI, G.P.: Arabidopsis Res. Pp.119-127 (1965)
- : J.Heredity 58, 229 (1967)
RÖBBELEN, G.: Planta 80, 237 (1968)

Nitrate reduction in Arabidopsis thaliana

Fietje J. OOSTINDIER-BRAAKSMA and W.J. FEENSTRA

(Institute of Genetics, University of Groningen, Haren-Gn., The Netherlands)

received March 1974

The second nitrate reductase-less mutant mentioned in our previous report (OOSTINDIER-BRAAKSMA and FEENSTRA 1973 a), which we will denote by its isolation number B25 until more definite data about its localization are obtained, appears to be different from the chl-2/chl-2 type isolated earlier (VAN DER LAAN et al.1971; OOSTINDIER-BRAAKSMA and FEENSTRA 1973 b). Whereas the chl-2 type exhibits some nitrate reductase activity when grown on a medium with nitrate, though at a lower level than the wildtype, B25 has no enzyme activity at all; decrease in growth on a medium with nitrate as the only nitrogen source and chlorate resistance of B25 are more extrem as compared to chl-2.

In complementation tests B25 and the chlorate-resistant mutants of the chl-1 type and the chl-2 type B25 showed complementation with both other mutants indicating that the mutation in B25 probably effects a new gene.

Preliminary linkage studies showed that the mutated gene in B25 is probably linked with chl-2, though not so closely as might be expected with two genes in operon. Linkage experiments with marker lines are presently also being pursued.

Young B25 plants appear to grow rather poorly when cultivated under continuous illumination of about 10.000 lux (condition used previously for the isolation of mutants). Much better growth is obtained when the mutant is grown till shooting under lowered light intensity and a daily light period of 16 hours.

When the conditions for growing selected chlorate-resistant M2 plants to maturity were changed accordingly, several more mutants showing a lowered level of nitrate reductase activity were isolated. Presently experiments are being carried out to establish whether still other genes are involved besides those found previously.

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LAAN, P.H. van der, Fietje J. OOSTINDIER-BRAAKSMA, and W.J. FEENSTRA: Arabidopsis Inf.Serv.8, 22 (1971)
OOSTINDIER-BRAAKSMA, Fietje J. and W.J. FEENSTRA: Arabidopsis Inf.Serv.10, 33 (1973a)
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Laser irradiation-induced damages in the seeds of Arabidopsis thaliana (L.)HEYNH.

O.H. YULDASHEV, L.B. RUBIN, P.D. USMANOV

(Institute of Plant Physiology and Biophysics, Acad.Sci.of the Tajik SSR, Moscow University, U S S R)

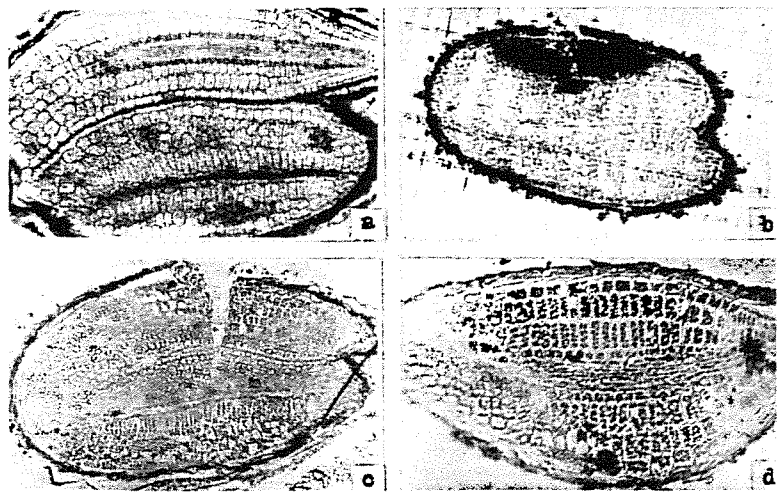
A technique has been elaborated for obtaining microtome sections (glass knife) of Arabidopsis thaliana seeds.

$C_6H_{14}O_2$ was used for the polymerization; as the initiator served the DDSA, 20 ml per 10 ml of butylmetacrylate. The polymerization took place at 60°C in the course of 72 hours. The seeds were fixed by 1% OsO_4 in the phosphate buffer. The fixation by osmium with the following dyeing by methyl green provided a distinct picture of the zone (Fig.1b) and the nature (Fig.1c) of damages in the seeds as a result of the laser irradiation (λ_2= 6943 Å, $\tau = 10^{-8}$ sec.). The density of the irradiation varied between 3 and 7 J/cm^2. Fig.1 represents the sections of the control and the experimental material.

The experiment has shown that osmium incorporated inside the seeds due to the destruction of the envelope and a part of inner components caused by the laser irradiation. In addition, the dark area depended upon the dose of the irradiation. Fig.1c shows the section dyed by methyl green. We observe the break of the envelope, the coagulated aleurone grains, representing some store protein, and the disconnection between the cells. On this basis it was concluded that the detonation wave and temperature might be possible factors of laser irradiation on the seeds.

As a result of the irradiation we observed empty cells in the seed sections. The nature of these empty cells is not clear, and further electron-microscopic study would give probably the explanation of this phenomenon. It is also noteworthy that empty cells as well as the break of contact between the cells and the coagulated aleurone grains occur too in the sections where the seed peel (testa) has not been damaged. This fact indicates, that apart from the detonation wave and temperature, the damage effect is also related to the wave length of the laser irradiation.

Figure 1: Microtome sections of A.thaliana seeds irradiated by a ruby laser (x 560)



a - control
b - Area of damage revealed by osmium
c - Break of the seed peel, the coagulated aleurone grains, empty cells revealed by methyl green
d - The damage takes place with the seed peel safe

References:
O.H. YULDASHEV, A.S. IVANOV, P.D. USMANOV, L.B. RUBIN: The effects of the capacity and density of the ruby laser irradiation on the seeds of Arabidopsis thaliana (L.) HEYNH.

The combined effects of the laser- and x-ray-irradiation on the seeds of Arabidopsis thaliana (L.) HEYNH.

O.H. YULDASHEV and P.D. USMANOV

(Institute of Plant Physiology and Biophysics, Acad.Sci. of the Tajik SSR, Dushanbe, U S S R)

received March 1974

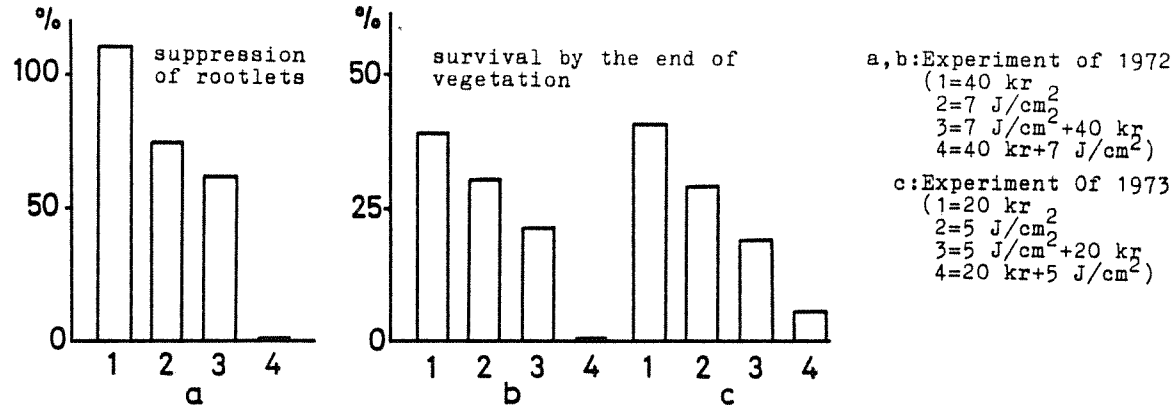
Two series of experiments have been performed on the combined effects of the ruby laser (λ = 6943 Å, $\tau = 10^{-8}$ sec.) and x-ray-irradiation.
Variants of the experiments:

1. X-ray-irradiation, the dose 40 kr, 20 kr.
2. Ruby laser, density of the irradiation P=7 J/cm^2, 5 J/cm^2
3. Laser + x-ray-irradiation
4. X-ray-irradiation + laser

In the two series of irradiation the capacities of the x-ray-irradiation were different. The following parameters have been estimated: The survival by the end of the vegetation; depression of the first rootlets on the 8th day of growing in agar medium.

As shown in Figure 1, no additivity has been observed in the variants"x-ray + laser" and "laser + x". The effect of the depression of all the parameters in the variant "x-ray + laser" was higher, than in "laser + x-ray" variant under the same doses of x-ray-irradiation and density of the laser irradiation.

Figure 1: The survival and suppression of growth of the first rootlets in per cent to control after the combined x-ray-ruby-laser-irradiation of A.thaliana seeds.



This fact might probably be explained by investigating the interactions between the laser irradiation and free radicals formed by the x-ray-irradiation.

The authors are indebted to Dr.L.B. RUBIN for the cooperation in irradiation of the seed material.

References:

O.H. YULDASHEV, A.S. IVANOV, P.D. USMANOV, L.B. RUBIN. The effects of the capacity and density of the ruby laser irradiation on the seeds of Arabidopsis thaliana (L.) HEYNH.

The induction and the cytological analysis of fertile plants of lethal mutants of Arabidopsis thaliana (L.) HEYNH.

P.D. USMANOV, H. ABDULLAEV, V.G. TULAKIN

(Institute of Plant Physiology and Biophysics, Acad.Sci.of the Tajik SSR, Dushanbe, U S S R)

received March 1974

Mutant embryos (Fig.1A) have been isolated from pods in five heterozygote strains of A.thaliana which segregate in the lethal chlorophyll mutations of the albina (9-al) and xantha(8-xy,79 P 10/43 xa, 127-xa) type. Their further cultivation on the specially composed artificial media demonstrated great biomass of the increasing, proliferating callus (Fig.1B), and from the callus the organogenesis has been induced followed by the formation of normal pods (Fig.1C-H)

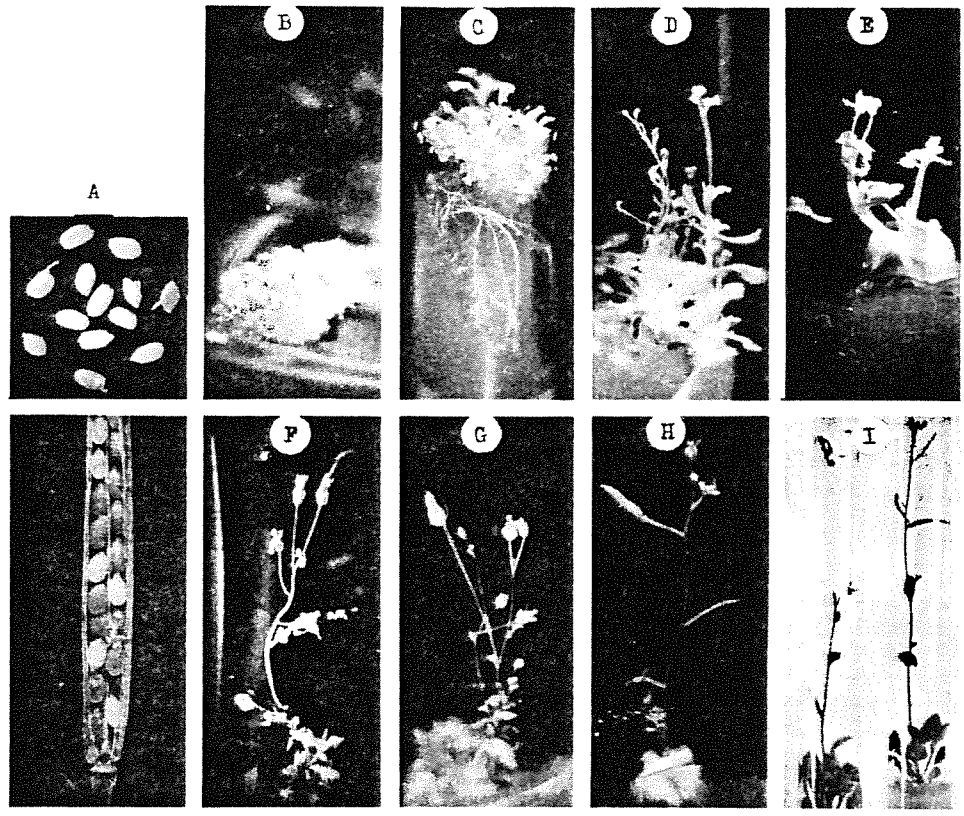


Figure 1: Embryos and plant regenerants of the lethal mutants.
A. The opened pod and its embryos
B. The increasing tissue culture of the germinating embryos on the 15thday
C. Organogenesis in the tissue culture of lethal mutants
D-E. The formation of the stem buds and shoots in the mutants 9-al and 8-xa in tissue culture
F,G,H. Flowering and fruiting of plant regenerants, obtained in the tissue culture of lethal mutants P 10/43-xa, 9-al and 79-xa
I. Plants of the wild race

This data being somewhat tempting, it is worth noting some details of cultivation of the mutant material. Rapid accumulation of the callus mass (for 8-10 days) has been observed on the medium containing: vitamins (according to JACOBS), casein 0.01%, glucose 2%, 2-4D 1mg/l, kinetin 1.5mg/l. The plant regenerants have been obtained on the media: a)vitamins (JACOBS), succhrose 2%, casein 0.01%, kinetin 1mg/l, IPA 0.03 mg/l; b) succhrose 2%, adenine (JACOBS) IAA 2mg/l, kinetin 2mg/l, casein 0.01%, DPN 4mg/l, NADP 1mg/l, thiamine 50mg/l, D-biotin, i-inositol, thiamine chloride, folic acid (according to JACOBS), alanine, DL-serine, L-valine, L-tryptophan, aspartic acid, glutaminic acid (JACOBS).

Differences have been observed in the colour of leaves, i.e.in the range from white and light-yellow to pale green, and the rate of development (from 30 to 45-50 days).

Electron-microscopic analysis revealed that growth and development of the lethal mutants occured exclusively without the activities of the photosynthetic apparatus, and the energy processes, probably, were exercised mostly by mitochondria. This is indicative by the increasing number of mitochondria obtained in the ultra-thin sections:

Table 1: Electron-microscopic analysis of the number of mitochondria in lethal mutants.

strains	number of plastids (M±m)	number of mitochondria
Enkheim	8 ± 0.14	9 ± 0.13
79 - xa	3 ± 0.23	15 ± 0.20
127 - xa	3 ± 0.10	16 ± 0.17

These results may be of interest in solving different biological problems.

Somatic and genetic effects of N-nitrosemethylbiuret in plants of Arabidopsis thaliana

P.D. USMANOV, Sh. SAHIBNAZAROV

(Institute of Plant Physiology and Biophysics, Acad.Sci.of the Tajik SSR, Dushanbe, U S S R)

received March 1974

It has been recently reported about the high efficiency of the new chemical mutagen N-nitrosomethylbiuret (NMB,I)inducing mutations in insects, fungi and microorganisms. At our request Dr.I.A. RAPPOPORT has kindly given us the opportunity to investigate the effects of NMB in plants.

Air-dried seeds of the race Enkheim of Arabidopsis thaliana were soaked in solutions of the mutagen of different concentrations 16,32,63,125,250,500,1000 and 2000 µM during 18 hours at t = +20±0.5°C (pH=5.9). The action of the mutagen was terminated by washing the seeds in tap water. The plants were grown in the soil in boxes of the mountain station Sieh-Kuh. In M_1 we estimated the somatic and in the embryo stage of the seed development the genetic effects by many parameters (Table 1).

Table 1: The effects of N-nitrosomethylbiuret on the frequency of recessive embryonic mutations in A. thaliana.

Concentration µM	0	63	125	250	500	1000
Number of M_2-embryo studied	26900	18404	19662	16085	16298	2467
Lethal mutations m_c (p.c.) *	0.34	0.65	1.00	3.83	17.89	53.17
Chlorophyll mutations, m_c (p.c.)**	0.00	0.11	0.16	0.69	3.35	10.85

* excluding the chlorophyll mutants

** excluding the lethal mutants

At the concentration 2000 µM the plants did not mature.

In order to evaluate the mutagen efficiency of NMB it has been compared to the effects of N-nitrosomethylbiuret (NMU).

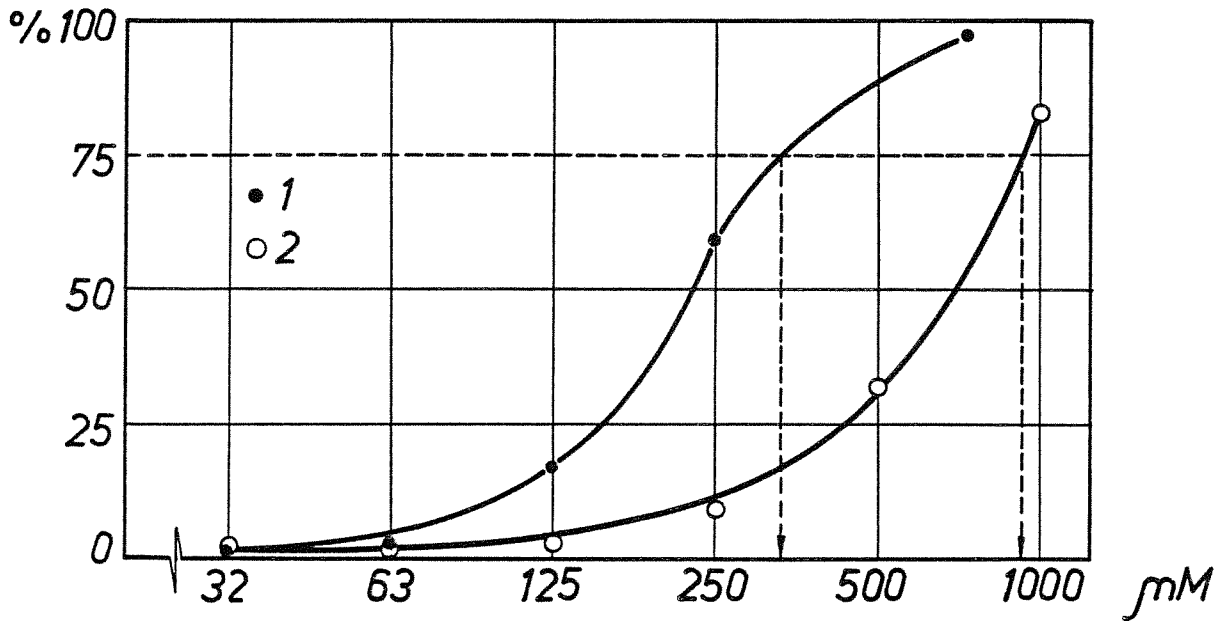
In figure 1 curves are shown indicating the differences in the rate of sterility in M_1-plants after treating the seeds by NMU and NMB (similar experimental conditions). The point of curvature at 75 p.c.-rate of sterility is corresponding to 350 µM NMU concentration, and for NMB approximately 1000 µM. For these different concentrations (but similar in inducing the sterility)the values have been found by the graphs.

They characterize the mutability and the frequency of plants with chemomorphosis in p.c.:

Mutagen	m^c	m'^c	Chemomorphosis
NMB	53	11.0	6
NMU	14	1.2	14

Such a comparison quite clearly shows the priority of NMB in the induction of mutations. NMB induces less of the sterile plants (Figure 1) and plants with chemomorphosis and this indicates its lower toxicity than NMU.

Figure 1: The rate of sterility of M_1 plants at different concentrations of NMU (1) and NMB (2). The rate of sterility has been estimated according to MÜLLER (1964)



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On the chimerism in generative tissue of Arabidopsis after x-ray-irradiation of seeds.

L.I. GRINIKH, V.V. SHEVCHENKO, G.A. GRIGORIEVA, L.Y. DRAGINSKAYA

(Institute of Developmental Biology, Moscow, U S S R)

received March 1974

The problem of chimerism in generative tissue in A.thaliana is of much importance (MÜLLER 1965, BALKEMA 1970, HARLE 1972 and others). In this paper the pattern of the occurence of the mutated tissue in different inflorescences has been analysed using MÜLLER's embryo test.

X_1-plants were grown from x-ray-irradiated seeds (50 kr) in soil under continuous illumination at 24±2°C. Since chimerism occured already within the range of the first to the sixth pods, the inflorescences with more than six pods were taken. Plants having normal main inflorescences were removed.

In 19 out of 61 x_1-plants (=31.2±5.03 p.c.)there were mutations. They were separated into two groups: i) with chimeric, ii) with non-chimeric main inflorescences of apical shoot. The average mutated sector size of the first group of plants was constant in the main, lateral order I and order II inflorescences: 62.6, 63.2 and 60.2p.c. resp. The rate of the chimeric inflorescences decreased with increasing of their order: 100.0, 40.0 and 19.6p.c. resp., i.e.the separation of mutated and non-mutated tissues between lateral inflorescences occured without any advantage of the tissues. The second group of plants were also non-chimeric in lateral order I and order II inflorescences.(Table 1).

Fasciated main inflorescences have been analysed in a different way: viable mutations in x_2 and lethal mutations in x_3 were scored among the progeny of every pod. The same mutation segregated frequently in both parts of the inflorescence, i.e. they had a common initial cell(s).

Evidence has been obtained that additional shoots (1.3 per plant in our experiment) originated from the same initial as the apical shoot.

Table 1: The mutated sector size (number of pods) in generative tissue of apical shoots with chimeric main inflorescences.

Plant No.	Inflorescences													
	main		lateral I order			lateral II order								
			number from the shoot base			number of inflorescences from the shoot base								
						1		2		3		4		
	normal	mutant		normal	mutant	normal	mutant	normal	mutant	normal	mutant	normal	mutant	
1	8	7	1 2 3	7 0 8	6 12 0	10 0 7	0 15 4							
2	9	11	1 2 3 4 5 6	0 12 6 0 15 6	11 1 0 11 1 0	0 6 3	8 3 4	0 1	6 5					
3	3	13	1 2	0 1	9 7	0 0	8 12	0	6					
4	4	23	1 2 3	13 0 1	2 25 9	8 0 15	0 6 0	0	6	0	15	0	16	
5	6	11	1 2 3 4	4 2 4 2	4 8 3 6									
6	9	10	1 2 3	0 11 0	12 3 25	7 0	0 10	10	0					
7	12	15	1 2 3 4	1 0 28 0	8 21 0 20	0 8 0	8 0 7	0 10 0	8 0 10	0 6 0	11 0 8	8	0	
8	10	9	1 2 3	0 13 13	6 2 0	8 15	0 0	10 5	0 2					
9	7	8	1 2 3	0 0 6	30 18 22	0 5 4	14 2 6	0 0 4	9 10 5	0 2 0	11 4 6			
10	3	12	1 2 3 4	0 12 0 15	11 0 15 0	6 0 10	0 10 0	7 0	0 14	0	13			
Σ total	71	119	35	180	309	lateral inflorescences: 51 pods: 190 normal, 287 mutant								
	62.6±3.56			63.2±2.17		60.2±2.00								
	average mutated sector size in p.c.													

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Quantitative studies on heterosis in Arabidopsis thaliana (L.) HEYNH.

Miss Hanane EL ASMI

(Génétique-Biométrie, Faculté des Sciences Campus Universitaire, Tunis)

received April 1974

Studies on heterosis had been realised with Arabidopsis thaliana for many traits as earliness of flowering (VAN DER VEEN, J.H. 1965) or heterosis expression under effects of different temperature levels (LANGRIDGE, J. 1962)or in various conditions of environment (GRIFFING, B.and ZSIRES, E.1971) and many others. In relation with quantitative point of view we have realised an analysis of the rosette diameter length at different stages of the growth.

Five inbred lines of Arabidopsis thaliana had been chosen; A_5 (Dijon, France), A_9 (Eastland Baltique), A_{64} (Edinburgh, Scotland), A_{51} (Bologna, Italia), A_{80} (Turin, Italia). Observations of flowering time of these lines show that A_{51} and A_{80} are the latest. The experiment had been designed as incomplete diallel crosses, disribed by GRIFFING, B.(1956)method 2, including the 5 foregoing lines taken as parents and their 10 direct combinations. Reciprocal crosses are not used here.

F_1-generation of all these crosses and parents are arranged according to a sixteen complete and randomised blocks design (finally 16 repetitions for each parent or F_1), disposed under a light varying from 6000 to 8000 lux, 18 hours a day. Plants are grown in "giffy-pots" (6 cm of diameter) with a mixture of vegetable mould and sand.

Many characteristics had been examined and will be presented elsewhere. This paper presents only the results of analysis made on the rosette diameter length at an early stage, 19 days after sowing, exposed in table 1.

Table 1: Rosette diameter length in cm (mean value 19 days after sowing, parents are diagonal)

♀ \ ♂	A_5	A_9	A_{51}	A_{64}	A_{80}
A_5	1.96	2.47	2.99	2.94	2.43
A_9		2.88	3.51	3.02	3.70
A_{51}			2.39	3.40	3.44
A_{64}				2.45	3.44
A_{80}					2.38

Data of table 1 show a real superiority for a large number of the hybrids in relation to their parents. These differences had been analysed by using the quantity of heterosis expressed according to FALCONER, D.S. (1960)which is as follow:

If $\frac{1}{2}(m_1 + m_2)$ = mean value of the parents P_1 and P_2

and m_{F_1} = mean value of the F_1 between P_1 and P_2,

the quantity of heterosis expressed on phenotype is measured on the rosette diameter by the difference:

$$H_{F_1} = m_{F_1} - \frac{1}{2}(m_1 + m_2)$$

The significance of this difference had been examined with a STUDENT t-test at a 5 p.c.-level of probability.

Table 2 summarizes the quantities H_{F_1} obtained with this material disposed according to an increasing arrangement as well as the gain of the hybrid (in per cent relatively to the mean parent), and the results of the t-test at a 5 p.c.-level of significance.

Hybrids	A_5xA_9	A_5xA_{80}	A_9xA_{64}	A_5xA_{64}	$A_{51}xA_{80}$	A_5xA_{51}	A_9xA_{51}	$A_{51}xA_{64}$	$A_{64}xA_{80}$	A_9xA_{80}
H_{F_1}	0.04	0.26	0.36	0.73	0.80	0.82	0.88	0.98	1.04	1.16
p.c.	1.53	11.80	13.37	33.12	33.67	37.40	33.32	40.38	43.22	44.30
t	N S	N S	S	S	T S	H S	H S	H S	H S	H S

Table 2: Analysis of the quantity of heterosis (N S = not significant S = significant at 5 p.c., T S = at 1 p.c., H S = at o.1 p.c.)

According to these results we may conclude that there is an evidence for phenomena in rosette diameter length, whose expression is effective at the early stages of the growth, and magnitude is varying in relation to the parents crossed. Further observations on this material have indicated a persistance of this effects on the rosette diameter length until the end of its growth, (about 50 days after sowing).

Hypothesis of genetic causes of the quantitative differences observed seems to be plausible. In respect to this hypothesis the analysis of the data by the diallel cross method (GRIFFING, B.1956) indicates clearness of high specific combining ability. On the other hand some other characteristic such as the spike length present the same kind of result.

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Electrophoretic variation in invertase of Arabidopsis thaliana

E.P. MAHER

(Department of Genetics, University of Aberdeen, Aberdeen, Scotland, G.B.)

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Polyacrylamide gel electrophoresis of invertase has been developed for use in studies of the biochemical genetics of invertase production in Arabidopsis. Both disc gels and vertical slab, gradient gels have been used successfully and have produced essentially similar banding patterns. Only the latter method will be described here since it gives more consistent results and allows direct comparisons of up to fourteen different samples on one gel.

A Uniscil four cell electrophoresis apparatus (Universal Scientif Ltd.) was used with Gradipore continuous concentration gradient polyacrylamide gels (4 to 26%). The box buffer was Tris-glycine, pH 8.3, externally cooled to 4°C; gels were pre-run for 45 minutes before samples were applied. Upwards of 100 mg of frozen rosette leaves or roots were ground in cold 0.2 M Tris-HCl buffer, pH 8.5, containing 0.1% w/vß - mercaptoethanol and 10% glycerol at 100u l per 100 mg tissue. The supernatant was applied to the gel (25 to 45u l aliquots) and a constant voltage of 200 V applied for 18 to 20 hours.

After electrophoresis, gels were incubated in Mc ILVAINE citrate-phosphate buffer, pH 5.4, for 30 minutes, followed by 45 minutes in o.2 M sucrose in the same buffer at 30°C. After washing the gels were stained by the method of GABRIEL and WANG (1969) for aldose sugar reaction products, using 2, 3, 5-triphenyltetrazolium chloride which produces red bands.

The method was established using rosette leaves of race Est and two bands of activity were consistently located at positions corresponding to gel concentrations of approximately 11% and 20% respectively. A similar two-band pattern was also found after disc gel electrophoresis, but the faster moving band sometimes barely discernible suggesting that it is more labile than the slower band.

Traditionally, invertase has not been recognised as an amenable enzyme for electrophoretic study and therefore few comparisons with other sources of the enzyme are available. However, BERGGREN (1970) has analysed purified invertase from brewer's yeast on starch gels. He found a broad, slowly-moving band with a number of faster inactive bands. It is possible that the slower band from both yeast and Arabidopsis represents the intact, high molecular weight, carbohydrate-containing form of the enzyme while the faster, more labile bands are low molecular weight derivatives and not necessarily different protein species.

The enzymes from roots and leaves of several races been analysed in this system and some variation in mobility has been discovered. The results are summarised below and in Figure 1.

1) Leaves: The four strains tested so far Est, Ei, En, and St, have identical first band mobilities. The second band of Est may be fractionally slower than the second bands of the other strains, but any difference is very small and requires further analysis.

2) Roots: Two distinct first bands, both faster than the first leaf band have been found amongst the six strains tested. The faster of these two (R 2) is produced by roots of Est, Dj and La (erecta mutant) while the slower band(R 1) is found in roots of St, Ei and En. Again there may be slight differences between R 1 band strains which require further study.

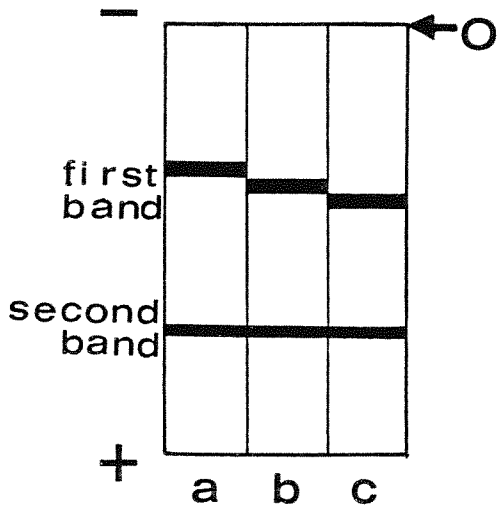

Figure 1: Diagram of invertase electrophoretic variants in leaf and root extracts of Arabidopsis thaliana.

a. leaves
b. roots - R 1 strains
c. roots - R 2 strains

It is now necessary to extend this analysis to other strains, to discover if this variation is genetic in origin and to establish if the variation is related to the physiological role of the enzyme.

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Thanks are due to Miss Carol INNES for technical assistance and Dr.G.E. HOBSON for advice on invertase electrophoresis.

An in vivo assay for invertase

Ms E.S. D'SOUZA and E.P. MAHER
(Department of Genetics, University of Aberdeen, Aberdeen, Scotland, G.B.)

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As described elsewhere in this issue, we have devised a screening procedure to detect apparent invertase-less mutants of Arabidopsis. In order to confirm the lack of hydrolytic activity in the isolated seedlings and to attempt to distinguish heterozygotes within individual M_2 families, we have also developed an in vivo technique for assaying invertase activity. The results we report here are of an experiment to determine the time course of the assay and the reproducibility of the technique.

Wild type seeds of race Est were germinated aseptically on 0.75% agar containing 0.1% KNO_3 at an angle of 60°, 10.000 lux, 25°C. 5 day old seedlings were measured and incubated under sterile conditions in 1 ml 2% sucrose (Aristar) for varying periods of time at 25°C. Controls included seedlings incubated in water only and substrate incubated without seedlings. At the end of each incubation period the reducing sugar content of the bathing solution was determined by the method of NELSON (1944).

Figure 1 shows the time courses obtained for single and double seedling incubations. Each point is the mean of 5 replicates. The results were subjected to orthogonal polynomial analysis which showed a highly significant linear function in both cases ($P<.001$)and negligible cubic and quadratic functions. Lines of best fit were obtained by regression analysis, the regression coefficient for the single seedling incubations being 5.48 (S.E. = 0.419, t = 13.1***, d.f.= 23) and 12.06 (S.E. = 0.683, t = 17.6***, d.f. = 23) for the double seedling incubation.

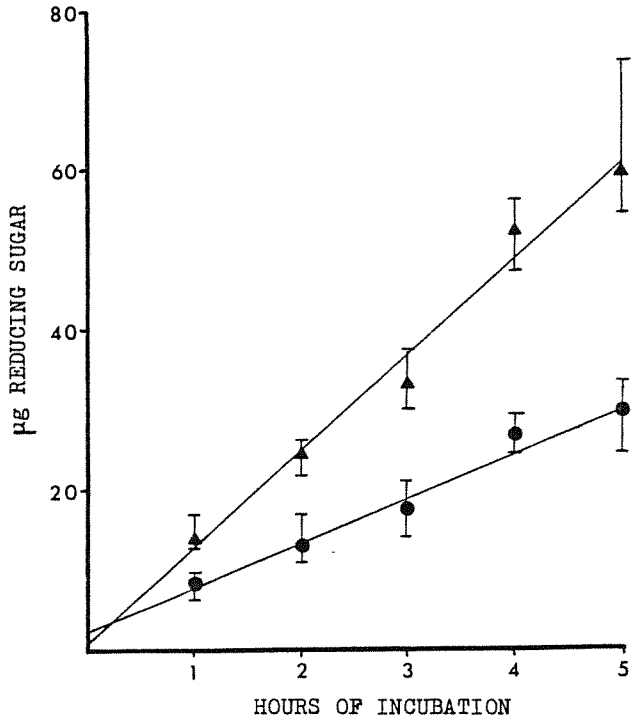

Figure 1: Time course of the in vivo assay of invertase activity.

● Single seedling incubations

▲ Double seedling incubations

This technique therefore presents a reliable and convenient way of determining the level of invertase in individual seedlings and it should be possible to identify heterozygotes if they have lowered levels of the enzyme. After completion of the assay seedlings may be transplanted aseptically to agar containing mineral salts and appropriate carbohydrate. Such seedlings have been successfully grown to maturity on 2% sucrose or glucose.

*** = $P_{.001}$

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E.S. D'S. is supported by a Science Research Council studentship.

Phytochrome-mediated photoperiodic reactions in two genotypes of Arabidopsis thaliana (L.) HEYNH.

M. HEHL and A.R. KRANZ

(Section Biology, Botany, University Frankfurt/Main, GFR)

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Arabidopsis thaliana is a facultative long day plant without sharply defined day length and variable to a certain extent in its natural populations (LAIBACH 1951, HUSSEY 1954, CLAUSS and RAU 1956, RÉDEI 1970). LD is more inductive to flowering and there is the reinforcement of the photophase which is more important for the induction of flowering than the alterations of the photo- and scotophases. LAIBACH (1943) observed that the red and blue parts of the spectrum were most stimulating to flower induction. Red-infrared antagonism was observed in the night break reaction (MEJER 1957, BROWN and KLEIN 1968). In some varieties neither red nor green light induced flowering (MEJER 1959).

Therefore one can postulate that the phytochrome as well as blue light absorbing pigment are participating in the flower initiation of Arabidopsis. But, there is still the question of the genotypic control of these responses to light and also other photoperiodically controlled characters besides flowering.

We cultivated the standard line En-2 and the mutant ch^1 in a growth chamber (20±2°C) under long day-(LD 16:8 hours) and short day-conditions(SD 8:16 hours). A light break of 15 minutes red or far red was given directly after finishing the light phase or in the middle of the dark period. The photoperiodic reactions were studied at the number of flowering plants (at least 1 flower per plant),i.e.the anthocyanin content of the leaf stalk, the length of the shoot and the leaf stalk, and the leaf area-index (length:width).

Table 1: Different photoperiodic and phytochrome-mediated reactions of 2 genotypes (En-2 and ch^1)under long day- and short day-conditions with a red(R) or far red(FR)light-break given at the end of the day or in the middle of the night in relation to the control (LD+R = 100 p.c.)

	number of flowering plants		anthocyanin content		height of the stem		length of the leaf stalk		leaf area-index	
	En-2	ch^1	En-2	ch^1	En-2	ch^1	En-2	ch^1	En-2	ch^1
LD+R	100	100	100	100	100	100	100	100	100	100
LD-R	90	76	108	90	96	76	100	119	79	97
SD+R	0	0	0	0	0	0	180	154	77	85
SD-R	0	0	0	0	0	0	260	140	44	76
SD+FR	0	0	0	0	0	0	240	144	47	85
SD	0	0	25	25	0	0	160	130	89	81

Table 1 shows the results of the experiments compared to the control which is the long day-treatment LD+R followed by the 15 minutes red light break, the latter probably being the best timed induction for a long day-plant. We can see that flowering occurs only during long day exposure in both genotypes. The red light break in the middle of the dark period of the long day exposure shows a considerably higher reduced number of flowering plants in the mutant ch^1 than in the standard line. The anthocyanin content of the leaf stalks and the length of the shoots is lower in the mutant. Under short day-conditions the leaf stalks are substantially lengthened but much less in the ch^1 than in the En-2. The light break given as red under long day-conditions promotes these characters in the mutant more than in the standard line which shows no differences to the control. After short day-treatment the leafs of the En-2 do not reach the leaf area-index of the ch^1, which always seen in relation to the control. We got the same result with the long day-treatment using a red light break in the dark phase. Looking at the leaf characters last mentioned it is most important in which phase of the light dark-cycle the red or far red light break is given. The phytochrome P_{fr} activated by the 15-minute-red-experiment can induce positive as well as negative photomorphoses. These photoinductions are quantitatively dependent on the genotype.

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Isolation of mutants defective in certain phytochrome-mediated photomorphosis.

A.R. KRANZ

(Section Biology, Botany, University Frankfurt/Main, GFR)

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After a selection of mutants defective in phytochrome-mediated photomorphogenesis we have isolated 6 of these mutants out of 30 lines of Arabidopsis thaliana (L.)HEYNH. These show an increased seed germination by 5 minutes red after 10 minutes far red (FR+R) in contrast to 10 minutes FR and 5 minutes R plus 5 minutes FR (FR+R+FR). Two of the selected mutants originated from the wild type which was obviously not stimulated by R. One mutant resulted from the chlorophyll b-defective mutant ch3, whose germination is slightly increased by FR+R, and there are also two other mutants which are the homoallelic mutants ch^1 and ch^2. Whereas the mutant En-2 f_{1y+b} selected from the wild type shows an increase in the hypocotyl length and the development of the cotyledons after FR plus R (figure 1), the mutant ch3 shows only an increase of the hypocotyl length. This effect is not significant in the mutants V81 v_2 and

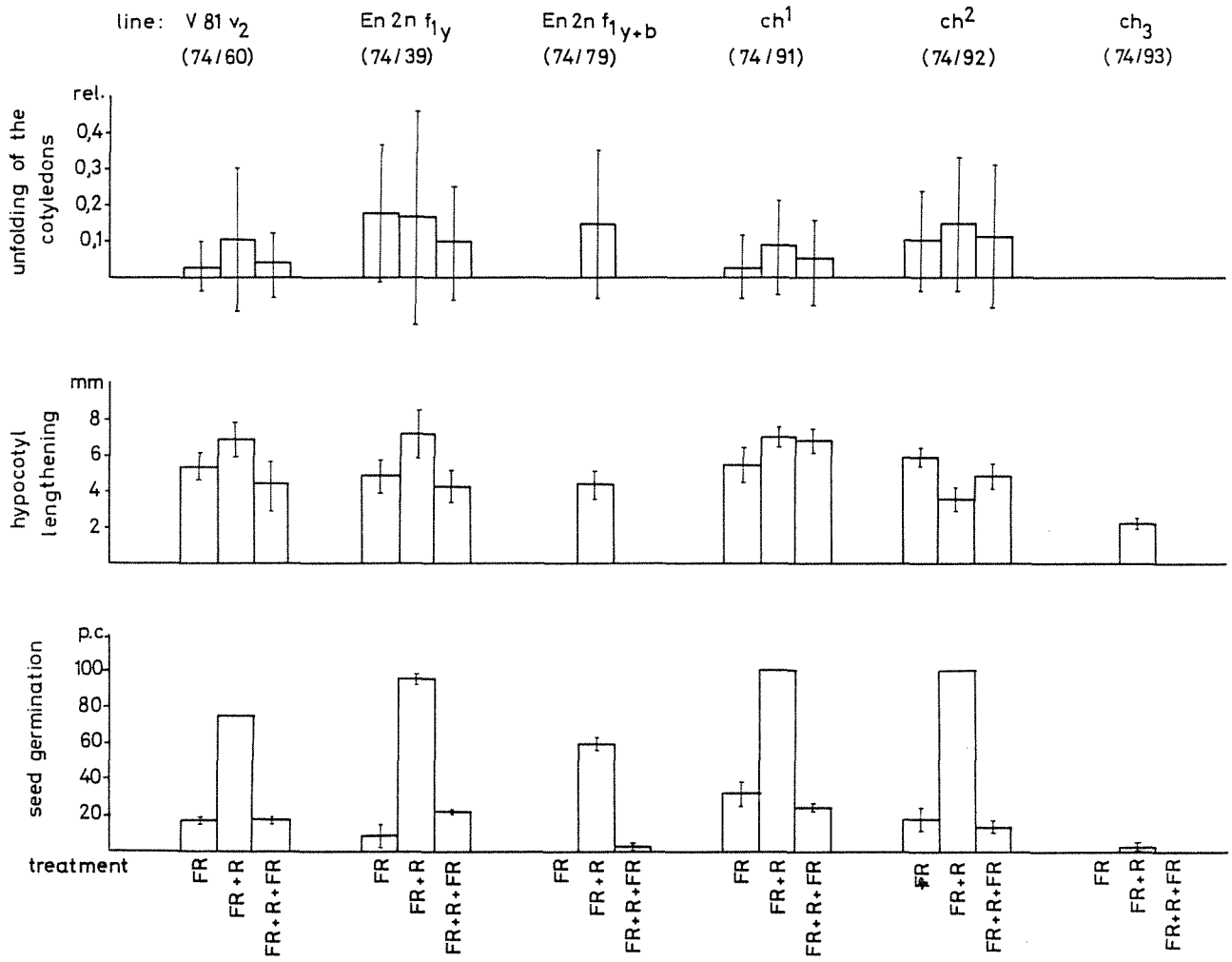

Figure 1: Differences in certain phytochrome-mediated photomorphogenetic reactions in 6 mutants of Arabidopsis thaliana.
(R = red light with λ_{max}= 658±60 nm and 0.95×10^4 erg.cm^{-2}.sec^{-1}
FR = far red light with $\lambda_{max} \geqq 725$ nm and $\lambda_{min} \leqq 680$ nm and 0.65×10^4 erg.cm^{-2}.sec^{-1})
for methods see DIEKMANN and KRANZ (1973)

En-2f1g(standard errors are overlapping). On the other hand hypocotyl lengthening is significantly reduced after FR+R-compared with FR- and FR+R+FR-treatment in the mutant ch^2.

These results indicate that after FR+R the amount of activation of the phytochrome to P_{fr} may be dependent on the specific genotype, and thus very different photomorphogenetic phenomena are produced. This leads to the following hypothesis for future work: The differences in photomorphogenesis observed originate from 1) different primary effects of various P_{fr}-forms, and/or 2) from different efficiencies of one active P_{fr}-form. In both cases certain genes need to be activated for control of the further differentiation.

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The experiments have been supported by a grant of the Deutsche Forschungsgemeinschaft.

Intra-cellular localization of L-(U)-^{14}C-leucine in Arabidopsis and barley leaves

M. BOUNIAS

(Laboratoire d'Histochimie-Autoradiographie, CEN, Cadarache, 13115, FRANCE)

received October 1974

Previous work gave evidence to the role of L-leucine on the greening of Arabidopsis and barley leaves, both in chlorophyll mutants and control plants (BABADZHANOVA et al. 1971, BOUNIAS 1972a). Moreover, L-(U)-^{14}C-leucine is easily incorporated into different metabolic compounds,such as sugars, amino acids, chlorophylls and carotenoids (FALUDI-DANIEL 1969, BOUNIAS 1973).

These results led us to study the sites of incorporation of L-(U)-^{14}C-leucine into Arabidopsis and barley leaves.

M e t h o d e s

Plants were grown aseptically in tubes on sand and liquid mineral medium. L-(U)-^{14}C-leucine was given to the roots during 5 hours, just before tissue fixation. The specific activity of ^{14}C-leucine was 500 μ-Ci/m.mole and its concentration in the medium 5 mM. The leaves were then cut off and either fixed in cooled acetone or directly included by the "freeze drying" technique.

The plates were coated with ILFORD G5 or L4 emulsion (6 μ thickness) and kept for exposition during 2 months. Biochemical analysis showed that the radio-activity observed was actually due to L-leucine, other amino acids being only slightly marked in the experimental conditions.

Individual chloroplasts have been separated from the other cell-constituents using a sedimentation technique in non-polar solvents: with a gradient mixture of petroleum-spirit + carbon tetrachloride, pure chloroplasts are isolated at a density of 1.42.

In both Arabidopsis and barley cells, L-(U)-^{14}C-leucine is mainly located in chloroplastic grana: Fig.1a shows leucine localizations in Arabidopsis cells, with the presence of leucine into the chloroplasts and on peripheric sites. Fig.1b shows the same results at a higher magnification on individual chloroplasts isolated by the gradient sedimentation technique.

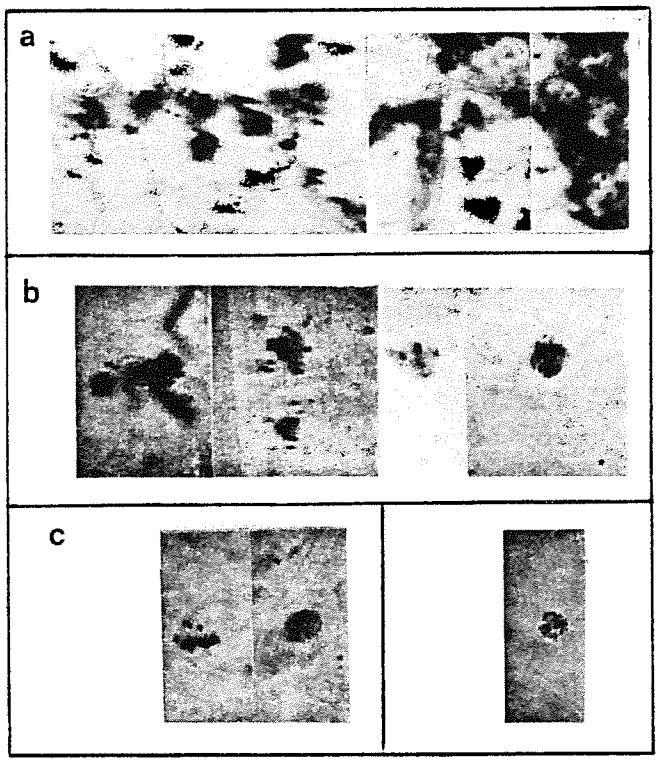

Figure 1: Intra cellular localization of ^{14}C-leucine and alkaline phosphatase activity(E.C.3.1.3.1.)in Arabidopsis and barley leaves.

1a - ^{14}C-leucine in Arabidopsis cells:localization in chloroplasts (x 720)

1b - ^{14}C-leucine localization into isolated chloroplasts of Arabidopsis (x 900)

1c - ^{14}C-leucine into an isolated chloroplast of barley (x 720)

1d - alkaline phosphatase activity into an isolated chloroplast of barley (x 720)

These first experiments have shown an interesting similarity between Arabidopsis and barley, for the chloroplastic localization of L-^{14}C-leucine, and we know that L-leucine is able to increase or decrease the photosynthetic-pigment content of Arabidopsis according to its concentration and that in mutants with low level of leucine, the alkaline phosphatase activity is significantly increased (BOUNIAS, 1972b). So that it is now possible to express the hypothesis that in Arabidopsis leaves, as in barley, alkaline phosphatase activity should be largely located in chloroplasts: This is a call for Arabidopsis-histo-chemists'investigations!

S u m m a r y:
L-(U)-^{14}C-leucine incorporated into Arabidopsis and barley cells is mainly localized in chloroplastic sites, as internal and peripheric grana. Experiments in barley have shown a similar localization for alkaline phosphatase activity which could explain the role of L-leucine on this enzyme. A hypothesis is thus expressed for the chloroplastic localization of alkaline phosphatase in Arabidopsis.

R é s u m é:
La L-leucine-(U)-^{14}C incorporée dans les cellules d'Arabidopsis et d'orge est essentiellement localisée au niveau des chloroplastes sous forme de grana intra et péri chloroplastiques. Des expériences réalisées chez l'orge ont montré une localisation de même type pour l'activité phosphatasique alcaline, ce qui pourrait expliquer le rôle régulator de la leucine à l'égard de cette enzyme. On émet alors l'hypothése d'une localisation également chloroplastique de la phosphatase chez Arabidopsis.

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The Author is greatly indebted to Dr.L. KHAU-VAN-KIEN for highly valuable suggestions and encouragements; the main part of this work was done in his laboratory.

B. TECHNIQUES

Improvements in screening mutations

G.P. RÉDEI

(Department of Agronomy, University of Missouri, Columbia, Mo. USA)

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An impressive endeavor has been devoted to studies of mutation in Arabidopsis. In the majority of experiments MÜLLER's (1963, 1965) embryo test has been used and the induced mutations were screened by the examination of 1-5 siliques of the M_1 plants, and the mutants were discarded. When the mutants have to be saved for genetic studies, the seed of the M_1 plants is carried to M_2. The number of the plants needed in M_1 generation will be determined by the (a) mutation rate, (b) level of ploidy, (c) the number of cells in the germline at the time of the treatment and (d) by the effectiveness of the recovery of the mutation induced. When parameters (a), (b), (c) are known (LI and RÉDEI 1969), by the use of the following formula the minimal size of the M_1 population (n) can be calculated (RÉDEI 1974) $n = \frac{\log \text{ desired probability}}{\log \text{ expected frequency}}$.
The size of the M_2 will determine how many of the actually induced mutations will be retrieved. If the M_1 is chimerical, the sector size has to be also considered (RÉDEI 1974) and the actual testing of the offspring of each sector may be necessary.

In the simplest case the following relations apply between M_2 size and the probability of the recovering at least one mutant in the progeny of a non-chimerical heterozygote.

Probability	0.25	0.44	0.68	0.90	0.99	0.999
No. of plants in M_2	1	2	4	8	16	24

The interpretation is simple, if the M_2 (or F_2) is tested with 24 offspring, 0.999 is the chance that at least one recessive mutant will be found. If we use only a single plant in the M_2 (or F_2), there a 0.25 probability for finding the same mutant. In other words at the expense of working with 24 fold larger population the chances of retrieving that mutant improves only 4 fold; similarly the use of only 8 seedlings in M_2 does not involve a serious risk of missing a particular mutation if the transmission and survival are normal.

Thus we can save substantial time and space by reducing the size of the individual M_2 population. The saving may make possible to grow a comparably larger number of M_1 plants. If we grow 1 heterozygous plant and test its progeny with 24 offsprings the maximum number of different mutations is 1, (and this may occur is ca. 6 copies). If we grow 24 M_1 plants and test their M_2 progeny with 1 offspring each, we have an equal chance to find 6 mutations and the probability is good that they are all independent. Thus, if the cost per plant is the same in M_1 and M_2, in the first example the cost of and independent mutation is 25/1 (25) while in the second, the same is 48/6 (8). Thus this simple example shows that the second procedure may cost 1/3 as much as the first.

If the M_1 is chimerical, depending on the number of sectors, the most desirable M_2 size may increase proportionally. According to the experiences of my laboratory, after dry seed treatment, there are two sectors in the M_1 plants in the majority of cases.

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Starvation procedure for the isolation of nutritional mutants

Fietje J. OOSTINDIER-BRAAKSMA and W.J. FEENSTRA

(Institute of Genetics, University of Groningen, Haren-Gn., The Netherlands)

received March 1974

Techniques used in fungal genetics for the isolation of nutritional mutants usually involve some procedure aimed at enrichment of the required mutants in the sample to be screened. One of these procedures is the so called starvation method of M^{c} DONALD and PONTECORVO (PONTECORVO et al. 1953) which takes advantage of the phenomenon that double mutants with two nutritional deficiencies sometimes survive longer on minimal medium than the single mutants involved.

We used an analogous method in our search for mutants of Arabidopsis, blocked in some step of the nitrate metabolism.

After mutagenic treatment of seed of a non-leaky thiamine requiring line, M_1 plants are grown on a medium supplied with thiamine. M_2 families are grown under continuous illumination at 28^{0}C in petridishes on perlite, without thiamine and with nitrate as the sole nitrogen source. After about 2 weeks most plants have died because of lack of thiamine. At this moment the substrate is supplemented with both thiamine and an ammonium salt. After some more days all growing plants are transplanted to soil and grown to maturity in a green house. M_3 progenies are tested for growth on a nitrate medium.

In an experiment started with about 700 M_1 plants and testing about 100 progenies of "rescued" M_2 plants, we found 7 mutants that grow much worse on a nitrate medium than on ammoniumnitrate. Since we are especially interested in the isolation of mutants blocked in the nitrate reduction, and since all 7 mutants do exhibit normal enzyme activities, the mutants have not yet been studied further.

This method can easely be adopted for the isolation of auxotrophic mutants by proper supplementation according to the requirement of the mutants selected for.

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Operative control over the state of collections of Arabidopsis thaliana

P.D. USMANOV and Z.D. USMANOV

(Institute of Plant Physiology and Biophysics, Institute of Mathematics, Acad.Sci.of the Tajik SSR, Dushanbe, U S S R)

received March 1974

It becomes extremely difficult in the course of time to exercise an operative control over the collections of A.thaliana because of their constant increasing. This control according to the specially worked out algorythm has been entrusted to the electronic computer. The contents of the algorythm is the following.

The collection of seeds of A.thaliana is considered as a dynamic system consisting of N lines. Each of the lines is representing the sum total of the genetically identical species. In the line two characteristics are comparable: 1) $\alpha(t)$, counted from the time moment t, the maximal duration of the storage period for the seeds in the line, after the expiry of which the per cent of the survival of plants in the process of restoration of the given line becomes lower than the level P known previously; 2) β, is the time interval since the moment of sowing up to the moment of the new generation appearing.

The elaborated algorythm of the state of the collection at any time moment t indicates the critical time $t+\tau$, when in the collection at least for one of the lines $\alpha(t) = 0$. An approach of this moment is a signal for the restoration of the corresponding lines. At the time moment $t+\tau$ the state of the collection is registered and the lines to be restored are indicated.

The present algorythm is also good for the control of the other plant collections.

A screening technique for mutants lacking invertase activity

Ms. E.S. D'SOUZA and E.P. MAHER

(Department of Genetics, University of Aberdeen, Aberdeen, Scotland, G.B.)

received October 1974

Sucrose metabolism is of fundamental importance to all plants, but thus for the literature, although extensive, provides little evidence on the genetics of the system. Due to the ability of Arabidopsis to grow on defined media supplemented with sucrose or glucose, it is the ideal plant in which to investigate the biochemistry and genetics of invertase production. We are currently trying to isolate mutants using a screening test based on their inability to hydrolyse sucrose.

EMS-treated seeds of the race Est are sown in soil and the M_2 seed are harvested. Seeds are sterilised in a 1:1 mixture of EtOH and H_2O_2 (20 vols) and washed several times with sterile water before being sown aseptically in single rows on 0.75% agar containing 0.1% KNO_3 in petri plates. After 4 days at 4°C to ensure uniform germination, the plates are transferred to a growth cabinet (continuous light, 10.000 lux, 25°C) for another 4-5 days. During the growth period the plates stand at an angle of 60° so that the seedlings are regularly orientated and their roots remain on the surface of the agar (CONTANT 1966).

The screening procedure, based on LEPESANT, KUNST, LEPESANT-KEJZLAROVÁ and DEDONDER (1972) is as follows: Using a sterile all-glass handspray (Quickfit) connected to an air-line with microbial filter incorporated, the row of seedlings on each plate is sprayed with a 50% solution of especially pure sucrose (Aristar) which was previously sterilised by ultrafiltration. Glucostat Special reagent (Worthington) is made up by dissolving the contents of an enzyme vial and a chromogen vial in 8 ml of sterile distilled water each and then mixing the two solutions. The seedlings are sprayed with this reagent 15 minutes after the addition of sucrose and the plates are subsequently placed in the dark at 25°C for 1 hour before scoring under a binocular microscope.

Due to the photosensitivity of the reagent the entire sprayed region developes a faint pink hue, but specific regions around the seedling show a much deeper red colour than the background, indicating areas of high surface invertase activity. These are at the root tip, around the cotyledons and to a lesser extent at the junction of the hypocotyl and root (see Figure 1).

Figure 1: Sprayed seedlings showing areas of invertase activity.

1. around the cotyledons
2. at the junction of hypocotyl and root
3. around the root tip

Individual seedlings which lack these three "spots" are picked out and transplanted aseptically into a mineral medium supplemented with 2% glucose and solidified with 0.8% agar. Such putative "null-enzyme" mutants survive for a long time (over 4 months), but their rosettes remain very small and do not develop inflorescences before they ultimately die. On the other hand wild-type seedlings which have undergone the same screening procedures grow normally and produce fertile inflorescences.

As these mutants have proved sterile our present efforts are being directed towards the isolation of heterozygotes from individual M_2 families for further genetic studies. We are also trying to establish callus lines from the mutants as we believe that the genotypes are more likely to survive in the undifferentiated state and it should be possible to collect sufficient callus tissue for biochemical studies.

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We wish to thank Professor W.J. FEENSTRA and Fietje J. OOSTINDIER-BRAAKSMA for their advice and encouragement when we started to work with Arabidopsis.
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Une methode specifique de restauration de croissance de mutants letaux d'Arabidopsis (type xantha)

M. BOUNIAS

(Laboratoire d'Histochimie-autoradiographie, CEN, Cadarache, 13115, FRANCE)

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Parmi les mutants chlorophylliens, les Viridis sont le plus souvent les seuls viables, à des degrés divers. Cependant, certains mutants, de type Albina en particulier, ont pu être rendus fertiles par addition à leur milieu nutritif de substances simples (amino-acides, sucres, vitamines) ou complexes (lait de coco). Ainsi, LANGRIDGE (1958) a restauré la fertilité de mutants albina d'Arabidopsis avec du glucose et du lait de coco et REDEI (1963) a isolé un mutant du même type nécessitant un apport de cystéine. Ces cas sont relativement rares et la survie, parfois obtenue par greffage (LEFORT 1959) n'entraîne pas toujours le reverdissement. Le greffage sur une plante saine de même espèce est sans doute la solution la plus efficace pour la survie des mutants: cette technique est malheureusement difficile à mettre en oeuvre chez Arabidopsis.

L'utilisation de ces techniques vise en fait, deux objectifs bien distincts:
- Rétablir la croissance d'un mutant dans le seul but de le multiplier en vue d'études ultérieures
- Etudier les déficiences biochimiques directement par l'analyse nutritionelle.

Notre but ayant été d'obtenir le plus grand nombre possible de mutants pour en effectuer l'étude biochimique, nous avons dû rechercher, en premier lieu, un moyen de les sauver.

P r i n c i p e e t e x p e r i m e n t a t i o n

Nous avons remarqué que les mutants ne meurent presque jamais avant d'avoir utilisé les réserves cotylédonaires; cependant, ces réserves pouvant elles-mêmes être insuffisantes du fait de la mutation, nous avons composé une solution susceptible de contenir les facteurs necessaires au déclenchement de la différenciation des tissus et des organites cellulaires. Nous avons supposé qu'il fallait fournir aux plantules lésées une solution nourricière s p é c i f i q u e d e l ' e s p è c e. Cette solution a été composée de la facon suivante:

a) des graines sont mises à germer pendant 24 heures environ; dès que la cuticule commence à se craqueler (c'est-à-dire que les processus enzymatiques destinés à libérer les réserves nutritives sont entrés en action) les graines sont broyées à froid dans du tampon phosphate M/15 à pH 7.5; la solution est centrifugée puis ajustée à volume équivalent à 1 cm^3 pour 100 graines; elle peut être alors incorporée au milieu de culture, soit directement, soit après stérilisation sur filtre de porosité 0.45 u.

b) Enfin, des unités carbonées simples sont ajoutées à la solution précédente sous forme d'acétate d'ammonium (0.015 M) qui constitue également un complément azoté.

R é s u l t a t s

Grâce à ce procédé, nous avons pu rétablir la fertilité de deux lignées de Xantha extrêmement dépigmentés obtenues en 2èmes génération (Xa-55 K). Dans les lots traités, seuls ont survécu les plants ayant reçu l'association de deux solutions précédentes. Dans l'un des cas, nous avons même obtenu un reverdissment et une rectification du défaut de différenciation des feuilles de la rosette: celles-ci, initialement allongées en lames fines, se sont arrondies et vascularisées pendant le verdissement (Fig.1). L-extrait de graines germées contient donc apparemment un ou plusieurs facteurs de différenciation des tissus. Cette observation permet de confirmer qu'il existe une relation étroite entre la morphologie de la plante (malformations foliaires) et sa physiologie (déficiences chlorophylliennes). Il s'agit d'une extension des liens déjà observés entre les altérations pigmentaires et le stade de différenciation des plantes (ERIKSSON et al.1961, BOYNTON 1967). La distinction entre mutants morphologiques et physiologiques s'avère donc très incertaine.

La figure 2 traduit la courbe de croissance restaurée de ce mutant. Dans d'autres cas, nous avons pu provoquer la formation des hampes, mais pas de graines dans les siliques. Le traitement devrait être étudié de manière plus approfondie et peut être ajusté à la croissance des plants en utilisant successivement des extraits complets de témoins du même âge (ou stade). Les concentrations à utiliser pourraient également varier d'un mutant à l'autre.

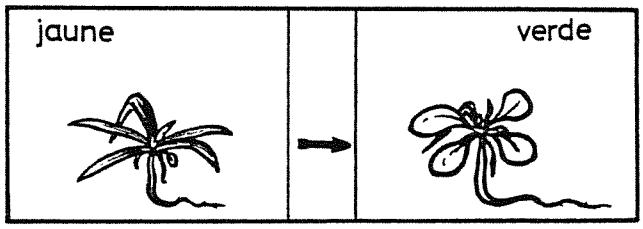

Figure 1: Rectification de la morphologie des feuilles du mutant Xantha Xa-55 K après de milieu nutritif complet specifique à base d'extrait des graines d'Arabidopsis en germination.

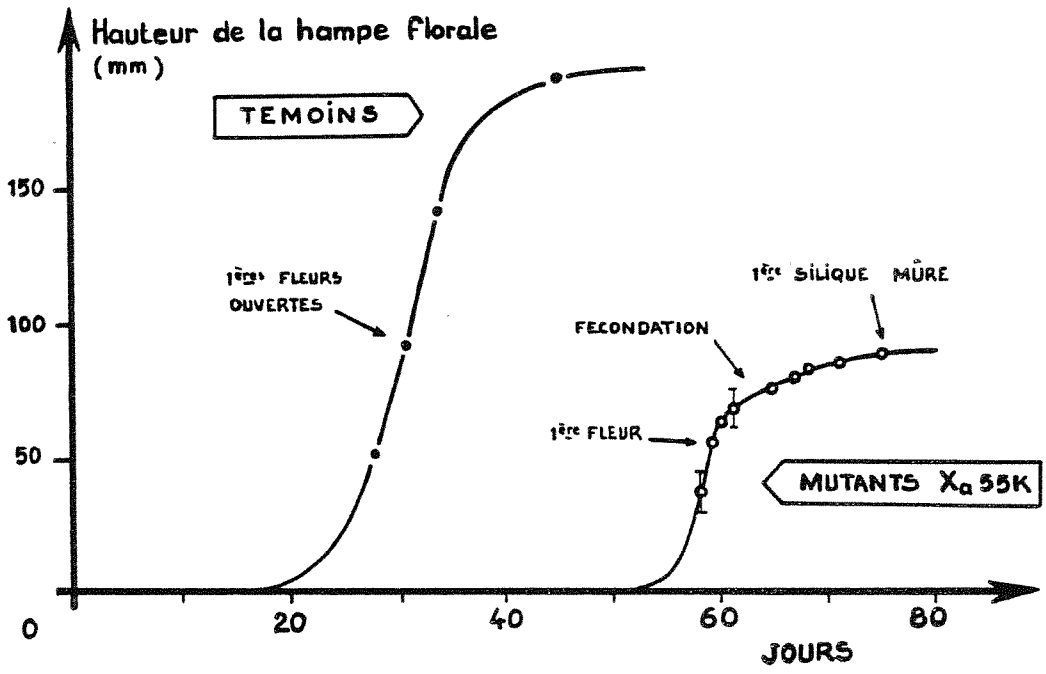

Figure 2: Vitesse et niveau de croissance du mutant Xantha Xa-55 K cultivé sur extrait de graines d'Arabidopsis en germination

D i s c u s s i o n

Ces résultats concordent avec les données bibliographiques et confirment l'hypothèse selon laquelle des mutants chlorophylliens présentant des altérations de degrés croissant pourront vraisemblablement être rendus viables à l'aide de facteurs de plus en plus complexes: les facteurs physiques purs (éclairement, température) et des métabolites simples peuvent convenir pour des mutants Viridis (BOUNIAS 1972) tandis que les Xantha et les Albina nécessitant le plus souvent des apports plus complets: vitamines, hormones, protéines, et à la limite: le greffage. L'emploi d'un extrait d'une plante saine de la même espèce devrait présenter sur les autres facteurs tels que lait de coco, l'avantage d'une efficacité plus probable et d'une action plus complète, se rapprochant de celle du greffage.

S u m m a r y

A method is proposed for growth restoration of Arabidopsis lethal mutants. The specifically required growth factors are given to the mutants as an extract of germinating seeds of sound plants of the same species in phosphate buffer and nitrogen and carbon supply added as ammonium acetate. A xantha lethal mutant was grown to flowering and fertilization by this way.

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C. INFORMATION

IMPORTANT EDITORIAL CHANGES

This year the costs for paper and printing have increased. Consequently the price of this volume must be raised to DM 7.00 (domestic) and DM 8.00 or US $ 3.00(foreign).

In order to save in the future costs and labour the following reminder is necessary:

i) no further requests for manuscripts and orders of AIS will be sent out annually. As indicated on the inside of the cover page of each volume your contribution will be expected not later than February 15, each year.

ii) the editor expects that the contributors will follow in detail informations given on that inside cover page. Costs and labour are substantially reduced, if you are typing your manuscript analogous to the size (DIN A 3) of the specimen mailed with AIS No.11.

BACK VOLUMES OF AIS

The supply of AIS No.1, 2, 3, 4, 6 and copies of "Arabidopsis Research" (Rep. Intern.Symp., Göttingen 1965) are entirely out of order. Only a few copies of the other AIS-volumes are still available. This will be distributed at cost price (DM 6.00 domestic, DM 7.00 or US $ 2.00 foreign) by the editor.

ADDRESSES (new and changed)

M. BOUNIAS, Dr. - INRA Department de Zoologie, Laboratoire de Biochimie, 84140 Montfavet, France

N. CHALBI, Prof.Dr.Directeur - Unité de Genetique-Biometrie et de Biologie Vegetale, Université de Tunis, Campus Universitaire, Tunis, Republique Tunisienne.

E.P. MAHER, Prof.Dr. - Department of Genetics, University of Aberdeen, 2 Tillydrone Avenue, Aberdeen, Scotland.

COMPUTERIZED BIBLIOGRAPHY

A colleague of mine has a computer program for vertical literature retrieval. We could help the Arabidopsis workers by having a computerized bibliography of all articles pertaining to Arabidopsis.

Alain F. CORCOS
Professor
Univ.College, Dep.Nat.Sci.
Kedzie Laboratory
Michigan State University
East Lansing, Michigan 48823

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