# FAST NEUTRON SENSITIVITY OF DRY AND GERMINATING TOMATO SEEDS

R. B. CONTANT

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PROEFSCHRIFT

ter verkrijging van de graad
van Doctor in de Landbouwwetenschappen
op gezag van de Rector Magnificus Dr.Ir. F. Hellinga,
Hoogleraar in de Cultuurtechniek,
te verdedigen tegen de bedenkingen
van een Commissie uit de Senaat
van de Landbouwhogeschool te Wageningen
op vrijdag, 5 juni 1970, te 16.00 uur

door

R.B. CONTANT

# STELLINGEN

I Op een aantal terreinen der fundamentele en toegepaste biologische wetenschappen is dringend behoefte aan een volledige inventarisatie en kritische her-evaluatie van alle ter zake dienende informatie, teneinde tot een doelmatiger voortzetting van het onderzoek te komen.

II De mogelijkheden van mutatieveredeling bij vegetatief vermeerderde gewassen worden in hoge mate bepaald door de beschikking over een methode tot adventiefspruit-vorming aan afgesneden bladeren.
C.Broertjes, B.Haccius & S.Weidlich. Euphytica 17 (1968), 321-344.

III Hussein's stelling dat 'In mutation breeding, more useful genetic variation can be obtained by chemical mutagens than by ionizing radiations' is een onjuiste generalisatie.
H.A.S.Hussein. Theorem I, Wageningen, 11th October 1968.

IV Selektie op hoge fertiliteit in de eerste generatie na zaadbestraling ($M_1$) is niet voor alle doeleinden aan te bevelen.
M.Mesken & J.H.van der Veen. Euphytica 17 (1968), 363-370.

V 'Reciprocal recurrent selection' is een efficiente methode voor de veredeling op bloemopbrengst en pyrethrinegehalte in Pyrethrum.

VI Bij de mutatieveredeling van door zaad vermeerderde gewassen in de tropen liggen de belangrijkste perspektieven in het mogelijk maken van nieuwe produktiepatronen en in de verbetering van kwaliteitseigenschappen.
M.S.Swaminathan. Induced mutations in plants, IAEA/FAO, Vienna (1969), 719-734.

VII Ontbossing heeft dusdanig ernstige en langdurige gevolgen voor de mensheid dat plaatselijke, nationale, en supranationale instanties in onderlinge samenwerking alle middelen dienen aan te wenden tot rigoureuze handhaving, en waar nodig herstel, van het voor de waterbeheersing en de bodembescherming benodigde bosbestand.

VIII Toereikend inzicht in de proeftechniek en het samenwerken met ervaren ter zake kundigen bij de voorbereiding van proeven zouden het rendement van het landbouwkundig onderzoek in Nederland met een waarde van verscheidene miljoenen guldens per jaar doen toenemen en het wetenschappelijke niveau van dit onderzoek ten goede komen. Verwezenlijking van dit doel vereist de spoedige instelling van een studierichting aan de Landbouwhogeschool, die de opleiding van landbouwkundig gevormde statistici beoogt.

IX Indien diverse maatregelen tot stimulering van geringe kinderaantallen in Nederland niet op korte termijn worden genomen en het gehoopte resultaat hebben, zal beperking der gezinsgrootte in de komende generatie onvermijdelijk geschieden door dwang.

X De mogelijkheden tot opleiding aan instellingen in de ontwikkelingslanden moeten worden uitgebreid, zo nodig ten koste van bedragen die beschikbaar zijn voor beurzen of studietoelagen voor opleiding in de ontwikkelde landen.

R.B.CONTANT
Wageningen, 5 juni 1970

Dit proefschrift met stellingen van

RUDOLF BERNARD CONTANT

landbouwkundig ingenieur, geboren te 's Gravenhage, 6 oktober 1935, is goedgekeurd door de promotor, Dr.Ir. S.J. WELLENSIEK, emeritus-hoogleraar in de Tuinbouwplantenteelt.

De Rector Magnificus der Landbouwhogeschool,

F. HELLINGA

Wageningen, 27 april 1970.

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(Communications Agricultural University, Wageningen, The Netherlands)

Aan Bep, mijn vrouw
Aan mijn ouders

CONTENTS

# 1. GENERAL INTRODUCTION

## 1.1. Motivation and aim

Mutation induction is an increasingly important tool in the genetic improvement of cultivated crops, especially of those with a long breeding-history; the breeder's aims are many and mutagenic treatment cannot yet be recommended for all applications. A thorough evaluation of the potentialities of mutation breeding in a given crop species is only possible by means of model selection and breeding experiments, with material subjected to various mutagens and dose levels at different stages of ontogenesis. This requires, among other things, both standardised methods and growth conditions, and suitable biological criteria to assess the effects of mutagenic treatments. Such standardisation is impossible without knowledge of the responses of a species to various kinds of mutagens, in terms of survival, growth, development, fertility and mutability. Though these responses are qualitatively similar in diverse species of higher plants, their quantitative relationships may differ considerably. This applies not only to the various kinds of damage at the cellular and subcellular levels but also to the macroscopic manifestations, which are greatly influenced by the anatomical and morphogenetical features of the species at all stages of development. For this reason, radiobiological investigations cannot be restricted to those species that are methodologically the most suitable for resolving fundamental problems (e.g. barley[57,69,85,142], *Vicia*[152,193], *Tradescantia*[59,112,139,159,179], *Arabidopsis*[5,153] and species with a 1-locus gametophytic self-incompatibility system[45]), but must be extended to the crop species in which mutation breeding is to be practised.

The choice of the tomato for the present study was based on its economic and nutritional importance and on the probability that mutation induction will be of substantial value to its future genetic improvement in both qualitative and quantitative traits[1,35,36,110,123,188].

The aim of this study was, in general terms, to contribute to the radiobiological and technical knowledge of the tomato needed to devise standardised methods and procedures of seed conditioning, irradiation and culturing; and to identify characters which may be

used as early indicators of radiation effectiveness, with a view to obtaining reproducible results in mutation induction experiments.

## 1.2. Material and variables

For all experiments, use was made of cv. 'Moneymaker', which was, until recently, one of the most important tomato cultivars grown in the Netherlands. In one experiment, a second cultivar, 'Glorie', was used for comparison.

The present experiments, carried out in the years 1965-1967, were concerned only with the effects of acute fast neutron irradiation on both dry and germinating seeds. They were followed in 1968-1969 by similar experiments in which the effects of fast neutrons were compared with those of high energy X-rays at different temperatures; the results of these will be published separately.

The restriction of this programme to the effects of ionizing radiations on seeds was justified on the grounds that the action of chemical agents on the tomato has been studied extensively by others[86, 87, 88], and radiation effects on other ontogenetic stages have been the subject of complementary research programmes of the Association EURATOM - ITAL[33,46,47,48,49], one of which involved a direct comparison with seeds[33].

Neutrons were chosen for the first series of experiments because their effects on dry seeds, in contrast to those of X- or $\gamma$-rays, are little modified by external factors such as temperature, moisture content, oxygen availability during irradiation, the addition of S-H containing compounds and post-irradiation storage [27, 42, 56, 74, 78, 104, 132, 141, 142,145]. This fact is ascribed to the high average density, and therefore the rapid interaction, of ions and radicals produced by neutrons[56,78,118,142,168]. As a result, this type of radiation is particularly suitable for studying the effects of endogenous variables.

Dry seeds are generally regarded as suitable material for radiobiological experiments and for mutation breeding. Little is known, however, about the relative mutagenic efficiency of radiations on prehydrated seeds[142] and all detailed information available comes from experiments with X-rays[99]. Several studies, concerned only with characters of the irradiated generation, have shown important differences between X-rays and neutrons with regard to the changes in sensitivity during hydration[104,169]. Therefore, the period of

seed hydration/germination prior to irradiation was chosen as the main variable in the present experiments.

The space and labour requirements of the tomato are considerable. Its relatively long life cycle, the need to pursue experiments up to the second generation ($M_2$) to obtain an indication of induced transmissible changes, and the large numbers of plants required per treatment imposed severe restrictions on the number of experiments. Nevertheless, the suitability of various methods of culturing could be explored by performing the successive experiments under partially different conditions.

## 2. GLOSSARY OF TERMS AND ABBREVIATIONS

| | |
|---|---|
| b | estimate of regression coefficient |
| chromosomal | concerning the integrity of the chromosomal structure |
| cv. | cultivated variety (cultivar) |
| d.f. | degree(s) of freedom |
| $D_i$ | irradiation treatment (series) of dry seeds in experiment i |
| $D_{H_2O}$ | fast neutron dose (dose rate) expressed as krad (krad/h) in water |
| dose | radiation -: a measure of the amount of radiation passing through a material; the unit of dose is the rad |
| DRF | dose reduction factor: factor by which the radiation dose, expressed as $D_{H_2O}$, required to produce a specified biological effect is reduced by a given prehydration/germination treatment, compared to dry seed irradiation |
| $ED_{50}$ | dose, expressed as $D_{H_2O}$, required to produce 50% of the maximum possible effect on a given character |
| efficiency | mutagenic -: the rate of increase per unit dose in the frequency of recessive mutations in relation to other, usually detrimental, biological effects such as reduction in survival, growth or fertility (cf.144) |
| effectiveness | - of radiation: the magnitude of the response of a given character per unit dose of radiation |
| electron | an elementary particle which is a common constituent of all atoms, having a mass of $9.1091 \times 10^{-28}$ gramme and unit negative electric charge |
| erg | $4.18 \times 10^{-7}$ calory |
| eV | electron volt: $1.6 \times 10^{-12}$ erg |
| 'fertile' | - plant: possessing $\geqslant 24$ seeds in each of the 2 trusses harvested (2 fruits per truss) |
| genetic | concerning the hereditary material in an unspecified manner, i.e. genic or chromosomal |
| genic | concerning the genes, i.e. the DNA codons, but not the structure of the chromosomes |
| germination | protrusion of the radicle from the seedcoat |
| GL | tomato cv. 'Glorie' |
| $H_i$ | irradiation treatment (series) of prehydrated seeds in experiment i |
| $H\frac{1}{2}$, H3, etc. | irradiation treatment (series) of seeds prehydrated for $\frac{1}{2}$ hour, 3 hours, etc. (exp.4 only) |
| IAEA | International Atomic Energy Agency, Vienna |

| | |
|---|---|
| initial cell | cell in the shoot or root meristem of an embryo or seedling at the time of irradiation which contributes to the formation of an organ or tissue |
| ionization | the liberation of an electron from an atom or molecule, resulting in a free electron and a positive ion |
| keV | $10^3$ eV |
| krad | $10^3$ rad |
| LET | linear energy transfer: the amount of energy deposited by a traversing quant or particle in the irradiated object per micron track length (keV/μm); the biological adequacy of this concept is challenged in the most recent literature(18, 65) but no satisfactory alternatives are yet available |
| log/normal probability paper | as normal probability paper (see below) but with log x as abscissa |
| $M_1$ | (of the) irradiated generation |
| $M_2$ | (of the) selfed offspring of irradiated individuals |
| MM | tomato cv. 'Moneymaker' |
| mutant | $M_2$ individual carrying in homozygous condition a mutation with visible expression |
| mutation | genetic change provoking, in the selfed offspring of an irradiated individual of an inbred line, a phenotypic segregation not readily distinguishable from that of a single gene |
| n | number in a group or sample |
| neutron | a nuclear particle having the approximate mass of a proton ($m_n = 1.6748 \times 10^{-24}$ gramme) and being electrically neutral |
| normal probability paper | a specially ruled graph paper with a variate x as abscissa and an ordinate y scaled in such a way that the graph of the distribution function, y, of the normal distribution, is a straight line(100) |
| P | level of statistical significance |
| proton | a nuclear particle having the mass of a hydrogen nucleus ($m_p = 1.6725 \times 10^{-24}$ gramme) and unit positive electric charge |
| quantal character | character giving an all-or-none response to a stimulus |
| $r_{i,j}$ | sample correlation coefficient between characters i and j |
| $\bar{r}_{i,j}$ | mean sample correlation coefficient, obtained after r,z transformation of individual r-values and back-transformation $\bar{z}, \bar{r}$ (64, table VII$_1$) |
| rad | a unit of radiation dose defined as the amount of radiation which leads to an absorption of 100 erg in 1 gramme of the material |

| | |
|---|---|
| recovery* | repair plus cell elimination and cell replacement, plus the diminution, in the course of growth and development, of the physiological consequences of damage to the early organs |
| repair* | all processes leading to the elimination of radiation injury at the intracellular level |
| restitution | the joining of chromosome fragments resulting from the same break(178) |
| reunion | = recombination: the joining of chromosome fragments resulting from different breaks(178) |
| root | primary root, unless otherwise stated |
| S.E. | estimated standard deviation |
| shoulder | that part of the dose/response curve corresponding to an absence of negative response |
| SR | sensitivity ratio: average effectiveness of fast neutrons on cv. 'Glorie' relative to cv. 'Money-maker' with regard to a given character |
| stem | hypocotyledon + stem up to the main shoot apex, unless otherwise stated |
| sublethals | $M_2$ seedlings of such low vigour that they are unsuitable for recognising distinct mutant characteristics; usually lethal at an early stage |
| sublethal dose | dose of radiation leading to a very high proportion of individuals with a permanently arrested or grossly disturbed main shoot apex |
| threshold dose | the highest radiation dose at which there is no detrimental effect on the character studied |

* A different terminology has recently been suggested by Alper et al. (4) to overcome the confusion in the use of the terms 'recovery' and 'repair', but the new terminology is not yet in general use.

# 3. IRRADIATION AND DOSIMETRY

## 3.1. Introduction

The interactions of fast neutrons with matter have been thoroughly described in most radiation physics textbooks[6, 61]. Consequently, only those characteristics which are of fundamental importance for understanding the action of fast neutrons on biological material will be discussed; for further information reference may be made to Lea[112], Giles[74], Sparrow[171] and Barendsen[9].

Fast neutrons, having, by definition, an energy >10 keV, interact in tissue mainly by elastic collisions with C, H, N and O nuclei. In most biological material the reaction with H, which produces recoil protons of varying kinetic energy, accounts for as much as 85-95% of the total dose absorption[92]. The recoil protons have a very low penetration (e.g.,600 keV protons travel only 15 microns in tissue having a specific density of 1.0) but on their path produce a dense track of ionisation, caused by ejections of electrons; these electrons in turn cause the ejection of low-energy secondary electrons with low penetration. Consequently, the path of a fast neutron is surrounded by a dense 'column' of primary and secondary ionisations at very close proximity. The heavier recoil ions of C, N and O have an even lower penetration and consequently produce even denser ionisation tracks. Other reactions are not relevant in the present context.

Until recently most workers applying neutrons to higher plants ignored the dose absorption characteristics of their material and the physical characteristics of the radiations used; dosimetrical data were given in a wide variety of units, frequently without reference to the methods employed. It is thus difficult to compare results in most of the literature dealing with fast neutron irradiations of plants. This difficulty is aggravated by problems arising from the widely different types of neutron source used. In principle, monoenergetic neutrons from an accelerator are the most suitable for biological experiments. Nevertheless, for both research and applied purposes, the most commonly available source, a nuclear reactor, must usually be relied on. This is in spite of the wide energy distribution and the $\gamma$-contamination of its beam and the resulting very complicated dosimetry and spectrometry. The comparison of experiments carried out in different reactors is hampered by differences in

neutron output, in neutron spectrum and in the proportions of contaminating radiation. Other complicating factors include differences in construction of the neutron facility which lead to differences in both the geometry of the irradiated specimens and the control of environmental conditions before, during and shortly after irradiation. Consequently, even with a full description of all relevant factors and circumstances, it may still be impossible to obtain an accurate reproduction of experiments with a different reactor. This problem can be partly overcome by monitoring with a standard biological material, such as Himalaya barley, for which a system is now being developed[92,93]. However, this presupposes a high degree of reproducibility of results at any given site, and this can be achieved only by rigorous standardisation of both the experimental procedures and the test material.

The aim of the present chapter is to provide dosimetrical data relevant to irradiated dry and germinating tomato seeds. As far as possible, the recent IAEA recommendations on dosimetry reporting, briefly enumerated below, will be followed. While descriptions of both the irradiation facility and the physical dosimetry were available in published papers, information on the characteristics of dry and germinating tomato seeds relevant to fast neutron dosimetry was lacking, and had to be obtained experimentally. The doses absorbed in the various irradiated materials could subsequently be calculated from the physical and biological data.

## 3.2. IAEA recommendations on dosimetry reporting

In 1967, the IAEA convened a study group to deal with both the problems of neutron dose measurement in biological experiments and the method of their reporting. This group recommended that the following information should be obtained and reported in all papers dealing with neutron seed irradiation[92,93]:

(a) the characteristics of both the neutron facility and the container or apparatus holding the seeds during irradiation; the irradiations must be performed in either an adequately isotropic field or a beam;
(b) the neutron flux densities and spectral distribution;
(c) the absorbed neutron doses (dose rates) expressed in rad (rad/h), either specifically for the objects irradiated, or estimated by reference to a material of known atomic composition, e.g. $H_2O$;

(d) the absorbed doses due to contaminating radiations expressed in rad (rad/h);
(e) the atomic composition of the irradiated objects, preferably the embryos rather than the whole seeds, for at least B, C, H, N and O in thermal neutron irradiations and for at least H (but if possible also C, N and O) in fast neutron irradiations;
(f) the correlation between the data under (b) and (c) and a standard biological effect such as the reduction in growth of the 1st leaf of Himalaya barley equilibrated at 13% moisture.

When the present experiments were conducted it was not yet possible to supplement the physical and chemical dose measurements with a biological monitoring system. Information on (f) is consequently lacking. The other data are given in the following sections.

## 3.3. Irradiation facility: reactor BARN

The 'Biological Agricultural Reactor Netherlands' (BARN) is a swimming pool reactor with light water as moderator and coolant, with 90% enriched $^{235}U$ as fuel, operating at a maximum permissible power of 100 kW; its most important feature is a 5x7 m climate controlled chamber below the reactor core, separated from the latter by a $D_2O$ diffusor, and by 2 bismuth shields to filter out most of the contaminating $\gamma$-radiation[14]. In the present experiments, the irradiations were performed with all $D_2O$ dumped from the diffusor. In this way, a fast neutron beam is obtained with only a small admixture of thermal neutrons. Most of the latter are captured by a boron layer, placed between the 2 bismuth shields.

The fast neutron spectrum of the BARN reactor does not differ radically from a fission neutron spectrum and it may be presumed that biological results obtained with these two spectra will be directly comparable[25,26].

## 3.4. Conditions of pretreatment and irradiation

The irradiation experiments were performed with both dry seeds and seeds prehydrated/germinated for various durations at $27\pm0.25^{\circ}C$ on a 3 mm layer of 0.75% agar + 0.1% $KNO_3$ in 9 cm polycarbonate petridishes, under ca 11,000 lux of Philips TL33RS+Philinea, measured inside the dishes. The $KNO_3$ was added to the agar in order to improve the uniformity of germination speed[30,94].

The dishes containing prehydrated or germinated seeds were sealed,

without lids, in thin polyethylene foil. The dry seeds were sealed directly in thin polyethylene foil and then placed in open petridishes with agar. In this way a comparable geometry was obtained for all specimens. The dishes were placed on a polypropylene box standing on a wooden structure in the centre of the irradiation chamber; the height from the floor was 1.20 m in exps.1-3 and 2.20 m in exp.4.

The fast neutron irradiations took place under ca 9000 lux of Philips TL33RS at 23°C, at a reactor power of 100 kW.

### 3.5. Physical dosimetry

The irradiation treatments were expressed initially as minutes exposure. These data were converted to rad doses in water on the basis of the most recent spectral and dosimetry data[25]. The fast neutron dose rates were measured using two types of ionisation chambers, and were also calculated from the spectral distribution determined with semiconductor sandwich detectors; these different methods yielded similar results. The γ-contamination was determined using a Mg-A ionisation chamber. All ionisation chambers were calibrated with a $^{137}Cs$ source. The dose rates, expressed as rad/h in water ($D_{H_2O}$), were shown to be:

| Height from floor | $D_{H_2O}$ (rad/h) | | Experiment |
|---|---|---|---|
| | fast neutrons | γ-radiation | |
| 1.20 m | 1000±5 % | 80 | 1, 2, 3 |
| 2.20 m | 2800±6½% | 160 | 4 |

### 3.6. Changes in germinating tomato seeds relevant to fast neutron dosimetry

#### 3.6.1. Introduction

In order to calculate the fast neutron doses actually absorbed by the irradiated specimens, the changes in water content, dry weight and elementary composition (C, H, O, N and Ash) of untreated tomato seeds and their constituent parts were determined during germination and early seedling growth. The pattern of water movement inside the seeds was also studied.

#### 3.6.2. Materials and methods

Seeds of cv. 'Moneymaker' reselection no. 83 (Nunhem Seeds Ltd., Haelen, Netherlands), equilibrated at 6.5% moisture and stored at

-23°C, were selected for uniformity in size, normal appearance and the absence of visible damage; their 100-seed weight was 363 mg.

These seeds were sown in 9 cm polycarbonate petridishes with 0.75% agar + 0.1% $KNO_3$, ca 120 seeds per dish, and incubated as in section 3.4 (p. 9 ). After 96 hours, the lids were raised to allow the seedlings to grow undisturbed, and water was added to prevent desiccation. The experiments were continued until 144 hours after sowing.

At regular intervals, 4 independent samples, each of 25 seeds or seedlings, were taken. The seeds or seedlings were dried of adhering water with filter paper. The specimens were then transferred immediately to a small weighing bottle, weighed on an automatic analytical balance, dried for 1 week under high vacuum (0.01 mg Hg) at room temperature and weighed again. The water contents were expressed as percentages of dry weight.

Water uptake in the embryo was studied by taking 4 independent samples of 20 seeds at each sampling time. Working in a room with high relative humidity, one seed at a time was taken from its petridish, blotted gently on damp filter paper and fixed in a clamp made of two 3x12 mm pieces of thin perspex connected by means of adhesive tape. The seed was then immediately cut in half along the 'horizontal' plane with a sharp razor blade. Both seed halves were laid on the damp filter paper, seedcoat down, and the embryo halves lifted out with a fine preparation needle. All embryos belonging to one sample were collected in a stoppered miniature weighing bottle (1 $cm^3$) and weighed. The seedcoat+endosperm halves of each sample were also collected, dried and weighed; their water contents were calculated from the corresponding data on whole seeds and embryos. The time needed to prepare one sample was 3-4 minutes.

The seedlings taken 72-144 hours after sowing were cut into 3 parts (roots, hypocotyledons and cotyledons) which were weighed separately. All samples were dried for 1 week under high vacuum at room temperature and weighed again.

The 4 replicates of the dried samples corresponding to germination times of 0, 24, 48, 72, 96 and 144 hours, were then combined to make one sample per germination time. The embryos (0-48 hours) were powdered in a watch glass with a spatula; the roots, hypocotyledons and cotyledons (72-144 hours) and the seedcoats (0-72 hours) were finely cut with a razor blade on a glass plate. The samples were then

kept under vacuum for at least 1 further day and analysed for their composition of C, H, N and Ash (W.P. Combé, Micro-analytical Dept., Lab. for Organic Chemistry, Agric.Univ., Wageningen). The determinations on N were done once, those on C and H were duplicated and those on Ash were done three times. The O contents could not be determined due to the presence of interfering elements, but their maxima ($O_{max}$) were calculated by subtracting the total percentage of the 4 components analysed from 100%.

The water movement inside the seed was studied by soaking seeds in water coloured with erythrosine or eosine; this was preceded by a 2 hour treatment in concentrated KOH to render the cuticle between seedcoat and endosperm permeable to the dyes. At various time intervals, seeds were blotted, cut in half and inspected under a binocular microscope.

3.6.3. Water uptake and germination

The water uptake in whole seeds (fig.1) was extremely rapid during the 1st hour. It then proceeded somewhat irregularly, at a much lower rate, until the 7th hour, after which the rate of increase diminished steadily until the 12th hour. Subsequent water uptake was extremely slow until ca 6 hours after the first visible germination (↑), after which it proceeded exponentially.

Fig.1 also shows the water uptake in the seedcoat+endosperm and the embryo separately. Water uptake in the seedcoat+endosperm was extremely rapid during the 1st hour, probably due chiefly to imbibition of the seedcoat proper. Nevertheless, in the embryo, a slight but significant increase in water content was noted as early as 1 hour after sowing, indicating that some water must have penetrated through the cuticle between the seedcoat and the endosperm (for tomato seed histology see Esau[58]). During the following hours, both the endosperm and the embryo imbibed gradually; this seemed to proceed linearly over the first few hours and then at a progressively lower rate. In the seedcoat+endosperm, the water content was almost stationary over the period 24-36 hours but this was followed by a slight further increase, probably due to a further imbibition by the endosperm, after the rupturing of the seedcoat.

Because the endosperm could not be separated from the seedcoat without disturbing the water content, the water uptake could not be determined for these components separately. However, the seedcoat,

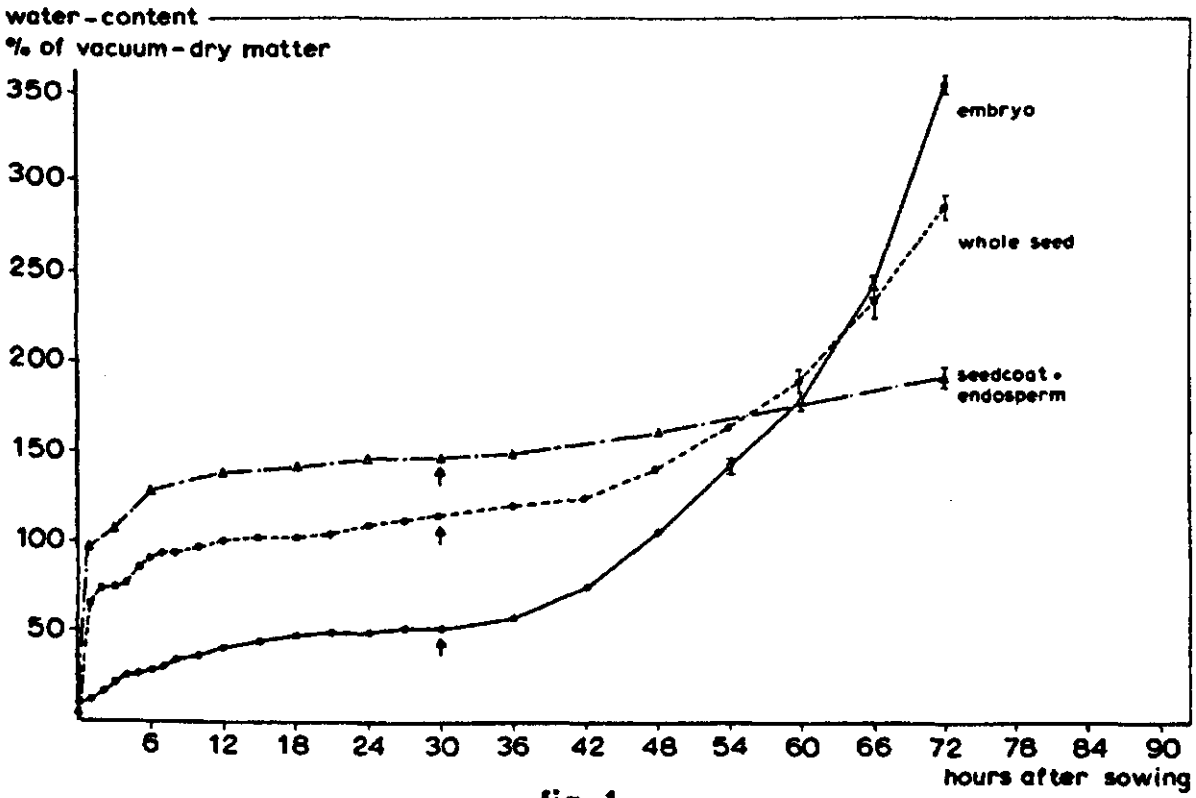
water-content
% of vacuum-dry matter
350
300
250
200
150
100
50
embryo
whole seed
seedcoat + endosperm
6
12
18
24
30
36
42
48
54
60
66
72
78
84
90
hours after sowing

fig. 1

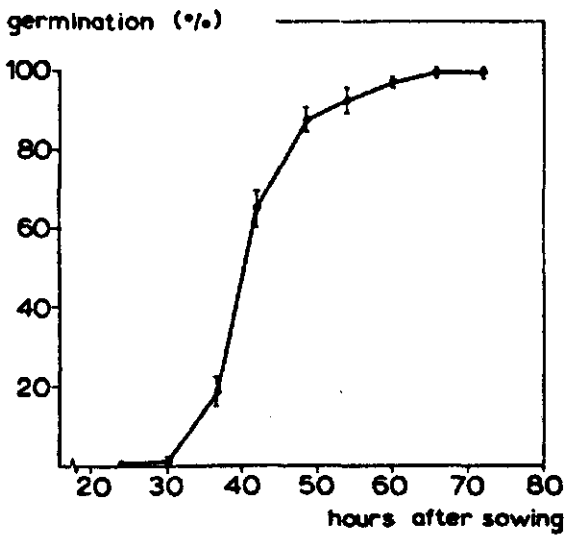
germination (%)
100
80
60
40
20
20
30
40
50
60
70
80
hours after sowing

fig. 2

which made up only ca 30% of the total dry weight of seedcoat+endosperm, was responsible for most of the water uptake during the first hour after hydration, i.e. for ca 2/3 of the total uptake in seedcoat+endosperm prior to germination. Thus, the seedcoat possessed a much higher water content than the endosperm at all stages prior to germination. The parallelism of the curves for seedcoat+endosperm and embryo from the 2nd hour of hydration until visible germination suggests that water uptake in the endosperm proper was similar to that in the embryo. The water uptake in the embryo proceeded approximately linearly over the first 4 hours and then at a progressively lower rate, to reach a plateau at 50-52%, approximately 21 hours after sowing. The end of this plateau, after 30 hours of incubation, corresponded with the germination of the first seeds (↑). From then on, the water content increased exponentially (table 1).

Table 1 : Water content (% of dry matter) of embryos/seedlings and their constituent parts up to 144 hours after sowing; 50% germination = 40 hours after sowing.

| Hours after sowing | Water Content | | | |
|---|---|---|---|---|
| | Whole seedlings | Roots | Hypocotyledons | Cotyledons |
| 30 | 51½ | - | - | - |
| 72 | 355 | 925 | 700 | 132 |
| 96 | 603 | 1835 | 1192 | 185 |
| 144 | 1646 | 1739 | 2422 | 954 |

It can be assumed that the different parts of the embryo have about the same water content at the onset of germination (50-52%). It may then be inferred from table 1 that the exponential increase in water content which follows germination commences first in the roots, then in the hypocotyledons, and only much later in the cotyledons. This sequence corresponds to the sequence of emergence of these organs from the seedcoat. After 48 hours, most roots but only a few hypocotyledons were visible. After 72 hours, all hypocotyledons were completely exposed, while the cotyledons were still enclosed. After 96 hours, the roots were 8-20 mm long and the hypocotyledons 15-20 mm, but in most seedlings only the lower parts of the cotyledons were visible. After 144 hours, the roots were up to 34 mm long and the hypocotyledons about 25 mm; the cotyledons were fully visible at this stage, but were held together at their tips by the seedcoat.

The restrictions in water uptake by the embryo before germination are chiefly of mechanical nature and are caused by its confinement within the seedcoat. This is shown by the fact that excised embryos absorb water at a higher rate than those in intact seeds. During the later stages of germination, however, metabolic processes lead to considerable forces in the embryo (due to increasing osmotic pressure and possibly the swelling of colloids) and the initiation of cell elongation[40]. This causes the rupture of the seedcoat and permits rapid water uptake. The importance of these forces is illustrated by the fact that the growth of weak seedlings is considerably improved by manual removal of the seedcoat.

The techniques for determining the water content of whole seeds and embryos proved satisfactory. The low standard deviations showed that 4 samples of 25 seeds were adequate to take into account the large variation in water uptake between individual seeds. These differences are ascribed to variation in the cuticle permeability, the embryo size, the amount of endosperm and the arrangement of the cotyledons within the seed.

The movement of the absorbed water inside the seed, inferred from the migration of water-soluble dyes, appeared to be gradual. It started at the point where the endosperm was the thinnest and there was no indication of rapid movement by capillary action to the shoot apex or to any part of the embryo.

Germination (fig.2, p.13) was rapid and uniform. This may be attributed to the high germinative power of the seeds, the germination-promoting effect of $KNO_3$[29,94,103,181], and the various advantages of an agar medium over other substrates, which are[29]:

(i) a strictly reproducible chemical composition and moisture content;

(ii) a homogeneous distribution of water and nutrients within the dishes, even when these are placed at an angle to promote uniformity of root growth;

(iii) a large reserve of potentially available water in each dish, without immersion of the seeds; this permits many seeds/dish without risk of desiccation;

(iv) a homogeneous physical composition and smooth surface which does not obstruct the root tips;

(v) transparency, which permits examination and recording with closed dishes;

(vi) freedom from root damage at transplanting, even when the roots have penetrated the substrate.

3.6.4. Increases in dry weight and changes in elementary composition

The average dry weights of seedcoat+endosperm and embryo are given in the right hand part of table 2 (left hand part to be used only for subsequent calculations).

Table 2: Mean water content (% of dry matter) and dry weight (mg) per seedcoat+endosperm (S+E) and per embryo or seedling (Em).

| Hours after sowing | Water content | | Dry weight per | |
|---|---|---|---|---|
| | S+E* | Em | S+E | Em |
| 0 | 4½ | 9½ | 2.13 | 1.29 |
| 1 | 96 | 12⅓ | 2.11 | 1.29 |
| 3 | 106 | 22 | 2.13 | 1.29 |
| 6 | 127 | 28½ | 2.11 | 1.30 |
| 12 | 137 | 41 | 2.11 | 1.29 |
| 24 | 146 | 49 | 2.10 | 1.30 |
| 36 | 149 | 57 | 2.13 | 1.31 |
| 48 | 161 | 105 | 2.09 | 1.33 |
| 72 | 192 | 355 | 1.51 | 2.02 |
| 96 | - | 603 | 1.02 | 2.64 |
| 144 | - | 1646 | 0.99 | 3.02 |

* calculated

No changes occurred until shortly after germination (36 hours after sowing). From then on, the dry weight of the seedcoat+endosperm decreased. The total dry weight reduction was 1.14 mg at 144 hours after sowing; the main reduction occurred between 48 and 96 hours. The dry weight of the embryo increased by as much as 1.71 mg over the same period. It may thus be concluded that the embryo has resorbed approximately 1.14 mg of dry matter from the endosperm (less the quantity lost by respiration). This amounts to 53% of the initial dry weight of seedcoat+endosperm or 75% of the initial dry weight of the endosperm. This resorption took place chiefly during the 48 hours following visible germination. During the greater part of this period the embryo also synthesized some new dry matter, notwithstanding the fact that the cotyledons were still enclosed in the seedcoat. This initial, de novo synthesis can be attributed to the hypocotyledon which under illumination forms chlorophyll as soon as it protrudes from the seedcoat. With the emergence of the cotyledons, the synthetic activity of the embryo increased greatly.

Differences occurred between the various organs (table 3).

Table 3 : Mean dry weight (mg and % of Em) of a single embryo or seedling (Em) and of its constituent parts: root (R), hypocotyledon (H) and cotyledons (C).

| Hours after sowing | mg | | | | % | | |
|---|---|---|---|---|---|---|---|
| | Em | R | H | C | R | H | C |
| 30 | 1.31 | 0.58 | | 0.73 | 44 | | 56 |
| 72 | 2.02 | 0.09 | 0.32 | 1.62 | 4 | 15 | 80 |
| 96 | 2.64 | 0.26 | 0.68 | 1.70 | 10 | 26 | 64 |
| 144 | 3.02 | 0.73 | 1.03 | 1.26 | 24 | 34 | 42 |

The dry weight of the root and the hypocotyledon decreased considerably after germination, up to ca 72 hours after sowing. From then onwards, the dry weight of the root increased exponentially, while that of the hypocotyledon increased rapidly at first and at a lower rate thereafter. In contrast, the dry weight of the cotyledons started to increase at a much earlier stage (ca 48 hours after sowing, cf. table 2) and reached a maximum at ca 96 hours (i.e. when the resorption of endosperm substances had been virtually completed); thereafter, it decreased markedly.

The initial loss in dry matter from the root+hypocotyledon after germination was undoubtedly due to respiration associated with the provision of energy for the germination processes and early growth. At this time, neither the transport of stored nutrients from the cotyledons nor photosynthesis had started. The data suggest that up to 72 hours from sowing all endosperm dry matter absorbed by the embryo was present in the cotyledons. Subsequently, however, there was an active redistribution of metabolites from the cotyledons to the other parts of the embryo, and this continued until at least 48 hours after the completion of endosperm resorption.

The results of the elementary composition analyses are shown in table 4. Changes were slight and inconsistent during the first 48 hours, i.e. until well after visible germination, except for noticeable increases in N which were due to $KNO_3$ absorption from the substrate. Subsequently (72-144 hours), the C and H contents decreased and the N, $O_{max}$ and Ash contents increased in both the seedlings as a whole and all components separately (except for N in the seedcoat+endosperm). These changes can be attributed partly to the oxidative breakdown of lipids and carbohydrates in the endosperm and the embryo (103,120,146) and partly to $KNO_3$ uptake.

Table 4 : Elementary composition of seedcoats+endosperm, embryos/seedlings and their constituent parts, in weight percentages of the dry matter.

| Material | Hours after sowing | C | H | N | $O_{max}$ | Ash |
|---|---|---|---|---|---|---|
| Seedcoats+endosperm | 0 | 51.7 | 7.8 | 4.8 | 32.3 | 3.4 |
| | 24 | 52.9 | 7.8 | 5.1 | 30.7 | 3.6 |
| | 48 | 52.5 | 7.9 | 5.2 | 30.9 | 3.5 |
| | 72 | 49.7 | 7.4 | 5.5 | 32.8 | 4.6 |
| | 96 | 48.2 | 7.1 | 5.1 | 36.6 | 3.1 |
| | 144 | 48.5 | 7.1 | 5.2 | 34.1 | 5.2 |
| Embryos | 0 | 59.1 | 8.8 | 5.1 | 20.9 | 6.0 |
| | 24 | 59.3 | 8.9 | 5.7 | 20.1 | 6.1 |
| | 48 | 58.5 | 8.9 | 5.8 | 20.8 | 6.0 |
| Whole seedlings* | 72 | 55.6 | 8.6 | 5.4 | 24.9 | 5.6 |
| | 96 | 51.1 | 7.9 | 5.9 | 28.6 | 6.6 |
| | 144 | 38.8 | 6.2 | 6.2 | 36.6 | 12.2 |
| Roots | 72 | 40.2 | 6.7 | 5.4 | 36.1 | 11.7 |
| | 96 | 36.8 | 5.8 | 8.1 | 34.7 | 14.7 |
| | 144 | 37.9 | 5.8 | 4.5 | 39.1 | 12.8 |
| Hypocotyledons | 72 | 53.3 | 8.5 | 4.8 | 27.0 | 6.3 |
| | 96 | 44.5 | 7.0 | 5.4 | 34.9 | 8.3 |
| | 144 | 33.5 | 5.6 | 5.9 | 36.7 | 18.3 |
| Cotyledons | 72 | 56.9 | 8.7 | 5.5 | 23.8 | 5.1 |
| | 96 | 55.8 | 8.6 | 5.7 | 25.1 | 4.7 |
| | 144 | 43.7 | 6.9 | 7.5 | 35.1 | 6.9 |

* calculated from constituent parts

However, many other metabolic changes are known to be involved. The increases in Ash resulted mainly from K uptake from the medium, although the resorption of various elements from the endosperm probably increased this effect. The increases in Ash are, however, exaggerated by the complete oxidation of these elements during the combustion of the samples. The $O_{max}$ contents were estimated by subtracting the C, H, N and Ash contents from 100%; these values may therefore include small proportions of halogens, P and some other elements. A more important source of error arises from the fact that some oxygen from the sample will be used in the ashing process. This leads to a progressive underestimation of the $O_{max}$ content with increasing germination time. This means that $O_{max}$ increased more than the data indicate.

In the seedlings as a whole, the main changes occurred from 96-144 hours after sowing, i.e. after the resorption of the endosperm and during the earliest phase of independent growth. In the roots, the

main changes seem to have occurred at a much earlier stage as C and H were already very low, and $O_{max}$ and Ash very high, in the 72 hour sampling (deviations from the general trends in the 96 and 144 hour samplings require confirmation). In the hypocotyledons, the most pronounced changes occurred at 72-96 hours, except for the major increase in Ash which occurred at about 144 hours after sowing. In the cotyledons, rapid changes commenced at 96-144 hours after sowing. The timing of these changes in the various organs is a measure of their metabolic activity and is closely related to the increases in water content (cf. table 1).

The composition of the seedcoat proper probably did not change appreciably during the sampling period (except for an increase in N). At the end of this period, ca 53% of the dry matter of seedcoat+endosperm had been resorbed by the embryo. On this basis it may be inferred from a comparison of the 0 and 144 hour data, that, in the resorbed part of the endosperm, the contents of C and H were somewhat lower than in the embryo, the N content was similar, the $O_{max}$ content was substantially higher and the Ash content was considerably lower.

The large differences in dry matter composition between the roots, the hypocotyledons at the specific stages considered are of obvious relevance to fast neutron dosimetry. However, their importance can only be assessed by considering the water contents also. This subject is discussed in section 3.7.

## 3.7. Dose absorption in the biological materials

The preceding data permit the calculation of the fast neutron doses absorbed in the various biological materials, relative to those in water ($D_{H_2O}$), as follows. The dry weight percentages of C, H, N and O (table 4) were multiplied by the following factors[93]:

| | |
|---|---|
| C | 0.0015; |
| H | 0.1120; |
| N | 0.0014; |
| O | 0.0012. |

The resulting products were added; the total, which represents the dose in rad in the specimen corresponding to 1 rad in acetylene, was divided by 1.35 to obtain the dose in rad in the specimen corresponding to 1 rad in water; let this be $\Delta$. Further, let the water content be $\alpha$, in % of dry matter (tables 1 and 2). The dose absorbed in the biological specimen is then:

$\frac{100\Delta+\alpha}{100+\alpha} \times D_{H_2O}$, where $\frac{100\Delta+\alpha}{100+\alpha}$ may be called the 'specimen dose correction factor'. This calculation was performed for all specimens in which the elementary composition had been analysed, and for the 1, 3, 6 and 12 hour samples. The dry matter composition of the 0 hour specimen was regarded as representative of the 1, 3 and 6 hour samples, whereas the mean composition of the 0 and 24 hour specimens was used for the 12 hour samples. The resulting values are shown in table 5, together with the same data expressed as percentages of the corresponding dry sample values.

Table 5 : Absorbed fast neutron dose relative to that in water, in the constituent parts of dry and germinating tomato seeds and young seedlings, and the same data as percentages of the dry sample values.

| Hours after sowing | Seedcoats+ endosperm | % | Embryos or seedlings (Em) | % |
|---|---|---|---|---|
| 0 | .752 | 100 | .841 | 100 |
| 1 | .868 | 115 | .845 | 101 |
| 3 | .874 | 116 | .857 | 102 |
| 6 | .886 | 118 | .864 | 103 |
| 12 | .889 | 118 | .877 | 104 |
| 24 | .892 | 119 | .886 | 105 |
| 48 | .902 | 120 | .916 | 109 |
| 72 | .899 | 120 | .956 | 114 |
| 96 | - | - | .964 | 115 |
| 144 | - | - | .977 | 116 |

| | Roots | % of $Em_0$ | Hypocotyledons | % of $Em_0$ | Cotyledons | % of $Em_0$ |
|---|---|---|---|---|---|---|
| 72 | .965 | 115 | .974 | 116 | .919 | 109 |
| 96 | .977 | 116 | .974 | 116 | .931 | 111 |
| 144 | .976 | 116 | .982 | 117 | .968 | 115 |

The lowest specimen dose correction values were those for the dry materials. Marked increases occurred in the course of germination; however, even the highest values were less than unity, indicating a lower energy absorption than in water.

In dry seeds, the absorbed dose was higher in the embryo than in the seedcoat+endosperm. This position was reversed within the 1st hour of hydration due to the rapid water uptake in the latter. However, 48 hours after sowing, the embryo had again the highest absorbed dose owing to its rapid water uptake following germination; from this time onwards, the absorbed dose in the seedcoat+endosperm became

constant or even declined. In young seedlings the absorbed dose was consistently higher in the roots and hypocotyledons than in the cotyledons. However, these differences became less with time. The % data relative to the dry sample values logically show the same trends. In conclusion, the data demonstrate that gross errors in both absolute and relative dosimetry may arise if the elementary composition of the biological targets is disregarded.

When considering these data in conjunction with those of the previous section, it appears that the dose absorption values are determined almost entirely by the water contents of the various specimens and the H contents of their dry matter. A similar conclusion was reached by Contant et al.[33] on the basis of a comparison of relative rad doses in dry embryos, small flower buds (85.5% $H_2O$) and dry pollen (2.8% $H_2O$) of tomato, which were 1.13, 1.28 and 0.94x the rad doses in acetylene (= 0.84, 0.95 and 0.70x $D_{H_2O}$), respectively. This conclusion is also in agreement with Constantin and Osborne[28] who performed analogous calculations on seeds of 10 different plant species and found that the conversion factors, which ranged from 0.83-1.23 relative to the tissue rad dose in air (= 0.911 $D_{H_2O}$) were almost perfectly correlated with H content.

Nevertheless, the C and O contents should not be disregarded in calculating rad doses. Although the contribution to the neutron dose per weight % of these elements is low, compared with that of H, their large proportions in biological materials (C especially in dry and O especially in hydrated objects[33]) cause a combined dose contribution of 8-9%. The N content is usually low and its dose contribution of minor importance. However, the following procedure accounts for all 4 elements discussed, without the separate determination of O and N, by making use of the fact that the dose contribution per weight % is very similar for these elements (p.19). The water content of the irradiated object and the C, H and Ash contents of its dry matter should be accurately determined; from these data the C, H and Ash contents in the 'fresh' samples can be calculated. The pooled N+O content may then be estimated by subtracting the sum of the former values from 100%. In calculating the absorbed dose the multiplication factor for O (0.0012) may be considered to apply approximately to the total content of N+O. Inaccuracies in the correction factors for dose absorption determined in this way are less than 1%, even when the estimated N+O content contains an error of several percent.

In addition to the relative absorbed dose, the relative amounts of radiation energy absorbed per meristem or per meristem cell may also be of value in interpreting the results of radiosensitization experiments. Because the changes in weight and elementary composition of individual meristem cells during hydration are unknown, they are represented by the changes occurring in the embryo as a whole. This is only possible prior to the rupture of the seedcoat, because thereafter the weight increase of the embryo is caused chiefly by the enlargement of differentiated cells. Therefore, no estimates are available for the 72-144 hour pregermination treatments; in fact, some bias may even be expected after 48 hours. In order to estimate the necessary parameters, the amounts of energy absorbed per embryo per krad in water were calculated first. This was done by multiplying the rad doses by 100 to obtain erg/g, and then by the fresh weights per seedcoat+endosperm or per embryo in g calculated from table 2. These products x 1000, which represent the energy (erg) absorbed per object per krad in water, are shown in table 6, together with the same data expressed as percentages of the corresponding dry sample values. These percentage data also represent the relative amounts of energy absorbed per cell.

Table 6 : Energy per krad in water (erg and % of dry sample value), absorbed per seedcoat+endosperm and per embryo or seedling.

| Hours after sowing | Seedcoat+endosperm | | Embryo or seedling | |
|---|---|---|---|---|
| | erg | % | erg | % |
| 0 | 168 | 100 | 118 | 100 |
| 1 | 359 | 214 | 123 | 104 |
| 3 | 384 | 229 | 135 | 114 |
| 6 | 424 | 253 | 144 | 123 |
| 12 | 445 | 266 | 160 | 135 |
| 24 | 461 | 275 | 172 | 146 |
| 48 | 493 | 295 | 250 | 212 |

The energy absorption/cell increased consistently in the course of germination, as expected. In the seedcoat+endosperm this increase was very rapid during the first hour up to a value of 214%, after which there was a further gradual rise to 295% after 48 hours. In the embryo, the increase was more gradual; a value of 146% was reached after 24 hours and probably a slightly higher value just before the onset of germination. After germination, the relative energy absorption/embryo increased rapidly because of the elongation of the

differentiated root cells but this was no longer a measure of the relative energy absorption per meristem cell, and the value of 212% after 48 hours is undoubtedly an overestimation.

These data will be used in connection with the changes in radiation effectiveness during germination which were observed in the experiments presented in the next chapter.

# 4. RADIATION EFFECTS

## 4.1. Introduction

The aim and scope of the present experiments were outlined in chapter 1. The main variable was the duration of seed germination prior to irradiation.

Four experiments will be considered in detail. By way of exploration, the conditions of culturing were varied between experiments after the petridish stage, because it was impracticable to investigate experimental conditions as a variable within each experiment. The experiments 1 and 2 were carried out in growth chambers but in containers of different size and depth entailing differences in nutrient supply; experiment 3 was performed in a greenhouse under optimal conditions. The methods and conditions of culturing of experiment 4 were based on conclusions derived from the preceding experiments.

Because of space restrictions, the experiments 1 and 2 had to be limited to the preflowering stage of the irradiated generation ($M_1$) and to one cultivar; experiment 3 could be pursued up to the seedling stage of the selfed offspring ($M_2$) and involved two cultivars. These experiments comprised, in addition to dry seeds, only one prehydration treatment. Experiment 4 was designed to elaborate the results of the preceding experiments and involved a wide range of prehydration/germination times.

Adequate knowledge of the shape of the dose/response relationships for various $M_1$ and $M_2$ characters, and of factors influencing these shapes, is essential for the efficient handling of seeds and plants irradiated with a view to mutation induction. In particular, any attempt to predict the mutagenic value of a radiation treatment from early $M_1$ characteristics requires a fair degree of constancy in the dose/response relationships. Accordingly, this subject is given considerable attention (section 4.3).

An analysis of the causes of differences in the effectiveness of radiation on characters with different dose/response relationships was not attempted. However, as only one mutagen (fast neutrons) was involved, the dose/response relationships of the various objects have essentially the same shape for a given character, so that relatively simple parameters are adequate for a quantitative comparison of radiation effectiveness (section 4.4).

The use of two cultivars in exp.3 aimed at a first exploration of

genetical differences in the radiation sensitivity of seeds, and at establishing whether the sensitivity relationship varies with the character studied. This latter question is directly relevant to the efficiency (defined in chapter 2) of mutation induction. In order to learn more about the nature of varietal sensitivity differences, and with a view to practical application, the possible relationships between these differences and certain biometrical characteristics of the seeds are also examined (section 4.5).

Studying the increase in neutron effectiveness with prehydration for a number of $M_1$ and $M_2$ characters will establish whether or not, and in which direction, prehydration changes the quantitative relationship between the various radiation effects. By analogy with the foregoing, this question has a direct bearing on the efficiency of mutation induction. Such a study also permits an evaluation of the proportion of effectiveness enhancement which is due to increased radiation energy absorption, using the data of section 3.7. Moreover, such information is conducive to a discussion on the various factors involved in sensitization, and on recovery in relation to the organisation of the embryonic shoot apex (section 4.6).

The degree of correlation between various characteristics of plants within a treatment is of great importance for practical mutagenesis. In particular, a high within-treatment correlation between early $M_1$ characters on the one hand and $M_1$ seed set or $M_2$ characters on the other hand would permit positive selection at an early $M_1$ stage. Alternatively, the absence of correlation would permit the early elimination of weak and semi-sterile $M_1$ individuals without any lowering of the frequency of recessive mutations. This question is examined in detail (section 4.7) because no suitable data pertaining to fast neutron irradiation have so far been reported.

The main practical implications of the evidence presented in this chapter will be emphasized in chapter 5.

## 4.2. Materials, methods, characters studied

### Experiment 1

The experimental material consisted of seeds of cv. 'Moneymaker', 100-seed weight 319 mg, 5.6% $H_2O$. These were irradiated both dry and at 48 hours hydration, which coincided with the germination of the first seeds (note the slower germination than in the experiments of section 3.6). For conditions of pretreatment and irradiation see sec-

tion 3.4.

The dry seeds received doses, expressed as $D_{H_2O}$, of 0, 1, 2, 3, 4, 6, 7, 9 and 11 krad ($D_1$ series); the prehydrated seeds received 0, 1, 2, 3 and 4 krad ($H_1$ series). Each treatment consisted of 100 seeds in one petridish.

After a total germination period of 4 days at 27°C under continuous illumination with 11,000 lux of Philips TL33RS+Philinea (interrupted in $H_1$ by irradiation at 23°C) the climatic regime was changed to 18/6 hours day/night, with temperatures of 23/17°C. These conditions were maintained until the end of the experiment. The young seedlings were transplanted into vermiculite in 50x40x8 cm wooden boxes at 5-8 days after sowing, depending on the speed of germination and growth; at this stage the experiment was laid out in 4 replicates. Nutrient solution, formula Hoagland[89], was supplied daily. Twenty five days after sowing, the seedlings were transferred to an aerated Hoagland solution. The experiment was concluded after 42 days.

The following characters were recorded on all individuals:

18 days after sowing
- presence or absence of at least 1 leaf >3 mm
- number of leaves >3 mm

25 days after sowing
- length of the primary root
- cotyledon length
- length of the 1st leaf
- length of the hypocotyledon + stem (= 'stem length')
- number of leaves >3 mm

The treatment means for length of the primary root, the cotyledons and the hypocotyledon + stem, i.e. of those organs that were already differentiated in the dry embryos, were converted to cumulative growth. This was done by subtracting the mean length attained in the lethal 6 krad treatment of $H_1$, where cell division was completely inhibited.

42 days after sowing
- ultimate length of the 3rd leaf
- ultimate fresh weight of the 3rd leaf
- length of the 1st axillary shoot
- fresh weight of the whole plant
- presence or absence of an apparently normal terminal apex

Experiment 2

This experiment was carried out with seeds of the same lot of cv. 'Moneymaker' no. 83 as used for the experiments in section 3.6. These seeds were irradiated dry and after 24 hours hydration as described in section 3.4.

The dry seeds received doses ($D_{H_2O}$) of 0, 1, 1½, 2, 3, 6 and 9 krad ($D_2$ series); the prehydrated seeds received 0, 1, 1½, 2, 3, 4 and 6 krad ($H_2$ series). In addition, a series of low doses was given to both dry and prehydrated seeds; these doses were 2, 10, 50, 200 and 500 rad.

The experiments consisted of 6 replicates of 25 seeds/treatment from sowing onwards. However, because of the long exposure times, all replicates of a given treatment had to be irradiated simultaneously, while the different treatments were irradiated in succession. The methods of culturing conformed to those in exp.1 except that the seedlings were transplanted into 22x22x5 cm earthenware seedpans and the substrate was wetted on alternate days with Hoagland nutrient solution and water. The experiment was concluded 25 days after sowing.

The following characters were recorded:

Petridish stage
- germination capacity
- days to germination
- length of the primary root, 4 days after 50% germination

These characters were recorded by taking photographs of the petridishes (lids removed) in a sterile room at 6 hour intervals; the root lengths were measured with the aid of a curvimeter on 40x enlarged projections of the negatives on a smooth white wall.

25 days after sowing
- as in exp.1

Experiment 3

The experiment was carried out with seeds of cv. 'Moneymaker' (100-seed weight 319 mg as in exp.1) and of cv. 'Glorie' (100-seed weight 279 mg); both seed-lots were equilibrated at 6.5% $H_2O$.

These seeds were irradiated dry and after 24 hours hydration as described in section 3.4.

The dry seeds received doses ($D_{H_2O}$) of 0, 1, 2, 3, 4, 6 and 9 krad ($D_3$ series) and the prehydrated seeds 0, 1, 2, 3, 4 and 6 krad ($H_3$ series). The dry seeds were stored for 3 days at room temperature

before being sown. The conditions of germination were as in exp.1. Shortly after germination, the seeds were planted into boxes with fertile soil, in a greenhouse with regulated temperature and under natural day-light (late spring). From this stage onwards the experiment consisted of 3 replicates. The conditions of culturing were very favourable so that growth was very rapid. At the age of 3½ weeks those seedlings considered to be incapable of satisfactory development were discarded. Of the transplantable seedlings, fixed numbers, increasing with dose, were taken at random from the various treatments, and planted into 9 cm pots. One week later they were transplanted into the greenhouse soil. The plants were topped above the 2nd cluster and pruned regularly.

Two adjacent fruits were harvested from the 1st and the 2nd truss of each $M_1$ plant, and the seeds extracted. The progenies ($M_2$) of all plants containing $\geq 24$ seeds in the least fertile truss were tested. These progenies, consisting of 24 seeds/truss, were sown directly in boxes with soil. They were screened for aberrations detectable at the seedling stage, 10 and 20 days after sowing when symptoms on the cotyledons and the first leaves, respectively, were the most easily recognised. Recessive 'visible' mutations were considered to be those events leading to 2-12 $M_2$ seedlings having clearly the same distinguishing marks; aberrant plants occurring single were disregarded or counted as sublethals, as previous experience had shown that a large proportion of these cases were not due to simple recessive mutations.

The following characters were recorded:

Petridish stage
- germination capacity
- days to germination

18 days after sowing
- cotyledon length, 12 seedlings/treatment; treatment means converted to cumulative growth as in exp.1
- fresh weight of the aerial parts, 3 seedlings/treatment

25 days after sowing
- number of plants suitable for transplanting

Maturity (all surviving plants)
- number and weight of seeds/fruit in 1st and 2nd truss, and mean over both trusses
- number of 'fertile' plants per treatment ($\geq 24$ seeds in the least fertile truss)

$M_2$ seedling stage (2 truss progenies of all 'fertile' $M_1$ plants)
- number of non-germinating seeds
- number of sublethal seedlings unsuitable for mutant screening
- number of recessive 'visible' mutations
- number of mutant seedlings corresponding to the 'visible' mutations

Spare $M_1$ seed was subjected to germination tests after 1 year of storage at room temperature, in order to study the effect of seed irradiation on loss of germinative power with storage.

To determine the 100-embryo weight, 100 vacuum-dry seeds of each cultivar were weighed, after which their embryos were removed as described in section 3.6 (p.11); the 100-embryo weight was obtained by weighing the seedcoat+endosperm halves and subtracting these weights from those for the whole seeds.

To determine the percentages of cells in the $G_1$ and $G_2$ stage of interphase, respectively (2C and 4C nuclei, i.e. before and after DNA duplication), 5 seeds per cultivar were soaked for 16 hours at low temperature to facilitate the removal of the embryos, which were then fixed in 3:1 alcohol:acetic acid (Carnoy) and stained with Feulgen. Photodensitometric DNA determinations were made on 500 nuclei per embryo (5 embryos per cultivar). These determinations were restricted to the strictly meristematic zone of the root apices (max.ca 150 µm from the root tips). As the distributions of the relative values for 2C and 4C nuclei do not overlap, the cell numbers at each stage are determined very easily.

Finally, to compare the average amounts of DNA/nucleus of the two cultivars, accurate measurements were made on 20 cells in each of 5 root meristems per cultivar, i.e. 100 measurements per cultivar. The results were expressed in scale units, and therefore constitute relative values. The standard errors were calculated on the basis of the means per meristem.

Experiment 4

The experiment was carried out with seeds of the same lot of cv. 'Moneymaker' no. 83 as used for exp.2 and for the experiments in section 3.6. The conditions of pretreatment and irradiation were as in section 3.4.

These seeds were irradiated dry (D series) and after ½, 3, 6, 12, 24, 48, 72, 96 and 144 hours of hydration/germination (H½, H3 .... H144 series). The irradiations were performed at a 2.8x higher dose

rate than in the preceding experiments, allowing shorter exposure times (see section 3.5). The exposure times and doses are shown in table 7. The dose ranges followed roughly the expected increases in neutron effectiveness with increasing prehydration time. In the series H48-H144 only very few treatments are available due to a too late discovered error in the execution of the irradiations.

Table 7 : Schedule of treatments (+ = $M_1$ seedling stage only; ⁎ = up to $M_2$ seedling stage). Experiment 3.

| Exposure (min) | $D_{H_2O}$ (krad) | Pre-irradiation germination period (hours) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0(D) | ½ | 3 | 6 | 12 | 24 | 48 | 72 | 96 | 144 |
| 0 | 0.00 | ⁎ | ⁎ | ⁎ | ⁎ | ⁎ | | | | | |
| 5 | 0.23 | | | | | | | ⁎ | ⁎ | ⁎ | ⁎ |
| 10 | 0.47 | | | | + | ⁎ | ⁎ | ⁎ | | | |
| 20 | 0.93 | + | ⁎ | ⁎ | ⁎ | ⁎ | ⁎ | | | | |
| 30 | 1.40 | | | | ⁎ | ⁎ | ⁎ | | | | |
| 40 | 1.87 | ⁎ | ⁎ | ⁎ | ⁎ | ⁎ | | | | | |
| 50 | 2.33 | | | | ⁎ | + | | | | | |
| 60 | 2.80 | ⁎ | ⁎ | ⁎ | + | | | | | | |
| 75 | 3.50 | | | ⁎ | + | | | | | | |
| 90 | 4.20 | ⁎ | ⁎ | + | | | | | | | |
| 105 | 4.90 | | + | + | | | | | | | |
| 120 | 5.60 | + | + | + | | | | | | | |
| 150 | 7.00 | + | + | | | | | | | | |
| 180 | 8.40 | + | | | | | | | | | |

The experiment consisted of 10 replicates, each of 10 seeds/treatment, from sowing until final transplanting. The seedlings were first transplanted into wooden boxes with fertile soil. This was done 4 days after sowing for all series up to H72 and 5 days after sowing for H96. In H144 the lids were removed from the petridishes after 96 hours and the dishes supplied regularly with water to avoid desiccation; this series was transplanted 7 days after sowing.

Until this time the climatic regime for all series was maintained as during pretreatment, and was then changed into a day/night rhythm of 16/8 hours with corresponding temperatures of 23/17°C. Transplanting into pots took place after 3½ weeks, followed by final transplanting into the soil of a greenhouse at 50x70 cm spacing 1 week later. At this stage only those treatments having a high percentage of transplantable seedlings (asterisks in table 7) were retained. These were planted in 8 randomised blocks, each containing 12 plants per treatment; gaps were filled with plants of corresponding vigour, in order to avoid bias due to spacing differences. Fruit harvesting,

fertility determination and study of the $M_2$ generation were carried out as in exp.3.

The following characters were recorded:

$M_1$ seedling stage (random sample of 20 seedlings/treatment, the same for all characters)

- cotyledon length, 12 and 19 days after sowing; treatment means converted to cumulative growth as in exp.1
- length of the 1st leaf, 19 days
- length of the 2nd leaf, 19 days
- number of leaves >3 mm, 19 days
- number of leaves with morphological or colour abnormalities

Flowering stage

- number of leaves below 1st inflorescence
- number of days to flowering

Maturity (all surviving plants)

- average weight of seeds/fruit in 1st and 2nd truss, and mean over both trusses
- number of 'fertile' plants per treatment

$M_2$ seedling stage (2 truss progenies of all 'fertile' $M_1$ plants)

- as in exp.3.

## 4.3. The shape of the dose/response relationships

In the preceding section the characters recorded were listed as much as possible in chronological order. In order to study the dose/response relationships they are now regrouped as follows:

- germination time;
- growth characters;
- developmental characters;
- $M_1$ fertility;
- $M_2$ characters.

Growth characters are defined as characters expressing the increase in length or weight of a given organ, whereas developmental characters are defined as those which concern the timing of the formation of new organs. Some characters, such as plant weight, are the result of both growth and development and their classification is somewhat arbitrary.

In exps.3 and 4, the correspondence between the various $M_1$ and $M_2$ characters with regard to the shape of their dose/response relationships was examined by means of the coefficients of correlation, based upon treatment means.

The presentation of the results is confined to a summing up of the main characteristics of the graphs. An interpretation is given in the subsequent discussion.

4.3.1. Results

Experiment 1

The data, expressed as percentages of the control values (table 8), are presented as graphs denoted $D_1$ and $H_1$; graphs denoted $D_2$ and $H_2$ in some figures will be considered later.

Table 8 : Control treatment means ± their S.E. Experiment 1.

| Character | Days | $D_1$ | $H_1$ |
|---|---|---|---|
| Leaf number | 18 | 2.8 ± 0.01 | 2.8 ± 0.01 |
| Root length (mm) | 25 | 82 ± 2.1 | 89 ± 2.4 |
| Cotyledon length (mm) | 25 | 30 ± 0.3 | 23 ± 0.3 |
| Length 1st leaf (mm) | 25 | 49 ± 0.6 | 39 ± 0.7 |
| Stem length (mm) | 25 | 41 ± 0.5 | 33 ± 0.5 |
| Leaf number | 25 | 4.3 ± 0.02 | 3.8 ± 0.01 |
| Length 3rd leaf (cm) | 42 | 23 ± 0.4 | 26 ± 0.5 |
| Weight 3rd leaf (g) | 42 | 49 ± 1.9 | 56 ± 2.6 |
| Plant weight (g) | 42 | 171 ± 8.7 | 295 ± 14.2 |
| Length 1st axillary shoot (cm) | 42 | 16 ± 2.0 | 19 ± 2.3 |

Growth characters

Cumulative root growth after 25 days (fig.3) responded almost linearly over most of the vital dose range, with a deviating tail at the highest doses in $H_1$.

Cumulative cotyledon growth after 25 days (fig.4) showed a discrepancy between the two series; the curve of $D_1$ was concave, whereas that of $H_1$ was convex in the upper part while tailing off at the high doses.

The curves for length of the 1st leaf after 25 days (fig.5) were slightly convex up to medium doses though in $D_1$ this trend was partly obscured by the relatively large scatter of the treatment means. There was no evidence of a tail at the highest doses in either series.

Cumulative stem growth after 25 days (fig.6) probably responded in both series with a very slight convex curvature at the lower doses; both curves showed a clear tapering off towards zero.

Length of the 3rd leaf after 42 days (fig.7) decreased approximately linearly in $D_1$ except for a dip in the 1 krad treatment, and

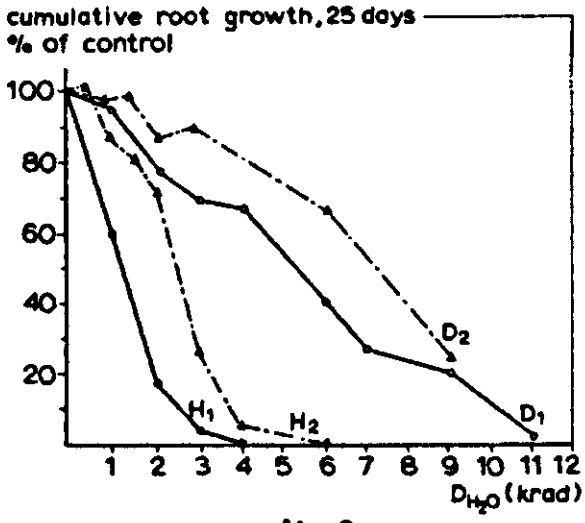


fig.3

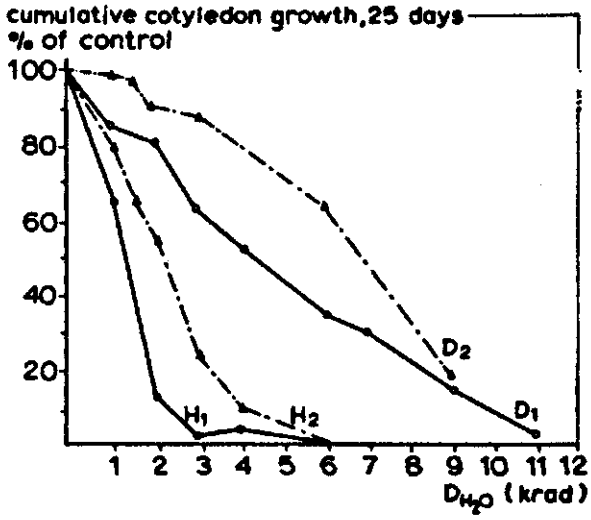


fig. 4

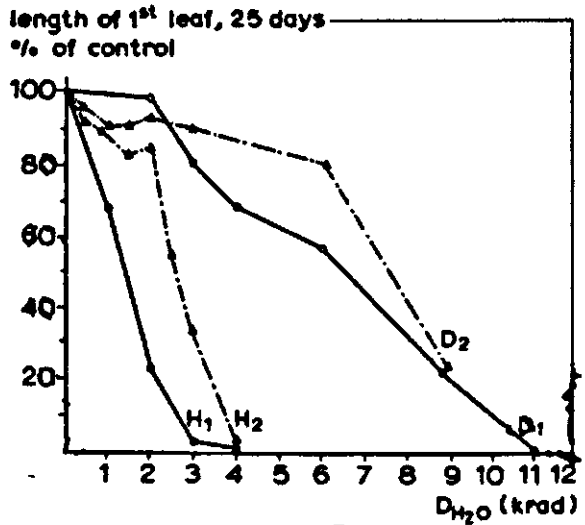


fig. 5

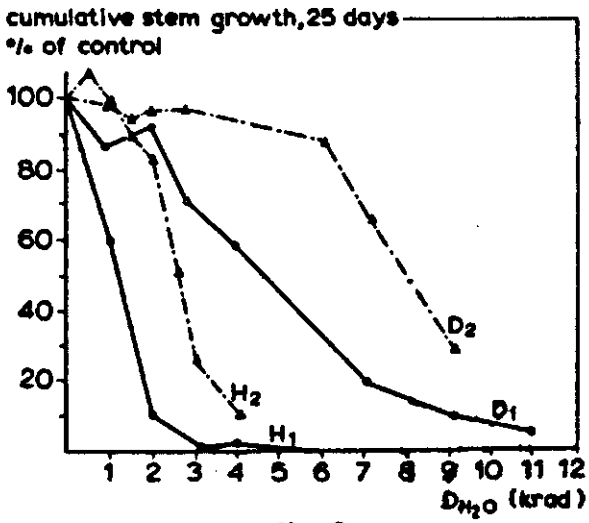


fig. 6

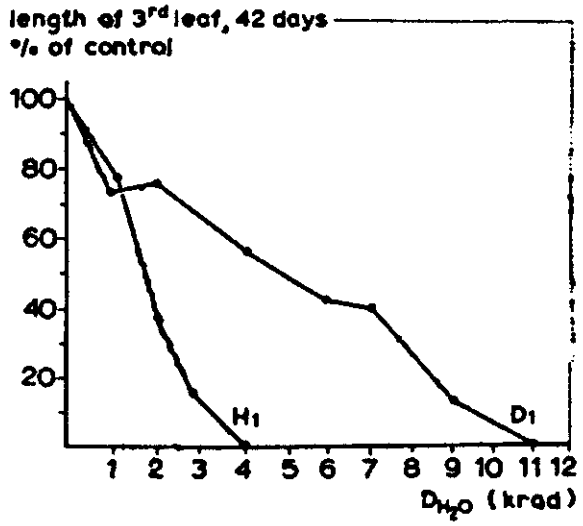


fig. 7

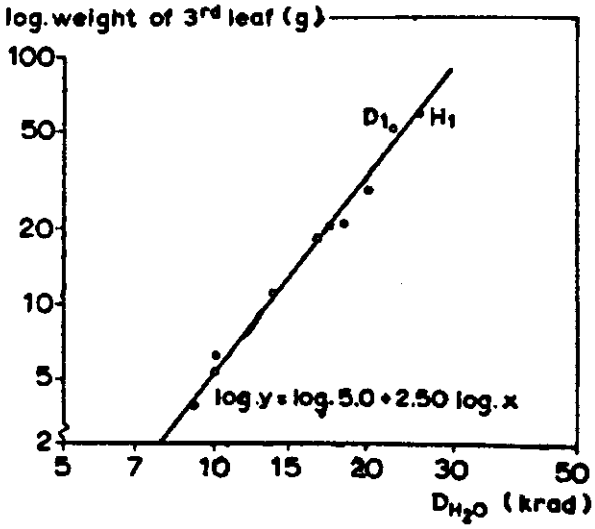


fig. 8

possibly somewhat sigmoidally in $H_1$. There was no pronounced tail in either series.

The relationship between length (x in cm) and weight (y in mg) of the 3rd leaf was exponential in both series (fig.8); the regression equations differed so slightly that $\log y = 5.0 + 2.50 \log x$ may be considered to apply to both sets of data, showing that the length/weight relationship of the leaves was not affected by an interaction between irradiation and seed hydration.

The best fitting linear and parabolic regressions on dose and the corresponding residual variances were calculated from the single plant data of the 0-7 krad $D_1$ treatments and the 0-2 krad $H_1$ treatments. This was done for all quantitative characters except weight of the 3rd leaf and length of the 1st axillary shoot.

The hypothesis that a parabolic regression would give a better fit than a linear regression was tested using the F-test. This test was highly sensitive because large numbers of plants were involved. Although for most characters there was some heterogeneity of variance between the treatments, the tests were performed as if the variances were homogeneous, firstly because it is a matter of experience that these procedures are reasonably tolerant to heterogeneity of variance, secondly because the relationship between the mean and the standard error for a given treatment (dose) is unknown and the data are insufficiently numerous to suggest a variance-stabilizing transformation.

The results (table 9) demonstrate a significant curvilinearity for cotyledon length, 1st leaf length and stem length after 25 days ($P \leqslant 0.05$) but not for root length after 25 days and length of the 3rd leaf or plant weight after 42 days ($P > 0.05$). The regression equations (not shown) confirmed that all curvatures were convex except for cotyledon length in $D_1$.

Table 9 : Probability P of a parabolic regression on radiation dose not yielding an improvement over a linear regression in fitting experimental data after irradiation of dry ($D_1$) and prehydrated ($H_1$) seeds. Experiment 1.

| Character | Days | $D_1$ (7 doses) | | $H_1$ (3 doses) | |
|---|---|---|---|---|---|
| | | P | d.f. | P | d.f. |
| Root length | 25 | 0.19 | 615 | 0.29 | 261 |
| Cotyledon length | 25 | 0.00 | 615 | 0.00 | 261 |
| Length 1st leaf | 25 | 0.00 | 615 | 0.00 | 261 |
| Stem length | 25 | 0.00 | 615 | 0.00 | 261 |
| Length 3rd leaf | 42 | 0.08 | 604 | 0.06 | 229 |
| Plant weight | 42 | 0.06 | 604 | 0.79 | 229 |

Developmental characters

The dose/response relationships of the percentage of plants having at least 1 leaf >3 mm after 18 days or a normal appearing shoot apex after 42 days (fig.9) seemed sigmoidal with a shoulder; the data suggest that the former character is a good indicator for the latter.

Sigmoidal responses were also observed for leaf number after 18 days (fig.10); a clear shoulder appeared in $D_1$ but was not demonstrated in $H_1$; the 2 krad $D_1$ treatment showed a slight increase over the control. Leaf number after 25 days (fig.11) showed similar patterns, except that $D_1$ had a higher threshold dose while increases over the control occurred up to medium doses.

Length of the 1st axillary shoot after 42 days (fig.12) showed remarkable increases over the control in the 2 and 3 krad treatments of $D_1$. This was due, at least partly, to an earlier initiation of axillary shoot growth in these treatments. No such increases over the control occurred in $H_1$. Plant weight after 42 days (fig.13) responded very irregularly in $D_1$, according to a pattern which was clearly influenced by the level of growth of the axillary shoots. It must be noted, however, that none of the treatment means of plant weight exceeded the control. This means that the increased lengths of the 1st axillary shoot at medium doses compared to the control must be attributed to effects on apical dominance rather than to real growth stimulation. The dose/response relationship for plant weight in $H_1$ was linear except for a tail at the highest dose. The last two characters are clearly governed by both developmental and growth processes.

Experiment 2

The data, expressed as percentages of their controls (table 10), are shown by means of the graphs denoted $D_2$ and $H_2$.

Table 10 : Control treatment means ± their S.E. Experiment 2.

| Character | Days | $D_2$ | $H_2$ |
|---|---|---|---|
| Root length (mm) | 4* | 36 ± 0.8 | 35 ± 0.7 |
| Root length (mm) | 25 | 69 ± 1.8 | 68 ± 1.9 |
| Cotyledon length (mm) | 25 | 17 ± 0.3 | 20 ± 0.4 |
| Length 1st leaf (mm) | 25 | 22 ± 0.6 | 23 ± 0.7 |
| Stem length (mm) | 25 | 24 ± 0.3 | 25 ± 0.4 |
| Leaf number | 25 | 2.7 ± 0.06 | 2.7 ± 0.07 |

* after 50% germination

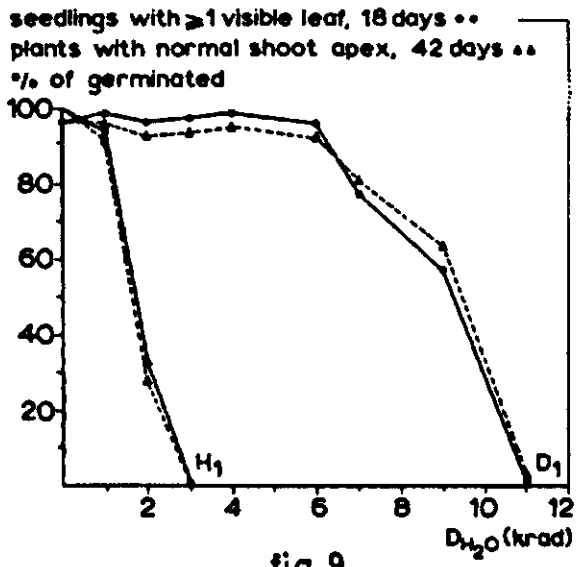


fig. 9

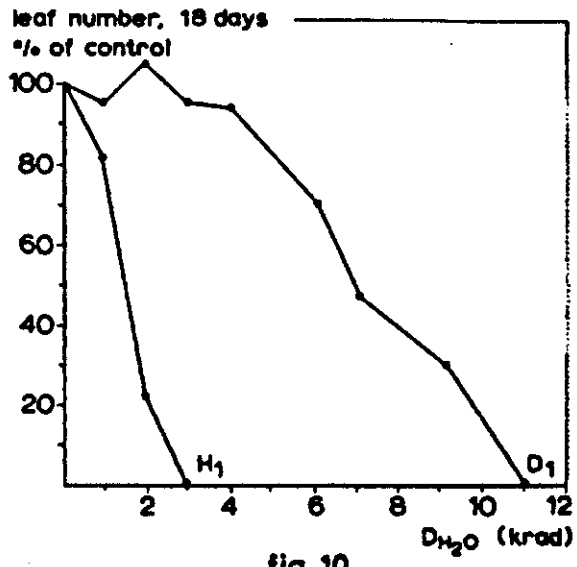


fig. 10

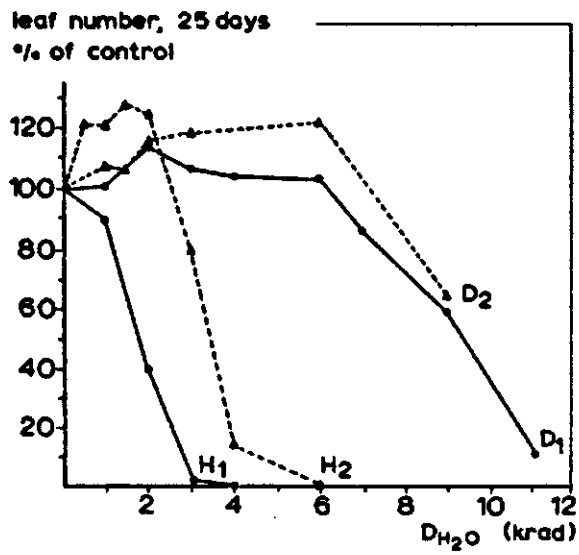


fig. 11

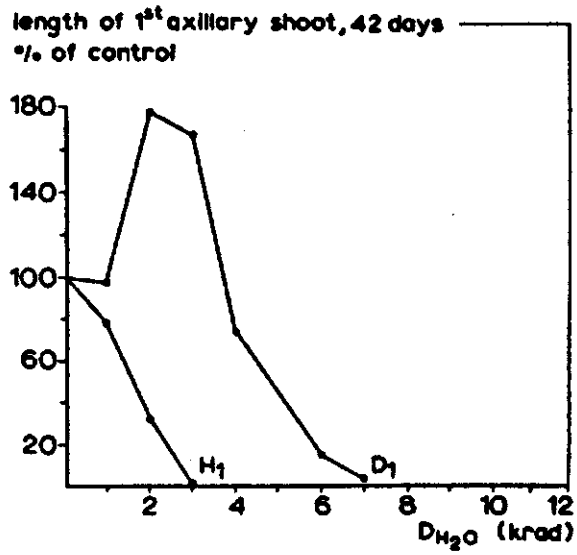


fig. 12

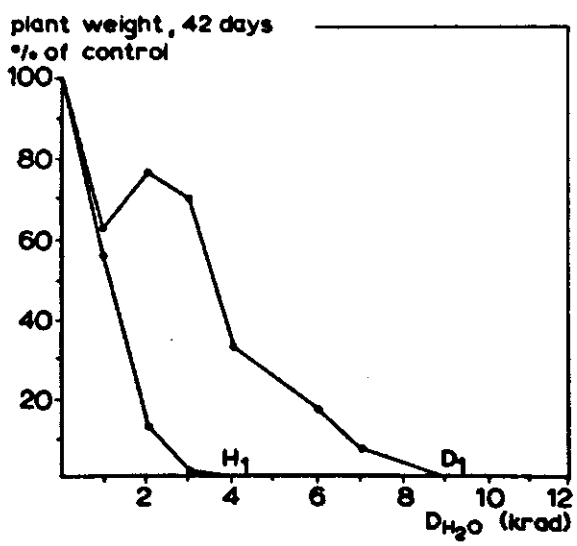


fig. 13

Germination time

The cumulative germination/time curves, some of which are shown in fig.14, were S-shaped for all treatments. These cumulative data, expressed relative to the final germination percentages which were unaffected by radiation, yielded approximately linear regressions on log/normal probability paper (not shown); the slope of these regressions did not change consistently with dose. The numbers of days needed to reach 50% germination were read from the transformed graphs and plotted in fig.15. Germination commenced earlier in $D_2$ than in $H_2$; this was because the temperature during irradiation (23°C), to which $D_2$ was exposed in the dry state but $H_2$ in the course of germination, was lower than the incubation temperature (27°C). The average germination time increased less than linearly with dose. A germination delay was observed even at a dose as low as 200 rad. The maximum delay observed was larger in $D_2$ than in $H_2$.

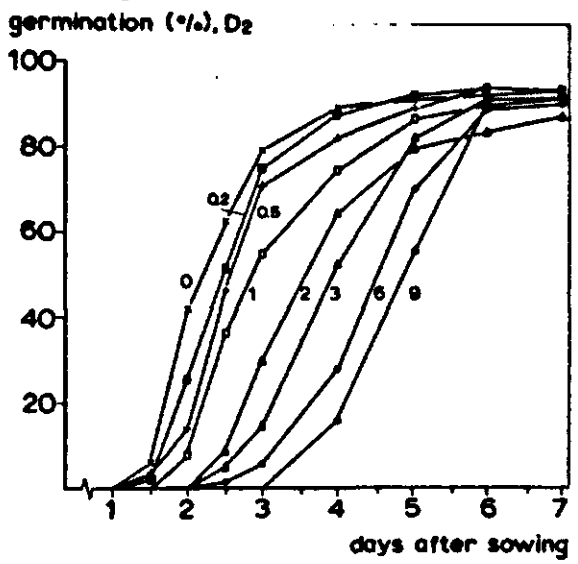


fig. 14

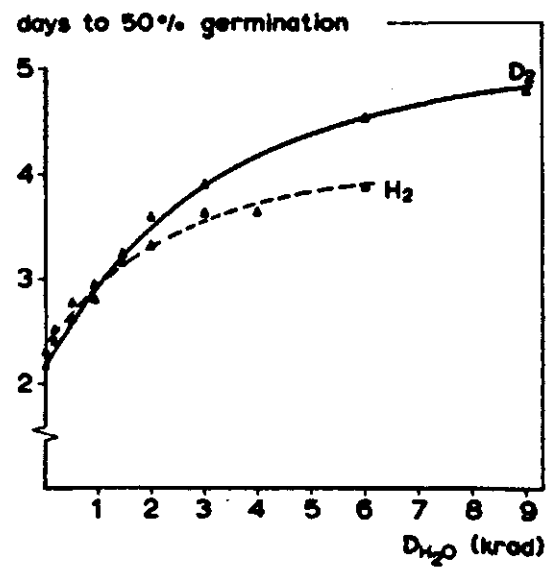


fig. 15

Growth characters

Cumulative root growth recorded in the petridishes 4 days after 50% germination (fig.16) decreased almost linearly up to medium doses; this was followed by a tail at high doses. The curves were approximately straightened on log/normal probability paper (fig.17), showing that they resembled sigmoids. Moreover, these transformed regressions were parallel, demonstrating that the increased neutron effectiveness on 24 hours prehydrated seeds was fully explained by dose modification.

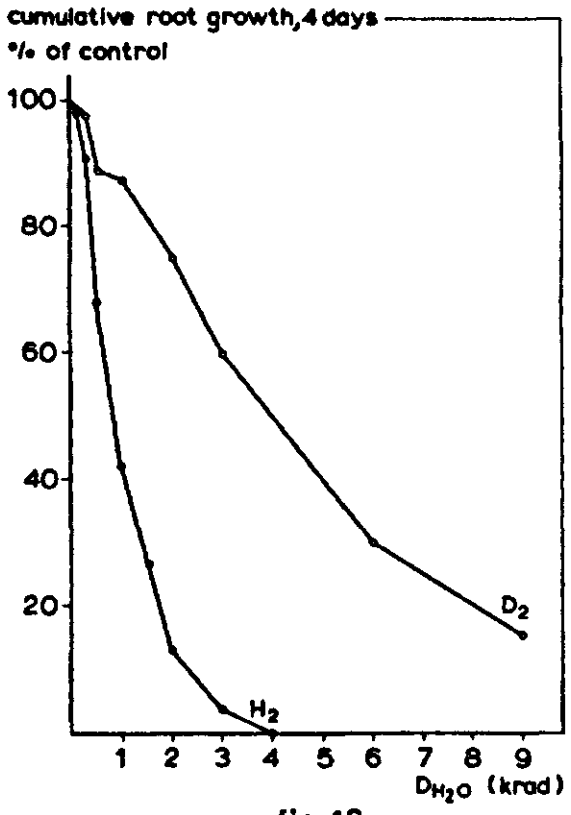


fig. 16

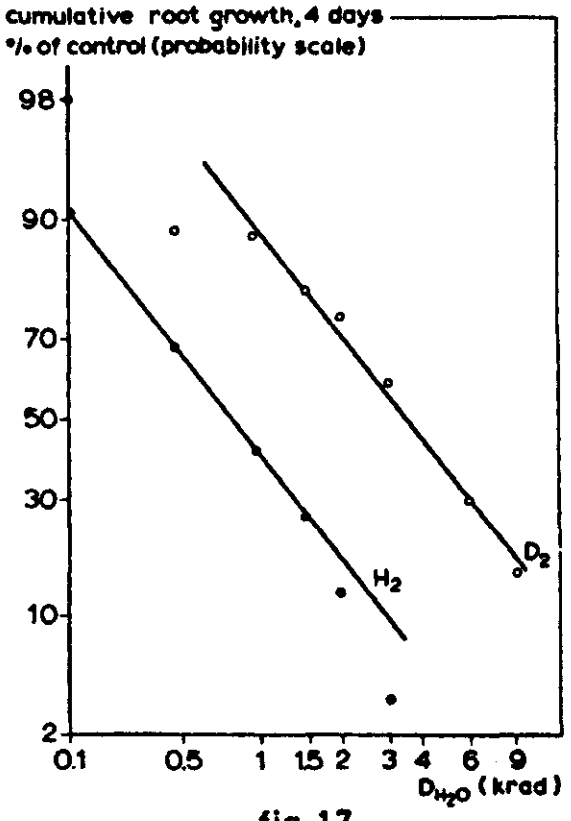


fig. 17

Cumulative root growth (fig.3, p.33) and cotyledon growth after 25 days (fig.4) responded in both series with a smooth but pronounced convex curvature up to medium doses; the data on $H_2$ suggested the presence of a tail.

Length of the 1st leaf after 25 days (fig.5) was affected only slightly up to medium doses, but decreased steeply and more or less linearly at higher doses, without a tail in either series.

Cumulative stem growth after 25 days (fig.6) showed a clear shoulder followed by a steep decrease; the 0.5 krad $H_2$ treatment had a higher mean than the control; the $H_2$ series suggested the presence of a tail.

Developmental character

Leaf number after 25 days (fig.11, p.36) increased substantially up to half the lethal dose in both series; at still higher doses it decreased sharply.

Experiment 3

The data, wherever applicable expressed as percentages of the control (table 11), are shown graphically. The solid lines refer to cv. 'Moneymaker', the broken lines to cv. 'Glorie'.

Table 11 : Control treatment means, $M_1$ characters (for $M_2$ characters see table 14, p.43). Experiment 3.

| Character | Days | $D_3$ | | $H_3$ | |
|---|---|---|---|---|---|
| | | MM | GL | MM | GL |
| Cotyledon length (mm) | 18 | 42 | 42 | 35 | 35 |
| Seedling weight (g) | 18 | 4.6 | 4.0 | 3.2 | 3.5 |
| Weight of seeds/fruit ± S.E.Mean (10 mg) | | 48±2.1 | 44±2.8 | 41±2.3 | 36±1.7 |
| Number of seeds/fruit ± S.E.Mean | | 145±6.2 | 139±8.9 | 127±7.2 | 120±5.6 |

Germination time

Plotting the cumulative germination percentages against time on log/normal probability paper generally yielded a straight regression from which the time needed to attain 50% germination was read. Variance analysis of these latter data showed highly significant germination delay due to storage time, radiation dose and their interaction. Because varietal differences were insignificant, only the means of both cultivars are given (fig.18). Germination time increased less than proportionally with dose in $H_3$, and in $D_3$ after 1 year of storage, but the relationship was about linear in the 'unstored' seeds of $D_3$ and a stimulation effect was observed in its 1 krad treatment.

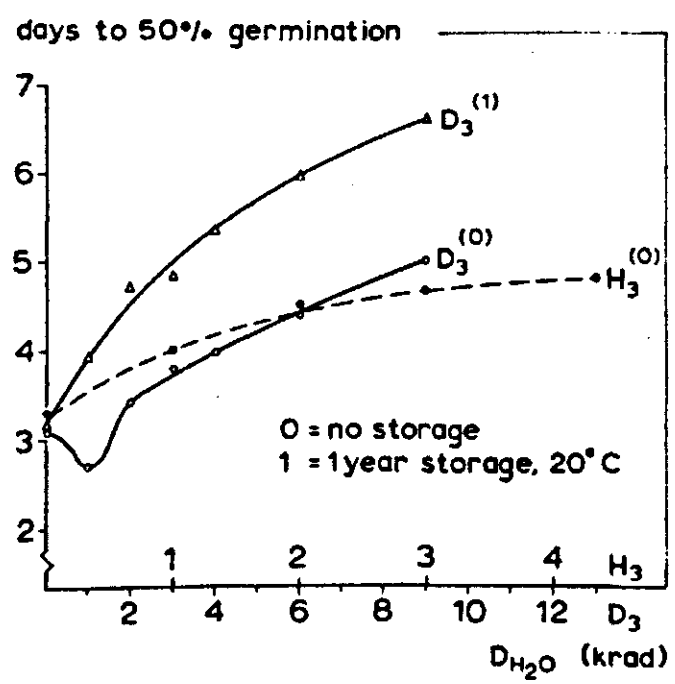


fig. 18

Growth characters

Cumulative cotyledon growth after 18 days (fig.19) responded sigmoidally to dose; approximately straight regressions were obtained on log/normal probability paper (fig.20).

Seedling weight after 18 days (fig.21) decreased with a slight convex curvature up to medium doses in $D_3$, though almost linearly in $H_3$; the curves tended to tail off at high doses, especially in $H_3$; these data conformed approximately to straight regressions on normal probability paper (not shown). The dose/response relationships of these quantitative seedling traits did not contain a shoulder.

Developmental character

The % transplantable seedlings (table 12, columns b) showed in all series a dose/response relationship characterized by a broad shoulder, followed by a consistent decrease which was fairly sharp in $H_3$.

Table 12 : Effects of seed irradiation on quantal characters in 'Moneymaker' and 'Glorie': (a) number of germinated seeds of each cultivar, retained at random; (b) % transplantable seedlings; (c) 'fertile' plants, % of flowering; (d) 'fertile' plants, % of (a). Experiment 3.

| | krad | | 'Moneymaker' | | | 'Glorie' | | |
|---|---|---|---|---|---|---|---|---|
| | | (a) | (b) | (c) | (d) | (b) | (c) | (d) |
| $D_3$ | 0 | 20 | 100 | 100 | 100 | 100 | 100 | 100 |
| | 1 | 20 | 100 | 100 | 100 | 100 | 95 | 95 |
| | 2 | 20 | 100 | 100 | 100 | 100 | 90 | 90 |
| | 3 | 45 | 100 | 96 | 96 | 98 | 86 | 82 |
| | 4 | 45 | 100 | 78 | 78 | 93 | 80 | 71 |
| | 6 | 180 | 100 | 58 | 58 | 90 | 44 | 39 |
| | 9 | 180 | 87 | 17 | 15 | 83 | 12 | 8 |
| $H_3$ | 0 | 30 | 100 | 100 | 100 | 100 | 100 | 100 |
| | 1 | 30 | 100 | 93 | 93 | 97 | 100 | 90 |
| | 2 | 90 | 94 | 75 | 69 | 82 | 57 | 43 |
| | 3 | 180 | 96 | 39 | 36 | 63 | 23 | 14 |
| | 4 | 400 | 55 | 20 | 10 | 23 | 10 | 2 |

$M_1$ fertility

The 'fertile' plants, expressed as a percentage of flowering plants (table 12, columns c) decreased in a manner similar to the % transplantable seedlings (columns b), but much more sharply.

Because all transplanted individuals reached the flowering stage, the products of the data in columns b. and c. represent the 'fertile' plants as a percentage of germinated (columns d and fig.22). These

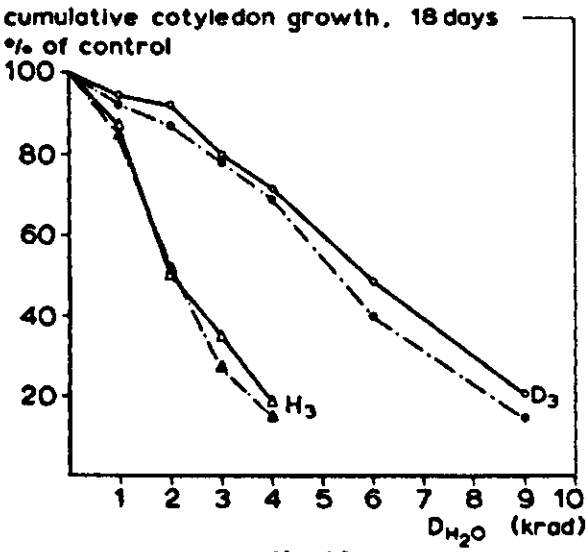


fig. 19

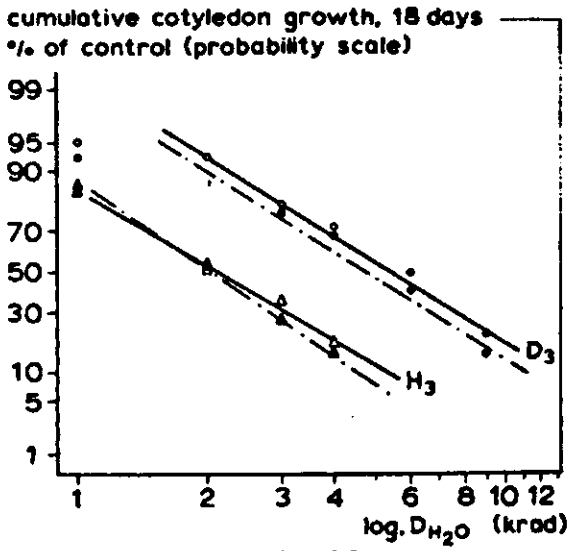


fig. 20

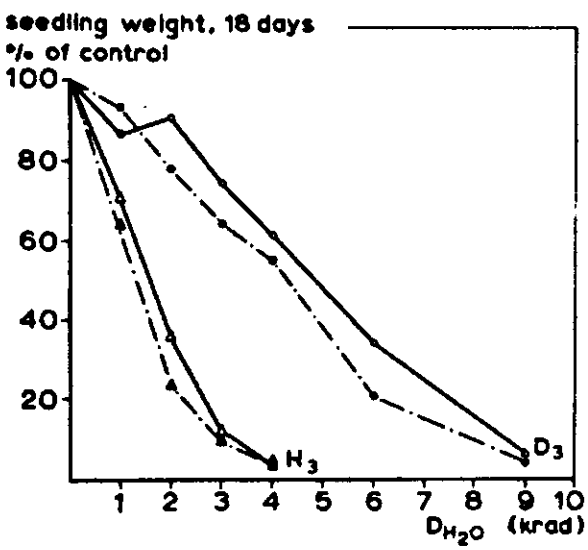


fig. 21

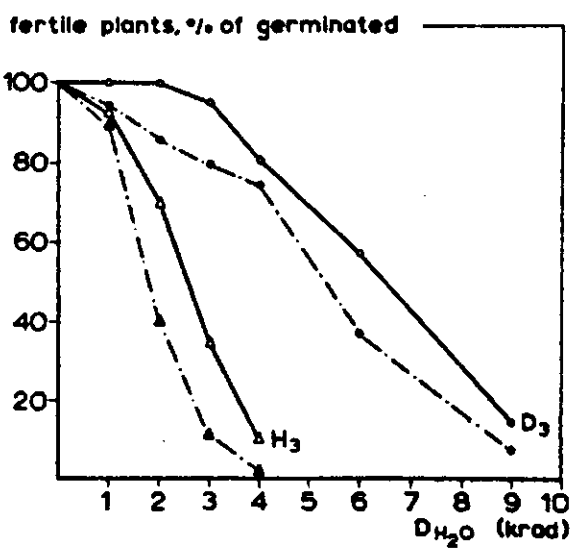


fig. 22

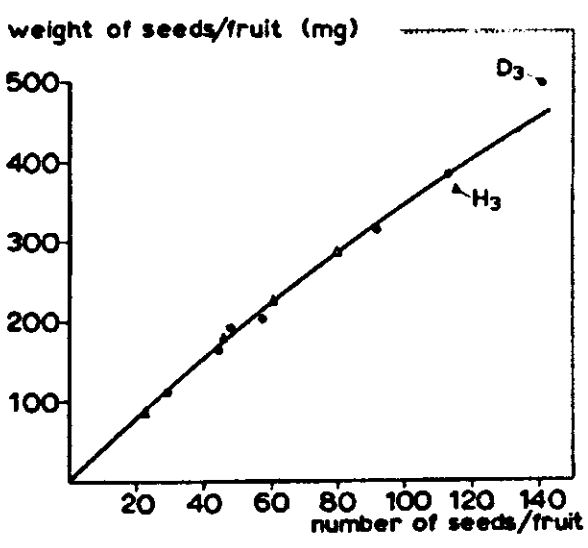


fig. 23

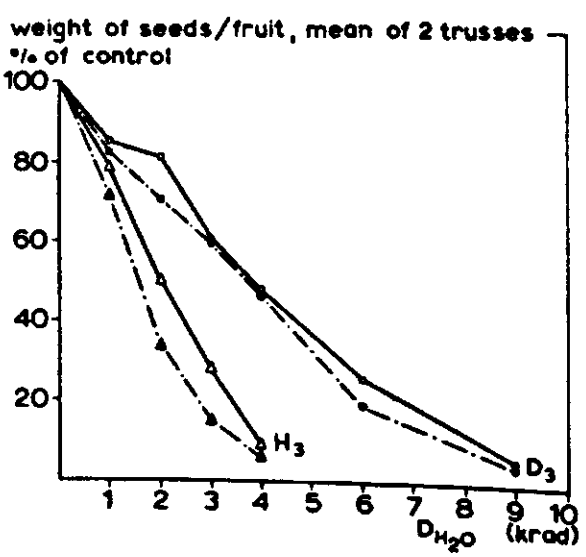


fig. 24

——— cv. 'Moneymaker'
–·–·– cv. 'Glorie'

data were the most suitable for judging the shape of the dose/response relationships. Approximately straight regressions were obtained on normal probability paper (not shown); the data were also subjected to probit analysis[63] and by means of a $Chi^2$ test found to be consistent with a probit model (P=0.25-0.86).

The relationship between number and weight of seeds/fruit was slightly curvilinear, as exemplified in fig.23 for the 1st truss of 'Moneymaker'. This was due to an increase with dose in the average weight per seed, up to about 120% of the control in semi-sterile treatments. This curvilinearity is apparently due to a normal physiological competition of seeds within a fruit and not specifically to radiation because a similar relationship between number and weight of seeds/fruit was found in unirradiated material (not shown). In spite of this slight curvilinearity the coefficient of linear correlation over the treatment means (n=12), for each cultivar and each truss separately, was >0.99 (table 13); the character 'number of seeds/fruit' can therefore be represented by the much more practical character 'weight of seeds/fruit'.

Table 13 : Linear correlation (r) and regression (y=a+bx) between number (x) and weight (y in mg) of seeds/fruit, based on treatment means (n=12). Experiment 3.

| Cultivar | Truss | r | y=a+bx |
|---|---|---|---|
| 'Moneymaker' | 1 | 0.995 | y = 13.4+3.40 x |
| | 2 | 0.990 | y = 13.6+3.14 x |
| 'Glorie' | 1 | 0.998 | y = 2.5+3.25 x |
| | 2 | 0.996 | y = 7.8+2.97 x |

Weight of seeds/fruit (fig.24) decreased almost linearly over most of the dose range in all series, but it tended to tail off at high doses.

$M_2$ characters

Table 14 shows the treatment means of all $M_2$ characters, of each cultivar. Only the shapes of the dose/response relationships will be considered in this section.

The % non-germinating seeds (columns b) tended to increase with dose; except for an absence of response after the lowest doses in $D_3$ of both cultivars, the dose/response relationships showed no consist-

Table 14 : Average $M_2$ characteristics following fast neutron irradiation of dry ($D_3$) and 24 hours prehydrated ($H_3$) seeds of 'Moneymaker' (MM) and 'Glorie' (GL): (a) number of $M_1$ plant progenies tested, each consisting of 2 truss progenies; (b) non-germinating $M_2$ seeds, % of sown; (c) sublethals unfit for mutant screening, % of sown; (d) number of mutations/100 $M_1$ truss progenies; (e) mutant seedlings, % of suitable for screening; (f) total % of all aberrant $M_2$ categories (b+c+e). Experiment 3.

| | krad | (a) Progenies tested | | (b) Non-germinating seeds (%) | | (c) Sublethals (%) | |
|---|---|---|---|---|---|---|---|
| | | MM | GL | MM | GL | MM | GL |
| $D_3$ | 0 | 14 | 15 | 5.1 | 2.5 | 4.4 | 5.8 |
| | 1 | 22 | 19 | 3.6 | 2.6 | 4.4 | 8.0 |
| | 2 | 22 | 18 | 5.2 | 5.9 | 4.8 | 8.1 |
| | 3 | 43 | 37 | 12.9 | 4.7 | 5.0 | 9.2 |
| | 4 | 35 | 32 | 17.8 | 9.4 | 6.6 | 7.0 |
| | 6 | 104 | 70 | 17.2 | 13.9 | 7.3 | 8.4 |
| | 9 | 27 | 15 | 26.6 | 16.0 | 7.3 | 10.2 |
| $H_3$ | 0 | 15 | 15 | 5.0 | 3.9 | 4.8 | 4.7 |
| | 1 | 28 | 27 | 14.0 | 5.2 | 4.6 | 4.6 |
| | 2 | 57 | 39 | 16.6 | 13.9 | 6.0 | 6.4 |
| | 3 | 65 | 25 | 16.5 | 15.1 | 5.6 | 8.2 |
| | 4 | 42 | 9 | 27.4 | 17.8 | 7.2 | 9.7 |

| | krad | (d) Mutations/100 truss progenies | | (e) Mutant seedlings (%) | | (f) All aberrant categories (%) | |
|---|---|---|---|---|---|---|---|
| | | MM | GL | MM | GL | MM | GL |
| $D_3$ | 0 | 0 | 0 | 0.0 | 0.0 | 9.5 | 8.3 |
| | 1 | 11 | 6 | 1.2 | 1.4 | 9.2 | 11.4 |
| | 2 | 14 | 28 | 1.6 | 4.2 | 11.6 | 18.2 |
| | 3 | 37 | 42 | 5.7 | 7.2 | 23.6 | 21.1 |
| | 4 | 29 | 53 | 6.1 | 10.9 | 30.5 | 27.3 |
| | 6 | 48 | 58 | 10.0 | 13.9 | 34.5 | 36.2 |
| | 9 | 43 | 67 | 9.2 | 19.6 | 43.1 | 45.8 |
| $H_3$ | 0 | 0 | 0 | 0.0 | 0.0 | 9.8 | 8.6 |
| | 1 | 30 | 37 | 6.0 | 6.3 | 24.6 | 16.1 |
| | 2 | 52 | 38 | 10.3 | 7.0 | 32.9 | 27.3 |
| | 3 | 65 | 50 | 12.1 | 8.7 | 34.2 | 32.0 |
| | 4 | 66 | 67 | 16.0 | 14.4 | 50.6 | 41.9 |

ent deviations from linearity (fig.25).

The % sublethals (columns c) was appreciable in the control and increased slightly with dose. These increases were irregular and no consistent deviations from linearity could be demonstrated.

The number of mutations/100 truss progenies (columns d) increased irregularly with dose, as is usual with this character. In the $D_3$ series of both cultivars these increases could not be distinguished from linear in the lower dose range but showed marked downward deviations from linearity

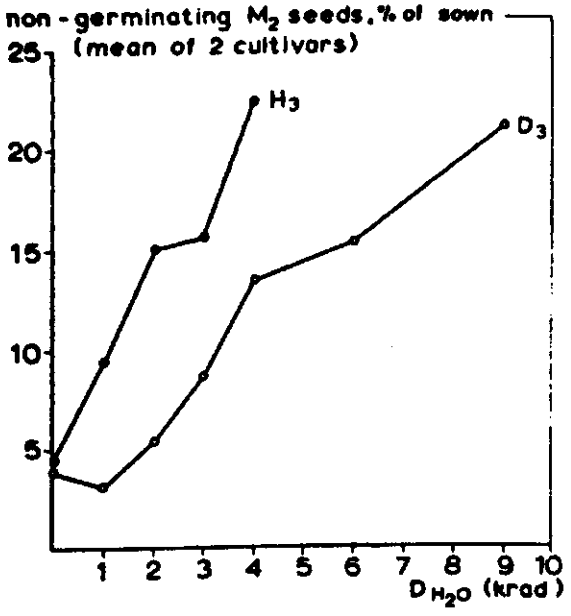


fig. 25

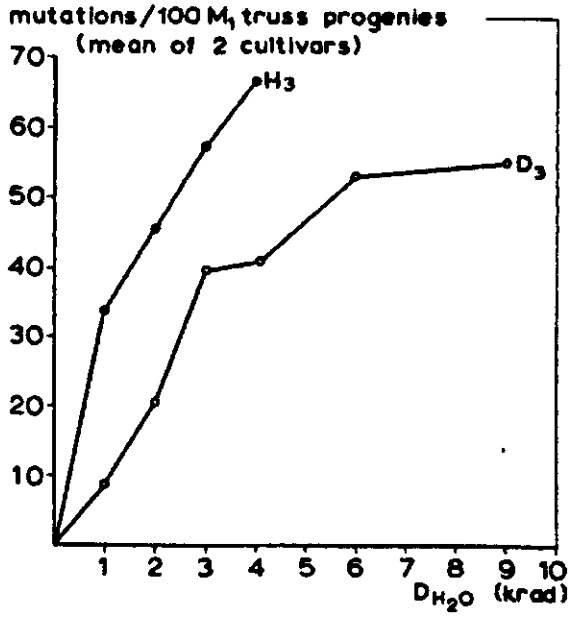


fig. 26

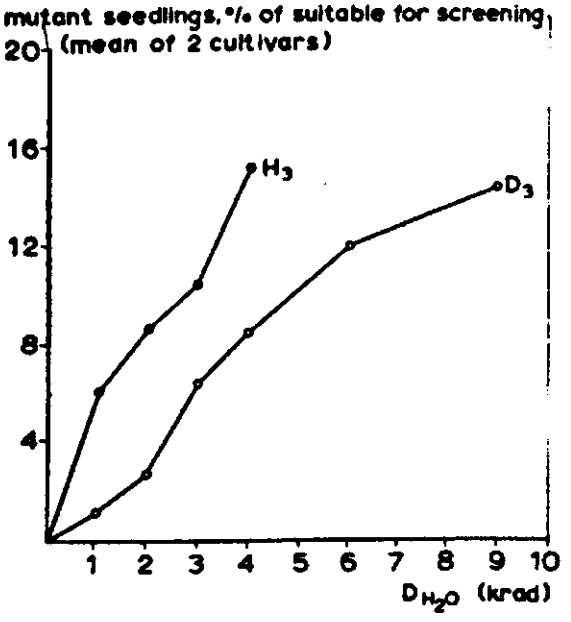


fig. 27

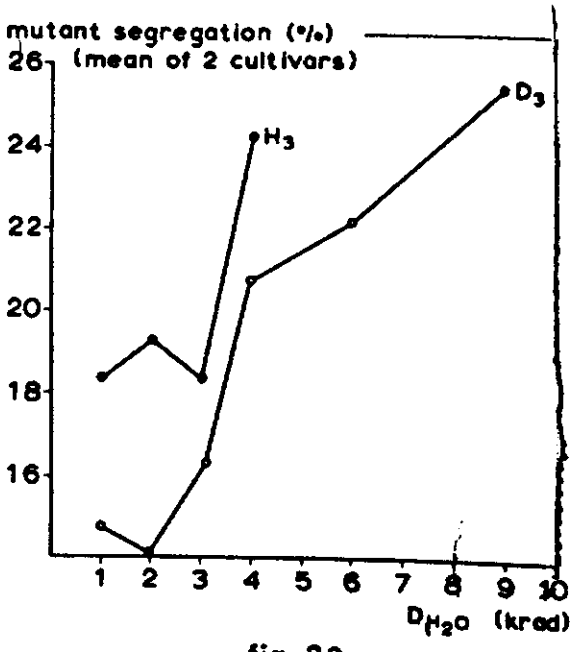


fig. 28

after 6 and 9 krad. In the $H_3$ series of 'Moneymaker' the increases showed a downward deviation from linearity, whereas in 'Glorie' they were irregular, although suggesting the same pattern. The trends became more clear when the data on both cultivars were pooled (fig.26).

The mutant seedlings were expressed as a % of seedlings suitable for screening because both non-germinability and sublethality mask the mutant phenotypes. This character (table 14, columns e and fig.27) showed a somewhat more regular and also more linear increase with dose than the preceding character, except in the $D_3$ series of 'Moneymaker'. This improved linearity resulted from a considerable increase with dose in the average % mutant seedlings per mutated progeny, from 14-18% at the lowest doses to 24-25% at the highest doses (fig.28).

The total % of all aberrant $M_2$ categories, i.e. of non-germinating seeds, sublethals and mutant seedlings pooled (table 14, columns f), yielded dose/response relationships which were similar in shape to those for non-germinating seeds, but somewhat more regular.

Correlation between responses of different characters

The coefficients of correlation between characters based on their treatment means reflect essentially the degree of correspondence in their dose/response relationships. These coefficients were calculated per cultivar and then averaged over both cultivars via the (r,z)transformation[65]; the results are shown in table 15.

In $D_3$, the coefficients of correlation involving ultimate cotyledon length (1) were slightly lower than those involving seedling weight (2), but in $H_3$ the differences were inconsistent. The coefficients of correlation among the $M_1$ characters (1-3) tended to be somewhat higher than those between the $M_1$ characters on the one hand and the $M_2$ characters (4-6) on the other; weight of seeds/fruit (3) showed the highest correlation with the $M_2$ characters. The coefficients of correlation involving the % non-germinating seeds (4) or the % mutant seedlings (5) were in most cases lower than those involving the total % of all aberrant $M_2$ categories (6).

Experiment 4

The results are shown graphically. Each graph is identified by the number of hours of prehydration. All graphs pertaining to a given character have a common origin, being the mean over all control values;this was possible because these values (table 16, p.47) did not differ significantly.

Table 15 : Coefficients of linear correlation between various characters based on the treatment means of the $D_3$ series (n=7) and the $H_3$ series (n=5), averaged over MM and G1. Experiment 3.

| | | $D_3$ | | | | | $H_3$ | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 2 | 3 | 4 | 5 | 6 | 2 | 3 | 4 | 5 | 6 |
| **$M_1$** | | | | | | | | | | | |
| Cotyledon length, 18 days | 1 | .992 | .966 | .958 | .945 | .963 | .981 | .988 | .957 | .949 | .957 |
| Seedling weight, 18 days | 2 | | .985 | .963 | .966 | .981 | | .985 | .936 | .955 | .955 |
| Weight of seeds/fruit | 3 | | | .966 | .973 | .988 | | | .978 | .969 | .978 |
| **$M_2$** | | | | | | | | | | | |
| % non-germinating seeds | 4 | | | | .945 | .985 | | | | .924 | .986 |
| % mutant seedlings | 5 | | | | | .984 | | | | | .972 |
| % all aberrant categories | 6 | | | | | - | | | | | - |

| | $D_3$ | $H_3$ |
|---|---|---|
| Critical value at P=0.05 : | .755 | .878 |
| P=0.01 : | .875 | .959 |
| P=0.001: | .951 | .991 |

Table 16 : Control treatment means. Experiment 4.

| Character | Days | Pre-treatment (series) D | H½ | H3 | H6 | H12 | Mean |
|---|---|---|---|---|---|---|---|
| $M_1$ | | | | | | | |
| Cotyledon length (mm) | 12 | 38 | 34 | 35 | 35 | 35 | 35.4 |
| Cotyledon length (mm) | 19 | 48 | 47 | 46 | 45 | 44 | 46.0 |
| Length 1st leaf (mm) | 19 | 80 | 77 | 78 | 77 | 76 | 77.6 |
| Length 2nd leaf (mm) | 19 | 90 | 83 | 83 | 83 | 82 | 84.2 |
| Leaf number | 19 | 5.3 | 5.3 | 5.3 | 5.2 | 5.3 | 5.3 |
| Abnormal leaves/plant | | 0.5 | 0.5 | 0.2 | 0.6 | 0.2 | 0.4 |
| Leaves below 1st inflor. | | 9.2 | 9.6 | 9.4 | 9.5 | 9.5 | 9.4 |
| Days to flowering | | 50 | 51 | 51 | 51 | 50 | 50.4 |
| Weight of seeds/fruit (10 mg) | | 43 | 45 | 42 | 44 | 46 | 44.0 |
| $M_2$ | | | | | | | |
| % non-germinating seeds | | 4.9 | 4.8 | 5.3 | 4.8 | 4.1 | 4.8 |
| % sublethals | | 1.2 | 1.1 | 1.0 | 1.0 | 1.0 | 1.1 |
| % mutant seedlings | | 0 | 0 | 0 | 0 | 0 | 0 |
| % all aberrant categories | | 6.1 | 5.9 | 6.3 | 5.8 | 5.1 | 5.9 |

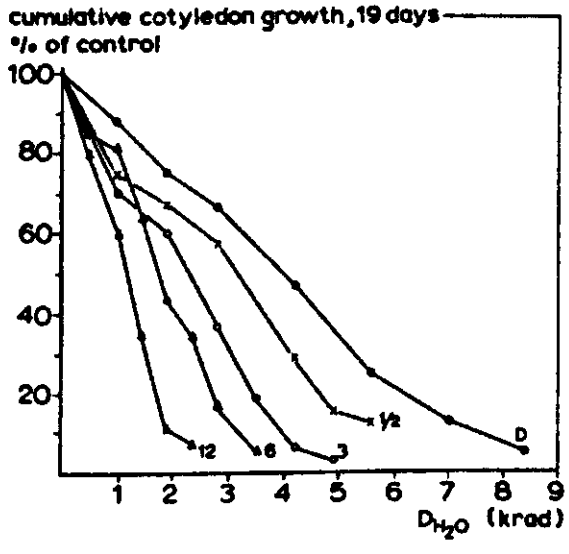


fig. 29

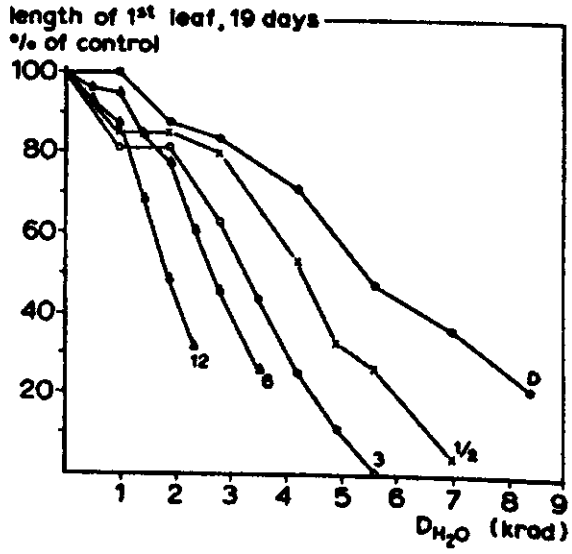


fig. 30

Growth characters

The percentages cumulative cotyledon growth after 12 days were virtually identical to those after 19 days and consequently only the latter are shown (fig.29). The dose/response relationships were approximately straight up to doses reducing growth to <10%; beyond this, they

tailed off. Any slight sigmoidal tendencies were overshadowed by irregularities.

In contrast, length of the 1st leaf after 19 days (fig.30) showed markedly convex responses at the lower doses, followed by linear decreases down to the lethal level, without evidence of a tail.

Length of the 2nd leaf (fig.31) showed a less curved dose/response pattern which was otherwise similar to that of the previous character.

Developmental characters

Leaf number after 19 days (fig.32) responded with a sharp linear decrease after a broad shoulder.

The number of abnormal leaves/plant, i.e. of leaves containing at least 1 visible abnormality (fig.33), tended to increase exponentially with radiation dose, except that the curves of H3 and H12 levelled off at the highest dose. Of all the treatments retained after transplanting the high dose samples of H3 and H12 revealed the most pronounced damage at the seedling stage. This suggests that the other curves would also have shown a flattening if higher doses had been used. This flattening of the curves apparently occurred at an average of 3-4 visibly abnormal leaves except after long pre-irradiation germination periods (H144) when this number must have been higher. The % transplantable seedlings was close to 100% in all treatments retained, except at the highest dose in the series H3 (80%), H48 (68%) and H72 (85%); therefore no dose/response curves could be drawn.

Leaf number below the 1st inflorescence (fig.34) increased approximately linearly with dose, though the D and H½ series suggested a slight upward curvature. Downward deviations from these trends occurred at the highest dose in most series; the absence of such deviations in D and H½ was possibly due to an insufficient range of doses. The tendencies towards saturation seemed to start at lower leaf numbers as pre-irradiation germination times were longer.

Flowering delay (fig.35) showed upwardly curved dose/response relationships except for a deviating point in H3 (0.93 krad); though an upward curvature was not pronounced in the D series, a parabolic regression nevertheless yielded a significantly higher coefficient of determination $r^2$ than a linear equation.

The difference in shape between the dose/response graphs of leaf number below the 1st inflorescence and flowering delay indicates an increase in the average plastochron at the highest doses studied and especially, according to fig.32, in those treatments that had after

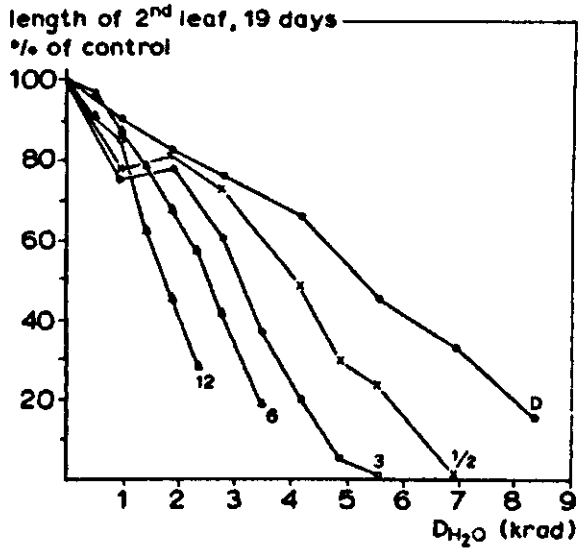


fig. 31

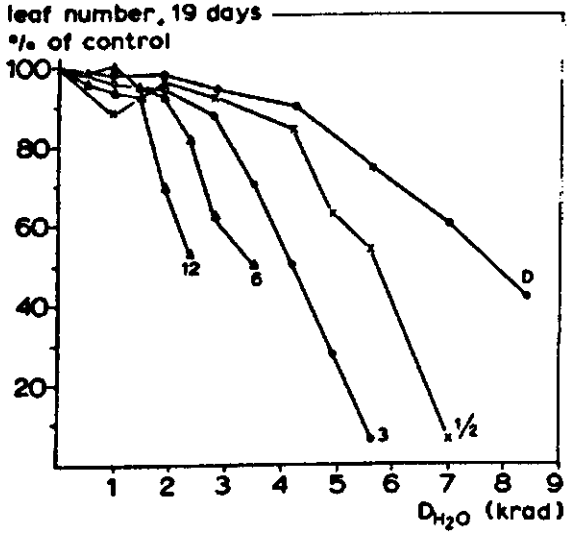


fig. 32

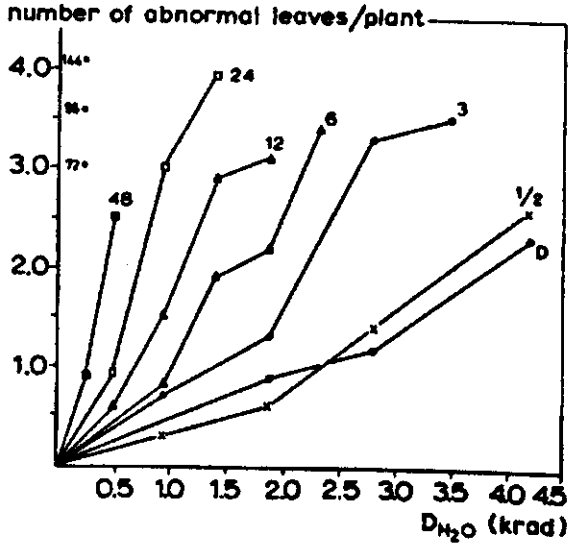


fig. 33

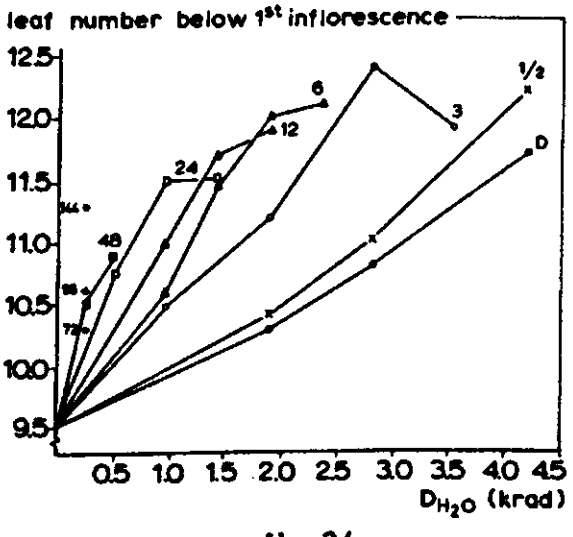


fig. 34

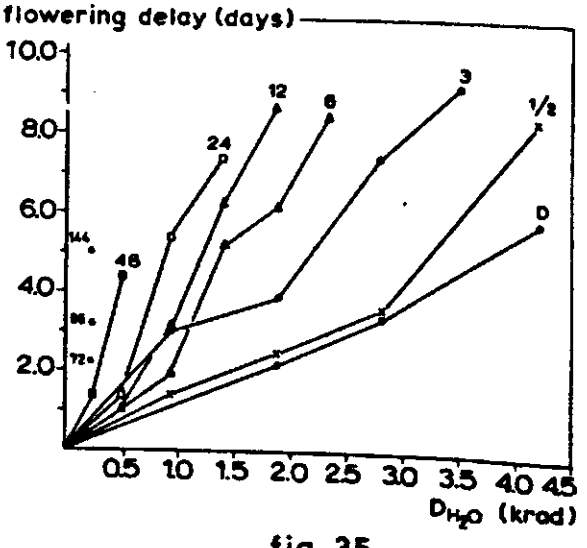


fig. 35

19 days a substantially lower leaf number than the control. This suggests that the 'extra' flowering delay at the highest doses should be attributed essentially to a retarded early development and not to a slower development throughout. This was analysed by dividing, per treatment, the flowering delay by the sum of the deficit in leaf number after 19 days and the increase in leaf number below the 1st inflorescence, both in relation to the control. The quotients (table 17) constitute the hypothetical average plastochron of those leaves that make up the difference between the treatment and the control since the 19th day, assuming the average plastochron of the other leaves to be the same as in the control. These quotients do not increase consistently with dose, showing, indeed, that the retardation in leaf differentiation must have been limited to the very early seedling stage.

Table 17 : Quotients representing flowering delay divided by the difference between an irradiated treatment and its control in respect of [ultimate leaf number minus leaf number after 19 days]. Experiment 4.

| Dose | Pretreatment (series) | | | | |
|---|---|---|---|---|---|
| | D | H½ | H3 | H6 | H12 |
| low ↓ | 2.44 | 1.75 | 2.50 | 2.00 | 1.87 |
| | 2.20 | 2.40 | 2.17 | 2.26 | 2.21 |
| | 2.18 | 1.90 | 2.11 | 2.18 | 2.52 |
| high | | 2.40 | 2.36 | 2.40 | 2.23 |

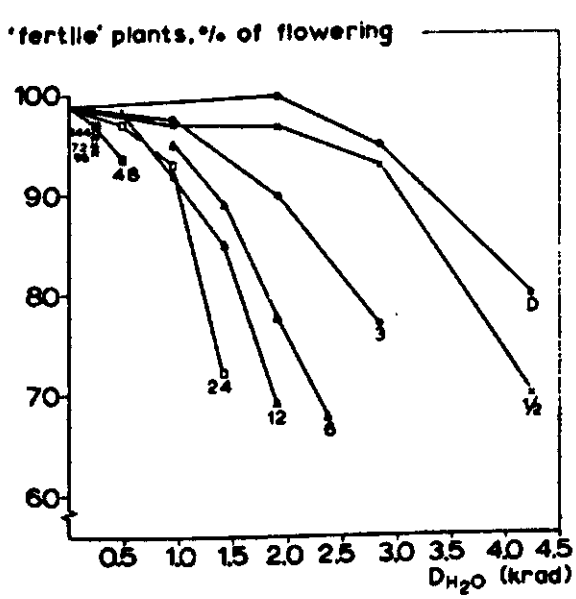


fig. 36

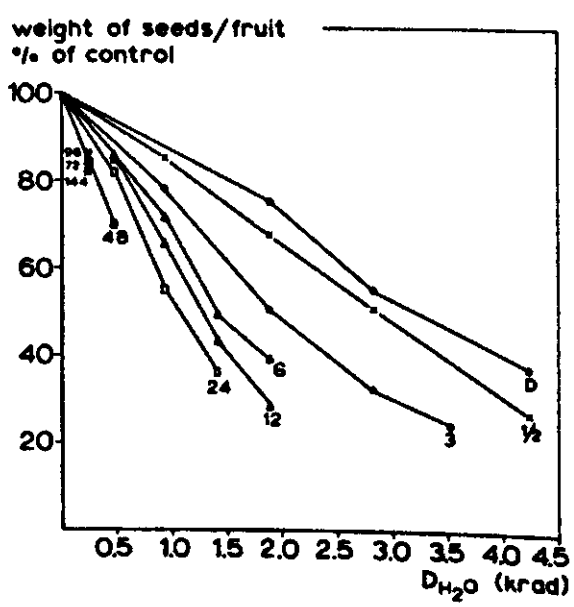


fig. 37

$M_1$ fertility

The % 'fertile' plants (fig.36) decreased steeply with dose after a pronounced shoulder.

Weight of seeds/fruit (fig.37) decreased linearly with dose, with possibly a slight sigmoidal tendency in some series. The dose/response relationships for this character were more regular than for any of the previous characters.

$M_2$ characters

The % non-germinating $M_2$ seeds (fig.38) and the % sublethals (fig. 39) both increased irregularly with dose; no consistent deviation from linearity could be demonstrated.

The number of mutations/100 $M_1$ truss progenies (fig.40) and the % mutant seedlings (fig.41) increased linearly with dose and more regularly than the preceding $M_2$ characters.

As expected from the foregoing, the total % of all aberrant $M_2$ categories (fig.42), i.e. of non-germinating seeds, sublethals and mutant seedlings, also showed linear dose/response relationships. These were more regular than for any single $M_2$ character; the only exception was the H6 series in which the 1.40 and 1.87 krad treatments had particularly high values resulting chiefly from unexplained, high percentages of non-germinating seeds and mutant seedlings.

Correlation between responses of different characters

The coefficients of correlation between the quantitative characters were calculated over the means of all treatments in the series D, $H_{\frac{1}{2}}$, H3 and H6 (table 18). Combining the data of these 4 series was justified by the fact (to be demonstrated later, section 4.6, p.83) that up to H6, the degree of sensitization by prehydration was similar for all characters, and that consequently the regressions between the various characters were not systematically affected. The high number of experimental points thus obtained permitted a much sharper comparison of r-values relating to different characters than in exp.3.

The coefficients of correlation involving cotyledon length after 12 days were almost identical to those involving ultimate cotyledon length recorded after 19 days, and the same set of r-values (1) represents both characters. Most coefficients involving length of the 1st leaf (2) or of the 2nd leaf (3) were lower than those involving cotyledon length. Also the coefficients of correlation involving the

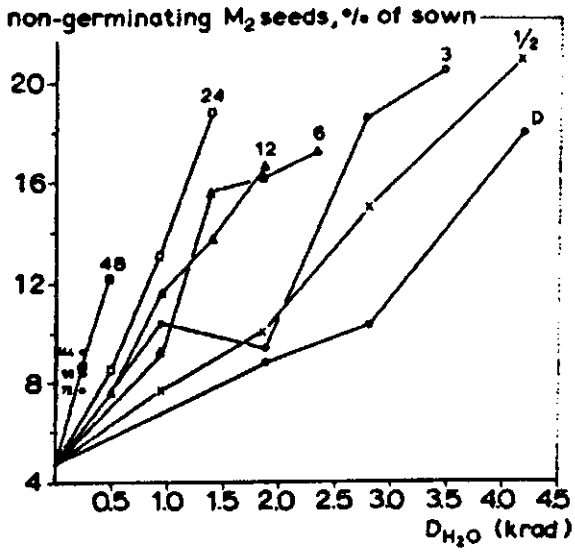


fig. 38

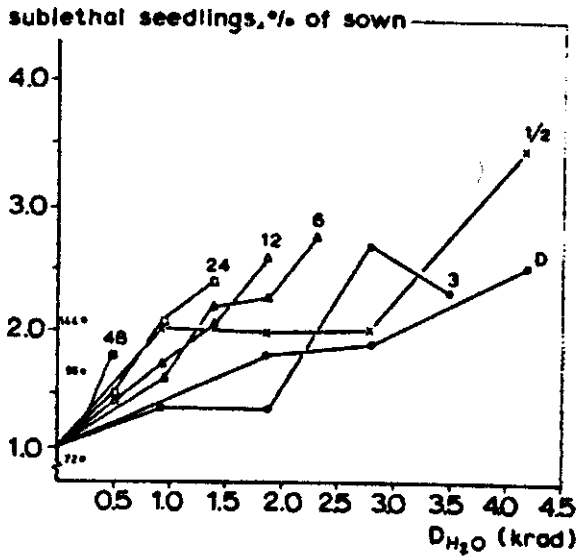


fig. 39

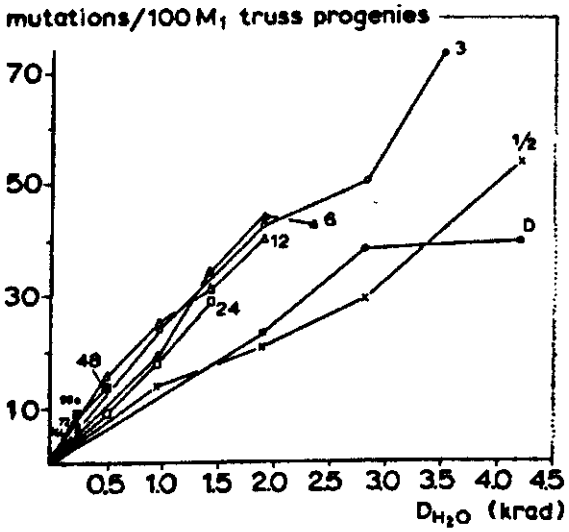


fig. 40

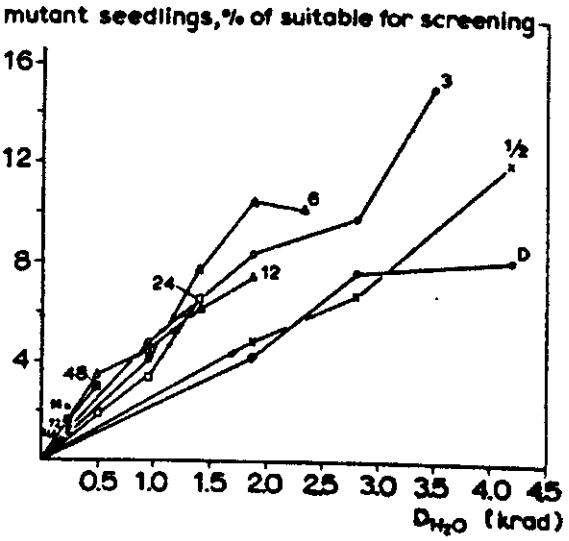


fig. 41

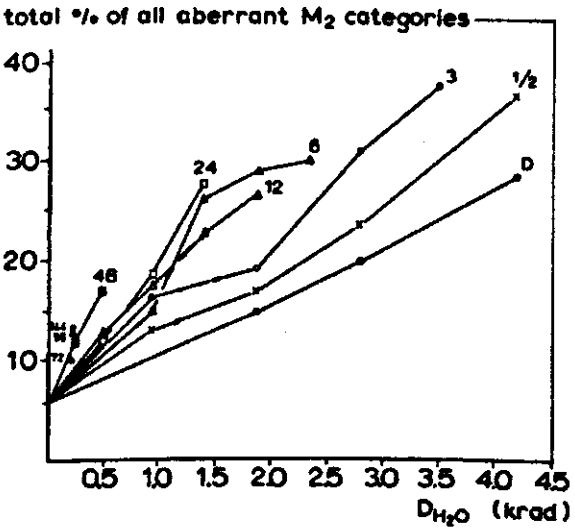


fig. 42

Table 18 : Coefficients of linear correlation between various characters based on the treatment means of the D, $H\frac{1}{2}$, H3 and H6 series (n=19). Experiment 4.

| | | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $M_1$ | | | | | | | | | | | | |
| Cotyledon length, 12 or 19 days | 1 | .96 | .97 | -.94 | -.95 | -.97 | .96 | -.94 | -.86 | -.96 | -.97 | -.97 |
| Length 1st leaf, 19 days | 2 | | .98 | -.91 | -.86 | -.95 | .89 | -.89 | -.86 | -.93 | -.93 | -.93 |
| Length 2nd leaf, 19 days | 3 | | | -.90 | -.88 | -.94 | .91 | -.91 | -.85 | -.94 | -.96 | -.95 |
| Abnormal leaves/plant | 4 | | | | .94 | .96 | -.95 | .95 | .88 | .90 | .93 | .96 |
| Leaves below 1st inflorescence | 5 | | | | | .96 | -.98 | .95 | .87 | .92 | .94 | .96 |
| Days to flowering | 6 | | | | | | -.97 | .96 | .89 | .95 | .97 | .98 |
| Weight of seeds/fruit | 7 | | | | | | | -.95 | -.87 | -.95 | -.97 | -.97 |
| $M_2$ | | | | | | | | | | | | |
| % non-germinating seeds | 8 | | | | | | | | .80 | .90 | .94 | .99 |
| % sublethals | 9 | | | | | | | | | .78 | .85 | .91 |
| Mutations/100 $M_1$ truss progenies | 10 | | | | | | | | | | .98 | .94 |
| % mutant seedlings | 11 | | | | | | | | | | | .98 |
| % all aberrant categories | 12 | | | | | | | | | | | - |

Critical value at P=0.001: 0.69

number of abnormal leaves/plant (4) were with some exceptions somewhat lower than those involving cotyledon length. Leaf number below the 1st inflorescence (5) usually yielded somewhat lower $\bar{r}$-values than days to flowering (6). The latter had generally the highest $\bar{r}$-values of all $M_1$ characters, closely followed by cotyledon length and weight of seeds/fruit (7).

The $\bar{r}$-values involving the % non-germinating seeds (8) were substantially higher than those involving the % sublethal seedlings (9), as would be expected from the irregular dose/response relationships of the latter. The $\bar{r}$-values involving mutations/100 $M_1$ truss progenies (10) were consistently lower than those involving the % mutant seedlings (11). Finally, the coefficients of correlation involving the total % of all aberrant categories (12) were generally slightly higher than those involving each category separately (8, 9 and 11). Actual numbers of non-germinating $M_2$ seeds, sublethals or mutant seedlings yielded considerably lower $\bar{r}$-values (not shown) than the same data expressed as percentages.

4.3.2. Discussion

Germination capacity and germination time

The absence of a radiation effect on germination capacity corresponds with similar observations on fast neutron irradiated barley seeds[128], thermal neutron- and X-irradiated Melilotus seeds[125], X-irradiated tomato seeds[37] and rice seeds, except at lethal doses [11] and seeds of other species. These findings are explained by the fact that the rupture of the seedcoat generally depends chiefly or entirely upon cell elongation, which is highly radiation resistant [79,81].

The consistently linear regressions of cumulative germination % against time on log/normal probability paper demonstrate that the distribution of log.germination time is about normal; consequently, the distribution of germination time itself is markedly skew. The fact that the transformed linear regressions were almost parallel for all treatments (exp.2) suggests that the germination delay of the individual seeds in a neutron irradiated sample is directly proportional to the germinative power of the same seeds when in an unirradiated condition.

The median germination time generally increased with a power <1 of the dose. The linear relationship in the $D_3$ series (exp.3), sown

3 days after irradiation, is apparently exceptional, because the usual curvilinear relationship was found with the same material sown after 1 year of storage.

This downwardly curved relationship was also observed with radiations of low ionisation density[37], and is similar to that found for the delay of first cleavage in sea-urchin eggs (Henshaw et al., treated by Lea[112 Ch.8], and for division delay in various other organisms. In Henshaw's experiment, the delay was due chiefly to a prolongation of prophase, which Lea attributed to the destruction by radiation of a nuclear constituent necessary for chromosome condensation, followed by recovery when this constituent was reformed as a result of metabolic activity of the cell. Whatever the exact nature and degree of specificity of the damaged sites or functions, the striking difference between the observed dose response pattern for these delays and the relationships commonly found for genic and chromosomal effects [8,112,171] suggests that germination delay results primarily from damage to non-genetic targets.

The rather high radiosensitivity of the systems concerned is indicated by the fact that a delay was observed at a dose as low as 200 rad (exp.2). This excludes the possibility of enzyme molecules being a major target, because such molecules are known to be highly radioresistant in vivo[8,186].

The proper regulation of the numerous processes involved in germination is ensured both by the seedcoat and by many intracellular and intraorganellar membranes. The seedcoat plays an important role in water uptake and gas exchange and provides mechanical resistance to untimely embryo expansion. Radiation is unlikely to have a major detrimental effect on these processes, except in special instances when inhibitory substances are mobilised. Bacq and Alexander[8], Alper[3], Goldfeder[76,77] and others assume on the basis of diverse evidence that, in addition to the hereditary material in the nucleus, intracellular membranes are a major target of radiation action. Radiation induced germination delay can then be attributed chiefly to the disturbed permeability properties of damaged membranes. This hypothesis is consistent with the many known examples in which the uptake or the release of substances is enhanced by radiation[2,8,167]. It could explain the high radiation sensitivity of germination speed, because enzyme 'leakage' disturbs the regulation of the biochemical processes. Further, membrane damage could explain the characteristicly less-than-

proportional increase in median germination time with dose, assuming that the disturbing effect is less than proportional to the number of 'leaks' produced, and that consequently the rate of recovery is more than proportional to the dose. With irradiation of prehydrated seeds, as opposed to dry seeds, a number of critical processes are likely to have taken place before irradiation; furthermore, repair of membrane damage probably begins even during irradiation, due to the high metabolic activity at the prevailing temperature (23°C). Consequently, germination delays will be smaller in prehydrated seeds than in dry seeds unless the above factors are outweighed by increased amounts of certain kinds of primary damage.

This was apparently not so in exp.2 in which maximum germination delays were much larger after irradiation of dry than of prehydrated seeds. The results of exp.3, which showed comparable delays in both series, are not necessarily contradictory, as in this experiment the dry irradiated seeds were sown after 3 days storage at room temperature. Contant and Dankert[31] have observed with Arabidopsis a significant reduction in γ-ray induced germination delay by storage for periods of up to 53 hours at -20°C, and an insignificant but consistent reduction in fast neutron induced delay. Consequently, it is possible that the delays in the dry seed series of exp.3 were lower than they would have been without storage, particularly as the seeds were stored at a much higher temperature than in the Arabidopsis experiment.

The extent of genetic damage induced by fast neutrons is usually unaffected by post-irradiation storage[42] or is slightly enhanced [31], whereas genetic effects of X- or γ-radiation may be strongly enhanced, depending on seed moisture content and temperature during storage[42, 83, 99, 136]. Thus, the above observation by Contant and Dankert provides further evidence for the non-genetic nature of radiation-induced germination delay when sowing takes place within days after irradiation.

In contrast, prolonged (1 year) post-irradiation storage at room temperature had a very detrimental effect on germinative power; this effect increased with dose, suggesting that radiation damage considerably increases the rate of ageing of the seeds, and vice versa. The changes occurring with age are many and involve both physiological and genetic components and strong interactions between them[43]. Such changes include accumulation of toxic substances, coagulation

of proteins, disappearance of stored foods[38,39], oxidation processes and reduction in enzyme activity[38,39], increased exudation[176], accumulation of chromosome aberrations[38,41,137,138,161], accumulation of point mutations[41,175], degeneration of the nuclei[38,39]. The interdependence of radiation damage and ageing has been demonstrated by several authors[34,127,140,161,162]. The frequently observed decrease, following prolonged storage, in the percentage mutants among the germinating seeds of segregating progenies[37], is probably an example. It can be assumed that both natural and radiation-induced ageing involve a decreasing integrity of cellular structures and increasing frequencies of chromosome aberrations and mutations, and that there are strong interactions between all these components of deterioration.

A stimulation of germination was observed only in the 1 krad dry seed treatment of exp.3; no stimulation at all occurred in exp.2 notwithstanding the wide range of high and low doses tested. This difference may be associated with the fact that the seed-lot used for exp.3 germinated more slowly than that used for exp.2. The inconsistency of these data is in agreement with general experience[91,102], and is due to inadequate control over the more important variables. The stimulating action of radiation on germination is generally held to be due to a shortening, or breaking, of seed dormancy[91,cf.190]. Dormancy is frequently associated with properties of the seedcoats which in many species act as selectively permeable membranes[39,103,120]. Various treatments which are able to reduce or break dormancy increase the permeability to water; other effects may also occur, such as improved gas exchange and the elimination of germination inhibitors[103]. It is quite possible that radiation could stimulate germination in similar ways, especially in those cases where fairly high doses are needed. However, changes in the permeability of intracellular membranes in both the endosperm and the embryo, and other activating mechanisms, may also be involved[8,91,167].

Because there is no radiation dose which produces exclusively favourable effects, the radiation stimulation of germination is likely to be very sensitive to the formation history of the seeds, the radiation dose, the duration and conditions of pre- and post-irradiation storage and the conditions of germination. Thus, the absence of stimulation in the 1 krad treatment of exp.3 after 1 year of storage suggests that the initially favourable balance of effects had been

gradually eliminated by the increased rate of ageing due to radiation damage.

Growth characters

The dose/response relationships of the characters recorded after 25 days in exp.2 were much more strongly curved up to medium doses than those of the same or similar characters in the other experiments, and of cumulative root growth recorded at the petridish stage in exp.2. These differences are probably related to the experimental conditions after transplanting, notably those of the rooting media, which were clearly sub-optimal in exp.2, more favourable in exp.1 and very favourable in exps.3 and 4 (cf. the control values). A given set of sub-optimal nutritional conditions imposes more severe growth restrictions on plants with a high growth potential than on those with a low growth potential. This means that, under sub-optimal conditions, the proportion of artificial growth restriction is the highest in the control and is progressively less with increasing dose.

As a result, the dose/response relationships must become increasingly more convex as nutritional conditions become less favourable, which is in agreement with the observed facts. In this connection, it must be stressed that the plant's requirements change throughout its life, and that a nutritional situation which is optimal at an early stage may be grossly sub-optimal later. Apart from the quality of the substrate, spacing is undoubtedly a major factor.

Such sub-optimal conditions may lead to shoulders in the dose/response relationships which would be absent under more favourable conditions; this is exemplified by cumulative stem growth in exp.2 as compared with exp.1. Changes of this kind will unavoidably affect the choice of mathematical functions for describing the dose/response relationships or for transforming these to linear regressions. Such lack of consistency hampers the estimation of the average genetic effectiveness of radiation treatments, and is particularly serious when an attempt is made to derive models on fundamental radiobiological mechanisms or cell proliferation kinetics from these dose/response relationships. This will be illustrated on p.64, using the developmental character leaf number.

Except for variables connected with the irradiation treatment, radiobiological experiments are usually performed under one set of cultural conditions which are often governed by convenience; the presence of growth limiting factors is by no means exceptional. Under

these circumstances, it is conceivable that the differences in the shapes of dose/response curves of comparable characters encountered in the literature and even published by one author[128,129,131], might be due partly to unnoticed interactions of this type. To avoid this risk, it is advisable to use both quantitatively and qualitatively optimal conditions at all stages of growth in all biological experiments involving radiation or any other variable which may cause differences in endogenous growth potential.

The dose/response data on cumulative root growth at the petridish stage (exp.2), cumulative cotyledon growth (exps.1, 3 and 4), and cumulative stem growth (exp.1) could be fairly well represented by straight lines when plotted on log/normal probability paper. In contrast, the data on leaf length (exps.1 and 4) and seedling weight (exp.3) yielded approximately straight lines on normal probability paper. This difference is associated with the observed tendency towards a more pronounced tail in the dose/response curves of the former characters than in those of the latter characters. It must be remembered that the former characters concern organs which are fully differentiated and of appreciable size in the mature embryo. These organs (the root, the hypocotyledon and the cotyledons) grow by means of (a) cell elongation prior to or in the absence of any cell division, and (b) cell division and subsequent elongation of the daughter cells. Although these two components are not strictly independent [154,165] it is assumed for the present purpose that each has its own dose/response relationship. The component (a) has a much higher radiation tolerance than (b) in which the relatively high sensitivity to the inhibition of cell division is the limiting factor. A model (fig.43) shows the effects of irradiation on total growth (a+b), under the assumptions that in the unirradiated control 20% of the total growth is due to (a) and 80% to (b), and that both growth components decrease with dose according to a normal sigmoid, i.e. linearly on normal probability paper, (a) reaching about zero after 12 krad and (b) after 4 krad. The curve for (a+b) is seen to have a strongly extended tail. The length, height and shape of the tail may be varied according to the proportion of total growth in the control attributable to (a) and the relative sensitivity of the 2 components. This model may be used as a tentative representation of cumulative cotyledon growth, as for example in the $H_1$ series. It must be noted that a 'reversal' at very high doses, as occurs in seedling height in

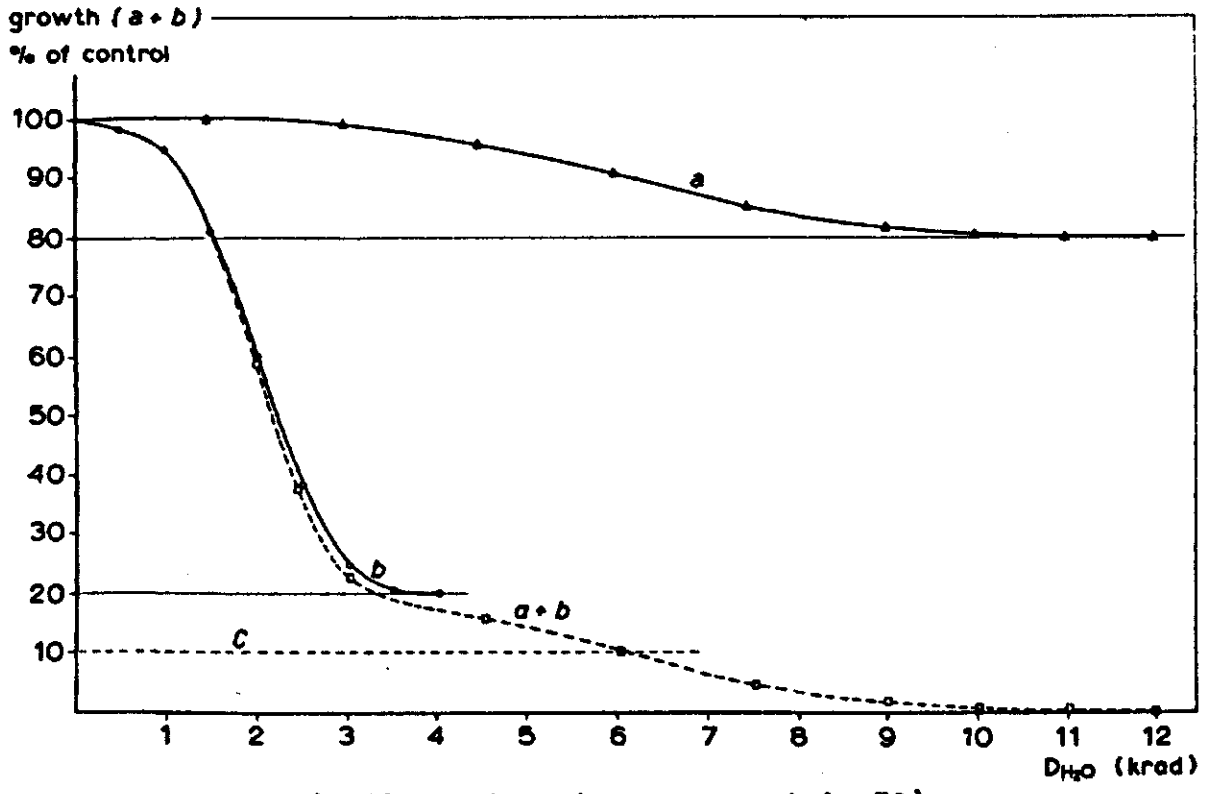


fig. 43 : explanation see text (p. 59)

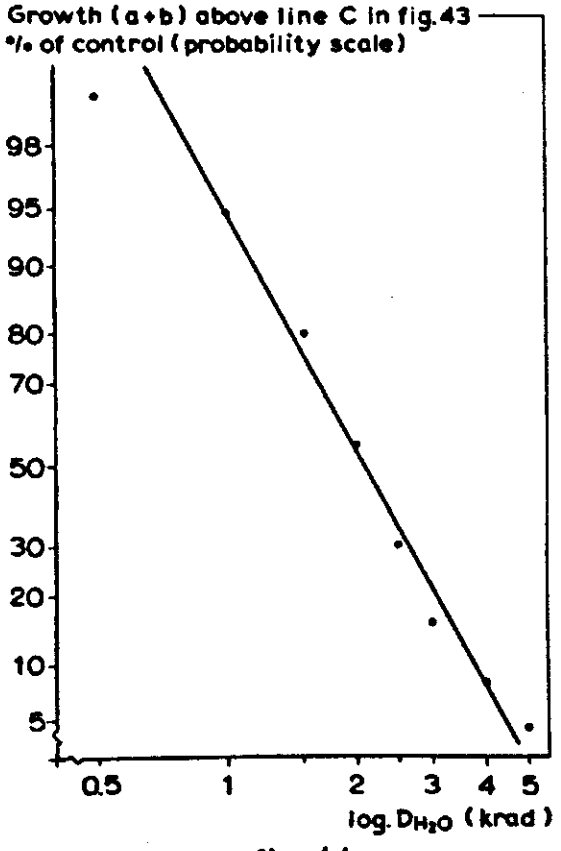


fig. 44

maize[165,170] or barley[134], has not been observed in tomato.

The dose/response curves for cumulative cotyledon growth in the present experiments were obtained by subtracting from all treatment means the mean length attained at a lethal dose (6 krad in $M_1$). If the same procedure is followed with the model in fig.43, a dose/response curve corresponding to that part of (a+b) lying above the line C, is produced.

In order to simulate the experimental procedure further, points on this part of the curve were plotted on log/normal probability paper (fig.44), after the y-axis had been adjusted in such a way that the intersection with C become 0%. The points appear to be well represented by a straight line, showing that it would require very small standard errors indeed to demonstrate that a log/probit transformation function is not appropriate to these data. In other words, this model shows that the growth of the primary root, the hypocotyledon and the cotyledons may well respond to radiation in the same manner as that of subsequent organs, i.e. according to a pattern described approximately by a normal sigmoid, and that the apparent discrepancy can be explained by the confounding of two or more growth components which differ considerably in radiation tolerance.

Applying transformations to obtain straight regressions with experimental data may be very useful for descriptive purposes or for estimating certain parameters such as the median effective dose or the steepness of the response. Though the transformation functions need not correspond exactly to biological reality, they must allow reasonably consistent results to be obtained. In this connection it is important to note that, in cases resembling that of fig.43, the shape of the curves will be altered by conditions or treatments which markedly affect one growth component but not the other. In such situations, detailed knowledge of the biological reality is essential if erroneous interpretation is to be avoided.

Further studies under standardized conditions would be needed to determine the optimal position of line C for a given character, i.e. of the 'cut off' point in the curve permitting the most accurate estimation of growth due to component (b). In the present experiments, the 'cut off' dose, chosen somewhat arbitrarily at 6 krad on prehydrated seeds, was almost certainly too high.

The extent to which the elongation of embryo cells contributes to the total length of an organ at the time of recording depends upon

the species and the organ concerned, the growth period prior to recording or to organ maturity, the radiation dose and the conditions of culturing. For instance, cotyledons of tomato cv. 'Moneymaker' have a length of 1½-2 mm in the embryo, of 6⅔-8 mm at the end of their growth in the absence of cell division (lethal dose), of 20-30 mm in the control under unfavourable conditions and of 40 mm or more under favourable conditions. With this and similar characters, failure to convert length to net growth before expressing the data as percentages of the control will lead to an erroneous representation of the dose/response curve, and, more seriously, to false estimates of radiation effectiveness parameters, such as the $ED_{50}$. Unfortunately, this error occurs in the otherwise admirable IAEA-sponsored international programme on the biological monitoring of neutron facilities. In this programme, based on a standard stock of Himalaya barley and on procedures employed at Washington State University[105], the main character recorded is the length of the 1st leaf after 5-6 days of growth, which is expressed directly as a % of the control[93,106]. The radiation effectiveness is usually characterized by what is believed to be the $ED_{50}$. As shown clearly in several communications of results obtained by this procedure[128,129,130,131], all curves pertaining to the % seedling height or root length (not % growth, as stated[128,129]) decrease asymptotically towards values varying between 10 and 30% of the control. Consequently, the doses corresponding to the 50% point on the ordinate are not only erroneous estimates (always overestimations) of the real $ED_{50}$'s, but are also not comparable with each other because of differences in the amount of error resulting from differences in 'residual length'. In future, this source of error may be reduced sufficiently for most purposes by subtracting from all treatment means the length at which the growth curve approaches the horizontal.

Although a model based upon normal sigmoid dose/response curves can not take into account all variables (see below), no attempts have yet been made to describe and interpret the effects of neutrons on organ growth by a model of the meristem cell population kinetics, as has been done for X-irradiated roots[101]. Any endeavour in this direction should be accompanied by cyto-histological studies of DNA synthesis ($^3$H-thymidine incorporation), mitotic frequencies and mitotic cycle times in the various zones of the shoot apex. Such studies are much more complicated than similar studies on the root apex, due

to the more varied destination of shoot meristem cells and the insufficiently understood and flexible delimitation of the meristematic zones generating the successive leaves. The absolute need for both standardized and optimal conditions must be stressed again in this connection, not only with regard to nutrition, but also to light intensity in view of its profound influence on the degree of cell elongation.

At present it must suffice to say that the shapes of the dose/response curves for growth characters are determined by (i) cellular repair at the lower doses, (ii) cell death and replacement at higher doses, (iii) between-cell variation in radiation sensitivity, (iv) the interdependence of cell division and elongation in relation to the dose[cf.154,165] and (v) statistical considerations[98].

Developmental characters

The presence or absence of at least 1 leaf > 3 mm after 18 days, a normal terminal apex after 42 days (exp.1), sufficient vigour for transplanting (exp.3), and ⩾ 24 seeds/truss (exps.3 and 4) are quantal response characters. Each plant can be characterized by its radiation tolerance; this is the dose under a given set of conditions, below which the quantal effect does not occur and above which it does occur[63]. If the distribution of these tolerances is normal, the dose/response relationship of the cumulative % individuals showing the effect is a normal sigmoid, and a straight regression is obtained on normal probability paper. As far as could be ascertained, the dose/response relationships of all quantal characters were consistent with this model. This corresponds with common experience, both in pharmacological and toxicological assay[62,63] and in radiobiology[21,27,55,121]. The width of the shoulder in the curve depends chiefly upon the sensitivity of the material and the nature of the character; the lower the vitality requirements for classification as normal, the wider the shoulder.

The leaf number after 18 or 25 days (exps.1 and 2), and 19 days (exp.4) belongs to a category of developmental characters which may be termed 'quantitative discrete', having a multinomial distribution within each treatment. Its dose/response relationships had marked shoulders reflecting a relatively low radiation sensitivity. The occurrence of mean values exceeding the control in exps.1 and 2 clearly distinguishes the curves from true sigmoids.

These increases were not associated with increased vigour because

length of the 1st leaf (exps.1 and 2), the 3rd leaf or plant weight (exp.1) were reduced in all treatments. The increases over the control were small in exp.1 and very large in exp.2; no increases were found in exp.4. This strongly suggests an inverse relationship between the magnitude of this phenomenon and the nutritional conditions (see p.58). Two subsequent experiments[37] have confirmed the absence of any increase in leaf number under optimal conditions.

At any given time, sub-optimal nutritional conditions cause the greatest amount of physiological growth restriction in material having the largest endogenous growth potential, i.e. the control. This restriction becomes less with decreasing growth potential, i.e. with increasing dose (p.58). Because growth inhibition by environmental factors also affects the rate of leaf differentiation, the observed increases in leaf number after irradiation are probably due to relatively strong restrictions in the control rather than to stimulation in the irradiated treatments. This interpretation is supported by the observation that the threshold dose for leaf number in both series of exp.1 increased from 18 to 25 days after sowing, i.e. as nutritional factors become more strongly limiting. Similarly, the absence of a peak in leaf number after 25 days in the $H_1$ series of exp. 1, as opposed to the $D_1$ series, may be attributed to the adequacy of nutritional conditions in the former series, due to its less advanced stage of growth as a result of a lower light intensity. This interpretation implies that at this stage light was not a limiting factor in any of the $H_1$ treatments. This does not exclude the possibility that differential effects of light intensity, e.g. in association with spacing, would have appeared at a later stage of growth.

Increased rates of leaf differentiation by the factors described above may lead to other effects which, if considered apart, might also be erroneously attributed to stimulation by radiation or, in less extreme cases, to an absence of response. This would apply particularly to the moment of transition to the generative stage, which in turn would determine both the timing and rate of axillary shoot formation and the number of days to flowering. A slightly advanced flowering, found in tomato plants grown in small pots after thermal neutron irradiation of hydrated seeds[30], may have been due to this relationship. The earlier onset of growth of the first axillary shoot in the 2 and 3 krad $D_1$ treatment of exp.1 also suggests a relationship with the advanced leaf differentiation after 25 days in these

treatments. The close spacing of 15x20 cm between the 25th and 42nd day is thought to have been responsible for the fact that a slight advance in axillary shoot initiation led to their markedly greater lengths after 42 days (etiolation). Clearly, none of the data suggests the involvement of true stimulation.

Increased axillary shoot formation and -growth has also been reported by Johnson for a large number of species, including tomato, after X-irradiation at the seedling stage[95,96], and for wheat after irradiation of dry and soaked seeds[97]. Similarly, MacKey has found a strongly increased tillering in X-irradiated barley and wheat[118] which he attributed to the improved light and spacing conditions among surviving plants due to the high killing rate at high doses. In contrast, he found no increase in tillering after neutron irradiation and explained this by the low killing and the uniform reduction in growth potential induced by this type of radiation as compared with X-rays. Gottschalk and Imam[80] found very considerable increases in tillering with dose in X-irradiated wheat species, but on good grounds attributed these chiefly to the disorganisation of normal correlative development by the destruction of cells in the meristem of the shoot apex, and only to a minor extent to the factor advanced by MacKey.

The interpretation of the present increases over the control is essentially in accordance with MacKey. In addition, it is shown that increases in axillary shoot formation need not be restricted to radiation treatments showing a reduction in plant survival but may be produced already by a reduction in vigour. Furthermore, the phenomenon is not necessarily connected with restrictions in light and spacing but also with restricted nutrient supply. Finally, it should be pointed out that MacKey's interpretation implies that in his experiments the conditions for development were grossly sub-optimal for the control and at the lower doses.

The present data do not permit an evaluation of the possibility that a partial disorganisation of the development-coordinating function of the shoot apex, as proposed by Gottschalk and Imam[80], might also have been involved.

It is concluded that stimulation should be studied both under convincingly optimal conditions and on the plant as a whole, rather than on one particular plant character (e.g. tillering or yield). Though the findings of single character studies may be of great practical value under the conditions of testing, they generally contri-

bute little to our understanding of the nature of radiation stimulation and may lead to misinterpretation.

It is considered inappropriate to use the term 'radiation stimulation' in those cases where the stimulation effects are the indirect consequence of radiation-induced damage at other sites in the plant, especially when this damage occurs at the organ or meristem level. If radiation stimulation is defined in this way, it will probably prove to be less common then is currently thought.

The number of abnormal leaves per $M_1$ plant is a result of both induced cytoplasmic and dominant genetic aberrations and of cell selection in the different zones of the shoot meristem. It was observed that abnormal sectors were both the smallest and the most numerous in the earliest leaves, and were progressively larger but less numerous in subsequent leaves. This is a logical consequence of the fact that the first leaves originate from relatively large numbers of predisposed embryo cells whereas subsequent leaves originate from progressively fewer initial cells.

The upward curvature in the dose/response relationships pertaining to the number of abnormal leaves/plant, up to an average of 3-4 such leaves, is explained by considering that the likelihood of detecting abnormal sectors increases with their size, i.e. from the 1st leaf upwards, and consequently also more than proportionally with dose owing to an 'interaction' between independently damaged initial cells in producing a visibly aberrant leaf. The subsequent saturation of the curves suggests that the 4-5th and subsequent leaves are formed from largely undifferentiated cells. Here, there are improved chances of cell replacement and those cells carrying gross aberrations are eliminated. These results and their interpretation are in agreement with Mertens and Burdick[123] and with Shapiro[166]. The latter author recorded the frequency of tomato plants with sectors of abnormal colour in the 1st, 2nd .. up to the 6th leaf, after irradiation of seeds heterozygous at specific loci governing leaf colour. He found a pronounced increase in frequency from leaf 1 to 4 which he attributed to an increasing likelihood of detection; this was followed by a marked decrease up to leaf 6, explained by the supposition that 'cells which are destined to become leaves 3, 4 and 5 can probably sustain a larger amount of damage than those cells that will become leaf 6 and onwards'. This supposition should be modified by placing greater emphasis on cell interrelationships in connection with

their degree of differentiation and position in the meristem. The proper analysis of these factors is extremely complex.

Leaf number below the 1st inflorescence (exp.4) is a developmental character marking the transition from the vegetative to the generative stage. It may be regarded as a growth character in so far as it is influenced by the growth and the ultimate size of the leaves. The increases in the lower dose range should be regarded as physiological consequences of permanent radiation damage which has affected leaf size. The tendency towards a levelling off at higher doses in most series may have resulted from a counteracting (i.e. flower promoting) effect associated with the earlier leaf maturation which accompanies reduced leaf growth after fast neutron irradiation. This hypothesis is in accordance with De Zeeuw[50] who found that an increased ratio of mature to growing foliage promoted the initiation of flowering in day-neutral species, including the tomato.

When the retardation in flowering at the highest doses could not be explained by increased leaf numbers, it was apparently due to a delay in the early development of the seedling. This can hardly be attributed to germination delay because the dose/response relationship of this character was shown not to correspond with that pertaining to the deficit in leaf number after 19 days. Cell division delay due to reparable causes cannot be invoked either, because it follows a dose/response pattern resembling that of germination delay (see p.55). The retardation in early development above a threshold dose is thus probably due to cell elimination resulting from an accumulation of chromosome damage, followed by gradual recovery of the meristem by cell substitution.

$M_1$ fertility

The general linearity of the dose/response relationships for weight of seeds/fruit suggests a closer connection with the $M_2$ characters than with the somatic $M_1$ characters. This is plausible, because both the failure of seed formation and the functional abnormality of $M_2$ seeds or seedlings are caused by aberrations induced in those few cells of the embryonic shoot apex which generate the sporogenic tissues of the 1st and 2nd truss.

An approximately linear reduction in seed set with dose has been reported for various other plant species[11,15,99,119,131], although spacing effects can interfere[80].

Generally, radiation-induced reductions in fertility are attri-

buted to chromosome aberrations. Gaul[67,69] using barley and Bora et al.[15] using Plantago ovata have found that cytologically detectable translocations explain a noticeable part of the radiation-induced sterility, though small deficiencies and inversions, not detectable cytologically, probably play an even more important part in both pollen and ovule sterility. On the other hand, the sterility induced by various chemical mutagens, such as ethyl-methane-sulphonate and diethyl-sulphate, is hardly associated with detectable aberrations[54,107,124,142,157]. With ionizing radiation, therefore, but not necessarily with chemical mutagens, $M_1$ seed set reduction can be regarded as a representative measure of the 'sum' of chromosomal defects that are able to persist in the somatic tissues but are eliminated during either gamete formation or early embryogenesis[69]. Even so, the possibility of maternal-physiological effects on gamete functioning cannot be altogether excluded. In fact, such a component would explain the slight convex curvatures in the lower dose region of some of the dose/response relationships for weight of seeds/fruit (exps.3 and 4). The tendencies towards a tail at the highest doses (exp.3), also found by others[11,191], could be due to a bias resulting from the elimination of very weak seedlings at transplanting. This assumes a slight positive relationship between vegetative vigour and the degree of fertility for which there is some evidence (section 4.7, p.105). Alternatively, these tails might have been either created or accentuated by the randomness of lethal events, causing an increasing wastage with dose on cells that had already been lethally affected[cf.8 Ch.3].

$M_2$ characters

The irregular nature of the radiation responses of the percentages non-germinating $M_2$ seeds and sublethals are partly due to their low mean frequencies, their heterogeneous origin and their dependence upon environmental conditions. Variation in response of all $M_2$ characters, including mutant frequency, is also due to the relatively small number of seeds tested per progeny and, in most treatments, to the restricted number of progenies. These limitations are imposed by practical considerations and are usually unavoidable.

The establishing of correct dose/response relationships is further complicated by the fact that their theoretical shape depends also upon the assumptions made. If, for instance, the non-germinating seeds and sublethals are assumed to be manifestations of the same

genetic events, both categories should be expressed as percentages of seeds sown; if on the other hand they are thought be to caused by independent events, the sublethals should be expressed as percentages of seeds germinated and this will affect the shape of their dose/response relationships. In principle, these alternative assumptions could be tested, because, in the case of neutron irradiation, all types of aberrations generally respond linearly to the dose (see below). Because the present data are too variable for this purpose, it must suffice to say that the dose/response relationship of non-germinating seeds and sublethals could not be shown to differ from linear (exps.3 and 4).

The number of mutations/100 $M_1$ truss progenies increased less than proportionally to the dose (exp.3). This was associated with sharp increases in the mutant segregation % and may therefore be attributed chiefly to a decreasing chimerism resulting from a progressive elimination of initial cells at higher doses. The greatly improved linearity over the whole dose range which was obtained when this character was replaced by the % mutant seedlings, is in agreement with Gaul[66] who has shown that this latter character, in contrast to the former, is insensitive to differences in the degree of chimerism.

However, considering that all semi-sterile $M_1$ plants (<24 seeds/truss) were excluded from progeny testing, a downward deviation from linearity in the dose/response relationship of the % mutant seedlings would still be expected in the event of a within-treatment association between recessive 'visible' mutations and aberrations causing sterility. The absence of consistent evidence of such a residual non-linearity (some may have occurred in $D_3$, fig.27) suggests that this association was largely or wholly lacking. Direct confirmatory evidence will be presented in section 4.7 (p.106) where the findings of other authors will be considered.

The present linearity of the dose/response relationships for all phenotypic $M_2$ categories (excluding bias in mutation frequency) corresponds with the general experience, that all types of genetic aberrations, and consequently their phenotypic manifestations, induced by fast neutrons or other high-LET radiations increase as a linear function of the dose. This is plausibly explained by the assumption that the high ionization density of these radiations causes even chromosome interchanges to be produced by one and the same ionizing particle [73, 74, 112, 171]. With high-LET radiations therefore, the average com-

plexity of the induced disturbances at the chromosomal level cannot be deduced from the shapes of the dose/response relationships.

The fact that the pooled percentages of non-germinating seeds, sublethals and mutant seedlings showed the most regular pattern of response, especially in exp.4, suggests a partial complementarity of these categories. This is not illogical because an aberration resulting from one or more unstable lesions is always produced at the expense of any other type of aberration which might, in principle, have resulted from these lesions. Furthermore, although the genetic causes of early aborted seeds, non-germinating seeds, sublethals and mutant seedlings are highly diverse, they show considerable mutual overlapping (see p.93-94).

Correlation between responses of different characters

Because all characters respond to radiation, the coefficients of correlation between characters based on treatment means are always high. Essentially, they provide only a measure of the degree of correspondence in the dose/response relationships of the various characters pairwise.

The fact that the total % of all aberrant $M_2$ categories usually yielded higher correlation values than its separate components tends to confirm that within treatments the percentages individuals in the various aberrant $M_2$ categories possess a weak complementary tendency (see above). The finding that r-values involving the % mutant seedlings were higher than those involving the number of mutations/100 $M_1$ truss progenies is certainly due partly to a greater variation in the latter character and partly to the bias in this character at high doses (see p.69). Whereas in exp.3 the r-values among the $M_1$ characters were generally somewhat higher than those between the $M_1$- and the $M_2$ characters, this was not clearly demonstrated in exp.4. This discrepancy was possibly due to the much lower dose range in the latter experiment. The same explanation is thought to hold for the fact that in exp.3 the seedling characters showed slightly lower levels of correlation with the $M_2$ characters than did weight of seeds/fruit; whereas in exp.4 cotyledon length, days to flowering and weight of seeds/fruit yielded the highest and similar coefficients of correlation with the $M_2$ characters.

The evidence of all 4 experiments combined clearly indicates that cumulative root growth and cumulative cotyledon growth are, at least in respect of the shape of their dose/response relationships, the most

useful early indicators of the average genetic effectiveness of fast neutron treatments. Days to flowering and weight of seeds/fruit cannot, of course, permit an early evaluation of average radiation effectiveness.

## 4.4. Estimates of radiation effectiveness

### 4.4.1. Results

The chief aim of this section is to provide estimates of parameters suitable for comparing, per character, the effectiveness of fast neutrons on the various objects, i.e. 'Glorie' versus 'Moneymaker', and prehydrated versus dry seeds.

For those characters having either fixed or accurately known upper and lower limits, the parameter used was the dose causing 50% effect ($ED_{50}$). This is a measure of radiation tolerance, i.e. the reciprocal of radiation effectiveness. The $ED_{50}$ estimates were obtained, whenever possible, from straight regressions on graph paper with suitable scales.

For characters without fixed boundaries, or for which such boundaries would exist only in theory (e.g. 100% non-germinating $M_2$ seeds), the radiation effectiveness was estimated by means of the coefficient of linear regression on dose, except when the dose/response relationships were strongly non-linear. In the latter case (some $M_1$ characters in exp.4), the radiation effectiveness of different objects was compared graphically by applying linear dose-scale transformations until all sets of data were optimally represented by the same regression line.

It is important to note that all estimates are based on rad doses in water ($D_{H_2O}$).

In the present section, only differences in radiation effectiveness between characters will be discussed; differences between objects will be dealt with in sections 4.5 and 4.6.

#### Experiments 1 and 2

The $ED_{50}$ estimates and their methods of estimation are shown in tables 19 (exp.1) and 20 (exp.2), together with the quotients $ED_{50}(D_1)/ED_{50}(H_1)$ = DRF. The DRF's will be considered in section 4.6.

Table 19 : Estimated $ED_{50}$'s (krad $D_{H_2O}$) after irradiation of dry ($D_1$) and 48 hours prehydrated ($H_1$) seeds, and corresponding quotients $ED_{50}(D_1)/ED_{50}(H_1)$ = DRF. Experiment 1.

Methods of estimation: from plots on either normal probability paper (p), log/normal probability paper (lp) or linear graph paper (gr), or from regression coefficients (b) based on the treatments 0-7 krad in $D_1$ and 0-2 krad in $H_1$.

| Character | Days | $ED_{50}$ | | DRF | Method |
|---|---|---|---|---|---|
| | | $D_1$ | $H_1$ | | |
| % seedlings with ⩾1 leaf | 18 | 8.75 | 1.85 | 4.7 | lp |
| Leaf number | 18 | 7.50 | 1.60 | 4.7 | gr |
| Cumulative root growth | 25 | 5.40 | 1.25 | 4.3 | b |
| Cumul.cotyledon growth | 25 | 4.90 | 1.20 | 4.1 | b |
| Length 1st leaf | 25 | 6.35 | 1.45 | 4.3 | p |
| Cumulative stem growth | 25 | 4.90 | 1.20 | 4.1 | lp |
| Leaf number | 25 | 8.80 | 1.75 | 5.0 | gr |
| Ultimate length 3rd leaf | 42 | 5.00 | 1.65 | 3.0 | b |
| Plant weight | 42 | 3.60 | 1.15 | 3.1 | b |

Table 20 : Estimated $ED_{50}$'s (krad $D_{H_2O}$) after irradiation of dry ($D_2$) and 24 hours prehydrated ($H_2$) seeds, and corresponding quotients $ED_{50}(D_2)/ED_{50}(H_2)$ = DRF. Experiment 2.

Methods of estimation as in table 19.

| Character | Days | $ED_{50}$ | | DRF | Method |
|---|---|---|---|---|---|
| | | $D_2$ | $H_2$ | | |
| Cumulative root growth | 4* | 3.70 | 0.80 | 4.6 | lp |
| Cumulative root growth | 25 | 7.30 | 2.50 | 2.9 | gr |
| Cumul.cotyledon growth | 25 | 6.90 | 2.30 | 3.1 | gr |
| Length 1st leaf | 25 | 7.70 | 2.65 | 2.9 | gr |
| Cumulative stem growth | 25 | 7.90 | 2.60 | 3.0 | gr |
| Leaf number | 25 | 9.75 | 3.35 | 2.9 | gr |

* after 50% germination

Experiment 3

The $ED_{50}$'s, their methods of estimation and the quotients $ED_{50}(MM)/ED_{50}(GL)$ = SR are shown in table 21. The SR values will be considered in section 4.5. Table 22 gives, for both weight of seeds/$M_1$ fruit and the $M_2$ characters, the coefficients of linear regression on dose, b, and the quotients $b_{GL}/b_{MM}$ = SR; the latter are discussed

Table 21 : Estimated $ED_{50}$'s (krad $D_{H_2O}$) after irradiation of dry ($D_3$) and 24 hours prehydrated ($H_3$) seeds of MM and GL, and corresponding quotients $ED_{50}MM/ED_{50}GL$ = SR. Experiment 3.
Methods of estimation as in table 19.

| Character | Days | | $ED_{50}$ MM | $ED_{50}$ GL | SR | | Method |
|---|---|---|---|---|---|---|---|
| Cumulative cotyledon growth | 18 | $D_3$ | 5.8 | 5.1 | 1.14 | | lp |
| | | $H_3$ | 2.10 | 2.00 | 1.06 | | |
| Seedling weight | 18 | $D_3$ | 5.0 | 4.4 | 1.14 | 1.14 | p |
| | | $H_3$ | 1.55 | 1.32 | 1.17 | | |
| % transplantable seedlings | | $D_3$ | - | - | - | | |
| | | $H_3$ | 4.21 | 3.52 | 1.20 | | p |
| 'Fertile' plants, % of flowering | | $D_3$ | 6.6 | 5.8 | 1.14 | | p |
| | | $H_3$ | 2.73 | 2.20 | 1.24 | 1.17 | |
| Weight of seeds/$M_1$ fruit | | $D_3$ | 4.1 | 3.6 | 1.11 | | b |
| | | $H_3$ | 2.00 | 1.65 | 1.21 | | |

Table 22 : Coefficients of linear regression on dose (b = % response/krad $D_{H_2O}$) after irradiation of dry ($D_3$) and 24 hours prehydrated ($H_3$) seeds of MM and GL, and corresponding quotients $b_{GL}/b_{MM}$ = SR. Experiment 3.

| Character | | b MM | b GL | SR |
|---|---|---|---|---|
| % reduction weight of seeds/$M_1$ fruit | $D_3$ | 12.5 | 13.9 | 1.11 |
| | $H_3$ | 25.0 | 30.3 | 1.21 |
| % non-germinating seeds ($M_2$) | $D_3$ | 2.25 | 1.64 | 0.73 |
| | $H_3$ | 5.03 | 4.25 | 0.84 |
| % sublethals ($M_2$) | $D_3$ | 0.384 | 0.647 | 1.68 |
| | $H_3$ | 0.525 | 0.825 | 1.57 |
| % mutant seedlings ($M_2$) | $D_3$ | 1.45 | 2.31 | 1.59 |
| | $H_3$ | 4.22 | 3.42 | 0.81 |
| % all aberrant categories ($M_2$) | $D_3$ | 4.08 | 4.60 | 1.13 |
| | $H_3$ | 9.78 | 8.50 | 0.87 |
| Total of all aberrations in sporogenic cells (see text, p.74) | $D_3$ | 16.58 | 18.50 | 1.12 |
| | $H_3$ | 34.73 | 38.80 | 1.12 |

in section 4.5. The regressions were calculated to pass through the average control values. This was done in order to minimize bias in the case of those characters whose radiation responses were irregular or somewhat curved. For weight of seeds/$M_1$ fruit the regression coefficients are shown, in addition to the $ED_{50}$'s, because the radiation responses of this character result chiefly from aberrations induced in those initial cells which also give rise to the $M_2$ generation.

A parameter which is roughly proportional to the total number of aberrations induced in the sporogenic cells is obtained by adding the regression coefficients pertaining to weight of seeds/$M_1$ fruit, non-germinating $M_2$ seeds, sublethals and mutant seedlings, assuming, among other things, that the events leading to these various categories segregate independently. The resulting coefficients, referred to as 'total of all aberrations in sporogenic cells' are shown at the bottom of table 22.

Experiment 4

The $ED_{50}$'s and their methods of estimation are shown in table 23 for various $M_1$ characters in the D and H12 series; those for the other series can, should this be desired, be calculated from relative values (DRF's) to be given in table 26 (p. 84).

The coefficients of linear regression on dose for weight of seeds/$M_1$ fruit and the $M_2$ characters are shown in table 24 for the D, H12, H24 and H48 series; those for the other series can be calculated as mentioned above.

Table 23 : Estimated $ED_{50}$'s (krad $D_{H_2O}$). Experiment 4.
Methods of estimation as in table 19.

| Character | Days | D | H12 | Method |
|---|---|---|---|---|
| Cumulative cotyledon growth | 19 | 3.87 | 1.13 | p |
| Length 1st leaf | 19 | 5.79 | 1.86 | p |
| Length 2nd leaf | 19 | 5.26 | 1.76 | p |
| Leaf number | 19 | 7.75 | 2.45 | gr |
| 'Fertile' plants, % of flowering | | (5.6) | (2.2) | p* |
| Weight of seeds/$M_1$ fruit | | 3.43 | 1.36 | b |

* extrapolated

Table 24 : Coefficients of linear regression on dose (krad $D_{H_2O}$). Experiment 4.

| Character | D | H12 | H24 | H48 |
|---|---|---|---|---|
| % reduction weight of seeds/$M_1$ fruit | 14.58 | 36.76 | 44.25 | 67.56 |
| % non-germinating seeds ($M_2$) | 2.57 | 6.95 | 9.20 | 16.10 |
| % sublethals ($M_2$) | 0.34 | 0.78 | 0.99 | 1.36 |
| % mutant seedlings ($M_2$) | 2.28 | 4.60 | 4.20 | 6.45 |
| % all aberrant categories ($M_2$) | 5.20 | 12.33 | 14.39 | 23.91 |
| Total of all aberrations in sporogenic cells | 19.78 | 49.09 | 58.64 | 91.47 |

4.4.2. Discussion

The effectiveness of radiation on a population is defined only with reference to a given character under a given set of experimental conditions and is then fully characterised by the dose/response relationship. The $ED_{50}$ refers to only one particular point of the latter and has therefore merely a descriptive value. It is only when the dose/response relationships of various traits or objects have the same shape that this parameter is sufficient to define the relative effectiveness of radiation. Two curves have the same shape when it is possible to transform one to coincide with the other by linear x-scale (dose) transformation.

Within a given series, the levels of the $ED_{50}$'s for the different characters are closely related to the degree of curvature as well as the presence and width of a shoulder in the dose/response curves (see graphs in section 4.3.1).

Thus, the high $ED_{50}$'s for both the quantal characters (e.g. % seedlings having $\geq$1 leaf) and the quantitative discrete characters (e.g. leaf number after 18 or 25 days) compared with the $ED_{50}$'s for the quantitative traits with continuous variation (e.g. cotyledon-, leaf- or stem length) are associated with the presence of marked shoulders in the dose/response curves of the former. The very high $ED_{50}$'s for the characters recorded after 25 days in exp.2, compared with those in exp.1, are a direct consequence of the strongly curved dose/response relationships in exp.2. Similarly, the fact that in exp.1 the $ED_{50}$'s for leaf number after 25 days were higher than after 18 days resulted from an increased 'shoulder' width in the dose/response curves after 25 days which must, according to section 4.3.2

be attributed to increasingly sub-optimal nutritional conditions with time, rather than to recovery from radiation damage.

For similar reasons, the low $ED_{50}$'s for cumulative root growth after 4 days in exp.2 cannot be usefully compared with the much higher values recorded after 25 days in the same experiment, because the former were obtained under favourable conditions (petridish stage) while the latter were derived from extremely curved dose/response relationships resulting from sub-optimal conditions. A more meaningful comparison, only possible with the D series, is between the $ED_{50}$ for cumulative root growth at the petridish stage in exp.2 (3.70 in $D_2$) and that for cumulative root growth after 25 days in exp.1 (5.40 in $D_1$). The observed increase in the $ED_{50}$ between the two times of observation suggests a recovery of root growth over this period, probably due chiefly to the gradual elimination of the most deleterious chromosome aberrations in successive cell division cycles(51 p.190,82), and possibly owing also to a diminution of the effects of initial division delay and other reparable ('physiological') types of damage. The practical conclusion based on this finding is that the estimated radiation sensitivity of an organ, as measured by macroscopic criteria, may depend greatly upon the growth stage in question.

The $ED_{50}$'s for the quantitative seedling characters in exp.2 did not follow entirely the same order as those in exp.1. Nevertheless, in both experiments the $ED_{50}$'s for cumulative root- and cotyledon growth were somewhat lower than those for length of the 1st leaf. This was confirmed in exp.4, where the $ED_{50}$'s for cumulative cotyledon growth were considerably lower than those relating to length of the 1st or 2nd leaf. Consequently, it is likely that fast neutrons had a greater effectiveness on root- and cotyledon growth than on the growth of the earliest leaves(cf.129). However, this statement requires comment. Firstly, like in other cases, the $ED_{50}$ differences between the characters concerned were connected with differences in the degree of curvature of the dose/response relationships. This implies that the relative radiation effectiveness on these characters is to some extent dose dependent. Secondly, as the $ED_{50}$ may increase during the growth of an organ (as in the case of root growth mentioned above), any conclusions based on such data must be restricted to the stages actually observed. Thirdly, the various organs have an entirely different histological and morphological constitution and physiological function. Finally, at the time of irradiation, these organs differ

greatly in both cell number and level of differentiation, and hence in the possibilities of recovery by cell substitution. Therefore, it would be incorrect to use macroscopic data for drawing any conclusions on radiation sensitivities at the meristem level, let alone at the cellular level.

The low $ED_{50}$'s for plant weight compared with those for length of the 3rd leaf, both after 42 days (exp.1), and the low $ED_{50}$'s for seedling weight compared to those for cumulative cotyledon growth, both after 18 days (exp.3), approximate to expectation, considering the 3-dimensional nature of the character 'weight'.

The low $ED_{50}$'s relating to the % 'fertile' plants compared with those for the % transplantable seedlings (exp.3) indicate that fast neutrons are much more effective in eliminating plants by reduction in seed set than by reduction in vegetative vigour. This conclusion is supported by the low $ED_{50}$'s for weight of seeds/fruit compared with those for the quantitative seedling characters in the dry seed series of exps.3 and 4; it is also in agreement with common experience[10,33,131,149]. However, this conclusion must be qualified, because the relationship between the $ED_{50}$'s for weight of seeds/fruit and certain seedling characters was altered or even reversed by seed prehydration in both experiments, which indicates a stronger enhancement of the effectiveness of fast neutrons on seedling growth than on seed set. This important fact will be discussed in greater detail in section 4.6, p.92-94.

Exps. 3 and 4 have shown that the % reduction in weight of seeds/$M_1$ fruit per unit dose was approximately 3 times greater than the % increase in the sum of all aberrant $M_2$ categories. Among the latter, the % non-germinating $M_2$ seeds increased much more sharply with dose than the % sublethals and, with one exception ($D_3$ of 'Glorie'), also somewhat more markedly than the % mutant seedlings. A similar result was obtained with thermal neutrons[30]. These data indicate that, on an average, recessive mutations induced by fast neutrons are accompanied by a very considerable incidence of disturbances which, after having persisted throughout the diplophase, either fail to pass meiosis or lead to early or late embryo abortion. These disturbances are partly transmissible[70,71,124], and are therefore of importance in mutation breeding (see section 5.2, p.110).

## 4.5. Varietal differences in fast neutron sensitivity

### 4.5.1. Results

The effectiveness of fast neutrons on cv. 'Glorie' (GL) relative to cv. 'Moneymaker' (MM) was estimated by means of the quotients $ED_{50}(MM)/ED_{50}(GL)$ and the quotients $b_{GL}/b_{MM}$ in exp.3. These estimates will be referred to as the 'sensitivity ratio' (SR).

The SR's for the various $M_1$ characters (table 21, p.73) varied between 1.06 and 1.24. The average over the vegetative $M_1$ characters was 1.14 and the average over the $M_1$ fertility characters 1.17.

The SR's for the $M_2$ characters (table 22) varied much more strongly than those for the $M_1$ characters. The SR for the total % of all aberrant $M_2$ categories was >1 in $D_3$ and <1 in $H_3$. This difference was the converse of that for reduction in weight of seeds/$M_1$ fruit. However, adding the regression coefficients pertaining to these characters resulted in SR estimates for the 'total of all aberrations in sporogenic cells' (cf. section 4.4.1, p.74) which were almost equal in both series (1.12).

The average 100-seed weight was 319 mg in MM and 279 mg in GL (ratio 1.14/1.00). The average 100-embryo weights were 210 and 183 mg, respectively (ratio 1.15/1.00). The numbers of root meristem cells in $G_1$ versus $G_2$, in 16 hours hydrated embryos, were: 2431 vs. 69 in MM (97.2% in $G_1$) and 2423 vs. 77 in GL (96.9% in $G_1$). The amount of DNA per $G_1$-nucleus was $12.65 \pm 0.37$ units in MM and $12.03 \pm 0.22$ units in GL.

### 4.5.2. Discussion

According to the SR values, GL was more radiation sensitive than MM in respect of effects on the vegetative $M_1$ characters, reduction in $M_1$ fertility and the production of sublethal $M_2$ seedlings. In contrast, GL was less sensitive than MM as regards the production of non-germinating $M_2$ seeds. This suggests that the phenotypic spectrum of the radiation injuries was different in the two seed-lots. Moreover, irradiation of prehydrated seeds ($H_3$) as compared to dry seeds ($D_3$) increased the SR with regard to $M_1$ fertility reduction and $M_2$ non-germinability, while lowering considerably the SR relating to the recessive mutant seedlings visible in $M_2$. This suggests that seed prehydration, which, according to the SR's for the 'total of all aberrations in sporogenic cells', caused an equal degree of overall sensitization in both cultivars, also modified the relationship between these cultivars with regard to the various phenotypic manifestations of

injury.

It is reasonable to assume that gamete sterility and early embryo abortion, which cause $M_1$ fertility reduction, are on an average due to more severe chromosomal disturbances than is non-germinability, and that the latter category is on an average due to more severe disturbances than are either sublethality or the occurrence of recessive 'visible' mutations (see section 4.6.2, p.94). The data then suggest that seed prehydration increased the average severity of neutron-induced chromosome aberrations in GL relative to MM. Further experiments would be required to determine whether these differences in aberration 'spectrum' are due to the inherent properties of these two cultivars or to incidental causes.

The SR estimates GL/MM average 1.14 for the vegetative $M_1$ characters and 1.12 for the overall effects on $M_1$ fertility and the $M_2$ characters. These results correspond closely with the 100-seed weight ratio or the 100-embryo weight ratio MM/GL (1.14-1.15). Without attaching particular significance to the striking quantitative correspondence, it is nevertheless tempting to relate the higher radiation sensitivity of GL to its genetically determined[37] smaller seed- and embryo size. This is in agreement with Scarascia[164] who has demonstrated in Nicotiana (a genus related to Lycopersicum) a positive relationship between radiation tolerance and seed size. De Nettancourt and Contant[47] have shown that the tolerance of L.esculentum (cv. 'Moneymaker') and L.pimpinellifolium to acute irradiation is positively related to the size of the shoot apex. Subsequent work of De Nettancourt and Devreux[48] involving these species, their reciprocal hybrids and all backcross lines has confirmed and elaborated this conclusion. Also other authors have demonstrated[116] or suggested[44] a positive relationship between radiation tolerance and apical size. Constantin and Osborne[28] have found interspecific differences in fast neutron tolerance to be governed mainly by the size of the shoot apex and the average nuclear volume.

Although apical size was not determined in the present work, it may be expected to exhibit, within a given species, a close positive relationship with embryo size. According to the data, varietal differences in stage of interphase or DNA content per nucleus can be excluded. This was not unexpected as such differences were slight or absent even in comparisons between L.esculentum and L.pimpinellifolium [47,48]. Because the cells and nuclei of GL are not expected to be

larger than those of MM, the factor 'relative energy absorption per cell' cannot be used to account for the greater radiation sensitivity of GL.

It is tentatively concluded, therefore, that the sensitivity differences between MM and GL are governed chiefly by a positive relationship between radiation tolerance and the number of cells in the meristem of the shoot apex. The latter would determine the opportunities of cell selection and replacement.

It would be very difficult to prove unequivocally whether or not additional factors governing radiation sensitivity at the cellular level might also be involved in this particular comparison. Many authors, who have demonstrated the influence of genetic[12,13,72,80,109] and/or cytoplasmic[53,196] factors on the radiation sensitivity of closely related species or varieties, did not provide data on seed-, embryo- or apical size of the various genotypes. Consequently, it is often impossible to judge to what extent these genotypic differences in sensitivity were operating at the histological level. Further studies on this problem, similar to the work of De Nettancourt and Devreux[48], would be of value in Lycopersicum esculentum, using a series of genotypes covering a maximum range of embryo sizes.

## 4.6. Dose reduction factors associated with seed prehydration and germination

### 4.6.1. Results

The dose reduction factors associated with seed prehydration and germination (DRF, defined in chapter 2) were calculated from the $ED_{50}$'s and coefficients of linear regression on dose, in which the doses were expressed as krad in water ($D_{H_2O}$).

These DRF values require correction for differences in relative radiation energy absorption in order to yield unbiased estimates of the degree of sensitization caused by the various prehydration treatments. Nonetheless, the uncorrected values are given in order to leave the choice of correction factors open to discussion. These uncorrected DRF's are adequate for a preliminary examination of trends within and between series.

Two types of correction factors will be considered, by which the DRF's must be divided. These are (a) the dose absorbed in prehydrated embryo tissue relative to that in dry embryo tissue, i.e. the relative rad dose (table 5, p.20, last column); and (b) the energy absorption

per prehydrated meristem or meristem cell relative to that per dry meristem or meristem cell, inferred from the relative amounts of energy absorbed per embryo (table 6, p.22, last column).

Correction factors of type (a) are based on the dosimetrical procedures currently recommended for neutron irradiation studies on dry seeds of different species[92,93]. Correction factors of type (b) are related to the relative energy absorption per nucleus or per interphase chromosome which have been used to compare the X- or γ-radiation sensitivity of species belonging to different taxa[172,173]. Although the direct estimation of the energy absorption per nucleus would probably be preferable to the indirect estimation of energy absorption per cell, in practice it would be difficult to obtain mutually comparable data on nuclear size in microscopic preparations of embryos germinated for different periods.

Experiment 1

The DRF's (table 19, p.72) showed a decreasing trend in the course of vegetative development.

The only discrepancy was the DRF for leaf number after 25 days (5.0) which was higher than after 18 days (4.7). This was clearly due to a stronger increase, under the influence of growth-limiting nutritional conditions from the 18th to the 25th day, in the shoulder width of the dose/response curve of the $D_1$ compared to the $H_1$ series, in connection with the more advanced growth stage of the former (cf. section 4.3.2, p.64). Consequently, it is reasonable to assume that under optimal conditions the DRF for leaf number after 25 days would not have exceeded 4.7. It should be noted that the nutritional conditions were not limiting from the 25th day onwards (Hoagland solution).

In this experiment, only a few seeds had germinated before or during irradiation. Therefore the degree of hydration of these seeds resembled that of the 24 hours rather than of the 48 hours prehydrated seeds in chapter 3 (cf. fig.1, p.13). Accordingly, the applicable DRF correction factors are (a) 1.05 and (b) 1.46. The former value is negligible compared to the high DRF's. Correction by the latter value leads to revised DRF estimates averaging 3.2, 3.0 and 2.1 after 18, 25 and 42 days, respectively.

Experiment 2

The DRF's (table 20, p.72) were appreciably lower after 25 days than after 4 days, thus indicating the same trend as in exp.1. The

DRF correction factors associated with 24 hours prehydration are (a) 1.05 and (b) 1.46; using the latter value yields a revised DRF estimate of 3.2 after 4 days and an average of 2.0 after 25 days.

Experiment 3

The DRF's for the $M_1$ characters were obtained by taking the quotients $ED_{50}(D_3)/ED_{50}(H_3)$ from the data in table 21 (p.73), and those for the $M_2$ characters by taking the quotients $b_{H_3}/b_{D_3}$ from the data in table 22 (p.73), per cultivar, and after averaging over both cultivars. The DRF's are shown in table 25.

Table 25 : Dose reduction factors (DRF) associated with 24 hours prehydration, estimated from $ED_{50}$'s ($M_1$ characters, table 21) and linear regression coefficients ($M_2$ characters, table 22), for MM, GL and their mean (MM+GL). Experiment 3.

| Character | MM | GL | MM+GL |
|---|---|---|---|
| $M_1$ | | | |
| Cumulative cotyledon growth, 18 days | 2.8 | 2.6 | 2.7 |
| Seedling weight, 18 days | 3.2 | 3.3 | 3.3 |
| % transplantable seedlings | - | - | - |
| 'Fertile' plants, % of flowering | 2.4 | 2.6 | 2.5 |
| Weight of seeds/fruit | 2.1 | 2.2 | 2.1 |
| $M_2$ | | | |
| % non-germinating seeds | 2.2 | 2.6 | 2.4 |
| % sublethals | 1.4 | 1.3 | 1.3 |
| % mutant seedlings | 2.9 | 1.5 | 2.0 |
| % all aberrant categories | 2.4 | 1.9 | 2.1 |
| Total of all aberrations in sporogenic cells | 2.1 | 2.1 | 2.1 |

Varietal differences were either absent or small for most characters but were considerable for the % non-germinating seeds (MM<GL) and especially so for the % mutant seedlings (MM>GL). Whereas the DRF pertaining to the total % of all aberrant $M_2$ categories was considerably higher in MM than in GL, the DRF for the estimated 'total of all aberrations in sporogenic cells' (including the aberrations causing $M_1$ seed set reduction) was the same in both cultivars. These data express in a different manner the findings of section 4.5 (p.78), namely that seed prehydration has caused the same overall degree of sensitization to fast neutrons in MM and GL, but, at the same time, a marked shift in the spectrum of aberrant phenotypes both within and between these cultivars. To what extent this is due to real varietal

differences or to the large variation which is normal for $M_2$ characters could not be established.

The DRF's calculated from the means over both cultivars (MM+GL) were higher for the $M_1$ seedling characters than for the % 'fertile' plants (≥24 seeds/truss). The latter value in turn was considerably higher than that for weight of seeds/fruit, indicating that the within-treatment variation in fertility was greater after irradiation of prehydrated seeds than of dry seeds. The DRF's show a decreasing trend in the course of $M_1$ development, though less clearly than in the preceding experiments.

The DRF's for the $M_2$ characters differed considerably, the value relating to the % non-germinating $M_2$ seeds being the highest and that for the % sublethals by far the lowest. The DRF for the total % of all aberrant $M_2$ categories was the same as that for weight of seeds/$M_1$ fruit.

The DRF's may be corrected by the same factor (b) as in exp.2 despite the differences in 100-seed weight and germination speed, because it is reasonable to assume that water uptake during the first 24 hours is proportional to initial seed weight and independent of the speed of visible germination. On this assumption, the revised DRF estimate is 2.0 for the damage expressed in the seedling characters and 1.4 for the permanent damage to the sporogenic cells.

Experiment 4

The DRF's were calculated from the best fitting linear or curvilinear regressions, whichever was applicable, through the data in figs.29-42. The regressions were calculated through the average control values as these represented the origin with good accuracy. Because the H72, H96 and H144 series each consisted of only one treatment, whose means varied considerably, an 'average' DRF over these series was calculated for each character. All DRF estimates are shown in table 26.

The DRF's for all characters increased almost consistently with the pregermination period. Within the prehydration treatments (i.e. per column) marked differences between $M_1$ characters were absent up to H6. The slight differences within the $H\frac{1}{2}$ column were associated with the fact that $\frac{1}{2}$ hour prehydration was short in relation to the exposure periods. Because the range of doses (exposure periods) was wider for the characters recorded after 12 or 19 days than for subsequent characters (table 7, p.30) the 'effective hydration periods'

Table 26 : Dose reduction factors (DRF) associated with ½-144 hours prehydration/germination, estimated either from $ED_{50}$'s or by linear x-scale transformation ($M_1$ characters) or from linear regression coefficients ($M_2$ characters); and correction factors (a) and (b). Experiment 4.

| Character | H½ | H3 | H6 | H12 | H24 | H48 | H72-144 |
|---|---|---|---|---|---|---|---|
| $M_1$ | | | | | | | |
| Cumulative cotyledon growth, 12 or 19 days | 1.4 | 1.8 | 2.3 | 3.6 | - | - | - |
| Length of 1st or 2nd leaf, 19 days | 1.3 | 1.8 | 2.2 | 3.1 | - | - | - |
| Leaf number, 19 days | 1.4 | 1.9 | 2.3 | 3.2 | - | - | - |
| Abnormal leaves/plant | 1.1 | 1.7 | 2.3 | 3.2 | 4.8 | 9.7 | 16.2 |
| Leaves below 1st inflor. | 1.2 | 1.8 | 2.5 | 3.1 | 4.6 | 7.1 | 10.7 |
| Days to flowering | 1.2 | 1.8 | 2.4 | 3.1 | 3.8 | 7.0 | 11.5 |
| Weight of seeds/fruit | 1.2 | 1.6 | 2.2 | 2.5 | 3.0 | 4.6 | 4.6 |
| $M_2$ | | | | | | | |
| % non-germinating seeds | 1.4 | 1.7 | 2.3 | 2.7 | 3.6 | 6.2 | 6.2 |
| % sublethals | 1.5 | 1.1 | 2.0 | 2.3 | 2.9 | 3.9 | 6.4 |
| Mutations/100 $M_1$ truss progenies | 1.1 | 1.8 | 1.9 | 2.0 | 1.8 | 2.8 | 2.9 |
| % mutant seedlings | 1.2 | 1.8 | 2.2 | 2.0 | 1.8 | 2.8 | 2.8 |
| % all aberrant categories | 1.3 | 1.7 | 2.3 | 2.3 | 2.9 | 4.7 | 4.8 |
| | | | Correction factors | | | | |
| (a) Relative rad dose | 1.01 | 1.02 | 1.03 | 1.04 | 1.05 | 1.09 | 1.14-1.16 |
| (b) Relative amount of energy absorbed per 'average' cell | 1.04 | 1.14 | 1.23 | 1.35 | 1.46 | 2.12 | - |

were also longer, and the DRF's somewhat higher, for the former characters than for the latter characters. In H3 and following series this trivial factor may be disregarded.

Conspicuous differences between $M_1$ characters appeared from H12 onwards. In particular, the data show a decreasing DRF in the course of $M_1$ development. The differences in DRF between characters increased considerably with the pregermination period.

In interpreting the DRF values for leaf number below the 1st inflorescence and flowering delay it must be kept in mind that these characters reflect the entire history of the plant's growth and vegetative development and consequently cannot be regarded as truly late $M_1$ characters. This explains why the DRF's for these traits are inter-

mediate between those for the earlier somatic traits and those for weight of seeds/fruit.

The DRF's for the % non-germinating seeds were higher than for the % sublethals, except in H½ and H72-144 where both values were about equal. Disregarding the values for H72-144 which were based on few data, the difference in DRF between these characters tended to increase with the germination period (except H3, see later). The DRF's for both the % non-germinating seeds and the % sublethals exceeded those for the recessive 'visible' mutations/100 $M_1$ truss progenies and the % mutant seedlings, except in H3 where the opposite occurred. From H6 onwards, the differences in DRF between the former two and the latter two categories increased considerably. This indicates that, with increasing pregermination time, the effectiveness of neutrons with regard to the production of non-germinating seeds and sublethals increased more rapidly than with regard to the production of recessive 'visible' mutant seedlings.

The DRF for the % sublethals in H½ was higher than in H3. This deviation from the general trend was accompanied by a relatively low DRF for the number of mutations/100 $M_1$ truss progenies (and for the % mutant seedlings) in H½, and a fairly high value in H3. These results are most readily explained by random variation, considering that severely abnormal individuals occurring singly in a progeny were always counted as sublethals even though some of them might have been due to recessive point mutations, especially at low doses where a high degree of chimerism was to be expected.

In H6, the DRF for the % mutant seedlings was higher than for mutations/100 $M_1$ truss progenies. This was due to relatively high mutant segregation percentages in this series for which there is no explanation. The DRF's for the total % of all aberrant $M_2$ categories were very similar to those for weight of seeds/fruit, as in exp.3.

The correction factors are also shown in table 26. The factors (a) increased consistently with the pregermination period but explained only a small fraction of the increases in DRF. The factors (b) explained a greater proportion. Nevertheless, it may be calculated that also on this basis only 13-30% of the increases in radiation effectiveness (=DRF-1.0) relating to weight of seeds/fruit or to the total of all aberrant $M_2$ categories could be attributed to the increases in energy absorption per cell (=correction factor-1.0). The DRF estimates pertaining to H3-H48, corrected by the factors (b), are shown

in table 27. Because the (b) value for H48 was probably an overestimation, the corrected values for this series are probably somewhat too low. No sensitization estimates are available for H72-144 (see section 3.7, p.22).

Table 27 : Dose reduction factors (DRF) after correction for differences in the amounts of energy absorbed per 'average' cell. Experiment 4.

| Character | H3 | H6 | H12 | H24 | H48 |
|---|---|---|---|---|---|
| $M_1$ | | | | | |
| Cumulative cotyledon growth, 12 or 19 days | 1.6 | 1.9 | 2.7 | - | - |
| Length of 1st or 2nd leaf, 19 days | 1.6 | 1.8 | 2.3 | - | - |
| Leaf number, 19 days | 1.7 | 1.9 | 2.4 | - | - |
| Abnormal leaves/plant | 1.5 | 1.9 | 2.4 | 3.3 | 4.6 |
| Leaves below 1st inflor. | 1.6 | 2.0 | 2.3 | 3.1 | 3.3 |
| Days to flowering | 1.6 | 1.9 | 2.3 | 2.6 | 3.3 |
| Weight of seeds/fruit | 1.3 | 1.8 | 1.8 | 2.0 | 2.2 |
| $M_2$ | | | | | |
| % non-germinating seeds | 1.5 | 1.9 | 2.0 | 2.5 | 2.9 |
| % sublethals | 1.0 | 1.6 | 1.7 | 2.0 | 1.8 |
| Mutations/100 $M_1$ truss progenies | 1.6 | 1.5 | 1.5 | 1.2 | 1.3 |
| % mutant seedlings | 1.6 | 1.8 | 1.5 | 1.3 | 1.3 |
| % all aberrant categories | 1.5 | 1.9 | 1.7 | 2.0 | 2.2 |

4.6.2. Discussion

The most important finding was an increase in radiation effectiveness with increasing prehydration/germination time. This must be attributed to an interplay of the following, and, possibly, other factors:

Increase in energy absorption

The rad dose correction factors (a) were found to explain only a very small fraction of the increases in radiation effectiveness with prehydration/germination. This was to be expected, considering that water uptake increases not only the amount of radiation energy absorbed per unit weight of tissue but also the weight of the embryo and its constituent cells.

Organ growth is determined chiefly by the reproductive capacity of cells in the apical zones and this capacity is influenced only

slightly by radiation absorbed in the differentiated parts of the organism[81]. Therefore, it is mainly the changes during hydration/germination which take place in the apical meristems that have to be considered.

Whaley[192], who has provided extensive data on meristem parameters in tomato throughout the vegetative part of the life cycle, did not find a consistent change in shoot meristem size during the first 6-7 days after sowing. However, this result may be biased, firstly by a possible interaction between the stage of imbibition and the degree of shrinkage due to the fixation technique used (CRAF), secondly by the fact that the seeds were grown without external nutrient supply for 6 days. These considerations would also explain his improbable observation of a decreased volume of the apical cells (excluding the dermatogen layer) 2 days after sowing, i.e. prior to mitosis, though the observed decreases after the onset of mitosis are undoubtedly valid.

Because of the risk of bias, the increases in energy absorption per meristem or per meristem cell (with increasing prehydration time prior to germination) were not estimated on the basis of measurements on fixed and sectioned apices. Instead, they were inferred from the increases in the amounts of energy absorbed per embryo (correction factors b). On this basis, 13-30% of the increase in neutron effectiveness with prehydration could be attributed to increased radiation energy absorption per meristem or per cell. These estimates are correct on the assumption of an equal and fairly synchronized rate of water uptake and a similar elementary composition in all parts of the embryo until the rupture of the seedcoat. This assumption is probably only partly valid, because the meristem cells are known to differ considerably in structure and function from those elsewhere in the embryo.

A further complication in dosimetry arises from the fact that the cell does not constitute a uniform target for the various effects of radiation[2,3,8 p.263-268]. The concept of energy absorption per cell disregards intracellular differences both in the rate of water uptake and in the changes in elementary composition and specific density associated with the swelling of macromolecules and structures in the nucleus and the various cytoplasmic constituents. Whaley[192] found that the nuclear volume generally increased during the first 2-4 days after sowing, and then gradually decreased; his data also indicate

that the proportion of the apical cell volume occupied by the nucleus increased over a long period and even during the first 2 days, suggesting a more rapid hydration of the nucleus than of the cytoplasm. This and other, more subtle, intracellular differences are undoubtedly responsible for a part of the observed enhancement of radiation effectiveness in the course of germination. It is in this realm that progress in dosimetry must be sought (section 5.1).

Increase in metabolic activity

Under normal physiological conditions, hydration leads to increased respiration, RNA and enzyme synthesis, and activation of many metabolic processes[120]. The role of metabolic activity in the increase of radiation sensitivity with pregermination has been stressed repeatedly[22,55,108,113,142]. A high metabolic activity implies a high mobility of structures, molecules, ions and radicals, both in the nucleus and in all other cell constituents. This increased mobility with pregermination is likely to reduce the time available for the restitution of potential chromosome breaks, thus increasing both the number of unrepaired chromosome aberrations per unit dose and, by increased proportions of errors in rejoining, their average severity. This hypothesis is supported by the observed shift with prehydration, towards greater proportions of the more drastic phenotypic aberrations in the $M_2$. It is probable that these spatial considerations, which apply strongly to low-LET radiations[74,112] also play a role in the effects of high-LET radiation, at least on hydrated systems[55,56].

For the same reasons, prehydration may also increase both the number and the average size of lesions produced in other cell structures, including membranes, and the amount of biochemical reactions in the cytoplasm, thus causing an increased physiological component of damage. This component is small with neutron irradiation of dry seeds (cf.p.2) and its relative importance in prehydrated seeds has not been evaluated.

Transition of cells through the mitotic cycle

The cell cycle is commonly divided into $G_1$, S, $G_2$ and M; the first 3 stages constitute the interphase, while M is composed of the various stages of actual mitosis. In the resting tomato embryo, all cells in the shoot apex and most of the cells in both the cotyledons and the root apex are in $G_1$[48]. If the true $G_1$ stage is considered to be characterized by a high metabolic activity in preparation for DNA synthesis

and cell division, the period of hydration of embryo cells might be referred to as a $G_0$ stage, in analogy to certain types of mammalian cells with a very long presynthetic stage[133]. Undoubtedly, the sensitivity to radiation increases during hydration, possibly followed by a plateau in $G_1$ proper. In vitro, the radiation sensitivity of $G_1$ is the lowest of all stages and fairly constantly so, except for an increase towards the end[60]. The end of $G_1$ and the subsequent stage S, during which DNA synthesis takes place, are usually characterized by a high sensitivity. Irradiation at S causes a decreased rate of DNA synthesis and hence a strong prolongation of S[177]. Within this stage, marked sensitivity differences occur, cells in early S being more sensitive than those in late S[115]. In HeLa cells (a commonly used strain of animal cells), S is the most sensitive of all stages of the division cycle[177]. However, in many materials, both zoological and botanical, cells in $G_2$ are more sensitive to aberration induction than those in S[115,150,158]. Also within $G_2$, the sensitivity to the induction of true chromosomal (chromatid) aberrations varies greatly, being highest at the early stages and decreasing towards late $G_2$[115,150,158]. The types of aberrations also differ between stages: prior to DNA synthesis chromosome aberrations are produced; after DNA synthesis chromatid aberrations prevail[59,194].

Another sensitivity maximum occurs in early to mid prophase. In Tradescantia microspores, this maximum is by far the highest of the division cycle[163]. At later stages of prophase, the sensitivity decreases markedly[112,163]. Subsequent stages of M, notably metaphase-anaphase, were shown to be extremely sensitive to the production of chromosome rearrangements[51 p.198,163] but Sax[163] noted that the analysis of aberrations at these stages is complicated by physiological effects (stickiness and clumping of chromosomes).

Sax[160,163], Giles[74], Lea[112], Wolff[193] and others have stressed that the differences in sensitivity to the production of chromatid aberrations may not result from differences in breakability of the chromosome strands but rather from differences in rejoining, associated notably with alterations in the spatial relationships between the chromosomes. This is also the view of Dubinin and Tarasov[52] who noted that the periods of maximum sensitivity coincide with considerable alterations in chromosome structure, first during S, then during early to mid prophase when the chromosomes become spiralised. According to Evans[60], all chromosome aberrations are

probably a consequence of misrepair. Of the many factors involved in repair one is certainly the time available for rejoining or restitution and another the amount of energy available. Lozzio[115] postulates that both these factors may be small in $G_2$ because this stage is the closest to mitosis.

Accordingly, there is likely to be firstly a considerable increase in sensitivity in the course of embryo hydration, secondly a peak in sensitivity during S and thirdly another peak at the boundary of $G_2$ and prophase.

The duration of the various stages has been studied intensively in mammalian cells in vitro and in vivo[133]. $G_1$ was found to vary between a few hours and several days even within a given cell population, depending on external and physiological conditions. For almost all mammalian cell types, S usually varies between 6 and 10 hours and $G_2$ between 2 and 5 hours. In embryonic cells, S may be shorter (3-4 hours) and $G_2$ longer (10-12 hours). In plant cells, the S period varies considerably, values of 4-5 hours being reported in pea root meristem cells[182,184] and of 10-11 hours in Tradescantia roots[185] in which, furthermore, $G_2$ was found to last 1.6-2.6 hours, M ca 2 hours and $G_1$ 3.3-5.8 hours. The duration of S increases considerably with irradiation (due to an inhibition of DNA synthesis and possibly also to blockage at the boundary between S and $G_2$[183]) but the length of $G_2$ is little affected[60].

Little is known about the kinetics of cell division in germinating tomato seeds. Van der Meij and De Nettancourt[180] found cyclic changes in mitotic frequencies in root tip cells suggesting a division cycle of 10-15 hours in the control and of 13-22 hours after a fairly high fast neutron dose. The apparently large heterogeneity in mitotic cycle time within the root meristem corresponds with data of other authors[e.g.82]. The moment of entry into first division is also highly variable. In the unirradiated seeds of the lot used for exp.4, the first mitoses in the root apex were observed ca 8 hours after visible germination and in the shoot apex 8 hours later. This roughly corresponds with De Nettancourt and Devreux[48] who found, in the same material, that 48, 72, 96, 120 and 144 hours after sowing 0, 8.3, 89.8, 74.5 and 66.2% of cells were in mitosis in the meristematic zone of the root apex and 0, 0.5, 10.2, 7.7 and 16.4% in that of the shoot apex. While these data do not provide any information on mitotic cycle time, they do suggest both a greater mitotic activity

and a higher degree of synchrony in the first division of cells in the root apex than in the shoot apex.

Combining the foregoing data with those on germination speed (median germination time ca 40 hours) it is reasonable to expect that 24 hours after sowing all cells were still in $G_1$; on the other hand, 48 hours after sowing (H48) the root apices contained a fair percentage of cells in S and $G_2$ and possibly some in M, while the shoot apices of most embryos must still have been in $G_1$ or S. At later stages (H72-144), all apices must have contained considerable proportions of cells at first mitosis or subsequent stages.

The large degree of asynchrony among cells of the root and shoot meristems and the suspected heterogeneity in mitotic cycle time within and between treatments add to the difficulty of assessing the quantitative relationship between cell stage and radiation sensitivity in germinating seeds. Nevertheless, the foregoing clearly indicates that the transition of the meristematic cell populations through the successive stages of the mitotic cycle and, in H72-144, the increased cell numbers in the apical zones, are responsible for a considerable if not the major part of the DRF increases in the course of seed hydration.

Resorption of endosperm by the embryo

From 48-96 hours after sowing, most of the endosperm dry matter was resorbed by the embryo, via the cotyledons (section 3.6.4, p.16). Part of these materials, e.g. nitrogen, breakdown products of starch, DNA and RNA[120], undoubtedly serve as substrates for an intensive synthetic activity in the cotyledons. The remainder, and the synthesized products, are distributed to other parts of the embryo. The cotyledons play an important role in early plant growth. For example, they provide almost all the phosphorus in the growing root tip[90]. During early seedling growth they also manufacture vitamins, including vitamin C[155] which plays an essential role in regulating the redox potential in the seedling[189]. Radiation causes the formation of toxic or mutagenic substances in the endosperm, by genetic alteration, membrane damage, enzyme inactivation, atomic transitions and other events. It is also likely to affect the conversion of stored nutrients. Those phenomena in the endosperm influence the metabolic activity of the cotyledons and, hence, they also influence early seedling growth. Furthermore, endosperm damage can also cause genetic effects in the embryo. This was demonstrated by Meletti et al.[122] and

Avanzi et al.[7] who grafted unirradiated embryos onto irradiated endosperm in durum wheat.

Consequently, with seed irradiation, a part of the damage sustained by the embryo is caused indirectly by toxic or mutagenic substances produced by a disturbed metabolism in the endosperm. This component is maximal prior to germination and it decreases in the course of endosperm resorption, i.e. over a 48 hour period following visible germination (section 3.6.4). On the other hand, the endosperm substances, once resorbed, contribute directly to radiation energy absorption in the embryo. Because damage induced in the endosperm undoubtedly has less effect on the embryo than directly induced damage, the presence of the endosperm might have contributed to the high DRF's for the vegetative $M_1$ characters in the H72-144 series.

The second important finding was a more pronounced sensitization by prehydration (DRF increase) in respect of the seedling characters than of the later $M_1$ characters, except during the first 6 hours of prehydration in exp.4. In other words, within each series from H12 onwards, the DRF decreased in the course of $M_1$ development and this decrease became more prominent with increasing prehydration/germination time. To explain this, it must be remembered that the effectiveness of radiation on the growth of organs (after seed irradiation) is determined not only by the radiation sensitivity (including the capacity of repair) of individual cells in the different parts of the apical meristems, but also by (i) the cell numbers and their degrees of differentiation or predisposition at the time of irradiation, (ii) the possibilities of substitution of lethally affected cells, (iii) the numbers of cell divisions normally involved in the growth of the different organs and (iv) the physiological interrelationships between cells and organs. Because of all these intervening secondary factors, differences in the effectiveness of radiation as measured by macroscopic criteria cannot be related to differences in sensitivity at the cellular level (see also p.76-77).

Nevertheless, because there is a general increase in sensitivity as cells pass through the cell cycle, it may be expected that the early dividing cells in the peripheral zones of the shoot meristem (which generate the first leaves) are sensitized more rapidly than the more centrally located cells (which give rise to the later somatic tissues and the generative tissues) which start dividing both later and at a lower rate. This factor may offer a partial explanation of

the sharper increases in the DRF's for the 'early' compared to the 'late' characters, especially from H24 or H48 onwards.

For the remainder, only secondary factors can be considered. The damaging effectiveness of a given amount of energy per cell is different in different parts of the embryo. In this connection, the choice of characters studied is important. For instance, hypocotyledon growth depends chiefly upon the radioresistant process of cell elongation and is only slightly affected at doses which cause a severe inhibition of cotyledon growth. Cotyledon growth relies to a considerable extent on cell division without any possibility of replacement of lethally affected cells from outside. A reduced cotyledon growth in turn affects, indirectly, the early growth of subsequent organs. The growth of the first leaves, starting from predisposed groups of cells in the flanks of the shoot meristem, may benefit from a certain amount of stem-cell substitution after lethal damage. This opportunity is even greater for subsequent leaves which are undifferentiated at the time of irradiation. These factors, which influence the effectiveness of radiation on the growth of the various organs after irradiation of dry seeds, are also involved in the greater increase with prehydration in the effectiveness of radiation on early than on late characters. Finally, the fact that the physiological impact of damage inflicted upon the early organs (especially the cotyledons) diminishes in the course of development, also contributes to this trend.

A third observation (see especially exp.4) was that prehydration increased the effectiveness of fast neutrons more strongly with regard to $M_1$ fertility reduction and the production of non-germinating $M_2$ seeds than with regard to the induction of recessive 'visible' mutations. This 'spectral' change is explained as follows. After seed irradiation, many types of chromosomal aberrations are able to persist until meiosis[151]. Most of these are then eliminated by haplophasic selection, failure of fertilization, and early embryo abortion due chiefly to dominant lethal factors in the male and/or female gametes [33]. These events lead to a reduced number of normal sized seeds/fruit. Failure of these seeds to germinate may be attributed to dominant or recessive late embryonic lethal factors, though early embryo abortion with normal endosperm development may also be involved. There is no sharp boundary between non-germinability and early seedling (sub)lethality and the phenotype of certain aberrations may vary between both categories depending upon the growth conditions. On the

other hand, several seedlings scored as sublethals were undoubtedly due to recessive 'visible' mutations segregating with a large mutant deficit as a result of chimerism. The causes of each of these aberrant phenotypic categories are undoubtedly highly diverse at the chromosomal level. Even the recessive 'visible' mutations do not constitute a uniform category at the chromosomal level. Most, if not all of them must be ascribed to structural alterations, probably chiefly microdeficiencies, rather than true gene mutations[21,111,143,171 ch.VII, 174]. Notwithstanding a considerable mutual overlapping between the causes of different phenotypic categories, there is good reason to believe that the radiation-induced aberrations responsible for both fertility reduction and non-germinability are on an average more drastic than those leading to post-emergence mutant phenotypes[15,33,69, 135,see also section 5.4,p.114]. Consequently, the 'spectral' change referred to above indicates that, with prehydration, there is a shift towards a greater proportion of translocations and large deficiencies. Though this shift might, in principle, be due to increasingly drastic primary molecular lesions with prehydration of the nucleus, it is more likely to result from increasing proportions of errors in the rejoining of unstable primary lesions. This might be due to an increasing metabolic activity and chromosomal mobility which would lead to shorter rejoining times and a progressively more rapid repair and fixation of radiation damage. It may be recalled that the trends in the DRF's for the $M_1$ characters led to the same interpretation (p.88). It can now be postulated that the recessive 'point' mutations derive, at least partly, from imperfect restitution of chromosome breaks.

Maternal effects were unlikely to have played a significant role in the 'spectral' shift in $M_2$ phenotypes because (i) the vegetative development of the plants, especially at later stages, was not greatly disturbed at the relatively low doses used in exp.4, (ii) the plants, grown at commercial spacing, were pruned above the 2nd truss to ensure a very adequate nutrient supply to the developing fruits, (iii) the observed increases in leaf number below the 1st inflorescence with increasing dose buffer the plants against detrimental effects of somatic damage on $M_1$ fertility, and (iv) neutrons cause only a relatively slight reduction in vegetative vigour for a given pronounced reduction in $M_1$ fertility (cf.section 4.4.2, p.77). Direct evidence against the importance of maternal influences will be presented in section 4.7 (p.105).

Finally, a comparison of the DRF's pertaining to the prehydrated seed series of the successive experiments (H24 in exp.4) is worthwhile. This reveals important differences. For instance, the corrected average DRF for the seedling traits was 3.0 in exp.1, 2.0 in exps.2 and 3, and 3.3 in exp.4. The corrected DRF pertaining to weight of seeds/fruit was 1.4 in exp.3 and 2.0 in exp.4.

These differences cannot be associated with the seed-lots used, because a good agreement would then be expected between exps.1 and 3, and between 2 and 4. Water content, elementary composition and fresh weight/embryo can be invoked only in so far as the correction factors (b) might not have been entirely applicable. Such scepticism is unjustified in exps.2-4. In exp.1, a slight underestimation of the correction factor is probable (see p.81) and the DRF's might have been somewhat overestimated.

The DRF differences between exp.1 on the one hand and either exp. 2 or 3 on the other hand, however, are probably related to the state of the cells at the time of irradiation. In exp.1, the prehydrated seeds were about to germinate at the time of irradiation; in exps.2 and 3, the seeds were irradiated at a physiologically much earlier stage, though at approximately the same water content. Furthermore, in exp.1, many cells must have been in the S period, whereas in both exps.2 and 3, most, if not all, cells were still in the more resistant $G_1$ stage.

The relatively high DRF values in the H24 series of exp.4 cannot be explained by these factors. They might be associated with the 2.8 times higher dose rate in this experiment. This variable, which does not influence the effectiveness of densely ionizing radiation on dry seeds[24], may have an appreciable effect on the radiation response of hydrated and actively metabolizing material. This has been demonstrated with non-dividing plant tissue[19] and with mice[16], and may also be inferred from dose fractionation experiments on cultured human cells which have shown that recovery ('repair' in the present terminology, 'sparing' in the terminology of Alper et al.[4]) is possible after irradiation with 15 MeV neutrons[17]. In view of the paucity of evidence on this subject and the scanty knowledge of the physical and biological parameters involved, it would be of interest to show whether indeed the response of germinating seeds to fast neutrons, preferably of different energies, can be modified by dose rate.

Other variables which may have influenced the degree of sensitization by prehydration are: (a) inadequate control of environmental conditions during the transport to and from the reactor; (b) differences in the age of the seed-lots and (c) differences in culturing conditions after transplanting from the petridishes.

Notwithstanding these differences between experiments, the present DRF estimates did not vary by more than a factor 1.5-1.8. This indicates that it should ultimately be possible to obtain a fair reproducibility of the degree of sensitization to fast neutrons as a function of prehydration/germination time. This, however, may only be expected when all conditions of seed production, storage and experimentation are both favourable and rigorously standardized.

## 4.7. Within-treatment correlations between characters

### 4.7.1. Results

The within-treatment coefficients of correlation (r) between the various characters are of interest because they are an expression of the kinetics of the plant's growth and development. They were calculated from the single plant data for all treatments in exps.1 and 2, and for some characters and treatments in exp.4. Because the plants belonging to any one treatment were planted in rows (1 row per replication) the observations were not necessarily independent and it is not certain that the r-values are unbiased estimates of the relevant correlation coefficients. However, a visual study of graphs, made by plotting the values for each pair of characters against each other for a number of treatments, gave no evidence of a replication effect on the pairwise relation between the character-values; nor did the points appear to be scattered systematically with the position within rows. It is therefore reasonable to regard the r-values as a measure of the degree of correlation between the various pairs of characters. On the other hand, tests for significance were not considered justified.

In order to simplify the presentation of the data, the r-values pertaining to the series $D_1$, $D_2$ and $H_2$, which comprised many treatments (doses), were averaged over 3 groups of doses by means of the r,z transformation[64]. The (sub)lethal treatments in exps.1 and 2 are omitted from the tables as they led to disturbed distributions and meaningless r-value estimates.

Experiment 1

The r-values of the $D_1$ series are shown in table 28; most of these were low.

Table 28 : Within-treatment correlation coefficients relating to the $D_1$ series. Per character-pair: mean over 0-1 krad (a), 2-4 krad (b), 6-7 krad (c); n=98-80 per treatment. Experiment 1.

| Character | | | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|---|
| 25 days | | | | | | | | | |
| Root length | 1 | (a) | .27 | .31 | .17 | .33 | -.08 | .36 | .26 |
| | | (b) | .21 | .44 | .48 | .40 | .01 | .29 | .34 |
| | | (c) | .26 | .60 | .58 | .42 | .11 | .00 | .10 |
| Ultimate cotyledon length | 2 | (a) | | .56 | .27 | .28 | .35 | .29 | .58 |
| | | (b) | | .57 | .42 | .18 | .36 | .22 | .41 |
| | | (c) | | .44 | .55 | .31 | .16 | .09 | .20 |
| Length 1st leaf | 3 | (a) | | | .37 | .47 | .31 | .39 | .53 |
| | | (b) | | | .64 | .37 | .42 | .30 | .56 |
| | | (c) | | | .64 | .49 | .14 | -.03 | .16 |
| Stem length | 4 | (a) | | | | .34 | -.05 | .35 | .26 |
| | | (b) | | | | .50 | .03 | .57 | .63 |
| | | (c) | | | | .57 | .13 | .18 | .29 |
| Leaf number | 5 | (a) | | | | | -.15 | .53 | .32 |
| | | (b) | | | | | -.23 | .37 | .41 |
| | | (c) | | | | | .09 | .12 | .29 |
| 42 days | | | | | | | | | |
| Ultimate length 3rd leaf | 6 | (a) | | | | | | -.09 | .44 |
| | | (b) | | | | | | -.15 | .22 |
| | | (c) | | | | | | .18 | .32 |
| Length 1st axillary shoot | 7 | (a) | | | | | | | .67 |
| | | (b) | | | | | | | .64 |
| | | (c) | | | | | | | .27 |
| Plant weight | 8 | | | | | | | | |

Among the seedling characters (1-5), most r-values increased with dose; however $r_{1,2}$, $r_{2,5}$ and $r_{3,5}$ changed little, while $r_{2,3}$ decreased at the higher doses. Within this group of characters, the coefficients involving cotyledon length (2) were consistently lower than those involving length of the 1st leaf (3) which were the highest.

Between characters 1-5 on the one hand and later characters (6-8) on the other hand, most coefficients either decreased with dose or increased up to medium doses and then decreased. However, $r_{1,6}$, $r_{4,6}$ and $r_{5,6}$ were negative in the control but tended towards positive with increasing dose. The coefficients of correlation between 2 or 3

on the one hand and 6-8 on the other hand were fair up to medium doses. The same was true for $r_{5,7}$ and $r_{5,8}$. However, at the higher doses, these between-group coefficients of correlation were much lower than among the characters 1-5.

The r-values involving weight of the 3rd leaf (not shown) were very similar to those involving its length (6), as expected. Nevertheless, the coefficients of correlation between these two characters decreased slightly with dose, from 0.93 to 0.78. The coefficients $r_{6,7}$ were negative at the lower doses but positive at the higher doses (cf. $r_{5,6}$); $r_{6,8}$ tended to decrease with increasing dose whereas $r_{7,8}$ decreased consistently.

In the $H_1$ series (table 29), the coefficients of correlation among the seedling characters (1-5) increased markedly with dose, except for an inconsistency in $r_{2,3}$; the coefficients involving 2 were lower than those involving 3, which were the highest.

Table 29 : Within-treatment correlation coefficients relating to the $H_1$ series. Per character-pair: control (a), 1 krad (b), 2 krad (c); n=97-41. Experiment 1.

| Character | | | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|---|
| 25 days | | | | | | | | | |
| Root length | 1 | (a) | .43 | .44 | .21 | .35 | .23 | -.02 | .39 |
| | | (b) | .45 | .62 | .58 | .54 | .01 | .21 | .19 |
| | | (c) | .79 | .82 | .67 | .69 | .14 | .02 | .10 |
| Ultimate cotyledon length | 2 | (a) | | .61 | .19 | .36 | .28 | -.16 | .30 |
| | | (b) | | .56 | .37 | .39 | .30 | -.10 | .18 |
| | | (c) | | .77 | .63 | .67 | .19 | .08 | .14 |
| Length 1st leaf | 3 | (a) | | | .32 | .43 | .40 | .03 | .54 |
| | | (b) | | | .60 | .69 | .30 | .02 | .14 |
| | | (c) | | | .71 | .89 | .30 | .13 | .08 |
| Stem length | 4 | (a) | | | | .38 | .12 | .09 | .23 |
| | | (b) | | | | .53 | -.12 | .20 | .05 |
| | | (c) | | | | .64 | -.05 | .14 | .04 |
| Leaf number | 5 | (a) | | | | | .27 | .08 | .34 |
| | | (b) | | | | | .03 | .13 | .10 |
| | | (c) | | | | | .19 | -.00 | .00 |
| 42 days | | | | | | | | | |
| Ultimate length 3rd leaf | 6 | (a) | | | | | | .61 | .79 |
| | | (b) | | | | | | .02 | .33 |
| | | (c) | | | | | | .30 | .37 |
| Length 1st axillay shoot | 7 | (a) | | | | | | | .60 |
| | | (b) | | | | | | | .44 |
| | | (c) | | | | | | | .39 |
| Plant weight | 8 | | | | | | | | |

The coefficients of correlation between the seedling characters (1-5) on the one hand and length of the 3rd leaf (6) on the other hand tended to decrease with increasing dose, although there were several exceptions. The degree of correlation between 1-5 and length of the axillary shoot (7) was particularly low and not clearly related to the dose. In contrast, the coefficients of correlation between 1-5 on the one hand and plant weight (8) on the other were fair in the control and showed a consistent decrease with increasing dose. The coefficients between 2 and the later characters (6-8) were not consistently lower than those between 3 and 6-8.

The degree of correlation between length and weight of the 3rd leaf decreased slightly with dose from 0.90 to 0.82 (not in the table). The coefficients of correlation between the characters 6-8 were strongly reduced by irradiation; $r_{6,7}$ was always positive, in contrast to the situation in $D_1$.

Experiment 2

The $\bar{r}$-values are shown in table 30.

Table 30 : Within-treatment correlation coefficients relating to the $D_2$ and $H_2$ series.
Per character-pair: $D_2$, mean over 0-0.5 krad (a), 1-3 krad (b), 6-9 krad (c)
$H_2$, mean over 0-0.5 krad (a), 1-2 krad (b), 3-4 krad (c)
(n=ca 150 per treatment). Experiment 2.

| Character | | | $D_2$ | | | | $H_2$ | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 2 | 3 | 4 | 5 | 2 | 3 | 4 | 5 |
| Root length | 1 | (a) | .32 | .28 | .18 | .11 | .32 | .29 | .22 | .20 |
| | | (b) | .32 | .30 | .21 | .20 | .43 | .42 | .36 | .33 |
| | | (c) | .39 | .42 | .39 | .18 | .41 | .44 | .16 | .30 |
| Ultimate cotyledon length | 2 | (a) | | .51 | .39 | .21 | | .69 | .48 | .45 |
| | | (b) | | .56 | .45 | .23 | | .67 | .51 | .49 |
| | | (c) | | .60 | .46 | .30 | | .59 | .33 | .47 |
| Length 1st leaf | 3 | (a) | | | .55 | .44 | | | .66 | .62 |
| | | (b) | | | .58 | .50 | | | .67 | .64 |
| | | (c) | | | .56 | .54 | | | .31 | .72 |
| Stem length | 4 | (a) | | | | .35 | | | | .54 |
| | | (b) | | | | .38 | | | | .51 |
| | | (c) | | | | .25 | | | | .32 |
| Leaf number | 5 | | | | | | | | | |

In $D_2$, $\bar{r}$ increased slightly with dose up to the highest dose (9 krad), except that at the higher doses $\bar{r}_{1,5}$ and $\bar{r}_{3,4}$ decreased slightly, and $\bar{r}_{4,5}$ substantially.

In $H_2$, $\bar{r}$ increased up to 2 krad for all character-pairs except for $\bar{r}_{2,3}$ and $\bar{r}_{4,5}$; these increases were only appreciable for those characters involving root length (1). Most $\bar{r}$-values decreased at 3-4 krad, especially those involving stem length (4); however, $\bar{r}_{1,3}$ and $\bar{r}_{3,5}$ continued to increase slightly.

The correlation coefficients involving root length (1) were generally the lowest and those involving length of the 1st leaf (3) the highest in both series; even the highest $\bar{r}$-values were relatively low. Cotyledon length and length of the 1st leaf showed about equal correlation with root length ($\bar{r}_{1,2}$ vs. $\bar{r}_{1,3}$), but they differed in their correlation with stem length (4) and leaf number (5), and, of the two, length of the 1st leaf yielded the highest values.

Experiment 4

The results are shown in table 31.

Table 31 : Within-treatment correlation coefficients relating to the H3 series. Per character-pair: control (a), 1.87 krad (b), 3.50 krad (c); n=96-77. Experiment 4.

| Character | | | 10 | 11 | 12 | 13 |
|---|---|---|---|---|---|---|
| Leaves below 1st inflorescence | 9 | (a) | .85 | .27 | -.10 | - |
| | | (b) | .82 | .25 | -.11 | .01 |
| | | (c) | .09 | .06 | -.04 | .03 |
| Days to flowering | 10 | (a) | | .32 | -.01 | - |
| | | (b) | | .10 | -.17 | .06 |
| | | (c) | | -.24 | .12 | .03 |
| Weight of seeds/fruit | 11 | (a) | | | -.27 | - |
| | | (b) | | | -.35 | -.11 |
| | | (c) | | | -.46 | .03 |
| % non-germinating $M_2$ seeds | 12 | (a) | | | | - |
| | | (b) | | | | .07 |
| | | (c) | | | | .15 |
| Number of mutations ($M_2$) | 13 | | | | | |

The number of leaves below the 1st inflorescence (9) was highly correlated with the number of days to flowering (10) both in the control and at the medium dose. However, this correlation was completely absent at the high dose. Both these characters showed a slight posi-

tive correlation with weight of seeds/fruit (11) in the control and in the case of $r_{9,11}$ also at the lowest dose. However, $r_{9,11}$ was virtually nil at the highest dose and $r_{10,11}$ had even become negative. Most coefficients of correlation between 9 or 10 and the % non-germinating $M_2$ seeds (12) were even lower. The values of $r_{11,12}$ were higher and showed a slight increase with dose. There was virtually no correlation between any of the preceding characters and the number of recessive mutations visible in the $M_2$ (13).

4.7.2. Discussion

The observation that the correlations among the seedling characters generally became stronger with dose in both series of exps.1 and 2 (in $H_2$ only up to 2 krad) suggests a negative relationship between these correlation values and the level of growth and development at the time of recording. This is supported by the fact that in exp.1 the levels of correlation among the seedling characters were generally lower in the more advanced $D_1$ series than in the less advanced $H_1$ series. Support also comes from the fact that the levels of correlation between the seedling characters and both length of the 1st axillary shoot and plant weight after 42 days were higher in $D_1$ than in $H_1$, considering that the $H_1$ series had considerably outgrown the $D_1$ series after 42 days (table 8, p.32).

This postulated decrease in correlation in the course of development is plausible because the complexity of physiological interrelationships increases with an increasing number of organs in the plant. In this respect, radiation has acted merely as an external factor reducing the rate of development.

The stage of development of the root system may also be considered in this context. The low coefficients of correlation involving length of the primary root in exp.2, compared to exp.1, were associated with a greater abundance of lateral and adventitious roots in exp.2 than in exp.1. Thus, it must be concluded that the length of the primary root is progressively less related to the performance of the plant as the number and length of secondary roots increases. This is according to expectation.

For certain pairs of seedling characters the situation was the reverse and the r-values decreased with increasing dose. The negative relationship between the r-values and the stage of development was evidently overshadowed by influences acting in the opposite direc-

tion. The primary root, the hypocotyledon, the cotyledons and the meristematic regions from which the first two leaves originate have no cells in common. Therefore, radiation damage must be induced independently in all these organs. It is logical to conclude that this factor disturbs the normal physiological coordination of their growth and thus reduces the r-values.

The low levels of correlation between seedling characters (25 days) and later characters (42 days) at the higher doses in exp.1 are at least partly due to similar causes. Growth and development over the period from 25-42 days are based on cell lines derived from undifferentiated initials in the embryonic shoot apex. Because there are considerable opportunities for selection among these initials, the later organs tend to be more normal. The correspondence between early and later plant performance must therefore be expected to decrease with dose. These relationships were doubtless further complicated both by the transfer (after 25 days) from vermiculite to a liquid medium and by the complexity of events governing the moment of initiation of axillary shoot growth. In this connection it is according to expectation that the coefficients of correlation between seedling characters and either ultimate length of the 3rd leaf or length of the 1st axillary shoot were lower than between the seedling characters and plant weight, because this last character includes the weight of the early organs and is insensitive to the moment of growth initiation of individual organs.

The sharply decreasing levels of correlation between various characters at 3-4 krad in $H_2$ (exp.2) were probably associated with the small amount of growth in these treatments due to both the high doses and the sub-optimal growth conditions. The strong decreases in the r-values involving 'stem' length are explained in this way, because, with little growth of the stem proper, this character is determined chiefly by the length of the hypocotyledon. The hypocotyledon lengthens mainly by the radioresistant process of cell elongation, and its length cannot be expected to be strongly correlated with root- or cotyledon length, which both depend largely on cell division. This difference also explains the decreasing correlation between stem length and leaf number at high doses in both series of exp.2.

The fact that, among the seedling characters, the r-values involving cotyledon length were lower than those involving length of the 1st leaf (except for the correlations with root length in exp.2)

is explained by the fact that the cotyledons had attained their ultimate length at the time of observation. Consequently, their influence on both the later growth of roots and leaves, and on the initiation of new ones, was either constant, or diminishing due to senescence. The observation that cotyledon length and length of the 1st leaf did not differ consistently in their coefficients of correlation with the characters recorded after 42 days (exp.1) may be explained by assuming that, at this later stage, both the cotyledons and the 1st leaf had lost importance in determining further plant growth.

The coefficients of within-treatment correlation between certain seedling characters and later characters in the $D_1$ series (but not the $H_1$ series) of exp.1 showed a maximum at medium doses. This fact is thought to be related to the observed peak in axillary shoot length at a medium dose in $D_1$ (cf.fig.12, p.36). Such a relationship is not impossible because axillary shoot length after 42 days was governed predominantly by the moment of growth initiation of this shoot. Consequently, this character constitutes a sensitive measure of differences in the rate of development of plants within a treatment. This 'sensitivity', and hence the level of correlation with other characters, increases with an increasing proportion of plants having an axillary shoot length greater than zero, and was therefore the greatest at medium doses in $D_1$.

However, the observation that the levels of correlation between ultimate length of the 3rd leaf and both length of the 1st axillary shoot and plant weight were generally lower in $D_1$ than in $H_1$ does not correspond with this trend. This apparent discrepancy was undoubtedly connected to the very low and in some cases even negative coefficients of correlation between ultimate length of the 3rd leaf and several other characters in $D_1$. A negative relationship was unexpected and no ready explanation offers itself, except that it may have been caused by the transfer from vermiculite to a liquid medium after 25 days. In particular, the growth of the 3rd leaf may have been inhibited relatively severely in those plants that were the most advanced at the time of transfer.

The high levels of correlation between length and weight of the 3rd leaf are obvious. The reduction in this correlation with increasing dose suggests a partial disturbance by radiation of the relationship between the 3 dimensions of the leaf. This interpretation is supported by unpublished data (30) showing that radiation causes a much

greater percentage reduction in leaf length and width than in leaf thickness. Considering that such a disturbance operates within one organ it is only to be expected that it will be more important for the relationship between different organs, as suggested earlier.

To summarize the foregoing discussion of exps.1 and 2, the levels of simple within-treatment correlation between quantitative somatic characters tend to decrease

(1) with advancing stage of development at the time of recording
(2) with increasing genetic and non-genetic radiation damage, especially when the characters studied concern organs derived from different initials
(3) with increasing interval between the dates of recording
(4) with increasing time lapse between the moment when one of the characters has reached its ultimate value at the date of recording, assuming that the value of the other character is still increasing
(5) at early stages of growth, especially in the case of organs of which the initial growth is determined by cell elongation independently of cell division (hypocotyledon, cotyledons), or of which the moment of growth initiation is very variable
(6) under sub-optimal conditions of culturing
(7) by a change in culturing conditions, especially if this affects one character more than the other.

Radiation may increase the degree of within-treatment correlation if the effects of radiation on one character (e.g. rate of leaf differentiation) exert a direct influence on the second character (e.g. the moment of growth initiation of axillary shoots). Radiation may therefore be used as an external factor to identify causal physiological relationships between developmental characters.

The low coefficients of correlation in exps.1 and 2, and similar findings in Arabidopsis[32], warranted the expectation that the levels of correlation between seedling characters and characters of the generative stage would be particularly low. This is borne out by the low levels of correlation between late somatic $M_1$ characters and both weight of seeds/fruit and $M_2$ characters in exp.4. In view of this, the earlier suggestion of Verkerk and Contant[187] that fertility reduction after neutron irradiation is chiefly of physiological origin cannot be maintained.

Despite the very low correlation levels in exp.4, several trends merit discussion. Because the plants were grown during late summer, in a temperature-regulated glasshouse, fluctuations in light intensity caused a fairly large variation in leaf number before flower initiation, and concomitantly in the number of days to flowering. In so far as this component of variation was not associated with inherent differences in plant vigour, or was able to overcompensate such differences, it was expected to cause a positive correlation with weight of seeds/fruit and possibly with $M_2$ seed germination, because a higher leaf number provides in the absence of differences in leaf size a larger photosynthetic apparatus for fruit and seed development. Such a positive relationship was observed in the control while it also predominated after the lower dose. Although it doubtless also played a role after the high dose, the much lower r-values, some of which even had a reversed sign, suggest that, at this dose, the variations in leaf number and flowering time were determined mainly by reduced plant vigour resulting from genetic disturbances. In other words, the coefficients of correlation between the somatic $M_1$ characters and either weight of seeds/fruit or the % non-germinating $M_2$ seeds in the irradiated treatments, were probably the resultant of two relationships acting in opposite directions. There was a weak positive one due to environmental factors and a weak negative one resulting from genetic disturbances to the somatic tissues. It is probable that not only the positive but also the negative relationship is due to maternal-physiological factors, because vegetative tissues and reproductive cells are generally derived from different initials and genetic aberrations in these two kinds of tissues would therefore be unrelated. This is supported by the observation that the levels of correlation between the somatic $M_1$ characters and the % non-germinating $M_2$ seeds were even lower than between the somatic $M_1$ characters and weight of seeds/fruit. Because weight of seeds/fruit and, to a minor degree, also the % non-germinating $M_2$ seeds were influenced by physiological factors, it is impossible to decide the extent to which the slight increase with dose in the negative correlation between these characters was caused by maternal effects on both characters or by a slight genetic relationship.

A correlation between the somatic $M_1$ characters and the % recessive mutant seedlings was virtually lacking. This fact demonstrates unequivocally that genetic disturbances causing somatic $M_1$ effects have

little, if any connection with the presence of recessive mutations transmitted by the seed. The genetic events leading to $M_1$ seed set reduction were only slightly correlated with those leading to non-germinating $M_2$ seeds and virtually unrelated to the presence of recessive mutations detectable in the $M_2$. This last point confirms an earlier deduction (section 4.3.2, p.69) and has also been demonstrated in preformed tillers of X-irradiated barley[68,70], in X-irradiated rice[11], in X- and EMS-treated peas[191] and Arabidopsis[124,135]. This mutual independence of fertility level and the frequency of recessive 'visible' mutations has also been shown for EMS-treated tomato seeds except among the earlier germinating seeds[86] and is confirmed recently for this species after γ- and fast neutron irradiation of several ontogenetic stages, viz. PMC, pollen, both gametes after pollination, and dry seeds[33]. The yield of mutations for quantitative traits was also found to be independent of the degree of $M_1$ fertility[69,195]. Such independence may, of course, only be expected if all inflorescences studied within a treatment are derived from initials which do not differ in sensitivity in respect of the induction of both sterility factors and recessive point mutations[86]. This condition must have been approximately fulfilled in the present experiments, because the $M_1$ seeds were selected for evenness of size and homogeneous appearance and only the main shoot was studied (1st and 2nd truss separately).

The practical implications of these findings will be discussed in section 5.2 (p.110).

# 5. GENERAL DISCUSSION AND PRACTICAL CONCLUSIONS

## 5.1. Parameters in dosimetry

The data of section 3.7 have clearly shown the importance of correcting fast neutron doses on the basis of the C, H and O contents of the irradiated specimens. It is current practice to use the atomic composition of the whole embryo for making these corrections[92,93]. Unavoidably, this procedure is very crude, because the impact of radiation on the growth of the organism is determined predominantly by the energy absorbed in the apical zones and these constitute but a fraction of the total mass of the embryo. However, no suitable techniques are at present available which allow the determination, with good accuracy, of the water content and the atomic composition of specifically the cells in the shoot and root apices. Furthermore, it has become clear that the real problems in dosimetry are at the intracellular level and require complex biophysical approaches. For a long time to come, these problems can only be studied using the most simple biological systems, notably phages[197]. Consequently, it is not justified at this stage to spend a great effort at obtaining more precise estimates of atomic composition at the tissue level than those available at present.

Rather is it worthwhile to devote attention to the cellular and histological constitution of the embryo and its apices. The results of section 4.5 have shown that dry seeds of two cultivars which received equal rad doses differed in radiation sensitivity in a manner which suggested that the size of the shoot apex is the main factor involved, the greater apex conferring a greater radiation resistance. Neither the amounts of energy absorbed per cell nor the factors 'nuclear volume' or 'chromosome volume', which provide the highest degree of simple correlation with sensitivity when species of different taxa are compared[116,147,172,173], appear to explain the differences.

The data of section 4.6 have demonstrated that in seeds prehydrated/germinated for various lengths of time the relative absorbed doses accounted for only a small fraction of the observed differences in neutron effectiveness. On the other hand, in contrast to the above comparison of dry seeds of different cultivars, the parameter 'amount of energy absorbed per cell (or per meristem)', estimated from the weights per embryo, explained 13-30% of the differences in neutron effectiveness associated with prehydration. Because, according to

Whaley[192], nuclear size increased more rapidly during germination than did cell size, it is possible that a larger proportion of the increase in effectiveness could have been associated with the factor 'energy absorbed per nucleus', as proposed by Sparrow et al.[172]. Undoubtedly, the situation is complicated by the involvement of various other factors, and these are all confounded and difficult to evaluate. Such factors are: differences in the rate of swelling of different cell components, increases in the mobility of intracellular structures, molecules, ions and radicals and concomitant increases in metabolic activity, transition through the successive stages of the cell cycle (including DNA synthesis), and resorption of endosperm substances. Further complications are caused by the multicellular nature of the embryo and the interactions between its cells.

Progress in the analysis of the sensitization by prehydration requires, therefore, the determination of changes at the biochemical, ultrastructural, cellular and tissue levels. This is clearly a formidable task. In principle, with increasingly profound knowledge at each of these levels, all differences in sensitivity may ultimately be reduced to matters of biophysics and dosimetry. At present, it is unlikely that multicellular organisms can make a substantial contribution to the major advances in this field.

## 5.2. Choice of treatments and within-treatment selection in $M_1$

After neutron irradiation, most seedlings with grossly disturbed apical meristems are unable to produce leaves within the first 2 weeks after sowing and can be eliminated at an early stage. This is of practical importance because apical meristems may regenerate from more resistant cells and such cells are likely to contain lower frequencies of mutations.

More refined criteria are available to evaluate the mean effectiveness of radiation in the lower dose ranges. Among the seedling characters, average root- and cotyledon length (to be converted to cumulative growth), until fairly high doses, generally yield the most directly proportional response to radiation. Root length, recorded a few days after germination (see p.27), allows a particularly early evaluation of radiation effectiveness. Confounding with germination delay must be avoided by recording the root lengths in each treatment at a fixed number of hours after the moment of 50% germination. The recording at a fixed number of hours after the germination of each

seed is a still more accurate procedure, which is too laborious in routine work, but is of interest for studying the within-treatment relationship between mutagen-induced germination delay and growth reduction.

The use of cumulative cotyledon growth (not cotyledon length, see p.62) as a criterion of radiation damage permits direct sowing in soil and easier measuring. Recording as early as 12 days after sowing was shown to yield representative results without correction for germination delay. It must be stressed, however, that germination delay must not be disregarded after treatment with low-LET radiations and with certain chemical mutagens. This difficulty is best avoided by measuring cotyledon length at a time when growth of this organ has virtually ceased in all treatments.

The dose/response relationships for cumulative root- and cotyledon growth also show the highest degree of correspondence with those for weight of seeds/fruit and mutant frequency. In principle, this allows the prediction of the latter characters from the former, provided that the quantitative relationships between these characters are constant. However, the genetic events leading to the inhibition of somatic cell division and hence to reduced organ growth, are not the same as those involved in fertility reduction, nor are they connected with recessive mutations (see p.105). Therefore, the quantitative relationships between these characters may differ when different objects, pretreatments or mutagens are involved. This was demonstrated by the difference in the relative proportions of the various radiation effects between two cultivars (section 4.5) and by the pronounced shift in these proportions with increasing duration of seed prehydration (section 4.6). Consequently, the evaluation of radiation effects at an early $M_1$ stage find its main applications in enabling the repetition of neutron irradiation treatments with the same material under comparable conditions, for instance with a view to mutation induction, and, possibly, in the biological monitoring of neutron irradiation facilities. This, of course, presupposes a good reproducibility of the radiation responses under a given set of conditions (see section 5.3).

The use of species other than barley for the biological monitoring of neutron irradiation facilities is recommended by the IAEA[92,93]. The tomato would in principle be suitable for this purpose because it is a dicotyledonous species and is therefore in many respects complementary to barley, and because its radiation sensitivity is not too

high. The relatively small size of tomato seeds may constitute an additional advantage in certain applications, although, in the Standard Neutron Irradiation Facility designed and distributed under the auspices of the IAEA, the geometry of the larger barley seeds is satisfactory[20].

Superimposed on the question of choosing the desirable treatments is that of the selection of plants within treatments if the aim is mutation induction. The practical conclusion which emerges from the discussion of the within-treatment correlations (section 4.7) is that selection within a treatment on the basis of any $M_1$ character has no effect on the frequency of recessive mutations. This precludes the possibility of increasing the frequency of this type of mutations by selection in $M_1$. On the other hand, all badly growing or less fertile plants can be eliminated without any noticeable reduction in mutation frequency. This procedure, in addition to having practical advantages, also decreases the frequency of undesirable genetic side-effects, such as reduced fertility or seed inviability transmitted to the offspring. These findings add considerably to the efficiency of mutation induction when used for plant breeding[33,69,70,86,124,135,195].

It must be emphasized, however, that preselection for high $M_1$ fertility is appropriate only when progress in mutation breeding is sought by means of micro-mutations or recessive mutations with major expression. For genetic improvement which relies on the production of chromosomal rearrangements, the reverse procedure, i.e. the selection of partially sterile individuals is indicated[85].

## 5.3. Reproducibility

Although the neutron effectiveness estimates in the present experiments do not provide a measure of the limits of reproducibility, due to differences in experimental conditions, a study of their variation should nevertheless give a good indication of these limits. To this end, the $ED_{50}$'s and regression coefficients for various characters in the dry seed irradiation series of exps.1, 3 and 4 are shown together (table 32). The data of exp.2 are omitted in view of the discrepancies caused by sub-optimal conditions (see p.58).

The $ED_{50}$'s relating to the seedling characters correspond reasonably well, except for the low value for cumulative cotyledon growth in exp.4. The $ED_{50}$'s or regression coefficients relating to weight of seeds/fruit vary by ca 20% between experiments. The regression coef-

Table 32 : Estimated $ED_{50}$'s (krad $D_{H_2O}$) and regression coefficients on dose, b, for various characters in different experiments.

| Parameter | Character | Experiment number and cultivar | | | |
|---|---|---|---|---|---|
| | | 1 | 3 | | 4 |
| | | MM | MM | GL | MM |
| $ED_{50}$ | Cumulative cotyledon growth | 5.6* | 5.8 | 5.1 | 3.9 |
| | Leaf number, 19 or 25 days | 7.5 | - | - | 7.8 |
| | Length 1st leaf, 19 or 25 days | 6.4 | - | - | 5.8 |
| | Ult. length 2nd or 3rd leaf | 5.0 | - | - | 5.3 |
| | Weight of seeds/fruit | - | 4.1 | 3.6 | 3.4 |
| b | Weight of seeds/fruit | - | 12.5 | 13.9 | 14.6 |
| | % non-germinating seeds ($M_2$) | - | 2.3 | 1.6 | 2.6 |
| | % sublethals ($M_2$) | - | 0.4 | 0.6 | 0.3 |
| | % mutant seedlings ($M_2$) | - | 1.5 | 2.3 | 2.3 |
| | % all aberrant categories ($M_2$) | - | 4.1 | 4.6 | 5.2 |
| | Total of all aberrations in sporogenic cells | - | 16.6 | 18.5 | 19.8 |

* estimate based on linear regression

ficients pertaining to the various $M_2$ categories analysed separately show considerable variation between experiments (50-100% of the lowest value) but those relating to the total of all aberrant $M_2$ categories vary between the fairly narrow limits of 4.1 and 5.2 (30%). Those for the estimated 'total of all aberrations in sporogenic cells' (cf.p.74) vary only between 16.6 and 19.8 (16%).

These findings support the earlier suggestion (p.70) that there is a certain degree of complementarity in the percentages $M_1$ fertility reduction, non-germinating $M_2$ seeds, sublethals and mutant seedlings. In the present comparisons (dry seeds), the large variation in the regression coefficients pertaining to the various aberrant $M_2$ categories separately is undoubtedly due chiefly to an element of chance in the fate of chromosome aberrations. The regression coefficients for the % mutant seedlings are furthermore influenced by the fact that only a small proportion of all recessive mutations have a visible expression.

There are thus difficulties in obtaining reproducible estimates of the effectiveness of radiation on the various aberrant $M_2$ categories separately. To overcome these difficulties, large numbers of $M_1$ plant progenies, tested in replications, would be required.

A much higher level of reproducibility is achieved when comparisons are based on either the total of all aberrant $M_2$ categories or

the 'total of all aberrations in sporogenic cells' (including the effects of radiation leading to fertility reduction, see p.74). The disadvantage of this procedure is that possible differences in spectrum are ignored.

In conclusion, the present data justify the expectation that a rigorous standardization of material and procedures should lead to a reproducible overall genetic response to fast neutron irradiation treatments and consequently to a useful degree of predictability of this response on the basis of average $M_1$ seedling performance. The importance of using optimal culturing conditions, and of avoiding transplanting shocks, must be stressed in this connection, especially because unfavourable conditions in the $M_1$ have a much stronger influence on the dose/response relationships (and hence the radiation sensitivity estimates) relating to the somatic $M_1$ characters than on those relating to the $M_2$ characters.

## 5.4. Choice of stages and types of radiation for mutation breeding

### Stage

Fast neutron irradiation of dry seeds causes a relatively slight impairment of vegetative growth and development but has severe effects on $M_1$ fertility. This relationship was shown to be greatly altered when seeds are irradiated during germination (section 4.6). Pregermination leads to a much stronger sensitization with regard to seedling growth reduction than with regard to $M_1$ fertility reduction. It also causes a shift in the relative occurrence of non-germinating $M_2$ seeds, sublethals and mutant seedlings, towards greater proportions of non-germinating seeds. These findings, which are discussed on p.84-94, imply that dry seeds are more efficient (as defined on p.4) for the production of recessive 'visible' mutations by irradiation than are prehydrated or germinating seeds. Contant et al.[33] have shown that dry tomato seeds are also much more efficient for this purpose than pollen mother cells, dry pollen or both gametes after pollination. For these reasons, and because of the practical advantages of dry seeds over prehydrated seeds or flowering plants, dry seeds are unquestionably the most suitable stage for the production of recessive mutations with visible expression.

Most recessive mutations are known only by their phenotypic expression and their molecular-structural nature is unknown. As a result, the relationship between their phenotype and their nature cannot

be clarified. Nevertheless, the existence of such a relationship can be inferred from various data. Several authors, using different plant species and different types of radiation, have found an increase in the proportion of sublethal and lethal recessive mutations with increasing dose[33,34,54,75,121]. Gladstones and Francis[75] have observed higher proportions of lethal mutations after irradiation of seeds at high moisture contents than at low contents, at comparable mutation frequencies. These spectral shifts strengthen the opinion that most if not all visible recessives are small structural changes (see p.94) and also suggest that the differences in the degree of phenotypic disturbance are at least to some extent related to the 'size' of the changes at the chromosomal level. This interpretation is clearly supported by the increasing proportion of non-germinating seeds with increasing prehydration time in the present experiments.

Consequently, the present conclusion on the high efficiency of dry seeds is almost certainly true for the induction of recessive mutations in general, and especially for the induction of 'micro-mutations'.

As a corollary to this conclusion, prehydrated seeds are probably preferable to dry seeds for the induction and isolation of drastic chromosome aberrations.

Contant et al.[33] have found that, in tomato, at a given frequency of recessive 'visible' mutations in the $M_2$, fast neutron irradiation of dry seeds leads to a higher percentage $M_1$ fertility reduction (i.e. a greater load of more severe aberrations) than irradiation of both gametes. Gaul[69] found a similar relationship after X-irradiation of dry seeds and zygotes of barley. When these findings are connected to the present results it can be concluded that irradiation of prehydrated seeds leads to a considerably higher ratio of severe chromosome aberrations to recessive 'visible' mutations, in the generative stage of the $M_1$, than does irradiation of both gametes or zygotes. This is explained either by a difference in the aberration 'spectrum' at the cellular level, or by a less effective elimination of fertility-reducing damage after irradiation of prehydrated seeds than after irradiation of gametes and zygotes. In the absence of evidence in favour of the former possibility, the latter is preferred. This then suggests that the multicellular nature and the advanced developmental stage of the embryo permits a considerable mutual assistance between affected cells. It should be remembered that, within a treatment, the occurrence of recessive 'visible' mutations is virtually unrelated to the

presence of dominant and haplophasic lethal damage (see p.106). Although persistent, drastic chromosome aberrations have been observed in tomato both after irradiation of prehydrated and dry seeds[151] and after irradiation of both gametes[156], the above relationships indicate that germinating seeds are, of all stages mentioned, the most useful for the isolation of such drastic aberrations.

Type of radiation

The present study was concerned exclusively with fast neutron irradiation. In many respects, the results cannot be extrapolated to X- or γ-radiation. Neutrons produce, relative to X- or γ-rays, (i) more uniform biological effects[10,21,23,56,117], (ii) a higher rate of survival and a lower rate of seedling injury for a given reduction in pollen fertility[10], in seed set[56,117,149], and in the frequency of interchanges and fragments[21,23], (iii) a greater effectiveness for the production of cytologically detectable chromosome aberrations [59] and (iv) a lesser dependence upon environmental factors (see p.2). For instance, the response of dry seeds to neutrons is not or slightly influenced by their water content[27,30,56,132], whereas with X-radiation a marked influence of water content must be expected. Usually, the X-ray sensitivity is relatively high in very dry seeds and decreases with increasing moisture content; a minimum sensitivity is reached at a moisture content which is fairly constant for a given species but which varies between species[148]; above this water content, the sensitivity increases[75,99,114,126,132,142,148]. Also with prehydration of seeds, the sensitivity to X-radiation increases more markedly than that to neutrons[55,56,104,169].

These facts are attributed chiefly to a lesser damage to extrachromosomal elements ('physiological damage') with neutrons than with X- or γ-rays, due to the high average LET of the former[23,56,142]. In these respects neutrons have advantages over low-LET radiations, because reproducible results may be obtained with a less rigorous control of external factors.

Because of their high effectiveness (as defined on p.4) in producing drastic chromosome aberrations, fast neutrons lead to a lower ratio of mutations to fertility reduction than X- or γ- radiation [33,74]. These latter radiations, however, lead to relatively large and irregular incidences of early lethality, loss in vigour ('γ-plantlets') and non-flowering. The overall consequence is, that the maximum attainable mutation frequencies do not differ greatly between neutrons

and X- or γ-radiation[33,37].

The greater amount of sterility associated with induced recessive mutations after neutron irradiation is not an important disadvantage because most of it may be eliminated by selection for high fertility in $M_1$, owing to the virtual absence of a correlation between the degree of $M_1$ sterility on the one hand and the likelihood of the presence of recessive mutations on the other hand.

Considering only the recessive 'visibles' obtained with both types of radiation, their overall phenotypic spectra do not differ greatly [37, 142], but indications of specificity for certain loci have been obtained by several authors and are reviewed by Gustafsson[84]. Nybom [145] found no differences in the distribution of viability and yielding capacity between mutations induced by neutrons and X-rays but his material was too limited to generalize this finding. Therefore, a satisfactory evaluation of differences between radiations in respect of mutagenic efficiency or mutation spectrum, especially for those qualitative and quantitative traits desired in plant breeding, is not yet possible.

This evaluation is complicated by various factors. Firstly, few recessive mutations are directly beneficial in homozygous condition but may become so in an altered genetic background, i.e. after crossing with other genotypes. Secondly, recessive mutations are not the only changes from which practical benefits may be derived[85]. Chromosome rearrangements (translocations, inversions) generally reduce viability and fertility in the heterozygous condition but are mostly without conspicuous detrimental effects in the homozygous state. These structural aberrations should be of great value in mutation breeding, because they enable a considerable reshuffling of the genetic material by intercrossing followed by inbreeding and selection. Whereas X-radiation could be the more favourable for the induction of certain kinds of recessives, the use of neutrons is certainly indicated for the production of translocations.

In conclusion, it is still clearly advisable to use both high- and low-LET radiations in mutation breeding studies.

## 6. SUMMARY

A study was made of changes in fast neutron effectiveness during the hydration and germination of tomato seeds. The main findings and conclusions are the following:

Section 3.6

Samples of unirradiated seeds and their constituent parts (seedcoat+endosperm and embryo) were taken at short intervals up to 72 hours after sowing, and samples of roots, hypocotyledons and cotyledons from 72 to 144 hours after sowing. Their water content, dry weight and elementary composition (C, H, N, O and Ash) were determined.

At 27°C, the water content reaches a 'plateau' after ca 20 hours. Further uptake depends upon the rupture of the seedcoat as a result of internal forces developed by metabolic processes. This post-germination water uptake is exponential until the 'saturation' phase, but it commences much earlier in the root than in the hypocotyledon and only much later in the cotyledons. This sequence corresponds with the time of liberation from the seedcoat.

Appreciable changes in the dry weight of the embryo and the endosperm commence ca 8 hours after 50% germination. These comprise the resorption by the embryo of 75% of the dry matter of the endosperm (completed ca 60 hours after 50% germination) and *de novo* synthesis. At 104 hours after 50% germination, the former process has contributed 2/3 and the latter 1/3 to the total dry weight increase of the seedling. The increase in dry matter occurs first in the cotyledons (resorption of endosperm) and commences only ca 24 hours later in the other parts of the embryo. Approximately 56 hours after 50% germination, the dry weight of the cotyledons reaches a maximum after which it decreases; at this time the dry weight of the other organs increases rapidly.

The elementary composition of the dry matter is static until germination (apart from an early increase in N, by $KNO_3$ uptake from the medium). Subsequently, the C and H contents decrease markedly in all parts of the young seedling, while the N, O and Ash contents increase. These changes commence very soon after germination in the roots and ca 20 hours later in the hypocotyledons. Changes in the cotyledons evidently commence at the beginning of endosperm resorption, but become considerable only at 56 hours or more after 50% germination.

Section 3.7

The rad doses relative to those in water ($D_{H_2O}$) were 0.841 in dry embryos, 0.886 (105% relative to dry embryos) 24 hours after sowing, 0.916 (109%) 48 hours after sowing, i.e. 8 hours after 50% germination, and 0.977 (116%) 104 hours after 50% germination. The amounts of energy absorbed per embryo per krad $D_{H_2O}$ increased from 118 erg per dry embryo (100%) to 172 erg (146%) at 24 hours after sowing and 250 erg (212%) shortly after germination; until germination, the percentage values also represent the relative amounts of energy per 'average' embryo cell.

Section 4.3

Four irradiation experiments were performed. The shapes of the dose/response relationships were studied for various $M_1$ and $M_2$ characters.

Germination delay increases less than proportionally to the dose. The induced delays are probably less with 24 hours prehydration than with irradiation of dry seeds. These and other facts, which are discussed, indicate that non-genetic disturbances are mainly involved, including possible damage to intracellular membranes. Stimulation of germination was noted in one experiment only; possible reasons for the inconsistency of this phenomenon are given. Irradiation increases the rate of seed ageing; this is attributed to complex genetic-cytoplasmic interactions which are discussed.

Under optimal conditions of culturing, the dose/response relationships for all growth characters are slightly S-shaped, without evidence of a true threshold; the relationships for some characters are almost linear over most of the dose range. Approximately straight regressions are usually obtained on normal probability paper. An apparent discrepancy in the dose/response curves relating to the growth of those organs already differentiated in the embryo was resolved by showing that these curves result from at least two growth components, differing in radiation sensitivity. One of these depends solely upon the elongation of existing cells. The 'residual length' attained by these organs at a lethal dose should be subtracted from the lengths actually measured, in order to obtain net growth. Failure to do this may lead to large errors in the estimation of radiation sensitivity; in this respect, the methods employed in the IAEA-sponsored international programme of biological monitoring of neutron sources with barley seeds should be reconsidered.

Sigmoidal dose/response curves with a threshold are obtained for all quantal characters and are amenable to probit analysis. Similar curves are obtained for quantitative discrete characters, but the underlying distributions are different and probit analysis is not appropriate.

Sub-optimal culturing conditions may cause considerable exaggeration of curvature and/or increased thresholds in the dose/response relationships. Such circumstances also lead to higher radiation tolerance estimates. In extreme cases, and for certain characters, increases over the control may occur and these should not be confused with 'stimulation' effects. Such increases were never observed under optimal conditions of nutrient supply and spacing.

The characters leaf number below the 1st inflorescence and days to flowering show linear or upwardly curved increases with dose. In so far as flowering delays are not fully accounted for by the increases in leaf number (at the higher doses), they can be attributed to delays at the earliest stages of seedling development rather than to an increased plastochron at later stages.

The dose/response relationships pertaining to both weight (or number) of seeds/$M_1$ fruit and the various categories of aberrant $M_2$ individuals (non-germinating seeds, sublethals and mutant seedlings) are apparently linear, except for a tapering off at sublethal doses in the case of weight of seeds/$M_1$ fruit. These results are discussed.

Section 4.4

Radiation sensitivity estimates were obtained by means of $ED_{50}$'s or regressions on dose. The $ED_{50}$'s for different characters show a close connection to the degree of curvature and/or the presence and width of a shoulder in their dose/response relationships. Cumulative root- and cotyledon growth are more affected by radiation than are cumulative growth of either the 1st or 2nd leaf, all recorded after 25 days. Whilst such data are of descriptive interest, they do not permit conclusions on relative sensitivities at the meristem level, let alone at the cellular level. Even extrapolation to other stages of growth is hazardous, because it is shown that the effect of a given dose may depend greatly upon the growth stage considered.

With dry seed irradiation, the effects of fast neutrons (in % per krad) on seeds/$M_1$ fruit are much more severe than on vegetative growth and development, and are moreover ca 3 times the % increases in the total of all aberrant $M_2$ categories. Among the latter, the increases

in the % non-germinating seeds with dose are generally higher than those of the % visibly mutated seedlings.

The relative proportions of the various effects changed considerably with prehydration; the changes were examined by means of Dose Reduction Factors (see 4.6 below).

Section 4.5

The radiation responses of 2 cultivars, 'Moneymaker' (MM) and 'Glorie' (GL) were compared. Seeds of GL were ca 1.14 times as sensitive as those of MM. The 100-seed weight ratio GL/MM was 1.00/1.14 and the 100-embryo weight ratio 1.00/1.15 (difference highly significant). The size of the shoot apex probably differed to the same extent and the difference in sensitivity is attributed mainly to this factor. The percentage root meristem cells in $G_1$ was virtually the same in both cultivars. The average amount of DNA per nucleus was 5% lower in GL than in MM (difference insignificant). Seed prehydration caused in GL, compared to MM, a more pronounced sensitization with regard to both $M_1$ seed set reduction and the production of non-germinating $M_2$ seeds, and a lesser sensitization in respect of the induction of recessive 'visible' mutations.

Section 4.6

The Dose Reduction Factors (DRF) associated with the various stages of prehydration/germination were calculated for each character. The DRF's relating to the seedling characters increase much more rapidly with increasing prehydration time than those for late somatic characters and $M_1$ fertility. Furthermore, in the $M_2$, the proportions of non-germinating seeds, sublethals and mutant seedlings are shifted in the direction of non-germinating seeds.

Only a small proportion of the increases in neutron effectiveness (DRF-1.0) can be explained by increased rad doses. The amount of energy absorbed per prehydrated embryo relative to that per dry embryo, which can be considered fairly representative of the relative energy absorption per (meristem) cell prior to germination, accounted for only 13-30% of the increase in neutron effectiveness as judged by $M_1$ fertility and the total of all aberrant $M_2$ categories. Consequently, most of the effectiveness enhancement with prehydration, and also the 'spectral' shift among the aberrant $M_2$ categories, must be ascribed to other factors: (i) intracellular differences in water uptake and swelling (possibly more rapid hydration of the nucleus), (ii) increases in the mobility of intracellular structures and of molecules,

ions and radicals, and corresponding increases in metabolic activity, (iii) progression through the successive stages of the cell cycle, and increases in cell number and (iv) resorption of endosperm substances. These various factors are discussed.

The more pronounced prehydration sensitization with regard to the early, compared to the late $M_1$ characters is attributed to (i) the structure of the embryonic shoot apex, which allows an increasing amount of replacement of damaged cells towards the centre of the meristematic region, (ii) possibly a more rapid sensitization, during germination, of the peripheral cells of the shoot apex than of the more centrally located cells, owing to the more rapid onset and higher rates of division of the former, and (iii) a decreasing impact of damage to the early organs as development proceeds.

Differences in the degree of sensitization between experiments are tentatively ascribed to several endogenous and external variables, including dose rate. It is concluded that a good reproducibility is attainable if all conditions are standardized.

Section 4.7

An analysis of simple within-treatment correlation between various quantitative somatic characters has shown that r-values tend to decrease

(1) with advancing stage of development at the time of recording
(2) with increasing genetic and non-genetic radiation damage, especialwhen the characters studied concern organs derived from different initials
(3) with increasing interval between the dates of recording
(4) with increasing time lapse after the moment when one of the characters has reached its ultimate value at the date of recording, assuming that the value of the other character is still increasing
(5) at early stages of growth, especially in the case of organs of which the initial growth is determined by cell elongation independently of cell division, or of which the moment of growth initiation is very variable
(6) under sub-optimal conditions of culturing
(7) by a change in culturing conditions, especially if this affects one character more than the other.

Radiation may increase the degree of within-treatment correlation if the effects of radiation on one character exert a direct influence on the second character.

Both the somatic $M_1$ characters and the weight (or number) of seeds/$M_1$ fruit are virtually uncorrelated with the presence of recessive mutations detectable in $M_2$.

Section 5.1

With a view to interpreting differences in radiation sensitivity it is necessary to devote increasing attention to the histological constitution of the shoot apices of irradiated objects, and to cellular and intracellular parameters, rather than aiming at more precise estimates of the atomic composition at the tissue level. Whereas all sensitivity differences may ultimately be explained in terms of biophysics and microdosimetry, it is unlikely that major advances in this field will come from studies on multicellular organisms.

Section 5.2

Cumulative root- and cotyledon growth are suitable characters for an early evaluation of the mean effectiveness of neutron treatments and allow the repetition of mutagenic treatments, though only with a given material and under comparable circumstances. Under optimal and standardized conditions, tomato seeds could probably be used, in addition to barley seeds, for the biological monitoring of neutron irradiation facilities.

The virtual absence of within-treatment correlation between $M_1$ and $M_2$ characters allows the elimination of all badly growing and partially sterile $M_1$ plants without a noticeable reduction in the frequency of recessive mutations. This is of value in mutation breeding by means of micro-mutations. However, when chromosomal rearrangements are desired, the reverse procedure, i.e. the selection of partially sterile individuals, is indicated.

Section 5.3

A good reproducibility of neutron effectiveness estimates is possible with regard to the seedling characters, $M_1$ fertility, and the sum of all aberrant $M_2$ categories (non-germinating seeds, sublethals and mutant seedlings) but probably not with regard to these $M_2$ categories analysed separately.

Section 5.4

Dry seeds are the most efficient for the induction of recessive mutations. Germinating seeds are probably the most efficient for the production of drastic chromosomal rearrangements.

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