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

G. RÖBBELEN

A. BRIEF NOTES

Variation in 'after-ripening' of seeds of Arabidopsis thaliana and its ecological significance

Jane EVANS and D. RATCLIFFE

(Department of Botany, University of Leicester, U.K.)

Many races of Arabidopsis thaliana have seeds which are dormant when freshly harvested but which show good germination after a period of 'after-ripening' in dry conditions. In the field, in Britain and other parts of its range, germination does not usually occur until early autumn even if the soil is sufficiently moist for long enough to allow germination. The adaptive significance in this context is obvious: germination at an inappropriate time is less likely.

Within the range of Arabidopsis thaliana there is considerable variation in summer rainfall, both from place to place and year to year. It would seem likely that differences in the after-ripening period are of importance in the adaptation of the species to the local environment. We have therefore investigated variation both between and within populations in order to clarify the degree of genetic control and the extent of the correlation with local climate.

A large and thriving population on a disused railway track near Husband's Bosworth, Leicestershire was used to study within-population variability. Freshly ripened seeds were collected from 22 individual plants and samples set to germinate at 20°C in constant light after various periods of dry storage. There was considerable variation between the single plant samples in the proportion of seeds which germinated early. It was possible to classify each sample into one of three groups with a high, intermediate or low proportion of early germinators.

Table 1: Mean percentage germination of progeny of three groups of plants from Husband's Bosworth, Leicestershire. Figures in brackets are standard errors

Time from harvest. (weeks)	Proportion of early germinators		
	High	Intermediate	Low
4	13.14 (± 4.27)	2.25 (± 0.25)	0
7	14.57 (± 6.03)	2.50 (± 0.74)	1.14 (± 0.59)
45	42.29 (± 12.77)	39.50 (± 8.73)	20.57 (± 9.58)
83	88.57 (± 4.49)	75.00 (± 7.24)	59.42 (± 10.42)

In addition the shape of the germination curves suggests that even within the progeny of a single plant there is variation between seeds but there is as yet no actual evidence of the degree of genetic differentiation. This heterogeneity will ensure that at all times there will be a reserve of seeds in the soil, and there may also be carry-over from years to year, ensuring continued survival of the population.

Large samples, from 25-30 randomly selected plants, were harvested from the Husband's Bosworth and other populations and germination tests conducted in the same way. Sub-samples of these bulk samples showed a smooth decrease of dormancy with increased time of dry storage in all populations. The time for complete after-ripening varied between populations from two to eight months. Moreover, there were greater differences between populations from different areas than between those from adjacent areas (Fig. 1).

Other experiments have shown that the optimum germination temperature for a population was related to the probable soil temperature at its point of origin at the normal time of field germination. This was followed up by tests at 5°, 10°, 15°, 20°, 25° and 30°C, both in constant light and in darkness. Sixteen races were investigated. Again a common pattern of germination was found. After two months dry storage performance varied greatly at the different temperatures with an optimum at 5°C. After four months germination at 5° and 10°C had declined slightly and had increased at higher temperatures (Fig. 2a). However the degree of after-ripening, as reflected in the rate and percentage germination, varied from population to population.

It is suggested that an additional control of germination time is achieved by this change of optimum germination so that little germination occurs until the ambient temperature falls (in the autumn) to a level near to the optimum temperature for germination.

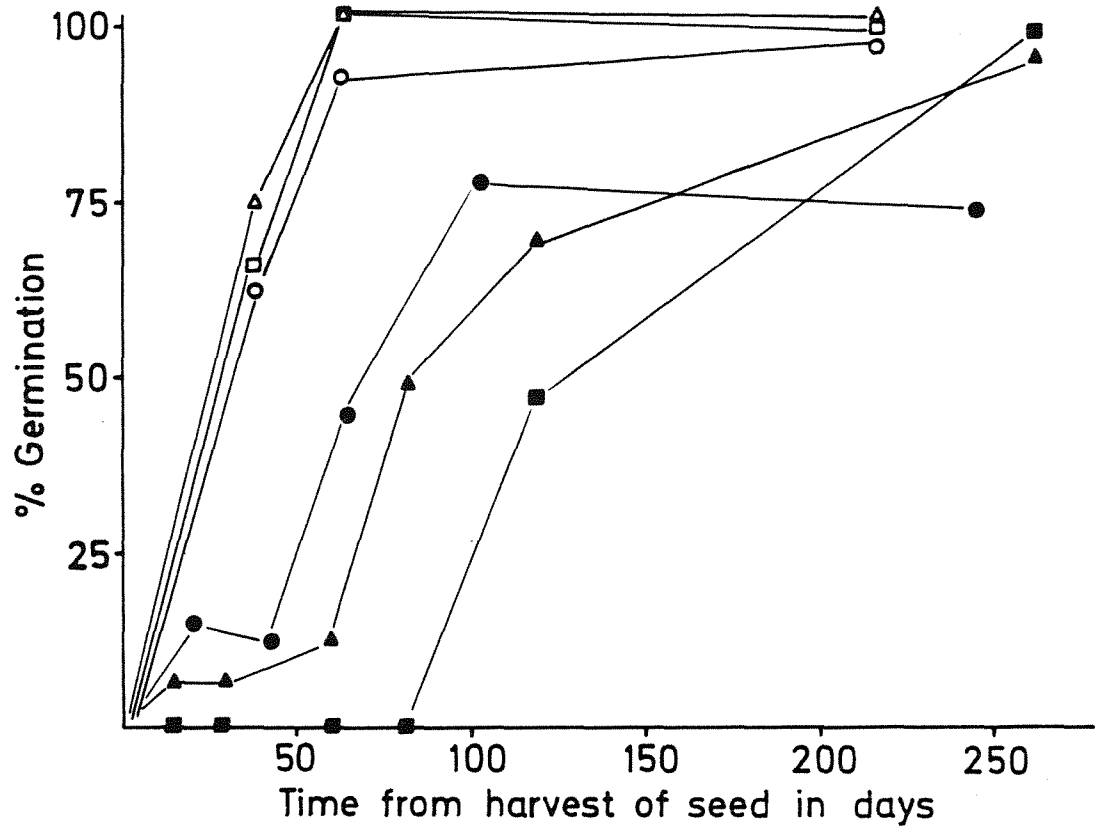


Figure 1: Germination of six populations of Arabidopsis thaliana after various periods of dry storage.
——■—— Aberdeen; ——▲—— Dirleton, East Lothian;
——●—— Husband's Bosworth; ——□—— Moorside, Derbyshire;
——○—— Parsley Hay, Derbyshire; ——△—— Cobbler's Nook, Derbyshire

In practice a combination of increased rainfall and lower average temperature would allow the characteristic autumn burst of germination observed in the field.

The variation between populations is significant. Populations with the shortest after-ripening period are those from areas with cool, moist summers and cold winters where seedlings are less likely to be killed by summer drought, e.g. Mickle Fell (Westmoreland), Ben Dhorain (Sutherland), Catterline (Kincardineshire), and Stockholm (Fig. 2b). Here early germination should allow maximum vegetative growth before the very low winter temperatures.

Populations with a definite inhibition of germination at higher temperatures after four months are those from areas with a rather dry, warm summer with periods of showery weather, where seedlings germinating too early are unlikely to survive e.g. St. Dogmael's Abbey (Cardiganshire), Husband's Bosworth (Fig. 2c), Whatton (Leicestershire), Woebley Castle (Glamorgan) (Fig. 2d), and Bush (Midlothian) (Fig. 2f).

There is one notable exception. The population from the Burren, County Clare, Eire (Fig. 2e), shows the reverse pattern: germination is high at high temperatures and lower at lower temperatures. There is a possibility that in this population germination occurs in the spring. Flowering probably does not take place until August or even September yet the seeds are dormant at low temperatures for at least four months and so may be prevented from germinating in the autumn.

Work is proceeding on other aspects of germination behaviour, particularly on variation in total percentage germination and differences in germination in light and dark, in relation to the possible carry over of seeds from year to year. It has been shown that dormancy is often more marked in the dark particularly at high temperatures and it may be that, in the field, buried seeds may survive more than

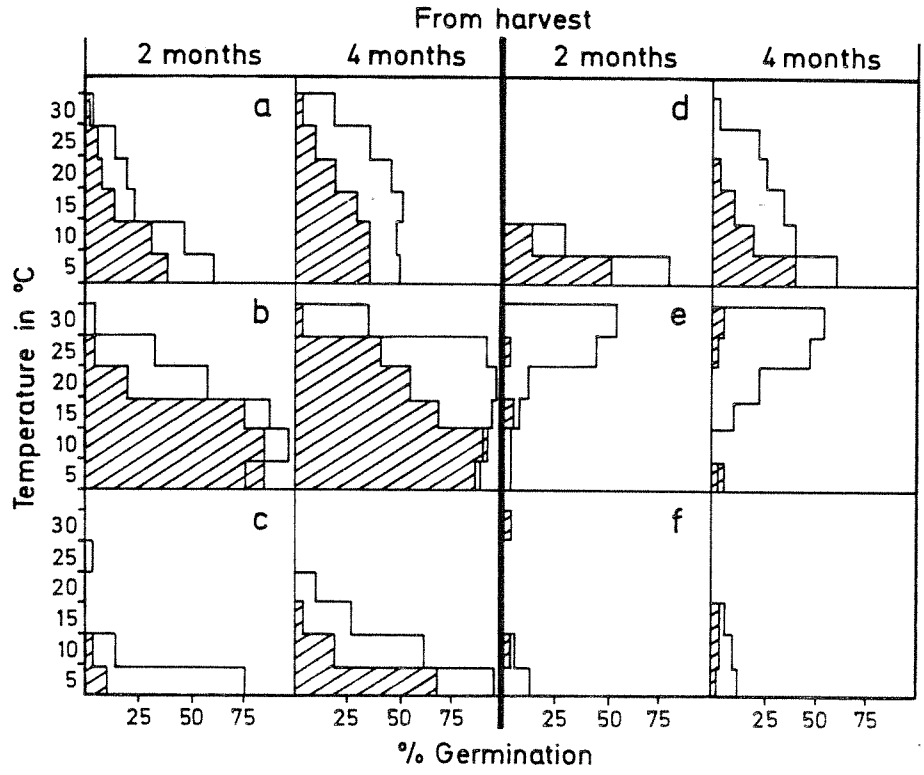


Figure 2: Germination of Arabidopsis thaliana at various temperatures after two and four months dry storage. Hatched area represents germination in constant dark. a, Mean of sixteen races; b, a Stockholm population; c, Husband's Bosworth; d, Woebley Castle, Glamorgan; e, Burren, Eire; f, Bush, Midlothian

one season. This mechanism would provide additional buffering of the population against catastrophic events, so contributing to long term stability.

Clearly there are many points at which selection can operate and the above results are being followed up by selection experiments.

On the physiological adaptation of species and ecotypes of Arabidopsis in Pamir-Alay

M.A. LOGINOV and P.D. USMANOV

(Institute of Plant Physiology and Biophysics, Acad.Sci.Tajik SSR, Dushanbe, USSR)

As previously was reported, large-scale genetic-physiological investigations of species and ecotypes of Arabidopsis are being carried out in Tajikistan (USMANOV, LOGINOV et al., 1970). Special attention was paid to study the processes of photosynthesis and its relationship to the environmental factors. In this connection, this work was designed mainly to study the behaviour of different species and ecotypes of different areal of habitat, if growing them for many years in the similar climate conditions and in different vertical zones of the Hissar mountain range.

Experiments were carried out in Dushanbe (740 m above sea-level) in the mountain station Siya Kuh (2500 m), and on the Anzob Peak of the Hissar range (3400 m). The results described in Table 1 show that magnitudes of the maximal potential photosynthesis, obtained at the optimal inner and outer factors, are practically equal. But species and ecotypes in different vertical zones showed different assimilative capacity. For instance, ecotype Rangon-Tau which grows not higher than 1100 m above sea level, most actively assimilated CO_2 in Dushanbe (250 $\pm$ 10 mg CO_2/g hr). On the opposite, on the Anzob Peak the activity of the assimilating apparatus in this ecotype is greatly reduced, that is probably the result of strong UV-radiation action, as well as the significant amplitude of temperatures.

Table 1: Eco-physiological characteristics of species and ecotypes of Arabidopsis

Species and ecotypes	Altitude above sea-level (m)	Maximal potential intensity of photosynthesis (mg CO_2/g hr) in relation to the habitat			Optimal T° for photosynthesis; Siya Kuh Station growing	
		Dushanbe, 740 above sea-level	Siya Kuh Station, 2500 m	Anzob Peak, 3400 m	1967	after 5 years
Arabidopsis pumila (Steph.) N.Busch	350-2100	260±10	225±10	180±7	24-28	20-25
A.thaliana (L) Heynh. Ecotype Rangon-Tau	1100	250±10	205±10	170±8	22-26	20-24
A.wallichii (Hook et Thoms) N.Busch	850-4000	230±12	225±11	220±15	18-22	18-21
A.korshinskyi Botsch.	3600-4000	190±9	230±10	250±15	15-17	18-20
Ecotypes of A.thaliana						
Rangon-Tau	1100	250±10	205±10	170±8	22-26	20-24
Harangon	1900	225±8	205±5	180±9	21-25	20-23
Shugnan	2700	170±9	200±8	200±10	19-22	19-22
Turkestan	3600	170±8	200±10	240±10	16-18	18-21

Growing for many years the species and ecotypes in the similar climate and environmental conditions on the mountain station Sija Kuh showed that zones of optimal temperatures for photosynthesis become almost equal with time. Thus, in the 1st year of growing the difference of temperature optimums for photosynthesis was 9-10°C, whereas after 5-year-growing this difference did not exceed 3-4°C.

Results of these investigations confirm once again the phenomenon of physiological adaptation of plants, which grow under similar climate conditions and belong to one florocenotype.

Reference:
USMANOV, P.D., M.A. LOGINOV, U. ISRAFILOVA, A.Ya. AHMEDOV, and S.Yu. YUNUSOV. Arabid.Inf.Serv. 7, 32 (1970

Nitrous acid effects on germination and mutation rate in Arabidopsis

Tomoo MABUCHI and T.J. ARNASON

(Biology Department, University of Saskatchewan, Saskatoon, Canada)

Although nitrous acid is a satisfactory mutagen when used on some microorganisms (MUNDRY and GIERER, 1958, and others) it is a weak mutagen when used on higher plants (MÜLLER, 1965; BROCK, 1971). We have tried various treatment modifications with Arabidopsis seeds with the object of getting high mutation frequency. Variations of dose, pH and length of presoaking time have been tried.

Seeds of A. thaliana (L.) Heynh. race Landsberg were presoaked in distilled water for 30 hours then treated with HNO_2 (from 0.03 M $NaNO_2$ in acetate buffer), pH 4.6 for 60, 90 or 120 minutes. Seeds were then soaked for 2 hours in phosphate buffer, pH 7.1 before sowing on nutrient-salt agar. Germination of the control was 100% at 1 day while all treated lots showed less than 50% germination. At 4 days, germination in lots treated for 60, 90 and 120 minutes was respectively 77.1, 48.3 and 29.3%. Based on these germination results at this concentration of HNO_2, 60-90 minute treatment time should be suitable for mutation induction test (roughly 50-75% seedling emergence).

The effects on germination of varying presoaking time in the 0-36 hour range showed that with HNO_2 concentration as described above, applied for 75 minutes, germination was near 100% when presoaking time was between 0-20 hours; with 37 hours presoaking the germination rate was 42%, dropping to 4% after 44 hours presoaking. We infer that beginning near 36 hours of soaking, seeds become increasingly sensitive to the effects of nitrous acid on processes related to germination.

To test the effects of varying pH, HNO_2 was prepared in acetate buffer at pH 3.6, 4.0, 4.4, 4.6, 4.8, 5.2 and 5.6. Control seeds were soaked in the buffer solution without $NaNO_2$. In this experiment 0.05 M $NaNO_2$ was used and seeds were presoaked for 18 or 35 hours. Germination scored at 7 days was reduced to less than 50% at pH 4.0 and to about 20% at pH 3.6. At pH 4.8 and higher no reduction of germination occurred. Acetate buffer alone had no inhibition effects. The effects produced in buffer plus $NaNO_2$ are attributed to HNO_2. To get maximal effects on germination and perhaps also on mutation induction the pH of solutions should be between 3.6 and 4.5.

Based on the germination tests the treatment methods for mutation induction were as follows. Presoaking 30 or 35 hours, HNO_2 at 0.03 or 0.05 M, pH 4.0 or 4.5, treatment time 60 or 75 minutes. One EMS treated lot was included for comparison. Seeds of M_1 plants were collected and sown separately. From each M_1 plant about 40 seedlings were scored for chlorophyll mutations and the frequencies of M_1 plants carrying mutations recorded. No sterility was found in HNO_2-treated plants but short siliques occurred in the EMS-treated plants indicating some sterility. Of 360 nitrous acid-treated M_1 plants only 3 (0.83%) were found to carry mutant genes for chlorophyll deficiency. Fourteen of 95 EMS-treated plants had new mutations. No mutants appeared in the control population. Treatment methods and results are shown in Table 1. Embryo and seedling poisoning by HNO_2 probably results mainly

Table 1: Germination rate, silique length and chlorophyll deficient mutations in M_1 resulting from nitrous acid and EMS. treatment

Mutagen	pH	Pre-soak (hr)	Conc. (M)	Treat. (min.)	Treated seeds: sown (no.)	Treated seeds: germ. (%)	Sil. length (mm)	M_1 plants: tested (no.)	M_1 plants: mut. (no.)
Control	6.8	36	-	-	327	100.0	12.7	45	0
NA*	4.5	30	0.03	60	240	83.7	11.5	51	0
NA*	4.5	30	0.03	75	240	56.2	12.1	51	1
NA*	4.0	36	0.05	60	500	85.2	-	258	2
EMS	-	20	0.095	120	378	100.0	9.8	95	14

*Molarity of $NaNO_2$ given

from attacks on amino groups in metabolically active cytoplasmic components rather than by deamination of bases in DNA. HNO_2 is obviously a very weak mutagen when used on Arabidopsis seeds.

References:
MUNDRY, K.W., and A. GIERER: Z.Vererbungslehre 89, 614-630 (1958)
MÜLLER, A.J.: Arabid.Inf.Serv. 2, 22-24 (1965)
BROCK, R.D.: Radiation Botany 11, 309-312 (1971)

Nutritional requirement of excised and non-excised roots of strain "Dijon G"

Ulrike WEILAND and A.J. MÜLLER

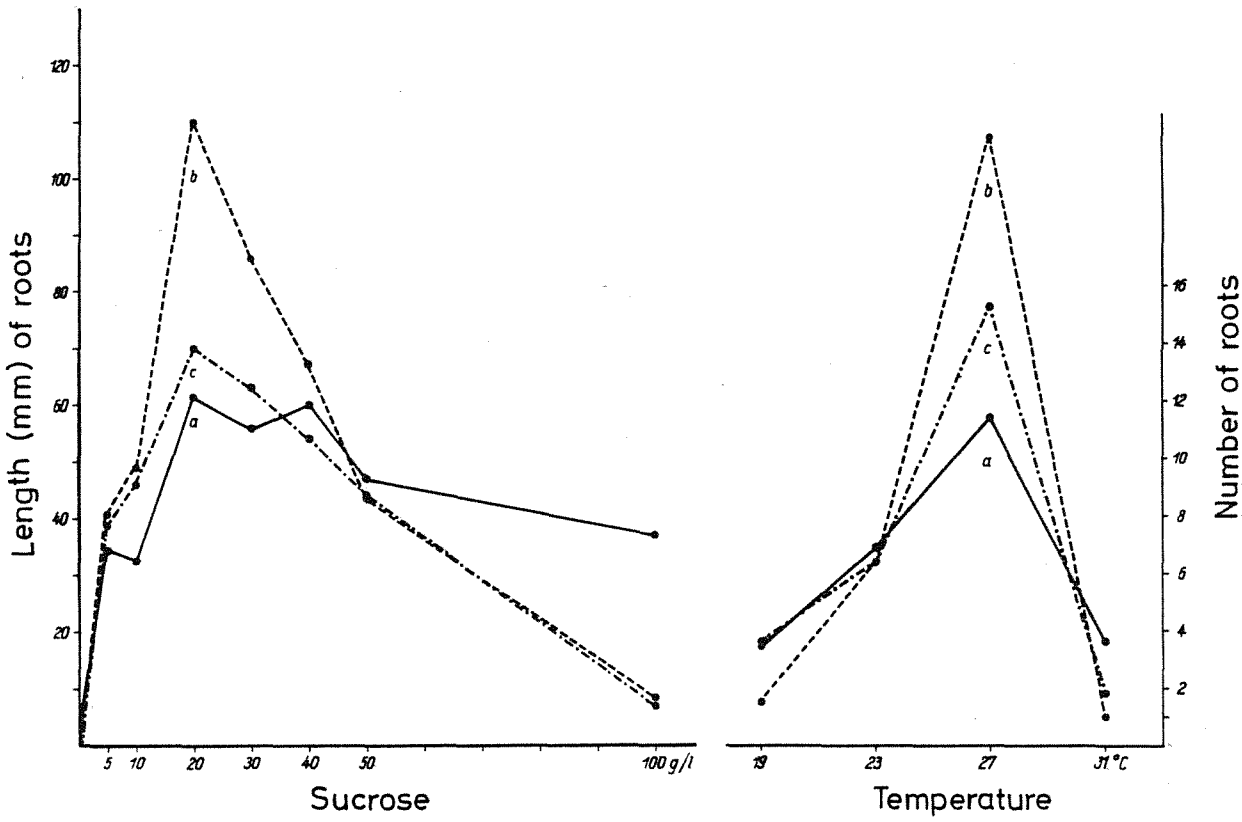
(Zentralinstitut für Genetik und Kulturpflanzenforschung, Gatersleben, Germany-DDR)

NEALES (1968a,b) has shown that excised root cultures of three wild-type strain (Estland, Pitztal, Blanes) of Arabidopsis thaliana differ in their vitamin requirements. We studied the effects of various organic medium components and of temperature on root growth of strain "Dijon G". Moreover, we tested to what extend the specific responses of excised roots are already expressed by the growth of non-excised primary roots in the dark.

M e t h o d s : For both modes of cultivation a liquid medium was used which included the inorganic components of White's medium and (if not otherwise stated) the following organic components: 20 g/l sucrose, 0.1 mg/l thiamin, 0.1 mg/l pyridoxine, 0.5 mg/l nicotinic acid, 3.0 mg/l glycin. Growth of non-excised primary roots was assessed after various periods of submerged culture of seedlings in the dark by measurement of the increase in length of the main axis and lateral roots and by counts of the number of laterals. Growth of excised roots was assessed after cultivation of a small portion (initial fresh weight: about 20 mg) of an established root clone by measurement of fresh and dry weight of the final root mass.

Figure 1

Figure 2



Figures 1 and 2: Effect of sucrose concentration (at 27°C) (Fig. 1) and effect of temperature (with 20 g/l sucrose) (Fig. 2) on growth of primary roots. Length of the main root (a), total length of the laterals per root (b) and number of laterals per root (c) after 7 days of submerged culture in the dark. Means of five replicates. Inocula: Seedlings with primary roots of 4-5 mm length

The r e s u l t s show that (as a consequence of suppression of photosynthesis and shoot growth) the roots of dark-grown seedlings behave in most respects like isolated roots:
(1) Sucrose concentration: Without an exogenous sugar supply the roots of dark-grown seedlings reached only a length of 3 mm. Their response to various sucrose concentrations is shown in Fig. 1. Excised roots responded in a similar way to variation of sucrose concentration. Thus, the roots of "Dijon G" proved to be very sensitive to 100 g/l sucrose, whereas NEALES (1968a) found that excised roots of "Estland" are unusually tolerant of high sucrose concentrations.

Table 1: Effect of vitamin addition to basal medium (with 20 g sucrose per l) on growth over 34 days at 27°C of excised roots. Means of five replicates. Inocula: 10-20 mg fresh weight

Treatment (mg/l)	Final fresh weight (mg/flask)	Final dry weight (mg/flask)
No vitamins	24.5	2.9
Biotin (0.1)	26.0	3.3
Thiamin (0.1)	141.5	15.2
Thiamin + Biotin	149.8	15.6
Complete medium	190.6	18.8

(2) Thiamin proved to be essential for growth of excised roots of "Dijon G" (see Table 1). Without an exogenous thiamin supply the roots of dark-grown seedlings showed only residual growth and reached a mean length of 13 mm (indicating a limited amount of preformed thiamin in the seedlings). "Dijon G" differs, therefore, from the strains "Estland" and "Pitztal" which do not require an exogenous thiamin supply for root growth (NEALES 1968a). A strong thiamin requirement of roots is typical for the majority of plant species.

(3) Other organic components: The results given in Table 1 show that growth of excised roots of "Dijon G" is stimulated by addition of pyridoxine, nicotinic acid and/or glycin, but not by addition of biotin.
(4) The effect of temperature on growth of non-excised primary roots (Fig. 2) has been found to be very similar to its effect on growth of excised roots reported previously (WEILAND and MÜLLER, 1971). Thus, roots of "Dijon G" are much more sensitive to deviations from optimum temperature than roots of "Estland" (NEALES, 1968a). It must be noted that the growth of whole plants of "Dijon G" is not inhibited at 19°C so strongly as the growth of isolated or dark grown roots is.

A detailed publication is in the press (Kulturpflanze 20, 1972).

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NEALES, T.F.: New Phytol. 67, 159-165 (1968a)
- : Austr.J.Biol.Sci. 21, 217-223 (1968b)
WEILAND, U., and A.J. MÜLLER: Arabid.Inf.Serv. 8, 34 (1971)

Asexual propagation of wild and mutant strains of Arabidopsis in liquid and solid media

A. CORCOS and R. LEWIS

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

We reported last year (CORCOS and LEWIS, 1971) that when whole plants were placed in liquid media containing Heller minerals, 2% dextrose and 6% V-8 juice, the plants usually grew into irregular balls 11 to 15 mm in diameter with numerous stems and leaves. Parts of the balls, when transferred in fresh medium grew into balls in about 2 weeks. Hence, healthy growth could be maintained by transferring into fresh medium every two weeks.

Since this report was made, we have found that plants put into a V-8 juice liquid medium will give rise to offshoots with numerous roots. These offshoots range in length from 1 to 10 mm and the long ones remind us of small strawberry rhizomes. They can be cut and propagated on solid media (containing VELEMÍNSKÝ minerals + 2% glucose) where in 2 to 3 weeks they flower and seed. No special precaution except sterility is necessary. The offshoot is simply laid on the agar.

Plants suspected of being mutants, that is those that are incapable of growing normally on standard mineral + glucose medium, have been transferred into V-8 juice media where they grew offshoots which were propagated asexually on appropriate media. By such a method we are hoping to propagate more morphological mutants which in the past have been lost because of failure to produce seeds.

References:
CORCOS, A., and R. LEWIS: Arabid.Inf.Serv. 8, 35-36 (1971)
VELEMÍNSKÝ, J., and T. GICHNER: Arabid.Inf.Serv. 1, 34 (1964)

Chlorate resistant mutants of Arabidopsis thaliana (II)

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

(Institute of Genetics, Biological Centre, State University of Groningen, Haren, The Netherlands)

Last year the isolation of a chlorate resistant mutant with a low nitrate-reductase content was reported (VAN DER LAAN, OOSTINDIER-BRAAKSMA, and FEENSTRA, 1971). This mutant had a yellow-green colour and a habitus somewhat different from wild-type. Based on the segregation of this phenotype it was stated that the chlorate resistance was recessive and monogenic (gene chl-2).

Further experiments showed, however, that the situation is more complex. From F_3 families two different resistant types could be extracted:
a. A line with normal plant habitus and only slightly lighter green leaf colour, at least when the plants are over 9 days old, but with a low nitrate reductase activity in extracts of rosette leaves, and chlorate resistant. Plants grow very badly on nitrate as the only nitrogen source.

b. A line with plant colour and habitus similar to that of the originally isolated mutant, with a moderate chorate-resistance, but with a normal nitrate-reductase activity in extracts of rosette-leaves.

We want to reserve the symbol chl-2 for the gene giving the phenotype described under a. and denominated the gene giving b. phenotype chl-8.

We do not know as yet what the reason is for the simultaneous isolation of the genes chl-2 and chl-8. The results of crosses do not suggest a close linkage between the genes. Further experiments have revealed that the frequencies with which mutants of the above types can be isolated are low.

Experiments showed that the chlorate resistance of the ch-2 type is not due to a lower uptake of chlorate, as is the case with our chl-1 and chl-3 mutants. A smaller amount of the chlorate is reduced by the plants. This is further support for the hypothesis that nitrate reductase is involved in the reduction of chlorate.

The nitrate content of chl-2 type plants appeared to be higher than that of the wildtype, so that the lower level of nitrate reductase is not caused by the absence of nitrate necessary for induction.

Reference:

LAAN, P.H. VAN DER, Fietje J. OOSTINDIER-BRAAKSMA, and W.J. FEENSTRA: Arabid.Inf. Serv. 8, 22 (1971)

Isozyme studies in Arabidopsis thaliana

N.S. GROVER and O.R. BYRNE
(Botany Department, Australian National University, Canberra, A.C.T.)

Arabidopsis thaliana has proved to be a very suitable organism for genetic studies particularly for quantitative and mutational research. With the development of electrophoretic techniques for studying isozymes, we have become interested in investigating the adaptive significance of these variants which often occur with high frequency in natural populations of plants and animals. A facet of our research is concerned with the effect of artificial selection for metrical characters on the gene frequency of particular isozymes. This note reports some of our preliminary findings on the incidence of some isozyme variants among some geographic races of A. thaliana and their inheritance.

Electrophoretic techniques and staining procedures followed closely those described by BREWBAKER et al. (1968). However, for acid phosphatases a 1:1 mixture of Fast blue RR and Fast garnet GBC salts resulted in a superior resolution of bands than when only Fast blue RR was used. Peroxidases were detected using a tris-citrate gel buffer (pH 8.6) and the electrolyte consisted of a boric acid - NaOH buffer (pH 8.2). The staining procedure involved the use of benzidine (3×10^{-3}M) in 50% solution of ethanol in 7% acetic acid. Hydrogen peroxide was added to make a 1.8×10^{-3}M solution (SMITH, 1970). This technique provides for the rapid appearance of blue coloured bands which, because of their instability, must be readily scored.

Material from different geographic races of A. thaliana has been made available to us from the Division of Plant Industry, C.S.I.R.O. The results obtained from the study of three of the races (arbitrarily designated as L, M and N) with respect to four loci are described here. Table 1 gives the genotypes assigned to these races.

Table 1: Genotypes of the races at four loci. (The subscripts define the relative mobilities of the enzyme bands)

Race	Acid phosphatase	Transaminase	Leucine amino-peptidase	Peroxidase
L	$Ap_{1.00}/Ap_{1.00}$	$Tr_{1.00}/Tr_{1.00}$	$Lap_{1.00}/Lap_{1.00}$	$Per_{1.00}/Per_{1.00}$
M	$Ap_{0.89}/Ap_{0.89}$	$Tr_{1.00}/Tr_{1.00}$	$Lap_{0.97}/Lap_{0.97}$	$Per_{0.81}/Per_{0.81}$
N	$Ap_{0.89}/Ap_{0.89}$	$Tr_{0.78}/Tr_{0.78}$	$Lap_{1.00}/Lap_{1.00}$	$Per_{1.00}/Per_{1.00}$

With the exception of peroxidases which have been determined from root extracts, the other enzymes were derived from extracts of rosette leaves.

A study of hybrids, F_2 and back-cross families involving these three races indicates a single gene difference for each of the enzymes. The peptidases and peroxidases are monomeric proteins with heterozygotes recognised by the presence of the two parental bands; however, heterozygotes for the acid phosphatase and transaminase

loci have an additional band of intermediate mobility which suggests that these proteins have a dimeric structure.

These and other races of Arabidopsis form parental genotypes for the development of segregating families of different gene frequencies with respect to isozyme alleles. Changes in these gene frequencies will be followed during selection programs for different metrical traits.

References:
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Isoenzyme variability in Arabidopsis thaliana. Genetic basis of the acid phosphatase and leucine aminopeptidase variation

M. JACOBS and F. SCHWIND

(Laboratory of Plant Genetics, Vrije Universiteit Brussel, Belgium)

The purpose of this work was to establish the genetic relationships between the different acid phosphatase and leucine aminopeptidase isozymes observed in Arabidopsis.

1. Acid phosphatase (AP)

Preliminary investigations have shown the occurrence of three main types of AP electrophoretic patterns (JACOBS, 1971). Using similar technical procedures we have accomplished a more extensive review of the existing polymorphism. Each of the tested 36 geographical races corresponds to one of the 3 types previously defined as fast (F), intermediate (I) or slow (S) (Table 1).

Table 1: Distribution of the tested races according to their electrophoretic pattern

Electrophoretic phenotypes				
Fast (F)	Intermediate (I)			Slow (S)
Chi	Wil	Oy	Bch	Zü
Rou	Dr	Co	Na	232 (St)
Stw	Tu	Te	Hi	
Gie	Pi	Ct	Gr	
Kn	Ta	Mr	Je	
Wa	Di	Lu	Mt	
In	St	Bl	Ei-5	
Rschw	öst	An	Ei-6	
	Gö	Tsu		

Crosses between these different types have been made and the analysis of F_2 progenies is given in Table 2. These results are consistent with the hypothesis that the phenotypic differences are controlled by three alleles of a single gene Ap_1, each specifying a set of isozymes with different rates of anodal migration in electrophoresis at pH 8.3. The third band of each set is the most intense. A certain number of isozymes belonging to different series were found to correspond in migration rates. In heterozygotes, the enzyme types specified by each allele migrate to the same positions as in the respective homozygotes. There is no evidence for the formation of hybrid enzymes (Fig. 1). The observed variation between the

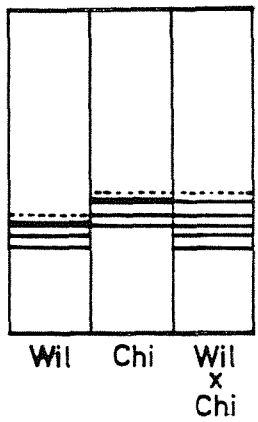

Figure 1:

Electrophoretic patterns of acid phosphatase from rosette leaves in Wil-2 (type I), Chi (type S) and in the F_1 hybrid obtained from crosses between these two races

Table 2: Segregation observed in progeny of crosses made to determine the genetic basis of acid phosphatase (AP) variation

Crosses		F_2 electrophoretic phenotypes					Probability of χ^2-value
Female Parent	Male Parent	F	I	S	Hetero-zygote	Total	P
F x F							
Rou	x Chi	14	0	0	-	14	-
F x I							
Rou	x Wil -2	3	2	0	6	11	$0.20 < P < 0.30$
Chi	x Wil -2	36	30	0	68	134	$0.10 < P < 0.20$
F x S							
Chi	x Zü	2	0	1	1	4	
I x F							
Öst	x Kn	6	7	0	13	26	$P < 0.05$
I x I							
Tsu	x Wil -2	0	22	0	-	22	-
I x S							
Wil -2	x Zü	0	16	15	36	67	$0.10 < P < 0.20$
S x F							
Zü	x Chi	8	0	9	16	33	$0.05 < P < 0.10$
232	x Chi	90	0	82	147	319	$0.30 < P < 0.40$
S x I							
Zü	x Wil -2	0	5	7	15	27	$0.30 < P < 0.40$

three AP patterns results thus from the action of three alleles of $\underline{Ap}_1$ gene that are expressed without dominance.

This investigation will be followed by further research on the nature of the differences between isozymes of one electrophoretic set controlled by one $\underline{Ap}_1$ allele and on the nature of the genetic control of their activity.

2. Leucine aminopeptidase (LAP)

LAP zymograms show two main zones of activity (F and S bands) and among 48 tested races, only one variant with a slower migration of the more anodal band has been found in the races An, En-1 and Sa (Sacavem). There is no evidence for the formation of hybrid enzymes in heterozygotes. In back crosses between F_1 hybrids and parental races, approximately half of the offspring showed the parental type and half the hybrid type. In the F_2 generation, the three phenotypes were represented in proportions which fit a 1:2:1 segregation (Table 3).

Table 3: Phenotypes obtained in progeny of self-fertilizations and of crosses made to determine the genetic basis of leucine aminopeptidase (LAP) variation

Crosses		LAP phenotypes			
Female Parent	Male	F	Hetero-zygote	S	Total
F/F	F/F	40	0	0	40
S/S	S/S	0	0	40	40
F/F	S/S	0	38	0	38
S/S	F/F	0	40	0	40
F/S	F/F	16	18	0	34
F/S	S/S	0	22	21	43
F/S	F/S	28	85	44	157

We can conclude that the two fast LAP isozymes are controlled by a single gene locus with two codominant alleles. This locus can be named $\underline{Lap}_1$ with the two alleles as $\underline{Lap}_1^1$ and $\underline{Lap}_1^2$.

Reference:

JACOBS, M.: Arabid.Inf.Serv. 8, 20 (1971)

Détermination des sucres libres dans les rosettes de feuilles d'Arabidopsis thaliana normales et mutées

M BOUNIAS*

(Laboratoire d'Histochimie-Autoradiographie - CEN-Cadarache - (13) - France)

Les sucres libres contenus dans les feuilles d'Arabidopsis thaliana, race Estland (Aimablement fournie par le Dr. J. LANGRIDGE) ont été analysés en vue de compléter l'étude biochimique d'un mutant viridis V-20 monofactoriel obtenu par irradiation γ de graines sèches. Les plantes sont cultivées en tubes à essais sur sable et liquide minéral "minimum", à 25°C et sous une photopériode de 19 H de jour (BOUNIAS, 1967). Les analyses ont été effectuées à divers stades: avant, pendant et après l'apparition des hampes florales, soit en particulier : 13 jours - 17 jours et 24 jours respectivement.

Le Glucose, le Fructose, le Maltose, le Lactose ainsi que deux polyholosides et l'amidon ont été séparés et dosés par chromatographie quantitative en couche mince (plaques préfabriquées KODAK - K 511 V) (BOUNIAS, 1969). Leur identification repose sur des techniques chromatographiques (séparations bi-dimensionnelles, superposition de sucres étalons aux extraits) et photométriques (spectre d'absorption et cinétique de coloration avec l'anisaldéhyde sulfurique). Le tableau ci-dessous donne les résultats au cours du développement des plants normaux et la comparaison avec les mutants au stade d'apparition des hampes (17 jours).

Tableau: Teneurs des rosettes de feuilles d'Arabidopsis en sucres libres (x 10^{-9} Moles/mg de poids frais)

Stades	Glucose	Fructose	Maltose	Lactose
Tém. 13 j.	3,15 ± 0,3	1,0 ± 0,03	0,27 ± 0,08	0,17 ± 0,02
Tém. 17 j.	1,83 ± 0,3	1,16 ± 0,03	0,28 ± 0,10	0,08 ± 0,01
T/M (%) 17 j.	76,5%	78,4%	126,6%	75,0%
Tém. 24 j.	2,65 ± 0,6	1,55 ± 0,15	0,42 ± 0,10	0,14 ± 0,06

Ces résultats montrent que le glucose est fortement consommé au début de l'apparition de la hampe florale, chez les plants normaux. Le mutant présente une réduction de teneur en sucres (sauf le maltose) consécutive à l'affaiblissement de sa fonction chlorophyllienne, mais proportionnellement moins importante, car les teneurs en chlorophylles (a) et (b) du mutant par rapport aux témoins sont de 48,7% et 34% respectivement.

Summary:
The free sugars present in Arabidopsis rosette leaves (normal type and viridis mutant) have been identified and determined. Glucose is heavily consumed at the stage of the beginning stem growth. In the mutants glucose, fructose and lactose are reduced and the maltose concentration is increased.

Références:
BOUNIAS, M.: D.E.A., Lyon, 12 p. (1967)
- : Chimie Analytique 51 (2), 76-82 (1969)

*Present address: Station de Zoologie, CRA d'Avignon - 84 - Montfavet - France

Relations entre les amino-acides libres et l'equipement pigmentaire photosynthétique chez Arabidopsis (plants normaux et mutants viridis)

M. BOUNIAS

(Laboratoire d'Histochimie-Autoradiographie - CEN-Cadarache 13 - France)

Au cours de recherches biochimiques sur les mutations chlorophylliennes de végétaux supérieurs, les relations entre pigments photosynthétiques et amino-acides libres ont été étudiées au niveau des rosettes de feuilles d'Arabidopsis thaliana, normales et mutées. Les plants normaux (race Estland) et les mutants viridis (V-20) sont cultivés aseptiquement dans des conditions précédemment décrites (BOUNIAS, 1967, et les métabolites sont analysés par chromatographie quantitative en couche mince (BOUNIAS, 1969-a).

Au cours du développement des plants normaux, les concentrations en pigments chlorophylliens passent par un maximum au moment de l'apparition des hampes (stade 17 jours), et la plupart des 22 amino-acides étudiés (excepté ceux du cycle de l'arginine) suivent des variations de sens opposé. De même, le mutant, qui contient deux fois moins de chlorophylles que les témoins, présente une accumulation importante d'amino-acides (BOUNIAS, 1969-b). Ces résultats sont en accord avec ceux de SVACHULOVA, 1967.

Une atténuation de lumière (de 2500 à 25 lux), pendant 5 heures, provoque une chute de teneurs en pigments, aussi bien chez les témoins que chez les mutants, et les concentrations en amino-acides augmentent alors à nouveau en conséquence (Tableau).

Les variations de la somme : leucine + Iso-leucine + Valine + Phényl-Alanine + Tryptophane + Lysine + Histidine + Ac. Aspartique + Asparagine + Ac. Glutamique + Glutamine s'expriment quantitativement en fonction des teneurs en chlorophylle (a) selon une décroissance logarithmique - (régression linéaire en coordonnées semi-logarithmiques - coefficient de corrélation : p = - 0,92) -

Tableau: Teneurs en amino-acides libres et en pigments chlorophylliens chez les Témoins et Mutants viridis d'Arabidopsis
($\times 10^{-9}$ M/mg de poids frais)
(T = Témoins M = Mutants N = Lumière Normale A = Lumière Atténuée)

Amino-Acides	TN 13 jours	TN 17 jours	TN 21 jours	TN 24 jours	MN — % TN	TA — % TN	MA — % MN
Lysine	0,077	0,046	0,133	0,051	236	233	175
Histidine	0,067	0,061	0,044	0,069	101	131	116
Ac. Aspart.	0,23	0,13	0,23	0,13	176	215	178
Asparagine	0,63	0,32	0,64	0,44			
Sérine	1,20	0,93	0,92	0,86	105	105	107
Glycocolle	0,38	0,50	0,29	1,90	60	54	79
Ac.Glutamique	1,27	0,90	1,0	1,25	112	125	117
Glutamine	1,1	0,82	0,92	1,1			
Thréonine	1,24	0,9	1,5	1,63	135	103	158
Alanine	0,68	0,57	1,13	0,75	147	146	113
Valine	0,12	0,087	0,09	0,121	111	362	337
Méthionine	0,063	0,048	0,151	0,035	179	117	131
Tyrosine	0,044	0,027	0,025	0,035	137	285	350
Iso-leucine	0,053	0,040	0,040	0,060	91	410	445
Leucine	0,068	0,056	0,059	0,060	81	410	425
Phényl-ala.	0,095	0,065	0,046	0,051	125	169	360
Tryptophane	0,023	0,014	0,005	0,025			
Chlorophylle a	0,75	1,02	1,01	0,84	48,7	68	69,7
Chlorophylle b	0,36	0,51	0,50	0,49	34	58,6	66,7

L'un des faits les plus importants est l'opposition entre l'augmentation considérable de teneurs en leucine et iso-leucine sous l'effet de la lumière atténuée chez les témoins comme chez les mutants, et leur teneur anormalement faible dans les mutants en lumière normale.

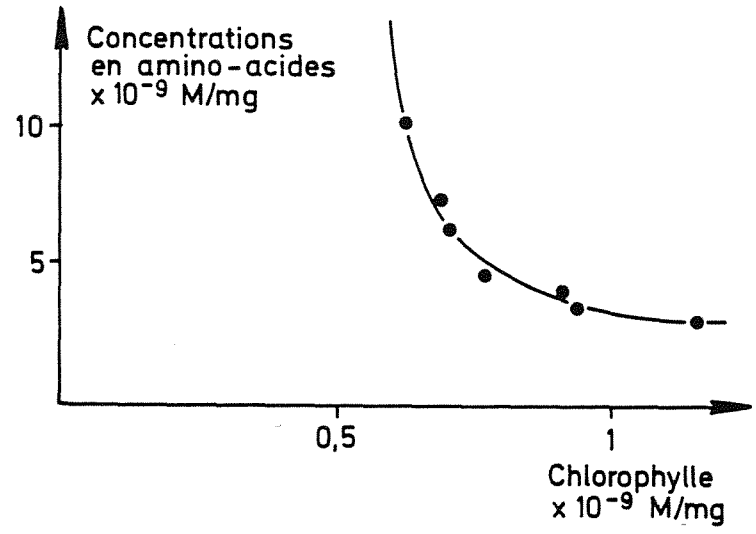

Figure: Variations des concentrations en 11 amino-acides libres en fonction des teneurs en chlorophylle (a) chez Arabidopsis thaliana, témoins et mutants

Summary:
Quantitative relationships between free amino-acids and chlorophyll pigments have been established in Arabidopsis mutants and normal plants. The amino-acid concentrations increase, when the chlorophyll pigment levels decrease, particularly under dim light where leucine and iso-leucine show very large accumulations. These two amino-acids, thus, are not accumulated in the chlorophyll mutants.

Références:
BOUNIAS, M.: D.E.A., Lyon, 12 p. (1967)
- : Colloque Intergroupe de Photosynthèse CEA - 4 p. (1969-b)
SVACHULOVA, J.: Biol.Plant. 2, 34-40 (1967)

Activité phosphatasique et teneurs en phosphates au niveau des rosettes de feuilles d'Arabidopsis normales et mutées

M. BOUNIAS

(Laboratoire d'Histochimie-Autoradiographie - CEN-Cadarache 13 - France)

L'activité phosphatasique alcaline et les teneurs en phosphates minéraux et organiques ont été déterminées chez Arabidopsis, race Estland en vue de l'étude biochimique de mutants chlorophylliens.

La cinétique et les paramètres d'activité ont été établis à pH 7,5, à l'aide d'une micro-méthode quantitative (BRETON et al., 1967) avec comme substrat chromogène : le para-Nitro-Phényl-Phosphate (pNPP) qui libère du para-Nitro-Phénol (pNP) coloré en jaune sous l'action de l'enzyme. Les plants normaux et les mutants viridis V-20 sont cultivés aseptiquement à $5^{o}C$ sous 19 H de jour et 5 H de nuit. Les résultats sont donnés avant, pendant, et après le stade d'apparition des hampes florales soit respectivement : 12 jours/16 jours - 18 jours/31 jours.

Tableau 1: Activité phosphatasique chez les plants normaux et mutés d'Arabidopsis : vitesse maximum : VM_{-} ($x10^{-3}$M/L/H de pNPP) et constante de Michaelis apparente : Km ($x10^{-5}$M/l pNP)
- concentration enzymatique : 20 mg de poids frais/ml

Stade	VM		Km	
	Témoins	Mutants	Témoins	Mutants
12 jours	1,32 ± 0,03	1,69 ± 0,3	5,1 ± 0,3	4,4 ± 0,5
16 jours	1,37 ± 0,05	-	5,5 ± 2,2	-
18 jours	1,70 ± 0,23	2,23 ± 0,25	4,7 ± 0,8	5,7 ± 2,8
31 jours	2,03 ± 0,50	2,63 ± 0,50	4,6 ± 1,1	5,8 ± 1,4

Le tableau 1 montre que la vitesse maximum augmente avec un point d'inflexion au stade d'apparition des hampes florales (17 jours). Les mutants atteignent une vitesse maximum supérieure à celle des témoins, mais ne présentent pas de variations significatives de la constante de MICHAELIS apparente, ce qui correspond soit à une augmentation de concentration en enzyme, soit à la levée d'une inhibition non compétitive par rapport aux plants normaux.

Les phosphates ont été dosés par colorimétrie (méthode du complexe phosphomolybdique (MARINI, 1965) sous les formes : phosphate minéral libre, phosphate organique hydrosoluble (extrait par l'acide trichloracétique) et phosphate organique éthérosoluble (extrait par l'éther di-éthylique).

Le tableau 2 montre que toutes les formes de phosphates varient chez les mutants dans un sens opposé à celui des témoins. Les mutants présentent un excès de phosphate libre et de phosphate organique éthéro-soluble. Dans ce dernier cas, le principal facteur d'accumulation a été identifié à un chroménol-phosphorylé (forme cyclisée de plastoquinone). Par contre, les phosphates organiques hydrosolubles (dont l'ATP et ses dérivés) tendent à s'épuiser.

La mutation chlorophyllienne V-20 se traduit donc par une déviation de la phosphorylation associée à une augmentation d'activité phosphatasique alcaline.

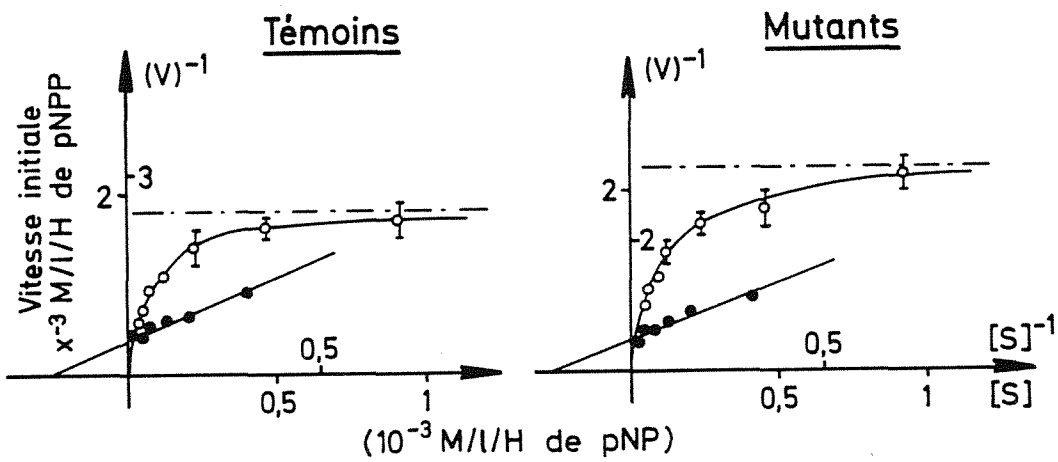

Figure: Activité phosphatasique chez Arabidopsis thaliana (Normaux et Viridis) : Variations de la vitesse initiale de réaction en fonction de la concentration en substrat S
Courbe de MICHAELIS:
Droite de LINEWEAVER et BURK:

Tableau 2: Teneurs en Phosphates chez Arabidopsis, en µg/g de poids frais : M = Mutants T = Témoins

Stades de croissance	Phosphate minéral libre:		Phosphates organiques:			
			hydrosoluble		éthéro-soluble	
	T	M	T	M	T	M
15 jours	214		++		16	
20 jours	232		22		58	
24 jours	149	174	25	68	68	60
1 mois	87	250	30	20	53	75

Summary:
The alkaline phosphatase activity in Arabidopsis rosettes increases at the beginning of the stem emergence. A viridis mutant (V-20) shows a deviation of the phosphate metabolism associated with an increase of the phosphatase activity without significant difference of the MICHAELIS constant.

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Rôle de la L. leucine dans la régulation de l'appareil photosynthétique de végétaux supérieurs : application au reverdissement d'un mutant d'Arabidopsis

M. BOUNIAS

(Laboratoire d'Histochimie-Autoradiographie - CEN-Cadarache 13 - France)

Des travaux antérieurs ont montré que la leucine est particulièrement sensible aux variations de teneurs pigmentaires (BOUNIAS, 1969 et 1972) et une étude histochimique a permis de mettre en évidence sa localisation à concentrations très élevées dans certains granules chloroplastiques (KHAU-VAN-KIEN et BOUNIAS, 1969). Une série de recherches effectuées sur l'Orge a montré que la leucine administrée à faibles doses induit un verdissement progressif suivi, à dose plus élevée, d'une inhibition de plus en plus forte de l'équipement pigmentaire. Le seuil d'inhibition est voisin de $8 \cdot 10^{-5}$M/l.

Tableau 1: Action de la leucine à doses croissantes sur l'équipement pigmentaire de l'Orge cultivé aseptiquement en tubes à essais

Pigments (nM/mg)	Doses de leucine administrées (x 10^{-3}M/l) 0	2	4	8	16	24
chlorophylle a	0,90	0,93	0,98	1,06	0,69	0,48
chlorophylle b	0,43	0,50	0,57	0,52	0,32	0,22

Ce double rôle de la leucine a été vérifié sur les plants d'Arabidopsis normaux et mutés. Les doses nécessaires sont, cependant, plus faibles que celles employées pour l'Orge.

Tableau 2: Influence de la concentration de leucine sur le verdissement des plants d'Arabidopsis normaux et mutés. Les résultats sont exprimés en % par rapport aux plants témoins cultivés sans leucine

Pigments	Dose faible $0,8 \cdot 10^{-3}$M/l		Dose inhibitrice $1,5 \cdot 10^{-3}$M/l	
	Normaux	Mutants	Normaux	Mutants
chlorophylle (a)	112%	123%	86%	92,5%
chlorophylle (b)	119%	129%	74%	77%

L'incidence des phénomènes d'activation et d'inhibition sur la synthèse des pigments a été vérifiée à l'aide de leucine-(U)-^{14}C. Les résultats ont montré que la L-leucine, selon les doses administrées, peut, tour à tour, stimuler ou inhiber sa propre incorporation dans les pigments photosynthétiques.

L'utilisation de L-leucine à dose stimulante a permis d'obtenir également le verdissement accru de plusieurs mutants d'Orge. De plus, il a été constaté qu'en phase activatrice, les mutants absorbent des doses de leucine plusieurs fois supérieures à celles absorbées par les plants normaux, de telle sorte qu'une concentration en leucine située à la limite de la stimulation pour les mutants, est déjà inhibitrice pour les témoins : ceci permet d'expliquer les résultats préliminaires de KAS'YANENKO dans lesquels la même dose de L-leucine, qui provoque un verdissement des mutants, entraîne un effet inverse sur les témoins (KAS'YANENKO, 1970).

Le rôle de la leucine vis à vis de l'équipement pigmentaire est confirmé par l'action de son antimétabolite : l'aza-4-leucine, qui provoque très rapidement, à dose faible, (10^{-6}M/l), une importante réduction des taux de pigments photosynthétiques.

Tableau 3: Variations de teneurs pigmentaires (en M x 10^{-9}/mg de poids frais) chez Arabidopsis (stade 13 jours) sous l'action de l'Aza-4-leucine (10^{-6}M/plant)

Pigments	Plants témoins	Plants normaux + Aza-4-leucine
chlorophylle (a)	0,674	0,41
chlorophylle (b)	0,36	0,28

La leucine se présente donc comme un facteur de régulation de l'équipement chlorophyllien. Les travaux précédents montrant l'augmentation d'activité phosphatasique chez le mutant Viridis V-20, associée à une semi-carence en leucine (BOUNIAS, 1972 a et b) laisse présager un rôle de cet amino-acide au niveau de la régulation de l'activité phosphatasique alcaline.

Summary:
Experiments with Arabidopsis and Hordeum, gave evidence that L-leucin is able to act as a regulator of the photosynthetic apparatus with an increase of the level of photosynthetic pigments at low dosages followed by an inhibition at higher dosages. These effects have been controlled by incorporation of leucin -(U)-^{14}C in chlorophyll pigments, and by the strong inhibition of the photosynthetic apparatus obtained with a leucin antagonist : 4-Aza-leucin. These results allowed us to control the greening of several chlorophyll mutants.

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Callus growth and differentiation in liquid media

A. CORCOS and R. LEWIS

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

One of the major questions in biology is how cells, tissues, and organs differentiate. With the availability of callus cultures, one could hope that the problem of vascular tissue differentiation might be approached quite directly.

The first step would be to obtain undifferentiated cells in callus and from callus. The second step would be to subject them directly to the proper conditions, including the proper environmental condition in order to induce them to differentiate into organs.

The last few months we have attempted with some success to make the first step. A small amount of callus (7 mg) obtained from previous cultures (CORCOS and LEWIS, 1971) was transferred into 2 media and grown on a horizontal shaker. The first medium, H19, contained Heller minerals 20 g of dextrose/liter, 90 cc of filtered V-8 juice, 120 cc of coconut milk, and 3 mg of 2-4D. The second medium, H20, contained the same components as H19, but 2-4D was omitted. In the H19 medium the callus grew to form rounded, nodular, yellow clumps of cells. These clumps of cells showed little internal differentiation. Sometimes tracheids were present, but not abundant. Individual cells were seen floating in the medium. The callus grew well and was subcultured with ease and was stored for a few months in the refrigerator and remained viable. In the H20 medium which did not contain 2-4D, the callus differentiated into root tips with root hairs. The callus turned brown with age and was tougher.

These experiments indicated that H19 was a good medium to grow Arabidopsis callus. However, this medium contains two complex organic components, coconut milk and V-8 juice, and this is a drawback to further exploration. Replacement of coconut milk by kinetin and V-8 juice by vitamins seemed to be a logic step. Hence callus and cell growth experiments were performed in the following media:

1. H19, a control, has been described.
2. H23, in which coconut milk had been replaced by 1 mg/liter of kinetin.
3. H30, in which coconut milk had been replaced by 1 mg/liter of kinetin and V-8 juice by 2 mg of glycine, 0.5 mg of niacin, 0.1 mg of pyridoxin HCl, and 100 mg of inositol.

The H23 medium supported faster growth than did H30 or H19 (Fig. 1). The callus was in the form of nodular clumps of cells. There were very few tracheids at first, but they sometimes became more numerous as the culture aged.

The growth of the callus in H30 medium was less rapid than in the H23, but faster than in the H19 medium (Fig. 1). The callus masses were irregular in shape with irregular growth points. They contained a large number of tracheids. As the culture aged, root primordia appeared. The cells had a wider range of shapes from circular to cubicle, to elongated in parallel groups.

Though the callus can be subcultured easily in the H30 medium, this medium is not satisfactory as the H23. More research must be done to obtain a better synthetic medium.

All these experiments indicated that the presence of 2-4D in the medium is necessary to keep cells undifferentiated. ANAND (1966) reported that "in a medium containing 10^{-7} M 2-4D and 15% coconut milk, Arabidopsis callus produced a large number of root primordia over the entire callus surface. In the course of two weeks these primordia grew as roots and after 2 months of growth their length was increased tremendously". He added, "This observation is of particular interest since TORREY and SHIGEMURA (1957) reported previously that 2-4D suppressed organization of roots". However, TORREY and SHIGEMURA used 2-4D at 10 times ANAND's concentration in their pea cultures and we used 2-4D at 100 times his concentration. See also STABA and LAMBA (1963).

We obtained root primordia from callus in H19 medium containing 0.5 mg of 2-4D instead of 3 mg. In addition we have been growing callus on solid media containing 2-4D, corn water, glucose, and minerals for months and the callus never produced any roots. Hence a high concentration of 2-4D permits growth but little or no differentiation.

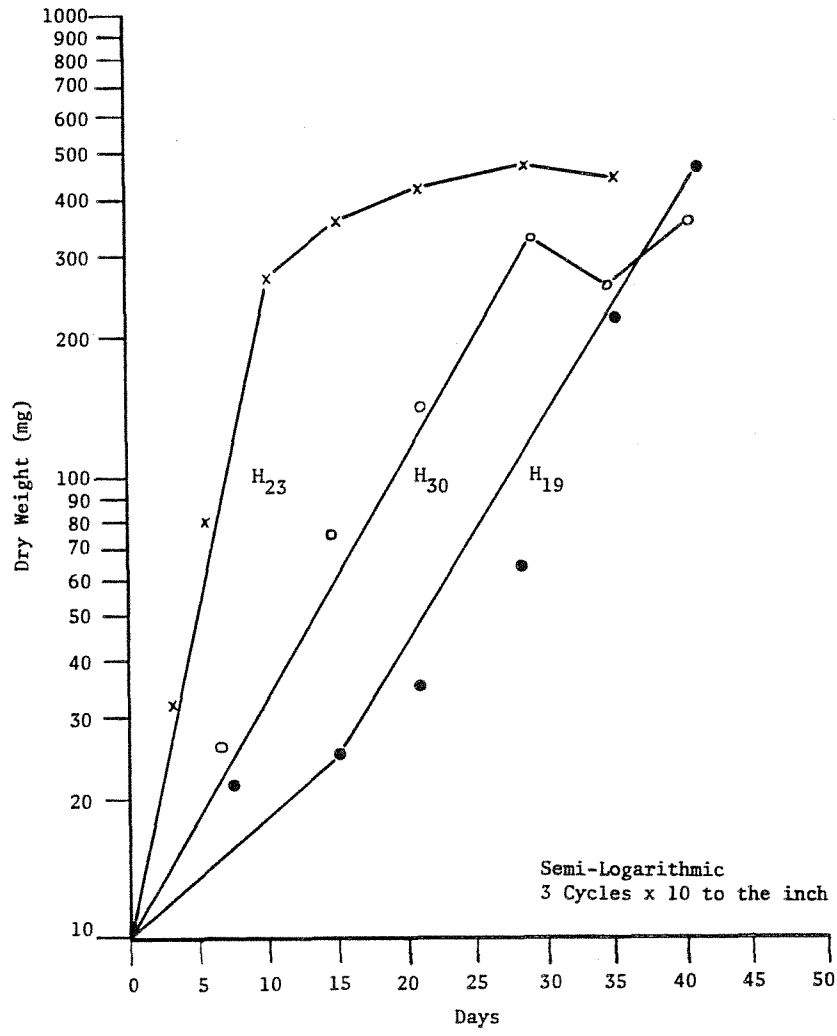

Figure 1: Growth curves of cells and callus in different media

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Difference and derivative spectra of chlorophylls in various ch genotypes of Arabidopsis thaliana

A.R. KRANZ

(Fachbereich Biologie, Universität Frankfurt/M., Germany-BRD)

The gene loci of two homoallelic mutants (ch^1, ch^2) and of a third mutant (ch_3) have primary effects on the biosynthesis of chlorophyll b (Cb) and secondary effects on the production of other chloroplast pigments (KRANZ, 1971). In agreement with preceding results the mutant ch_3 is found to be based on the recessive alleles of a second ch gene, which behaves complementary to the alleles ch^1 and ch^2 of the first gene, i.e. the double heterozygote $+ch_3/ch^1+$ resembles the green phenotype of the wildtype, and the double recessive genotype ch^1ch_3/ch^1ch_3 is lethal, whereas the two single recessives resemble the light green and yellow phenotypes of the parental mutants ch^1 and ch_3. Further information on the extent and time of gene action in the process of the chlorophyll formation will be obtained by refining the analytic methods for the detection of the pigment precursors.

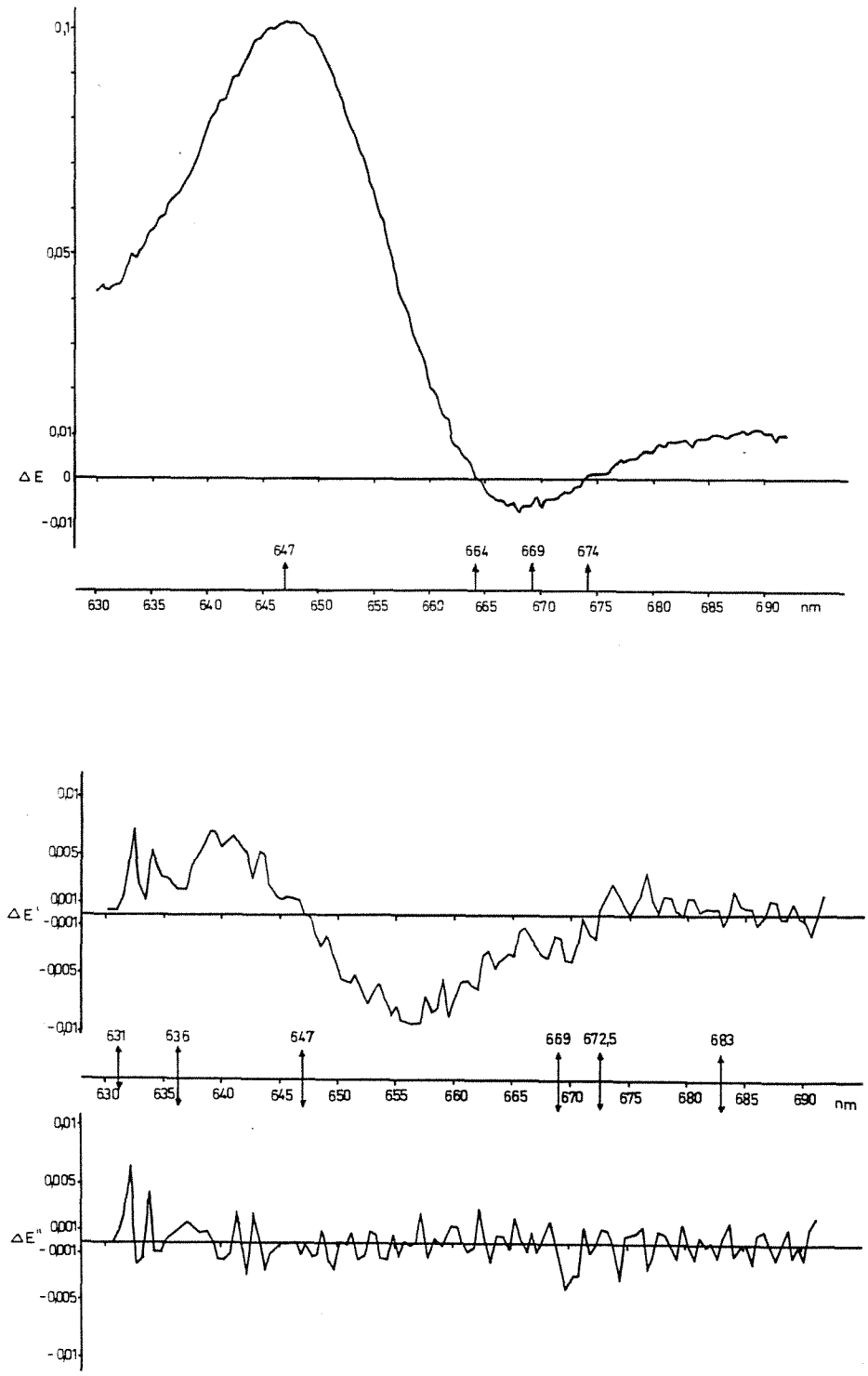

Figure 1: Difference (ΔE) (above) and derivative spectra (ΔE', and ΔE'') (below) of the double heterozygous genotype $\underline{+ch_3/ch^1+}$ minus the parental mutant $\underline{ch^1+/ch^1+}$ absorption

This analysis emphasizes the differences (ΔE) in the absorption spectra of the leaf pigments of the different genotypes and not the absolute values of the extinction (E). The derivation of ΔE is very effective in demonstrating small differences (SHIBATA, 1957; MEISTER, 1966). For that reason the extinction of the extracts (acetone 80 p.c.) of plants with one flower was measured with a Zeiss PMQ II spectrometer; the data were stored by punched tape, the difference was corrected for chlorophyll a (Ca) and its first and second derivative ($\Delta E'$, $\Delta E''$) was calculated by computer processing. In Figure 1 an example is given; its upper part showes the difference spectrum calculated from the extinction of the green heterozygote $\underline{+ch_3}/\underline{ch^1+}$ minus that extinction of the homozygous mutant $\underline{ch^1+}/\underline{ch^1+}$ without Cb. The lower graphs show the spectra of the first and second derivation. $\Delta E = 0$ signifies equal absorption of the same pigment at the wavelength concerned. $\Delta E' = 0$ and $\Delta E'' \longrightarrow$ minimum means pigments in excess in the green heterozygote.

The results so far obtained by this method are summarized as follows: 1) Besides the end products of the chlorophyll biosynthesis, Ca_{664} and Cb_{647}, five pigments can be observed at certain wavelengths. 2) In the green genotypes resembling the wildtype the content of the pigment for $\lambda_{\Delta E\ max}$ = 631 nm (P_{631}) exceeds that of the mutants $\underline{ch^1}$ and $\underline{ch_3}$. 3) When the difference spectrum is calculated from the absorptions of the heterozygotes minus the $\underline{ch^1}$, no excess in the pigment P_{636} can be observed. 4) Substracting the absorption of the $\underline{ch_3}$ from the heterozygotes the derivative spectra show more P_{636} in the heterozygotes than in the mutant. 5) Probably there are differences in the pigment C_{669} between the double heterozygotes from reciprocal crosses. 6) The double heterozygotes contain more pigment C_{684} than the mutants.

The results from this analysis show that the synthesis of P_{631} rsp. P_{636} is probably blocked to a certain extent by the mutant genes concerned. Complementary interactions between the first and the second $\underline{ch}$ gene on the other pigments can be observed. Further experiments will be necessary to confirm that the five pigments are transformation products in the biosynthesis of Ca_{664} and Cb_{647}. Hereby more precise information about the primary action of the different genes on the reactions during the chlorophyll formation can be expected.

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Genetic analyses of 11 chlorina mutants by diallel crosses

G. RÖBBELEN

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

Eleven chlorina mutants induced in the race En-2 of Arabidopsis, showing rosettes of uniform yellow-green colour and nearly normal morphology and growth had been earlier subjected to mutagenic treatment for a somatic reversion test (RÖBBELEN, 1969, 1972). Simultaneously, genetic analyses of these mutants were also taken up. Their first results were already reported (RÖBBELEN, 1970).

The accuracy of classification of F_1 p l a n t s by visual observation was compared with photometric determination of their pigments (using 80% aceton extracts) from whole rosettes shortly before the onset of flowering (cf. RÖBBELEN, 1957 , for procedure). The results confirmed our belief that visual classification of chlorophyll mutants is as good as any grouping based on photometric determinations. It is also simpler and quicker.

Some of the 11 mutants can be distinguished by their leaf colour (cf. the diagonal of the half chess-board in Fig. 1) and also by their size or minor characteristics of their rosettes. The mutants V 93 and V 95 are almost alike, and so are V 290 and V 295; while V 312 and V 81 are slightly and V 157 definitely "green hearted"; V 308 is often slightly "yellow hearted".

To establish linkage relationships, several mutants were crossed with RÉDEI's markers (cf. RÉDEI and HIRONO, 1964, and Arabidopsis Research, Rep.Int.Symp. Göttingen 1965, p. 210). The chlorina mutant character of V 157 was found to be linked with $\underline{an}$ (narrow leaves; group 1), the two being 12.6 Morgan units apart (calculated from 3705 F_2 plants of V 157 an x wild type at coupling phase). The non-complementary nature of F_1's of V 89, V 300, V 312, and V 318 indicated allelism of their mutant characters. This locus is linked to $\underline{vc_2}$ (shiny stems of inflorescence;

	En	V157	V89	V300	V312	V318	V95	V295	V308	V290	V81	V93
V93	543	532	498	544 563	586	493	563	454	517	493	556	304
V81	566	239	200	342	561	324	502	437 422	392	523	111	
V290	546	543	437	510	514 461		240 535	505	549	222		
V308	443	434	319	458	458 460	329 343	269	262	291			
V295	436	477	254 296	466	485 463	325	217 211	202				
V95	559	523	424 393	472	541	452	235					
V318	517	319	187	283	276	256						
V312	538	446	228 240	271 253	296							
V300	604	418	214	264								
V89	428	183	165									
V157	567	208										

Figure 1:
Leaf colour of F_1 plants.
● green, ◕ light green,
◑ yellowish green,
◔ yellow green and ○ yellow
(= mutant type). The figures in boxes give γ chlorophylls/g fresh weight of leaves (mean of at least two determinations). The top value gives the chlorophyll content of F_1's with the above written mutant as the maternal parent, and the lower value with that listed on the left side as mother.

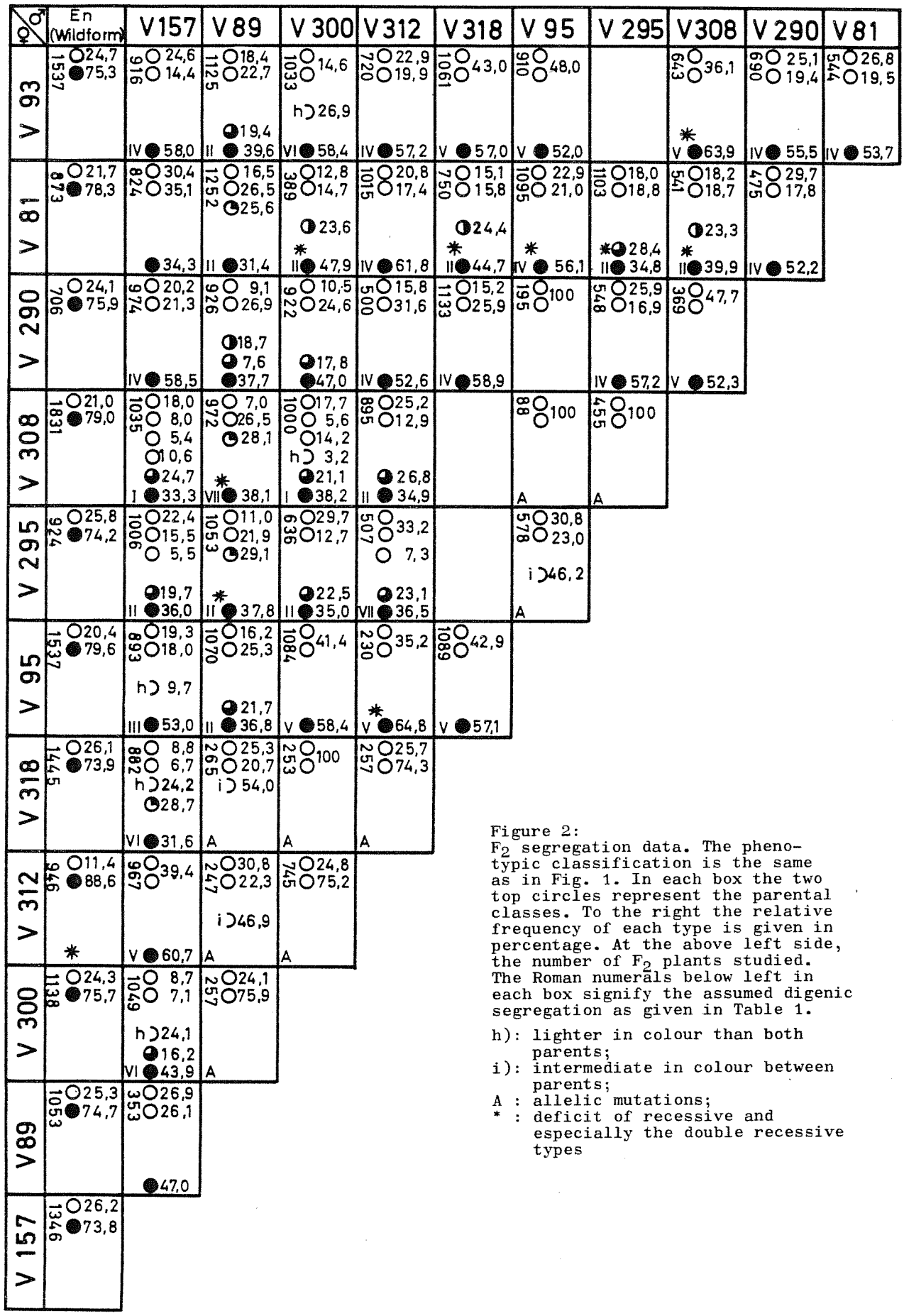

Figure 2:
F_2 segregation data. The phenotypic classification is the same as in Fig. 1. In each box the two top circles represent the parental classes. To the right the relative frequency of each type is given in percentage. At the above left side, the number of F_2 plants studied. The Roman numerals below left in each box signify the assumed digenic segregation as given in Table 1.

h): lighter in colour than both parents;
i): intermediate in colour between parents;
A : allelic mutations;
* : deficit of recessive and especially the double recessive types

group 6), but as yet segregation data from combinations in repulsion phase are only available. Another possible allelic group consists of mutants V 95, V 295, and V 308 (cf. Fig. 1). This locus and the mutated loci for V 290, V 81, and V 93 in Figure 1 did not exhibit closer linkage to each other nor to any of the used morphological markers of RÉDEI.

The non-allelic mutants are generally assumed to show full complementation in the F_1's. However, the data in Fig. 1 again clearly indicate at least partial n o n a l l e l i c n o n c o m p l e m e n t a t i o n in many instances of heterozygous combinations. Similar instances in other organisms (e.g. in Neurospora; FINCHAM, 1966) have been interpreted as indicating that such nonallelic mutants are concerned with the formation of the same heteropolymer. But other explanations may also be possible in our case as chromosome deletions and interactions of entirely unrelated metabolic pathways having a secondary influence on leaf pigment synthesis.

The F_1 V 290 x V 95 was exceptional. It was yellow with plants larger than the parental types. The reciprocal combination was green. The reason for this difference has not been understood.

The F_2 s e g r e g a t i o n data of the diallel crosses are given in Figure 2. The segregation data of crosses with the Enkheim (En) wild type show that all mutants are monogenic and recessive. Only V 312 showed about 50% deficit of recessives. Barring the before-mentioned cases of allelism (A) , hybrids between mutants produced variable classes of phenotypes. In many instances both the parental types appeared; in a few cases the double mutant type was also seen (I, III, VII). In all, dominant and epistatic interactions produced 7 different ratios (Table 1). They were sometimes modified by deficiencies of the recessives (marked by * in Fig. 2). This was specially true in crosses involving V 81. Here the double recessives were not obtained in majority of cases.

Table 1: Assumed digenic segregation

Genotype	Phenotypic appearance like:	Segregation ratios in %							
		I	II	III	IV	V	VI	VII	A
aa ++ 1x	one parent	18.7	25.0	18.7	25.0		6.2	37.5	25.0
aa +b 2x									-
aa bb 1x	double mutant	6.2		6.2		43.7	31.2	6.2	50.0
+a bb 2x									-
++ bb 1x	other parent	18.7	18.7	18.7	18.7		6.2		25.0
+a +b 4x	F_1 type	25.0	25.0				25.0	25.0	-
+a ++ 2x									-
++ +b 2x		31.2	31.2	56.2	56.2	56.2	31.2	31.2	-
++ ++ 1x	wild type								-

Chi square tests suggested several possible cases of misclassification of phenotypes. This may be understandable in view of the relatively high environmental influence on the expression of green-yellow gradations of leaf colour. For instance, several green segregates of the cross V 93 x V 89 were probably "light green" genotypically. Some segregates were difficult to classify in certain other crosses, like V 300 x V 157 and V 300 x V 295 also. In the cross V 290 x V 157 the excess of one of the parental types might have resulted from a random addition of the double mutants. In the cross V 308 x V 157, the fourth class from above did not probably represent a seperate genotype, but combined instead to the second parental class (increasing its frequency to 18.6%).Similarly, in the F_2's of V 308 x V 300 and V 295 x V 157, some of the yellow classes might have to be merged. Finally, observed segregations of V 290 x V 89, V 290 x V 300, and V 81 x V 157, cannot be explained without making progeny tests.

Two other cases remain to be mentioned. (1) The cross V 290 ♀ x V 95 yielded only a single class of yellow F_2 plants. The reciprocal has not been grown. (2) The F_2 from the yellow F_1 of the cross V 89 x V 157 segregated in the ratio 1:2:1, as if the mutated loci of the parents were closely linked. But, it has been seen that the mutated locus of V 89 is linked to group 6 and that of V 157 to group 1. Since the two mutated loci of the parents are nonallelic, the F_1 heterozygote should have appeared like the wild type. Thus this F_1 looks apparently like a case of complete noncomplementation.

In s u m m a r y , the F_1 and F_2 data from this study involving diallel crosses reveal a complex pattern of genetic interactions (even for allelic mutations) in spite of the phenotypic similarity of the selected 11 chlorina mutants. This supports the idea that different "chlorophyll mutations" (which in mutation

experiments are frequently grouped together) may be radically different in respect of their biochemical/physiological nature and genetic origin.

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On the nature of pigment mutations in Arabidopsis thaliana (L) HEYNH.

R.B. BATALOV, K.V. KVITKO, and P.D. USMANOV

(Institute of Plant Physiology and Biophysics, Leningrad State University, Dushanbe, USSR)

The genetic nature of several pigment mutant forms was studied by crossing them with the markers gl^1 an (glabra = absence of hairs on the rosette leaves - 3rd linkage group, angulosa = narrow, stretched leaf - 1st linkage group; RÉDEI, 1965). The Table 1 shows that mutant strains ch(B) and "morphological", induced from ch(B), differ from the other forms by their heterozygosis in respect to lethal factors, which display in the appearance of vana type embryonic lethal (MÜLLER, 1963) in 2/3 of the posterity; this indicates to us that these mutations may be of the chromosome type. Our conclusion is confirmed statistically by analysis of the genotypic ratios inside the hybrid posterity in F_2 for gl^+/gl^- and an^+/an^- at the probability ratios 3:1 using the χ^2 method.

Table 1: Characteristics of the investigated pigment mutants

Name of the mutant	Initial wildtype	Mutagene and moment of treatment	Segregation of chimeric plants	Segregation of mutant heterozygotes	Fertility	Embryonic lethals	Supposed character of the mutation
117/2-lutescens	En	1% EMS, seeds	45 : 7	393 wild : 132 mutant	100%	-	gene mutation
Spurio maculata	En	40 kR γ-rays, seeds	induced by selfsowing	2307 wild : 366 mutant	100%	-	?
24/45-viridi costata	Di	24 kR X-rays, pollen	45 : 11	255 wild : 52 mutant	100%	-	gene mutation
ch(B)-chlorina 80/2*	En	80 kR γ-rays, seeds	6 : 1	357 wild : 123 mutant	75,9%	24,1%	chromosome change
Morphological - viridis	80/2	15 kR X-rays, soaked seeds	69 : 5	260 wild : 20 mutant	68,3%	31,7%	chromosome change

* A detailed description of the mutant "ch(B)" is given by KASYANENKO, and USMANOV (1969).

The mutants described in Table 2 may be divided into two groups: (1) mutants 24/45, "morphological", and a half of families from crossings of the mutant ch(B) show declination from segregation 15:1, displayed in the high number of recessives and (2) 117/2, spurio maculata, and another half of families from ch(B), having no such declination. Mutants 117/2 and spurio maculata behave like true gene mutations. Thus it is assumed, that the mutants "morphological" and 24/25 belong to the chromosome type with lethal effects in chromosome 1 for 24/25 or chromosomes 1 and 3 for the "morphological" mutant.

Table 2: Results of the analysis of the hybrid posterity by the ratio of sums of dominant classes to the quanta of double recessives

Crossings and numbers of familie	Ratio of classes $\frac{gl^+/an^+ + gl^+/an^- + gl^-/an^+}{gl^-/an^-}$	Probability of conformity with 15:1 (χ^2)
$^{117}/_{2}$ x gl^1an (161, 163, 165, 166, 170)	218 : 19	0,99>P>0,95
Spuria maculata x gl^1an (25, 26, 27, 28, 29)	361 : 23	0,95>P>0,80
$^{24}/_{45}$ x gl^1an (71, 74, 76, 77)	465 : 59	0,01>P
gl^1an x ch(B) (1, 2, 5, 9, 10)	196 : 30	0,01>P
gl^1an x ch(B) (4, 6, 7, 8)	60 : 2	0,50>P>0,20
Morphological x gl^1an (31, 34, 36, 37, 40)	241 : 51	0,01>P

Double character of behavior of the mutants ch(B) in crossings is, probably, explained by different quality of gametes, formed in the process of meiosis (the change of the reciprocal translocation type is possible), as a result of that half of hybrid families from this crossing behaves in some cases as the posterity of gene mutation, in other cases as the posterity determined by the chromosome change.

More detailed description of the results of the genetic analysis is presented in the publication of BATALOV and KVITKO (1971).

Besides that, a scheme was elaborated on the genetic testing of lethal forms in Arabidopsis thaliana, which may help to reveal allelism and aid the genetic mapping in such mutant strains (BATALOV and KVITKO, 1971).

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Quantitative estimation of plastid microstructures in Arabidopsis thaliana

H.A. ABDULLAEV, P.D. USMANOV, S.V. TAGEEVA, and Y.S. NASYROV

(Institute of Plant Physiology and Biophysics, Acad. Sci. Tajik SSR, and Institute of Biophysics, Acad. Sci. of the USSR)

The range of variability of the plastid structural components was investigated by technique of quantitative estimation of microstructures using the linear integrater (TAGEEVA et al., 1970; ABDULLAEV et al., 1971). In 10 mutants of Arabidopsis thaliana (representatives of different classes: viridis, chlorina, variegata etc.) the rate of variability of matrix, lamellas, plastoglobules, starch granules and vacuoles was studied (Table 1). On the basis of this data the optimal per cent

Table 1: Limits of variability for areas of different chloroplast components in plants of Arabidopsis thaliana

Chloroplast components	Areas of chloroplast components in % Minimum	Maximum
Matrix	47.2	91.6
Lamellas	14.2	37.2
Vacuoles	3.1	27.3
Starch	2.4	24.0
Osmiophilic globules	0.2	6.5

ratios of volumes of chloroplast structure components were determined; the diagram was constructed representing the statistical model of chloroplast structures in the species of A. thaliana (Fig. 1).

Constructing such micromorphological models for chloroplasts of different taxons and phyletic lines is especially perspective for clearing the chloroplast phylogeny in the plant kingdom.

Morphometric analysis of ultrastructure as well as study of their functional peculiarities also provides the construction of morpho-functional models of chloroplasts.

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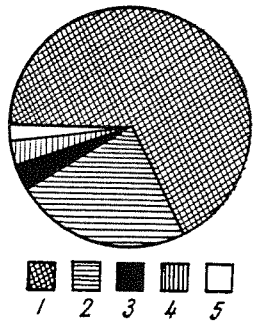

Figure 1: The supposed optimal ratios of areas of the chloroplast components in Arabidopsis thaliana.
1 - matrix; 2 - lamellas;
3 - plastoglobules; 4 - starch;
5 - vacuoles

Radiation induced shoot formation

Erna REINHOLZ

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

IVANOV (1967) reported on the formation of additional generative shoots after treatment of airdry seeds of Arabidopsis (race ENKHEIM) with ^{60}Co-γ-rays (10-160 kR). The percent of "multicaulous" plants increased with the radiation dose to a maximum of 58,7% after exposure to 110 kR. The number of additional shoots per multicaulous plant was very uniform in the whole dose range, i.e. 1.13-1.36. IVANOV had no explanation for this multicaulity-effect: "Our data offer no conclusive indication as to the possible nature of such damage".

In 1947 we observed a corresponding effect after 6 kR of X-rays on 38 h presoaked seeds of race ENKHEIM. Nearly 100% of the X_1-plants had 3-5 generative shoots coming out of the axils of the rosette leaf. It is well known, that the initials in the main shoot apex are very radiosensitive and will, therefore, be destroyed by relatively low doses. Consequently the apical dominance decreases. Thus the axillary buds, which are normally dormant, are able to grow.

(When a lot of seeds from a single plant is needed, the main stem of the inflorescence may be cut off for the same purpose after seed-harvest. Then the axillary buds of the uppermost rosette leaves are also sprouting in a basipetally direction, producing again many seeds.)

This explanation holds also true for the data of IVANOV: The higher the radiation dose was, the more multicaulous plants were observed; the maximum and the decrease after 110 kR are an expression of the lethality of the embryos irradiated with doses higher than kR. On the other side, the number of additional shoots is independent of the dose range and only determined by the physiological state and structure of the shoot apex. Thus I like to explain the discrepancy in the number of shoots per multicaulous plant (IVANOV: 1.13-1.36; REINHOLZ: 3-5) with the different number of initial cells of the shoot apex in airdry and presoaked seeds (embryos).

In many animals the vegetative and generative cells are separated during the first steps of development; in plants the "Keimbahn" is not so early determined. We are going to investigate the number of the initial cells of the embryonic shoot apex by testing conformity and discrimination of X-ray mutations in the different shoots of a multicaulous plant obtained by cutting off the main inflorescence stem.

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The distribution of induced mutations in the shoot apex of Arabidopsis seeds

Jiřina RELICHOVÁ

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

When mutations are induced in the shoot apices of seeds, the probability of simultaneous occurrence of two or more independent mutations in the same nucleus tends to increase with increasing the total frequency of induced mutations. The significance of this fact in the mutation breeding was demonstrated by HÄNSEL (1967) in barley. CETL (1969) and RIESOVÁ-KUČEROVÁ (1969) studied the role of double mutations in the modification of the type of dependence between the concentration of methyl-nitrosourea and the frequency of different types of mutations in Arabidopsis. In all spermatophyta, the situation is complicated by the complex organization of the vegetative shoot apex (cf. ESAU, 1960; POPHAM, 1963) so that LANGRIDGE (1958) was able to estimate, on the basis of mutation experiments with X-rays in Arabidopsis, that the mean number of apical initials per apex is two.

In the present experiments, current doses and/or concentrations of X-rays, ethyl-methanesulfonate (EMS) and methyl-nitrosourea (MNU) applied to dry seeds of Arabidopsis were used to induce lethal and vital chlorophyll and morphological mutations. In the M_2, individual M_1 families were studied separately. If more than one mutation appeared in the same M_1 family under such an arrangement of experiments, it could be due to two alternatives: (1) a double or a multiple mutation was induced in the nucleus of one apical initial, (2) two or more mutations originated in different apical initials. Both named cases could be, of course, combined in some families. By analysing corresponding M_2's (HÄNSEL, 1967) it can be distinguished between these two possibilities. An exact analysis requires, however, to take the complex organization of the shoot apex into consideration.

Our results (Table 1) showed that only one mutation appeared in each segregating M_1 family after X-irradiation. After EMS and MNU, on the contrary, also M_1 families segregating for two or more mutations were observed in the same proportion as those with only one mutation.

Table 1: Results of the analysis of M_1 families after different mutagenic treatment of dry seeds of Arabidopsis

Mutagene	No. of families tested	Segregation of M_1 families				Average No. of mutations per segregating M_1 family	Per cent of mutants in M_2	Per cent of single mutations in M_2
		one mut.type		two and more mut.types				
		No.	Per cent	No.	Per cent			
X 12 to 24 kR	84	0	17.9	0	0.0	1.00	2.13	2.18
EMS 7 to 28 mM	80	15	18.7	15	18.7	1.70	7.30	7.93
MNU 0.05 to 0,20 mM	90	25	27.8	26	28.9	1.72	11.30	12.99

Segregation ratios 3:1, 7:1, 11:1, 15:1, etc. were taken into consideration for estimating the number of one, two, three, four, etc. apical initials carrying a mutation in families with one mutation (LANGRIDGE, 1958). On this basis, a highly significant agreement with the 7:1 ratio (P = 70 to 95 per cent) was found in all mutagenes used. This result suggested that two apical initials, on the average, were involved in such families.

The given estimation of the mean number of apical initials in the shoot apex was used in further considerations: if two independent mutations are arising in one M_1 apex, they could be distributed in the relevant initials in two different ways, i.e., either both mutations in one initial while the other initial was without any mutation ("[2/0]"), or each mutation was located in another initial ("[1/1]") (see RELICHOVÁ and CETL, 1972). Then a hypothesis might be suggested that these two cases were equally possible, P[2/0] = P [1/1]. According to this hypothesis, a half of M_1 families segregating for more than one mutation would segregate in 18/32 non-mutants, 6/32 both "simple" mutants and in 1/32 double mutants. The other half of these families would segregate in 3/4 non-mutants and in 1/4 of both "simple" mutants.

This hypothesis was tested and the expected per cent of different mutants was very near to the observed ones (in EMS: expected 7.38 per cent, observed 7.63 per cent; in MNU: expected 10.31 per cent, observed 11.30 per cent). At the same time, the percentage of double mutations according to CETL (1969) was compared with the estimation of the frequency of double mutations in agreement with our hypothesis. This time, the former values were two or four times higher than the latter ones (in EMS: 0.63 per cent vs. 0.30 per cent; in MNU: 1.69 per cent vs. 0.44 per cent).

Various causes of this discrepance can be given but linkage between the two mutant alleles seems to be the most important: in M_1 apical initials, the mutant alleles are present in coupling so that the double mutant recessive homozygotes are the more frequent the more closely they are linked.

The experiments are continued by analysing the M_2 subfamilies in the M_3. The results yet obtained showed, that some M_1 plants segregating for two mutations carried really both mutations in the same initial ("[2/0/...]") while in the others, the two mutations were induced in two different initials ("[1/1/...]").

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An attempt to classify the distribution of induced recessive mutations in the complex shoot apex of Arabidopsis seeds

Jiřina RELICHOVÁ and I. CETL
(Department of Genetics, Purkyně University, Brno, Czechoslovakia)

In the considerations about the distribution of induced mutations in the complex shoot apex of Arabidopsis (RELICHOVÁ, 1972), we met with the necessity to classify the most probable situations. In the following survey, we summarize some of them with a suggestion to symbolize them:

Description	M_1 apex genotype	Symbol	M_2 segregation ratios
One mutation in a sole initial	$+m_1$	[1]	3:1
One mutation in one of two initials	$+m_1/++$	[1/0]	7:1
One mutation in one of four initials	$+m_1/++/++/++$	[1/0/0/0]	15:1
Two different mutations in one of two initials each	$+m_1$ $++/++$ $+m_2$	[1/1]	6:1:1*
Two different mutations in two of three initials	$+m_1$ $++/++$ $+m_1/++$ $++$	[1/1/0]	10:1:1*
Two different mutations in two of four initials	$+m_1$ $++/++$ $+m_2/++$ $++/++$ $++$	[1/1/0/0]	14:1:1*
Two different mutations in a sole initial	$+m_1$ $+m_2$	[2]	9:3:3:1*
Two different mutations in one of two initials	$+m_1$ $+m_2/++$ $++$	[2/0]	25:3:3:1*
Two different mutations in one of three initials	$+m_1$ $+m_2/++$ $++/++$ $++$	[2/0/0]	41:3:3:1*
Two different mutations in one of four initials	$+m_1$ $+m_2/++$ $++/++$ $++/++$ $++$	[2/0/0/0]	57:3:3:1*
Three different mutations, two of them in one initial, the other one in the second	$+m_1$ $+m_2$ $++/++$ $++$ $+m_3$	[2/1]	12:3:3:1:1*
Three different mutations in a sole initial	$+m_1$ $+m_2$ $+m_3$	[3]	27:9:9:9:3:3:3:1*

/ boundaries between initials and sectors
* if not linked

The segregation ratios given show that there are various difficulties to distinguish between different cases in the M_1 families especially if the family size is not too large. Even if the family size will be much larger than it is usual (IVANOV et al., 1966), it will be uneasy to differentiate topographically very different situations, e.g., [1/1] and [2/0]. Further complications can be due to (1) linkage what is very probable in Arabidopsis with its n=5; (2) diplontic selection between different sectors (GAUL, 1961); (3) differential gametic and zygotic viability of induced mutations; (4) overlapping of different sectors (MÜLLER, 1965); etc.

For this reason, it is necessary to work with very large M_1 families and to precisely analyse them in next generations. Only such very laborious experiments can answer the question how is the real distribution of one, two or more mutations

in the complex vegetative shoot apex of treated seeds in higher plants. This study might also throw light on the structure of the vegetative shoot apex itself (POPHAM, 1963).

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The influence of ionizing irradiation on some quantitative characters of Arabidopsis thaliana

O.G. STEPANENKO, A.M. FERSHTAT, and V.N. SAVIN

(Agrophysical Research Institute, Vaskhnil, Leningrad, USSR.)

Investigation of the influence of different irradiation doses on the plant development requires a complex application of statistical parameters. The aim of this paper is to study the influence of gamma-rays in the doses 10, 30 and 60 kR with the rate $3 \cdot 10^3$ R/min on the following characters of Arabidopsis thaliana plants: 1-the appearance of seminal leaves, 2-appearance of a rosette, 3-the beginning of buttoning, 4-the beginning of flowering, 5-the number of siliques on the main stem, 6-height to the first silique, 7-the number of side shoots.

As can be seen from Table 1, with the increase of the gamma-ray dose, the dates of the development phases occur with some delay. The range of ontogenetical variability of the last three phases (2,3,4) increases at the 30 and 60 kR dose of

Table 1: Influence of gamma-rays on quantitative characters of Arabidopsis thaliana

Characters	Mean				Variation coefficient (%)			
	Test	10 kR	30 kR	60 kR	Test	10 kR	30 kR	60 kR
1	3.5±0.08	3.8±0.11	4.4±0.13	4.6±0.09	16.4±1.6	20.9±2.21	20.9±2.21	14.2±1.43
2	5.9±0.10	6.5±0.10	7.9±0.17	8.3±0.19	11.4±1.12	10.5±1.02	15.2±1.55	15.9±1.57
3	14.9±0.15	15.0±0.11	15.3±0.16	16.5±0.25	6.9±0.71	5.0±0.51	7.2±0.71	10.2±1.02
4	20.8±0.19	21.8±0.21	21.7±0.35	23.9±0.43	6.4±0.51	6.9±0.71	11.1±1.12	12.2±1.23
5	9.3±0.50	10.8±0.50	8.9±0.43	8.6±0.48	37.0±4.21	31.7±3.55	27.0±2.92	31.0±3.42
6	10.7±0.27	10.4±0.13	8.5±0.27	8.0±0.33	17.0±1.71	18.8±1.92	18.0±1.87	23.0±2.44
7	2.3±0.21	2.0±0.22	3.0±0.23	3.3±0.22	63.3±8.53	74.4±10.82	53.0±6.69	46.0±7.69

irradiation. In the paper by FERSHTAT et al. (1971) a close correlation of the development phases of Arabidopsis plants was demonstrated, forming a correlation galaxy. In Table 2 the correlation matrixes of the development phases for Arabidopsis plants grown from the irradiated seeds are given. No reliable correlations of the development phases with characters 5,6 and 7 and between 5,6 and 7, as well, were observed: Irradiation of 10 kR delayed insignificantly the occurrance of the development phases, breaking at the same time the development phase correlations. Irradiations with 30 and 60 kR delayed all the development phases without violating the phase displacement conjugation.

Table 2: Influence of gamma-rays on the development phase correlation of Arabidopsis thaliana

Development phases	Correlation coefficient (R)											
	Test			10 kR			30 kR			60 kR		
	2	3	4	2	3	4	2	3	4	2	3	4
1	0.60 ±0.09	0.54 ±0.10	0.40 ±0.12	0.40 ±0.12	0.11	0.10	0.51 ±0.10	0.24	0.32	0.63 ±0.09	0.33	0.52 ±0.10
2		0.58 ±0.09	0.55 ±0.09		0.33	0.41 ±0.12		0.64 ±0.09	0.51 ±0.10		0.61 ±0.09	0.71 ±0.07
3			0.64 ±0.09			0.53 ±0.10			0.58 ±0.10			0.69 ±0.07

Reliable $R \geq 0.37$ at $P = 0.01$

It is not always possible to describe various factors influencing quantitative characters of plants in populations by the usually applied statistical parameters (M, σ^2, C, R). On the basis of the discrimination function (KALININ, 1969) some measure is introduced, characterizing the radiation influence on the investigated quantitative character. Let us introduce some "fictious" variable so as on the individuals of an unirradiated population it would take the values of 0, and on those of irradiated population = 1. Then the correlation coefficient of the investigated quantitative character with a fictious variable, the discriminating coefficient, will show the degree of population response on radiation.

Table 3: Discriminating coefficients of control and irradiated populations for the investigated quantitative characters of Arabidopsis thaliana

Characters	Discriminating coefficient					
	0-10 kR	10-30 kR	30-60 kR	0-30 kR	0-60 kR	10-60 kR
1. Appearance of cotyledons	0.15	0.35	0.12	0.48	0.64	0.73
2. Appearance of rosette	0.40	0.58	0.15	0.71	0.75	0.66
3. Beginning of buttoning	0.02	0.13	0.40	0.14	0.48	0.50
4. Beginning of flowering	0.08	0.17	0.38	0.22	0.56	0.53
5. Number of siliques on the main stem	0.21	-0.29	-0.04	-0.06	-0.08	-0.26
6. Height to the first silique	-0.10	0.62	-0.04	-0.54	-0.59	-0.66
7. Number of side shoots	-0.11	0.31	0.11	0.21	0.31	0.40

The application of discriminating coefficients makes it possible to take into account both the difference in the mean values and the dispersion ratio of control and irradiated populations, which allows to display the action of the factor when there are no reliable differences in the means. The analysis of discriminations shows that the most radiation-sensitive character is the time of the rosette appearance. The application of discriminating coefficients allows to estimate the influence of irradiation in the doses of 10 and 30 kR for the buttoning and flowering phases.

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The effect of concentration and exposure on leaf spotting in M_1 in Arabidopsis after HMU treatment

S.I. DEMCHENKO, R.Ya. SEROVA, and V.A. AVETISOV

(Institute of Chemical Physics, USSR Academy of Sciences, Moscow)

The dose of chemical mutagen is known to be expressed as concentration multiplied by the time, D = C x t (BLIXT, EHRENBERG and GELIN, 1966). To our knowledge, however, there are no experiments comparing the effect of lasting treatment by low concentrations with that of brief high concentration treatment, D = C x t = const. being observed. There are grounds to believe that a study of the differences in the effect of these two types of treatment may prove essential for the problem of dose-effect relationships.

Our study of the effects of treatment with N-nitrosomethyl urea of Arabidopsis thaliana HEYNH. dry seeds (race En-1) revealed the following regularities. Doses equal in value may prove both equal different as to their biological and genetic effects in M_1 and M_2. Doses in the range under investigation proved to be equivalent for germination capacity which is of little sensitivity. Germinating capacity was reduced down to 50% with respect to the control in all the tested products equalling 25 mM x hr, no matter by what components they had been formed. The same was true for less toxic doses.

For the effects of viability, sterility, leaf spotting in M_1 and mutation rate in M_2 non-equivalence of equal dose products of concentration multiplied by exposure time was revealed. The non-equivalence in the range of similar, equal-value doses manifested itself in the fact that the effect changed proportionally to exposure or concentration values. In fact, for the doses 12 mM x hr changes in viability were directly proportional to concentration values, for higher doses changed directly proportionally to exposure values. The effect of leaf spotting for all the tested doses changes in a direct proportion to exposure which appears of special interest and calls for further investigation. For that purpose the dose range,

Figure 1

t / c	,13	,22	,25	,29	,33	0,4	0,5	,66	0,7	,75	0,8	0,9	1	2	2,5	3	4	5	6	7	8	9	12	18	24
27		6		9																					
24	3		6		9																				
21				6		9																			
18					6		9																		
15						6		9																	
12							6			9			12												
9								6																	
6							3						6	12	15										
4,5														9											
3,0													3	6		9	12	15							
2,2																	9								
2,0																6									
2,7																		9							
1,5																			9						
1,2																		6		9	9				
1,0																3						9	12		
0,5																			3						
,26																							3		
,13																									3

Figure 2

t / c	,13	,22	,25	,29	,33	0,4	0,5	,66	0,7	,75	0,8	0,9	1,0	2	2,5	3	4	5	6	7	8	9	12	18	24
27		69		-																					
24	29		86		-																				
21				53		-																			
18					75		73																		
15						76		71																	
12							60			62			76												
9,0								70																	
6,0							10						42	62	33										
4,5														46											
3,0													2,6	21		43	28								
2,2																	28								
2,0																18									
1,7																		31							
1,5																	10		11						
1,2																		7,4		0	8,8				
1,0																0						4,0	32		
0,5																			0						
,26																							1,1		
,13																									1,3

especially of those inducing the given effect (3 mM x hr to 15 mM x hr) was tested for the widest possible set of factors. The actual values for the exposure are determined by the ability of the seeds of retaining viability after excessively long soaking, and mutagen solubility for concentrations. The solutions were prepared in phosphate citrate buffer pH 5.0, t = 22°. The exposure time was expressed in hours, NMU concentration in millimoles. The concentration and exposure values were placed by the sides of a square similar to Punnet lattice, in the points of crossing the values, doses being marked as products of both the values (Fig. 1). Equal values are thus found along certain diagonals of the square. After treatment the plants were grown in test-tubes in vegetation chamber. The effect under investigation, the rate of chlorophyll chimerae (leaf spotting) in M_1 were expressed as the ratio of the number of the plants with spots on the rosette leaves to all the plants at rosette stage in the given variant. Statistical treatment of the data obtained by means of dispersion analysis for one-factor complexes revealed that the results are highly significant ($P<0.01$). Figure 2 shows significant differences in the effect values along the diagonals, i.e. for doses equal in value. For $D = C \times t = \text{const.}$ the rate of chlorophyll chimerae in M_1 increases with an increase of the exposure duration and the corresponding decrease of the concentration. It can be concluded, therefore, that the effect is determined by the time factor. In addition, dose testing in a wide range made it possible to reveal an opposite direction of "dose curves" for two indices: the rate of chlorophyll chimerae in M_1 and mutation rate in M_2. The dose which provides the maximum of mutation rate and also more high doses connected with further decline corresponded to the dose range in which the effect of chlorophyll chimerae began to manifest itself and increased which is in keeping with some previously obtained data (GICHNER and VELEMINSKY, 1966). Both the above mentioned features give ground for certain assumptions concerning the nature of chlorophyll chimerae themselves. Besides the assumptions of plastid, gene and chromosomal mutations underlying this phenomenon (RÖBBELEN, 1965; BLIXT and GELIN, 1965) can be evolved another hypothesis. A tissue area with some chlorophyll defect can be regarded as somatic offspring of a changed embryonal cell which underwent a chlorophyll mutation under NMU effect connected with induced exchange. The above process which was previously referred to as muta-crossingover (RAPOPORT and DEMCHENKO, 1969), provides the transfer of the recessive mutation into homozygote and, thus, its phenotypical manifestation in M_1. We hope to support this assumption by means of spot tissue culture experiments. The transition of the changed somatic cell through the non-differentiating callus growth and further regeneration to the pathway of gametophyte will make it possible to analyse the alteration which took place in it. These experiments are underway.

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B. TECHNIQUES

An improved method for the culture of Arabidopsis on artificial media in petriplates

J.R. HARLE

(Erindale College, University of Toronto, Toronto, Ont., Canada)

During the course of investigations involving chemical mutagenesis, attempts were made to employ various published methods for the culture of Arabidopsis thaliana to maturity on soil and on agar media in test-tubes. Each of these methods proved to incorporate a number of disadvantages for this kind of study. It was difficult to sow seeds individually on soil, and the watering of the plants frequently resulted in accidental flooding, with the death of many individuals. In addition, the transplanting of mutant selections proved to be almost impossible. In test-tube culture on agar (VELEMÍNSKÝ and GICHNER, 1964), it was awkward to sow single seeds at the bottom of a 200 mm test-tube, and the flowers could not be reached to effect crosspollination. It was also hard to inspect the plants through the curved glass of the side of the test-tube.

A new method was developed which involved the culture of the plants entirely on agar medium in petri-plates. The method involved the use of standard 100 x 15 mm disposable plastic petri-plates, with additional modified lids. This arrangement provided both for the growth of 100 plants to the stage of flower bud appearance under the standard lid, and for the flowering and seed setting of one or a few plants under the modified lid.

The modified lids were formed by cutting out a 70 mm hole from the center of the 90 mm standard plastic lid. An inverted clear plastic drinking cup with a diameter of 75 mm and a height of 95 mm was cemented over the open hole in the lid (Figure 1), using a cement consisting of plexiglass shavings dissolved in carbontetrachloride (CCl_4). In removing the center of the standard lid, it was found necessary to use a machine designed for cutting letters in the surface of hard plastic sheets for the making of small signs, because other methods resulted in the shattering of the hard plastic of the petri-plate lids. This machine used a vertical rotating tool which cuts thin plastic neatly and quickly, guided by a pantograph from a template. One person could prepare 200 - 300 such lids in one day by this technique.

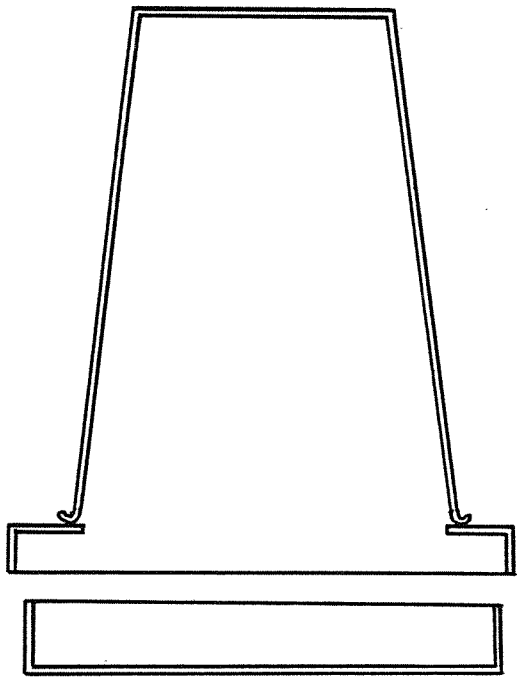

Figure 1: Structure of modified petri-plate, showing the standard lid with the center removed and the drinking cup cemented in place

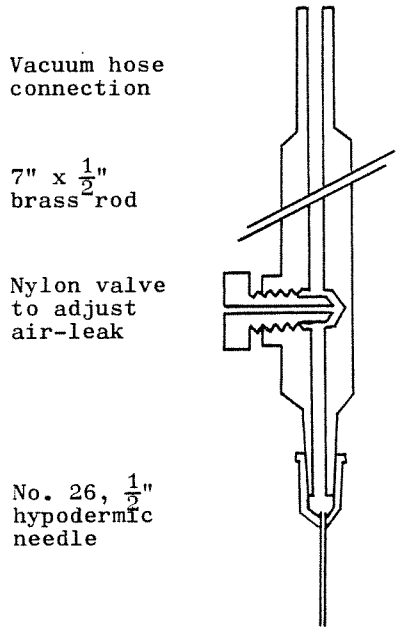

Figure 2: Vacuum pipette for seed sowing, showing the hypodermic needle and adjustable valve to control the vacuum at the tip

The minimal medium was prepared according to the recipe of JACOBS (1964), modified by the substitution of 0.06 g of Sequestrene as the source of iron. Sequestrene is a dry powder, a commercial preparation of Sodium Ferric Diethylene-triamine Pentaacetate obtained from Geigy Chemical Corp., Ardsley, N.Y.. Molybdenum was supplied as $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$, using 1.2 ml of a stock solution consisting of 0.50 g in 1000 ml of water. The quantities were adjusted to prepare 1200 ml of medium, for 40 petri-plates at 30 ml per plate. The medium was boiled for several minutes and then poured into presterilized disposable plastic petri-plates. Plates prepared in this way have proved to be very convenient for both the growth of Arabidopsis to maturity, and for storage at room temperature without the growth of fungi.

The seeds were sterilized by immersion in 2 ml of sterilant, consisting of equal parts absolute ethanol and 30% hydrogen peroxide, in a small funnel with a filter cut from commercial paper hand towels. (Laboratory filter paper disintegrated rapidly when exposed to the sterilant.) The seeds were picked up from the filter by a vacuum pipette (Figure 2), consisting of a # 26 hypodermic needle with the tip ground flat, and a valve to control the suction at the tip of the needle. The seeds were spaced at about 1/4" on the surface of the agar. Seeding was carried out in a small transfer chamber sterilized between planting periods by a U.V. sterilizing lamp. The seeded plates were stored overnight in a refrigerator to decrease variation in germination times.

The plants were allowed to grow at a temperature of 20-22°C under 1500 foot-candles continuous illumination with Grolux fluorescent lamps. After 10-14 days flower stem elongation commenced and the rosettes of leaves became crowded. The plants were examined at this time and selected individuals were transplanted by cutting out a small square of agar which was transferred to a fresh agar plate for further growth and seed production. The high lids described above were used at this time in place of the standard lids. Seed production under these conditions was excellent, aphid infestations were eliminated and the plants could be exposed readily for inspection and cross-pollination. The plants required no further maintenance prior to harvesting. Fungal contamination occurred rarely, usually only when a plant died and began to disintegrate, or when the medium was supplemented with glucose.

The seeds were harvested after the agar and the plant had both dried. The high lid was removed and the plant held above a stiff white card while the siliques were shattered. Separation of the ripe seeds from unripe seeds and chaff was obtained by holding the card at a slight angle and tapping it gently. The round seeds rolled off and the cleaned seed sample placed in a labelled gelatin capsule for storage.

One of the significant advantages of this method was that the plants were enclosed in the lidded petri-plates during the entire life-cycle, protected from accidental damage, and needed no routine maintenance.

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VELEMINSKÝ, J., and T. GICHNER: Arabid.Inf.Serv. 1, 34-35 (1964)

Mass seeding method on agar plates for intralocus recombination tests in Arabidopsis

Tomoo MABUCHI

(Biology Department, University of Saskatchewan, Saskatoon, Canada)

In higher plants very little work has been done on intralocus recombination because of the difficulties involved in collecting and planting hybrid seeds in large numbers to detect the rare recombinational events. While NELSON (1957) pointed out the advantage of using gametophyte characters, e.g. waxy/non-waxy pollen for study of fine structure of the gene in higher plants, very few such characters are available. For most genes we must use sporophytes. Here ease of getting large populations makes Arabidopsis more convenient than larger, slower growing plants. For example viable leaf color or auxotroph mutants have several advantages for study of intralocus recombination because viable "wild" green recombinants (one-half of the expected total) can be easily detected in the F_2 seedlings. It is desirable therefore to have several hundred seeds per dish. With this in mind a seeding method has been devised as follows: (1) Seeds are scattered on a round wax paper disc which is slightly smaller than the Petri dish and distributed uniformly by a small brush. (2) The cover of the Petri dish is removed. (3) The Petri dish is inverted over the wax paper and the glass plate. The seeds and the wax paper stick to the agar. (4) The wax paper is easily detached with the use of forceps from the

agar leaving seeds. (5) Place the cover on the Petri dish. By this method about 500 seeds per plate can be sown in five minutes and one hundred thousand seeds were sown in 16.6 hours. This population size should be large enough for estimates of the frequency of intralocus recombination to be made.

Reference:
NELSON, O.E.: Amer.Nat. 91, 331-332 (1957)

Micro-détermination de l'activité spécifique des pigments, sucres et amino-acides libres marqués au ^{14}C chez Arabidopsis

M. BOUNIAS

(Station de zoologie, C.R.A. d'Avignon, 84 - Montfavet - France)

Les composés marqués après incorporation d'acétate-^{14}C ou de leucine-^{14}C dans les plants d'Arabidopsis sont extraits et dosés par chromatographie quantitative en couche mince (BOUNIAS, 1969).

Les plaques sont ensuite exposées au contact d'un film Kodak (Kodirex). Après développement du film, les composés radio-actifs déjà séparés sur la plaque ont noirci l'émulsion à leur niveau, proportionnellement à leur radio-activité et les taches ainsi obtenues sont enregistrées quantitativement à l'aide d'un densito-mètre. Au cours de nos expériences, les taches de radio-activité enregistrées ont donné des pics dont l'intégration a fourni le résultat suivant : 1 mm^2 de surface de pic en 1 jour d'exposition correspond à une radio-activité de 34.10^{-15} Curies.

S u m m a r y : The specific activity of ^{14}C-labelled pigments, amino acids, and sugars is obtained by quantitative micro-chromatography of the compounds followed by quantitative autoradiography of the chromatographic plates.

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BOUNIAS, M.: Chimie Analytique 51 (2), 76-82 (1969)

Faster maturity in tube culture with plastic foam plugs

A. CORCOS

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

In some experiments performed last year, there were indications that plants in test tubes with elastic foam plugs* grew better than those in tubes with cotton plugs.

A randomized experiment was carried out to test the effect of plugs. Five treatments were used: (1) foam plugs only, (2) cotton plugs only, (3) foam plugs and aluminum foil caps, (4) cotton plugs and aluminum foil caps, and (5) aluminum foil caps only. For each treatment, 100 plants were seeded in 150 mm tubes on mineral medium and glucose and placed in a growth chamber under constant light and 21°C constant temperature.

Table 1 gives the results as to days to maturity. In tubes with elastic foam plugs, plants grew faster and as a group more uniformly. A possible reason which

Table 1: Days to maturity for the Estland variety under constant light and at 21°C temperatur

Treatment	Days to maturity	
	Average	Standard deviation
Plastic foam plug	31.9	3.0
Cotton plug	43.0	7.9
Foam plug & foil cap	49.7	14.5
Cotton plug & foil cap	50.2	12.6
Foil cap only	56.8	10.3

has not been tested is that the exchange of oxygen and CO_2 might be freer with foam plugs than with cotton plugs. This idea is supported because aluminum foil caps considerably delayed maturity.

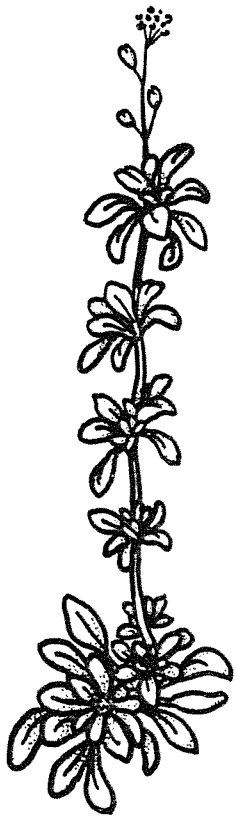
*The source of the elastic foam plugs is: Gaymar Industries, 701 Seneca, Buffalo, New York.

Vegetative reproduction

Erna REINHOLZ

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

To solve certain scientific questions a vegetative reproduction of Arabidopsis plants would be very useful. For example, it could be possible to preserve sterile mutations or detect somatic ones.



NAPP-ZINN et al. were anxious to cultivate cuttings of Arabidopsis leaves. They succeed in rooting a great lot of these leaves, especially by high light intensity and treatment with auxine (0.2 ppm NAA). But up to present they failed to induce shoot buds on the leaf cuttings. Thus the aim of their endeavour, the formation of a clone, could not be achieved.

We tried therefore, another way to solve this problem. We cultivated Arabidopsis plants (race ENKHEIM) under optimal conditions (18 °C and ≧ 12 h artificial light per day) in a mixture of compost and sand (1:1) in small pots. After development of the main inflorescence the vigorous plants were brought under short day conditions. Consequently the flower buds turned back into the vegetative phase and each flower started forming a small leaf rosette (see Figure). Such rosettes were detached carefully and planted in soil where they rooted after a short time. After transfer into long day conditions they formed normal inflorescences. The same photoperiodic treatment is effective with flower buds of shoots coming out of the axils of the rosette leaves.

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C. LABORATORY RESEARCH COMMUNICATION

A space flight experiment with Arabidopsis

Erna REINHOLZ

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

In April 1972 when Apollo 16 will start from the United States Eastern Test Range (Kennedy Space Center) to the moon it will carry also many thousand seeds of Arabidopsis thaliana.

The Manned Space Flight Experimental Board (MSFEB) of NASA has entered in the scientific program of the flight of Apollo 16 an european radiobiological experiment, called "Biostack", as Life Science Experiment M-211. It has been selected by four Commitees of American experts because of its scientific value and its technical fitness. The scientists participating in this experiment belong to the "European Working Group on Space Biophysics", Europarat.

The "Biostack" consists of a hermetically sealed aluminum cylinder which contains a series of monolayers of biological material (spores of Bacillus subtilis, eggs of Artemia salina, root tips of Vicia faba and seeds of Arabidopsis thaliana) with each layer sandwiched between several different types of cosmic radiation track detectors (nuclear emulsions and plastics). The purpose of this experiment is to obtain information on the biological effects of individual heavy nuclei of the cosmic radiation. The results may be important for the medical estimation of the danger of the manned space flight in future. The NASA hopes for an elucidation of the phenomenon of the "light flashes" noticed by the astronauts during the preceding space flights.

The fixation of the Arabidopsis seeds in foils from polyvinyl-alcohol (PVA) between the physical detectors enables an exact coordination between track and hit seed, consequently an evaluation of the effect of each individual radiation particle. Germination and growth of Arabidopsis is not inhibited by the embedding of the seeds in PVA alone, even after storage up to 18 months. The radiation effects will be studied after exposure during the Apollo flight by testing the germination of the seeds, the growth, development, and fertility of the X_1-plants, and the induction of morphological, physiological, and biochemical mutations in the X_1- and X_2 generations.

Already, the Biostack has passed with success the technical tests, the Qualification test (launch vibration, shock load, and vacuum) and the Acceptance test (dimensional check) performed by the Centre National d'Etudes Spatiales (CNES) in Paris and by the NASA Manned Spacecraft Center in Houston.

D. LATEST NEWS

The chm mutator locus

G.P. RÉDEI and S.B. PLURAD

(Department of Agronomy, University of Missouri, Columbia, Missouri)

Allelism of two independently obtained mutations involved in "plastom" alterations has been demonstrated by the method designed earlier (RÉDEI, 1971). Plants homozygous for factors gl[1] and chm were used as pollen donor and were crossed to females homozygous for as. The F_1 progeny which had normal cytoplasm and was heterozygous for all three genes was crossed as a pistillate parent with mutant 405-A-2090 as a pollen parent. The progeny displayed 14 normal plants and 12 with late appearing but clear chlorophyll deficient sectors indicating allelism. Expectation in case of allelism was 1:1. Thus chm was redesignated as chm[1] and to mutation 405-A-2090 the symbol chm[2] was assigned.

Linkage studies confirmed the chromosomal location of chm in linkage group 3 at approximately 37.5 map units from gene gl.

Light microscopic examination revealed that in plants homozygous for these alleles some cells contained more than one type of plastids. Electronmicroscopic studies permitted the identification of a number of different types of plastids within single cells. The development of plastids was often arrested at a very early stage and transitional types along with up to completely normal chloroplasts have been observed in a large number of cells. Many of these abnormal plastids were similar to those reported by RÖBBELEN (1966) yet a few types of different structural alterations have also been found. Some chloroplasts developed only a single but huge granum or two such "macrograna" have been observed. In some leaf sectors of rough appearance another type of chloroplast morphology was frequent (Figure 1). The cells containing these unusual plastids were generally of odd

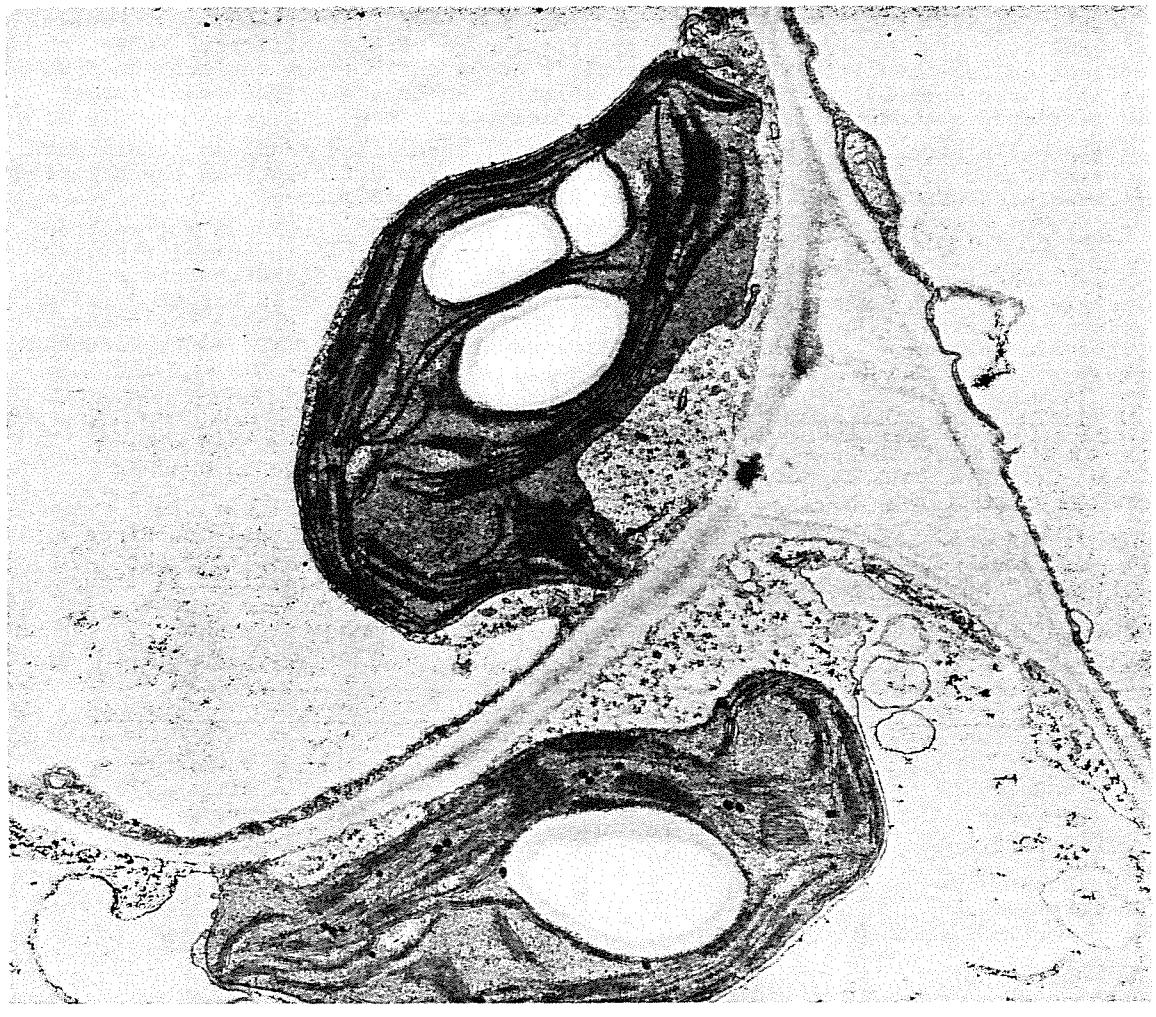

Figure 1: Atypical chloroplasts oberseved as an effect of mutator gene chm in the "rough" tissues of the leaves

shape rather than cubical. The chloroplasts in some of these cells were all irregular in shape and some of the thylakoids displayed an almost circular or hook-like arrangement. The stroma appeared unusually dense, the ribosomes seemed less dissimilar in size from those of the cytoplasm. In these abnormal chloroplasts clear areas were unusually frequent containing fairly articulate small fibers visible at high magnification. These fibers are generally regarded in plant chloroplasts as DNA (RIS & PLAUT 1962). In spite of these obvious structural modifications the photosynthetic activity of these plastids appeared excellent from the presence of the numerous large starch granules.

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Fructose intolerance in Arabidopsis, Cardaminopsis and Hylandra

G.P. RÉDEI

(Department of Agronomy, University of Missouri, Columbia, Missouri)

Sucrose, glucose and fructose all support the growth of the majority of plant species in aseptic culture - under certain conditions though there are significant differences among these three sugars (HILDEBRANDT & RIKER, 1949; STREET, 1959). On 3% fructose under continuous illumination and at light intensities between 300 and 500 foot candles at temperatures between 20-30°C the fresh weight of Arabidopsis (4n), Cardaminopsis arenosa (4n) and Hylandra suecica is reduced to 20-30% of that of plants grown without sugar on otherwise identical mineral agar medium. The reduction of dry weight is not as drastic though very significant. Under identical culture conditions but under only 8 hours daily light regimes, on fructose all three species produced more dry weight than on sugar-free media though they developed a conspicuous chlorophyll deficiency.

A generally recessive human metabolic anomaly, hereditary fructose intolerance, has been traced to fructose-1-phosphate aldolase enzyme deficiency in the affected individuals (FROESCH, 1966; PRADER, 1969; RIEU et al., 1971).

Plant biochemistry texts (BONNER and VARNER, 1965; DOBY, 1965) do not mention fructose-1-P aldolase, though the existence of such an enzyme in animal tissues has been long recognized (LEUTHARDT & WOLF, 1954). Fructose-1,6-diphosphate aldolase is common in apparently in all plant species. Aldolases in higher animals display 5 or more isozymic forms (LEBHERZ & RUTTER, 1969), in plants the number of isozymic species of aldolase is very limited (LEBHERZ & RUTTER, 1969; KAZUSUKE, 1969).

In Arabidopsis, Cardaminopsis and Hylandra only one main fructose-1,6-diphosphate aldolase is detectable by cellulose polyacetate electrophoresis. This band moves slowly toward the positive pole with a slight difference in these three species. In wheat both an anodic and a cathodic band could be detected under identical conditions.

Table 1: Aldolase assays on Arabidopsis, Cardaminopsis and Hylandra. Combined values of three experiments. Enzyme was extracted from plants aged 20, 31 and 33 days, respectively, grown under aseptic conditions in mineral-agar with the indicated supplements. Natural daylength in October and November, approximately 68°C temperature in greenhouse

Species and supplement to medium	Nanomole F-1, 6-P digested per mg protein per minute	Index	Statistics
Arabidopsis			
no sugar	41.6	100	F test : $P < 0.001$
2% glucose	43.1	104	Limits of significant
2% fructose	35.7	86	differences
Cardaminopsis			d Probability
no sugar	29.4	100	13.8 0.001
2% glucose	20.0	102	10.4 0.01
2% fructose	21.8	74	7.7 0.05
			6.4 0.10
Hylandra			
no sugar	32.5	100	
2% glucose	31.0	95	
2% fructose	17.5	54	

Fructose, 1,6-diphosphate aldolase assays (according to the method of PENHOET et al.) detected considerable activities in Arabidopsis, Cardaminopsis, Hylandra and wheat. Fructose-1-phosphate aldolase activity was practically absent however in Arabidopsis, Cardaminopsis and Hylandra and appeared extremely low in wheat.

A comparison of the fructose-1,6-phosphate aldolase activity in the three species was dependent on the conditions of culture (Table 1).

Obviously fructose repressed or other ways modified this enzyme in a specific manner since the activity of phosphohexose isomerase, an enzyme used as a general control, showed no difference under these conditions. Thus it appears that in fructose intolerance in these three species aldolase plays an important role.

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Obligate photoorganotrophy in submerged culture

G.P. RÉDEI

(Department of Agronomy, University of Missouri, Columbia, Missouri)

Last year we reported (RÉDEI and PERRY, 1971) that Arabidopsis can be very successfully cultured in shaken or aeriated submerged liquid medium supplemented with glucose. Recent experiments have shown (Table 1) that an appropriate carbohydrate source is indispensable for growth even under high light intensity. The carbohydrate cannot be replaced by acetate, and there is an obligate requirement for light even on sugar containing media.

Table 1: The seed (50/100 ml mineral medium) was dropped after disinfection in cotton plugged 250 ml Erlenmeyer flasks and placed on a shaker in the greenhouse. After 12 days the cultures were harvested. On sugar-free medium development ceased after germination

Species	Supplement to the medium	Fresh weight		Protein	
		mg/50 plants	index	microgram/ 50 plants	index
Arabidopsis	none	54.1	100	167	100
	2% glucose	1,361.6	2,517	7,230	4,329
	2% fructose	133.0	246	715	428
Cardaminopsis	none	169.0	100	431	100
	2% glucose	1,107.5	655	5,538	1,285
	2% fructose	475.0	281	2,537	589
Hylandra	none	111.0	100	310	100
	2% glucose	1,975.0	1,779	9,085	2,931
	2% fructose	896.6	807	5,331	1,720

Reference:
RÉDEI, G.P., and C.M. PERRY: Arabid.Inf.Serv. 8, 34 (1971)

Compatability of Arabidopsis thaliana with Cardaminopsis arenosa

G.P. RÉDEI
(Department of Agronomy, University of Missouri, Columbia, Missouri)

On the basis of morphological evidence and chromosome counts HYLANDER (1957) suggested that Cardaminopsis suecica (Fr.) Hiit (2n = 26) may be an amphiploid of Arabidopsis thaliana (L.) Heynh. (24 = 10) and Cardaminopsis arenosa (L.) Hayek (2n = 32). LÖVE, reexamining the case, proposed that Cardaminopsis suecica should be renamed as Hylandra suecica (Fr.) LÖVE and thus created a monotypic genus.

The origin of Hylandra according to the HYLANDER-LÖVE hypothesis appears logical since a cross between a tetraploid Arabidopsis and Cardaminopsis or if an occasional unreduced gamete of the diploid Arabidopsis is combined with the diploid gamete of Cardaminopsis arenosa a hybrid of the expected chromosome number may result.

Unfortunately direct proof for this phylogenetic hypothesis has not been produced during the 15 years elapsed since its inception. On the contrary LAIBACH (1958) who reported the first successful hybrids of Arabidopsis (Cardaminopsis) suecica with A. thaliana and with Cardaminopsis arenosa stated that "eine Kreuzung zwischen C. arenosa and A. thaliana nach unseren bisherigen Erfahrungen nicht möglich ist". Similarly MESICEK (1967) failed to obtain C. arenosa and A. thaliana hybrids though he made a successful cross between A. thaliana (2n = 10) and Cardaminopsis petraea (L.) Hiit. (2n = 16). BERGER (1968) demonstrated that fertilization may take place between C. arenosa and A. thaliana though she apparently failed to obtain hybrid plants from such a cross.

Recently we pollinated C. arenosa (obtained from Dr. G. RÖBBELEN) with tetraploid A. thaliana and viable seed was obtained in the reciprocal crosses. This information provides direct support to the HYLANDER-LÖVE hypothesis. It may be noted furthermore that under laboratory conditions we found both autotetraploid and autohexaploid forms of Arabidopsis without any specific treatment. Thus polyploidization may take place in nature thus providing an appropriate source of gametes. The spontaneous hybridization may be facilitated by the fact that C. arenosa, a species with fragrant flowers requires artificial (self-) pollination for seed production in the greenhouse and it is probably insect pollinated in nature.

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