A.M. van Harten

Institute of Plant Breeding, Agricultural University, Wageningen

Mutation breeding techniques and behaviour of irradiated shoot apices of potato

2061886

Centre for Agricultural Publishing and Documentation

Wageningen – 1978

2061886

Abstract

Harten, A.M. van (1978) Mutation breeding techniques and behaviour of irradiated shoot apices of potato. Agric. Res. Rep. (Versl. landbouwk. Onderz.) 873, ISBN 90 220 0667 0, (vii) + 132 p., 23 figs, 16 tables, 380 refs, Eng. and Dutch summaries.
Also: Doctoral thesis, Wageningen.

An attempt was made to produce a di(ha)ploid tester clone with marker genes in an heterozygous condition for studies on induced mutations in potato (*Solanum tuberosum* L.). Literature on potato mutations was reviewed. Formation of adventitious roots and shoots from potato leaves, leaflets and stem parts was studied in vivo. Roots formed easily, but adventitious shoots were very few. Techniques in vitro were more promising. After a review of literature on shoot apices, damage and recovery of irradiated potato shoot apices was studied in whole plants as well as from changes in tuber-skin colour caused by histogenic effects and from microscopic slides made during a 20-day period after irradiation.

Free descriptors: *Solanum tuberosum* L., adventitious bud, shoot apex, histogenic effects, histogenic layers, chimerism, di(ha)ploid, tester clone, marker gene, radiosensitivity, microscopy, tuber-skin colour, tuber eye morphology.

ISBN 90 220 0 667 0

The author graduated on 28 April 1978 as Doctor in de Landbouwwetenschappen at the Agricultural University, Wageningen, the Netherlands, on a thesis with the same title.

© Centre for Agricultural Publishing and Documentation, Wageningen, 1978.

No part of this book may be reproduced and/or published in any form, by print, photoprint, microfilm or any other means without written permission from the publishers.

Contents

1 Introduction

Every plant breeding programme starts with a search for suitable sources of genetic variation. Sometimes this part of the work simply consists of isolating an outstanding strain from a plant population; in other cases, promising parent plants are selected from existing cultivars and crossed consecutively. If genes for certain desired characters cannot be derived from such easily accessible sources, the plant breeder may, for example, turn to more distantly related cultivated species, or even to wild relatives. Breeding work will become increasingly difficult with more distant crosses, not only because of crossing barriers, which often exist between less related taxa, but also because undesired genes are introduced as well. Those undesired genes must then be removed at a later stage of the breeding programma, e.g. by repeated back-crossing, before an acceptable cultivar can be released.

For some plant species or for certain cultivars of a species, genetic improvement by crossing is even further limited and sometimes completely impossible, for example through absolute sterility. Especially then mutations may offer the breeder a way round the difficulties. Mutations can be roughly described as heritable changes in the genetic material. According to Stebbins (1950, p. 76), they are the ultimate source of all differences between two individuals.

Although unaware of the underlying principles, plant breeders have for centuries exploited spontaneous mutations. Their value for breeding work has been sufficiently demonstrated. Positive results have also been obtained during the last decades by artificial induction of mutations (e.g. Micke, 1976). Indeed one should not overestimate what is feasible, as has often been done. Induction of mutations seems, however, a realistic approach in solving certain breeding problems. Stadler (1930) considered that practical results from mutation induction could be expected, especially in vegetatively propagated crops. Although this statement referred in particular to fruit trees, it can be applied without many restrictions to other vegetatively propagated crops like many ornamentals, some grasses and potato. Some special problems arise when inducing useful mutations in crops like potato, as many characters of economic importance inherit in a quantitative way and, moreover, are often strongly affected by environmental conditions.

The potato (*Solanum tuberosum* L.), a crop of worldwide economic importance, is vegetatively propagated in common agriculture, but for breeding purposes mostly crosses are made. Breeding work in potato encounters many difficulties, which are caused by a complicated and stil largely unknown tetrasomic inheritance, by the occurrence of much heterozygosity, a low heritability of many important characters, poor flowering in many cases and often by the existence of sterility and incompatibility barriers, especially in crosses with wild or primitively cultivated species.

Today most potato breeders still roughly follow breeding procedures of half a century ago. These old methods are rather laborious and often lead to new cultivars by good

fortune only. Recently some progress has been made: improved methods of screening the material for certain resistances, the increased use of wild and primitively cultivated species and the growing insight in potato genetics.

Two now available approaches to potato breeding: the use of so-called dihaploids (i.e. haploids derived from tetraploids) and of induced mutations, have not yet been generally accepted, although both have been known for several decades. Already in 1935 Asseyeva & Blagovidova (1935) reported that X-ray treatment can provoke mutations in potato, but now, 40 years later, not much has been achieved in practice with this knowledge. Not only the limitations of the method, technical difficulties and inadequate small-scale experiments, it seems, have hampered the introduction of this method. Prejudices, irrelevant arguments and a considerable amount of conservatism among potato breeders and some scientists are responsible as well.

The most serious limitations and technical problems are generally considered to be: the low frequency of induced mutations, especially of positive genetic changes and the occurrence of many chimeric plants, i.e. plants composed of both mutated and non-mutated somatic cells or tissues. Moreover, in a number of publications reference has been made to the existence of a strong selection against mutated cells, which phenomenon has been indicated as diplontic selection (Gaul, 1959), or as intra-individual selection (Kaplan, 1953). The occurrence of undesirable pleiotropic effects, accompanying valuable mutations has also been reported on several occasions.

Reliable data on the frequency of mutagenic events are difficult to obtain. Dobzhansky (1970, p. 69), for example, pointed out that several factors may lead to either an overestimation or an underestimation of the mutation pressure if calculations are made either with the mutation rate of a single gene or with the total number of mutations in all genes of an organ.

Mutations are single-cell events and occur more or less at random in different plant parts. Only mutational events in those parts from which regeneration is possible, are of use to the breeder. Mostly reproduction takes place by sexual methods or via existing axillary buds, but the application of adventitiously induced shoots in this respect may be also considered. A complication, especially in vegetatively propagated plants, is the layered structure of young apices. For normal propagation of potato via tubers which develop after swelling of the apical end of subterranean stems (stolons), the different apical layers, which are commonly indicated as histogenic layers and denoted as L-I, L-II and L-III, take part in the formation of new organs to the same extent as they do in the parent plant. Thus a possible chimeric situation after a mutational event can be long lasting.

From a breeding point of view it would be highly preferable to have mutants in a solid (i.e. chimera-free) condition. Efforts to limit chimerism automatically imply the limitation of the number of cells from which a progeny arises. For generative propagation, this approach is relatively simple as each new plant developes from one single fertilized egg cell. Another advantage of generative propagation is that in the generative phase detrimental effects of the mutagenic treatment, such as gross chromosomal damage are selected against. In vegetatively propagated crops it is more difficult to obtain chimera-free mutants. The problem becomes less if whole plants are produced, tracing back to one histogenic layer only. It would be ideal if plants, derived from only one single cell, could be easily obtained and in sufficiently large quantities. When our work started, potato

plants from one histogenic layer could be produced in certain cases, but suitable single-cell methods were not available.

Does it make any difference to the mutation frequency (on the basis of mutational events per cell) which layer produces such single-cell plants? This question seems justified since (cells of) different histogenic layers have different physiological properties and thus, most probably, different radiosensitivity. Therefore the mutation rate per cell could be different. For a number of plant species, differences in radiosensitivity have been reported between different regions and also between different histogenic layers, but practically nothing is known in this respect for potato.

Differences in radiosensitivity between layers, moreover, may lead to rearrangement of layers and the like, a subject that has been little studied. One could imagine that cells of a rather radiosensitive layer, which probably carries more mutated cells, become inactivated or destroyed by a certain amount of radiation. Then cells of an adjoining, less radiosensitive part of the plant may take over. From a practical point of view the ultimate result could be a lower production of mutants per irradiated plant. It may well be that the type and amount of irradiation to which the plant material is exposed, plays a role here. Some authors have reported that the kind of histogenic effect is also affected by the treatment given.

Some of the aforementioned problems have been studied since 1961, at the Institute of Plant Breeding (IvP), initially by F.P. Ferwerda and coworkers and since 1969 by the author and coworkers. The intention was not to produce new potato cultivars, but to gain more insight into the different processes involved in mutagenic treatment and to try to bring the mutation technique within the reach of the practical potato breeder. This publication reports part of this work. In Chapter 2 the present stage of affairs in potato mutation work is evaluated and some suggestions for further investigation are made.

Efforts to produce a potato tester clone at the diploid (2n=2x=24) level are described in Chapter 3. The original idea was to use such a clone, carrying some marker genes in heterozygous condition, for all mutation experiments. Technical difficulties made it impossible to produce a suitable clone in time for the other investigations planned. It therefore became necessary to use other material from different sources.

Experiments to find a suitable in-vivo method to produce adventitious shoots in potato are reported in Chapter 4. The so-called adventitious bud technique is based on the fact that dissected plants parts, leaves, tubers and the like are often able to produce adventitious plantlets, which sometimes can be traced back to only one initial cell. If a mutation is induced in such a cell, the mutated plantlet will be (practically) chimera-free. The best-known early example of a one-cell origin of such plantlets was reported by Naylor & Johnson (1937) for *Saintpaulia ionantha*. Sparrow et al. (1960) observed that in this way solid mutants can be produced. However, Broertjes of the Institute of Atomic Sciences in Agriculture (ITAL), Wageningen, demonstrated the practical applicability of this method for mutation breeding work in a considerable number of ornamental plant species (mainly Gesneriads). The theory behind adventitious bud formation is summarized in the introduction to Chapter 4.

A review of present knowledge about the organization and behaviour of shoot apices, both during their normal life cycle as well as after irradiation is given in Chapter 5. Special attention is paid to the effect of irradiation on histogenic effects. In Chapter 6 the results of microscopic as well as morphological investigations on radiation-induced histogenic effects in potato are presented. They are preceded by a review of the related potato literature.

2 Survey of literature on mutations in potato

2.1 Spontaneous mutations

Several cases of spontaneous mutations (indicated as bud variations) in potato were reported by Darwin (1868) and even earlier by Carrière (1865). Darwin (1868, Chapter XI) defined bud variations as 'all changes in structure or appearance which occasionally occur in full-grown plants in their flower-buds or leaf-buds'. He attributed these changes in many cases to 'spontaneous variability', but he failed to indicate the cause of this variability. Fruwirth (1929), who worked with potato, mentioned that spontaneous variation occurs either as a result of irregular cell divisions, leading to genetically different somatic cells, or after rearrangement of tissues or layers. It is clear that in the latter case a visual change can only be expected if the plant already had a chimeric character. (The use of the word 'chimera' to indicate genetic changes in only a part of the somatic tissues of one (plant)species dates back to Baur (1909), who extended the meaning given to it by Winkler (1907).)

Already in 1907, Cramer (1907, p. 430) referred to some cases in which bud variations had led to cultivars of practical value, e.g. to an old example (Anonymous, 1857) concerning cv. White Fortyfold with white tubers, obtained from cv. Purple Fortyfold. Cv. White Fortyfold was reported to be completely similar to the then known cv. Regent. As only one eye of a purple tuber of cv. Purple Fortyfold had become white, contamination must be excluded.

During the first decades of this century, some scientists still doubted the occurrence of bud mutations (also referred to as bud variations, bud sports, vegetative segregations, vegetative mutations, somatic mutations and 'accidents'), let alone accepted that such mutations were of practical value to the breeder. Sutton (1918) stated: 'the more deeply the subject is investigated, the more convinced one becomes that there is no ground to believe that nature has ever given rise to any, new and distinct variety of potato by bud variation'. He claimed that bud mutations referred only to a change of tuber colour and must be looked on as variations, but not as new varieties. In my opinion, this is only a matter of terminology. It depends on the definition of a 'new' variety. Salaman (1926), also referring to Sutton (1918), expressed his opinion in the following way: 'it is not by way of bud mutations that we must look for new (potato) varieties, for such undoubted ones as have occurred amongst our domestic strains have failed to produce any form superior to their immediate parent'. A more optimistic view was presented by Dorst (1924) who, in an extensive review analysed many reported bud sports in potato, involving very different characters. According to Dorst the cause of bud sports is unknown, and there are no known methods for producing them. Large plant populations have to be screened to find them in nature. Dorst also presented some data on the use of certain bud sports in practice. During the period 1919-1929 about 2-3% of the area planted with the cultivars

Eigenheimer and Rode Star, was occupied by the bud sports Blauwe Eigenheimer and Bonte Rode Star in the Province of Friesland in the Netherlands.

According to Krantz (1951) 15% of the certified seed production of commercial cultivars in the USA in 1951 was reported to come from bud sports. Those sports displayed a change in tuber skin, i.e. either towards a russet structure or a different colour, more appreciable to the consumer. In 1959, the acreage of bud sports had risen to about 35% as Heiken et al. (1963) concluded from a survey by Turnquist (1960). A rough estimation of the situation in the Netherlands indicates that never more than 1% of the total acreage there has been covered with cultivars obtained from bud sports.

It is not feasible to record all reports about bud sports in potato. It may suffice to refer, in addition to the work already mentioned, to the publications of Salaman (1931), Miller (1954), Swaminathan & Howard (1954) and Heiken (1960). In general, most mutations referred to aberrations in general appearance, in leaves, flowers or tubers. Especially changes in tuber-skin colour and skin structure (russeting) have been reported. It seems that in early reports, symptoms of virus attacks and bud sports must have been occasionally mixed up. Already East (1908) mentioned this complication. In a later publication East (1910) suggested that careful observation of the plant might lead to the discovery of variation in other characters than, for example, colour of tuber skin. Dorst (1924) advocated selecting for bud sports with agricultural value during maintenance breeding.

As said before the frequency of spontaneous mutations is very low. East (1912) discovered only 12 clear cases in 100.000 hills. Folsom (1923) found only 5 leaf mutants in more than 350.000 plants. The frequency of mutations, of course, depends on the number and kind of characters examined and probably on the degree of heterozygosity of the characters studied (Dorst, 1924). Different characters may have different mutation rates. This subject was studied by Heiken (1960) who found spontaneous aberration rates ranging from 1.5×10^{-3} (for so-called bolters in certain cultivars) to 1.2×10^{-5} (for certain foliage mutants). Such data, of course, have only a limited value.

A point of scientific as well as practical importance was made by von Rudno (1925), who suggested that mutation of only one 'hereditary unit' might result in a change of more than one plant character and thus indicated the possible occurence of pleiotropic effects. In fact a sport with simultaneous loss of tuber-skin colour, change of sprout colour and change of leaf shape was reported as early as 1921 (McKelvie, 1921).

There is no doubt that in the past, but also today, many bud sports of possible agricultural value have been lost, either by negligence or because during breeding for maintenance it is common practice to discard all aberrant types (Miller, 1954).

2.2 Mutations and chimerism

As stated in the first chapter chimera formation is a common phenomenon after mutation induction. Especially in vegetatively propagated plants chimeric structures may be often very persistent and troublesome. The cause is found in the multicellular and layered structure of different plant parts in which mutations occur. The existence of a kind of layered structure in the potato shoot was suggested on anatomical grounds by Artschwager (1918, 1924) and later confirmed, e.g. in apices treated with colchicine (Baker, 1943).

Already Carrière (1865) reported that some bud sports cannot be reproduced by seed and East (1917) explained this finding by the existence of periclinal chimeric structures. He was aware that germ cells are restricted to the subepidermal layer. Dorst (1924) mentioned that only mutations present in the subepidermal layer can be propagated generatively.

The layered structure of the shoot apex and of tissues derived from it explains how a plant with a mutation affecting one cell usually becomes a more or less stable periclinal chimera via the intermediate stage of a mericlinal chimera (Jørgensen & Crane, 1927). Contrary to this opinion, Salaman (1925, 1931) believed in a mosaic arrangement of both mutant and normal cells. It is now accepted that such mosaics are very rare, as is also the case in vegetatively propagated plants with 'real' sectorial chimeras.

Asseyeva (1927) concluded that, assumedly, the overwhelming majority of bud sports in potato, and very probably also in other vegetatively propagated plants are periclinal chimeras. Asseyeva developed the eye-excision technique, a method for the production of new sprouts from endogenous plant tissues, thus revealing the genetic nature of such deeper zones. In this way the periclinal nature of certain mutated characters could be identified as such. The best known example of this is the so-called 'Kostroma' leaf-mutant in the, then well-known cultivar Richters Imperator. In a footnote Asseyeva added that mutations do not have to be confined to outer layers only. In a later publication Asseyeva (1930) stated that relatively more mutations occur in the outer layers, although she did not substantiate this remark.

2.3 Induced mutations

2.3.1 Preliminary remarks

After plants have been treated with mutagenic agents like X-rays, neutrons or chemicals, normally two different groups of effects are distinguished; firstly so-called primary injury or physiological damage and secondly genetic changes or mutations. Examples of the first group of effects are: direct damage to cell walls or cell contents, arrest of mitotic divisions, inhibition of apical dominance, low-dose growth stimulation and several effects qualified as histogenic.

Especially in early literature, it is sometimes difficult to make out whether after irradiation the author indeed obtained mutations or erroneously referred to effects of a non-heriditary nature. This complication applies particularly to literature on vegetatively propagated crops like potato. Even after some cycles of vegetative propagation, it is still hard to say whether real mutations were induced. As an additional complication scoring of mutations is often hampered by the fact that symptoms of different virus attacks may have the appearance of a mutation. In mutation experiments only healthy and virus-free plant material should be used, but this point has been neglected by many investigators.

2.3.2 Early reports

Potatoes were treated with X-rays for the first time by Jacobson (1923), Johnson (1928, 1937) and by Sprague & Lenz (1929). Jacobson (1923) reported considerable increase in yield and larger tubers in two different cultivars. Johnson (1928) who adminis-

tered a 'low' (whatever that may mean) dose of X-rays to tubers of cv. Early Ohio, observed increased tuberization but lower tuber weights. Sprague & Lenz (1929), treating tubers of the cultivars Irish Cobbler and Green Mountain with 400-1200 R of X-rays, on the other hand obtained fewer but larger tubers and a somewhat higher total yield. No data were given about the number of tubers that had been treated. Mutations for tuber – or foliage characters were not reported. Johnson (1937) in experiments with the so-called Colorado wild potato (*S. jamesii*) found increased tuberization and increased weight per hill and per tuber after exposure to 1500 R of X-rays. There is no definite proof that the effects described were of a permanent genetic nature.

The first reliable experiment was carried out by Asseyeva & Blagovidova (1935). Four cultivars were X-irradiated with doses between 500 and 8000 R. Per cultivar a total of 390 tubers was treated. Altogether 23 foliage mutations were obtained: 10 in cv. Prof. Wohltmann but none in cv. Epicure. According to Heiken (1960), Asseyeva & Blagovidova (1935) also tested the mutagenicity of several chemical agents, but apparently without succes.

If one excludes colchicine work and a report from Demidovic (1934), who claimed that storage of true potato seed for 5 years induces more mutations than any other method, no further mutation research on potato was reported till 1950. At that time Sparrow & Christensen (1950) described the inhibiting effect of X-rays on sprouting of potato tubers. Stanton & Sinclair (1951) treated their material with ^{32} P and described morphological changes in the leaves. They also reported that the apical area of the shoots displayed a higher radiosensitivity than other plant parts. Hagberg & Nybom (1954), who used ^{32} P and X-rays in small-scale experiments, obtained morphological changes lasting through different cycles of vegetative propagation (commonly indicated as vM_1, vM_2, etc.).

With the work of Heiken in Sweden around the sixties, which will be discussed in other sections, the period of non-directed orientation work can be considered at an end. With this work true start was made to apply mutation techniques in potato breeding.

2.3.3 The starting material

Practically all mutagenic treatments of potato in the past have been performed on whole tubers or tuber halves (the other half being used as control). Tubers are mostly divided longitudinally according to the method of Asseyeva (1927). Nayar & Dayal (1970) on the other hand used rose (apical) and heel ends. Howard (1970) has repeatedly used rose ends only. Dormant as well as sprouting material is treated. The use of dormant tubers normally leads to a lower amount of chimeric plants, but sprouting tubers may produce higher mutation frequencies (Heiken, 1960).

There is a great deal of variation about the age of the treated tubers. Sometimes very small, premature tubers are used, sometimes tubers are irradiated immediately after harvesting. Occasionally experiments are conducted with tubers that have been stored for several months. Gradually researchers found that treatment of smaller structures like young tubers, tuber parts, single-eye pieces, cuttings or adventitiously developed shoots, is more advantageous from several points of view: the amount of 'bulk' to be handled is reduced, large quantities of material are treated more uniformly and chimerism is reduced.

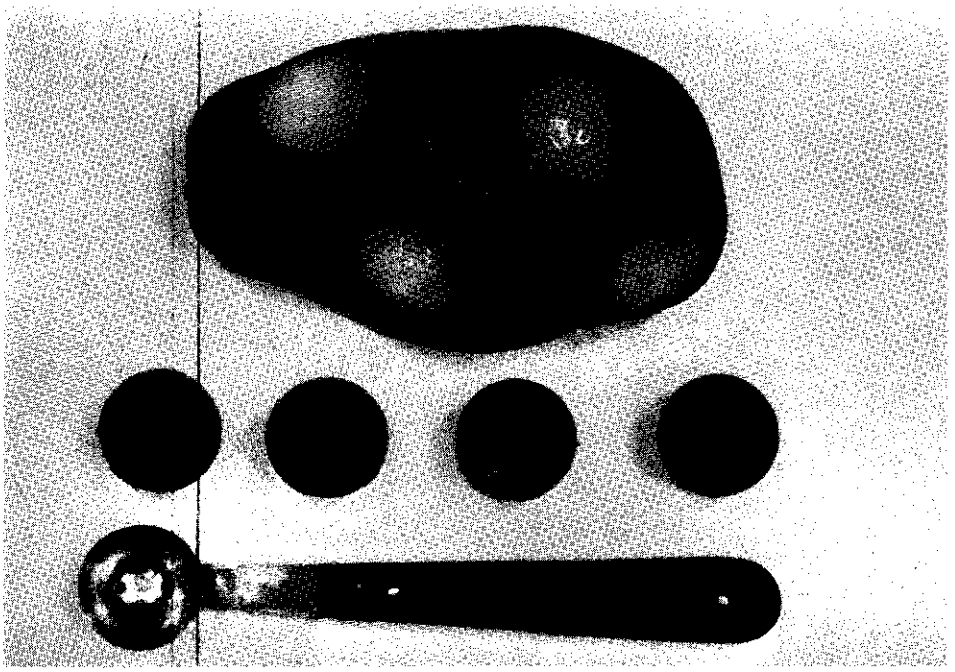

Fig. 1. Scooping spoon, scooped-out tuber and tuber eye-pieces as used for mutation experiments at the Institute for Plant Breeding (IvP), Wageningen.

Single-eye pieces (see Fig. 1) were used by Ferwerda (1965), Umaerus (1966), van Harten (1970), van Harten et al. (1973) and Upadhya & Purohit (1973)[1]. In experiments on chimerism, van Harten et al. (1972) started from eye-excised tubers which were sectioned into an apical, middle and stolon part in order to check the behaviour of the respective sections. The advantage of using eye-excised material is the initiation of (adventitiously developed) organs from a reduced number of cells. Miedema (1973a) irradiated tubers slices on which young sprouts initiated adventitiously on roots which themselves had been developed adventitiously on the tuber slices. Rooted cuttings were used by McCrory & Grun (1969).

Since 1974 unrooted cuttings from which the root area is inserted into a piece of lead during irradiation, are used at the IvP. This method is to be preferred if tuber production, for example of dihaploids, is low and time has to be saved. Another positive point of using cuttings is the uniformity of the starting material. In addition to vegetative parts also seed and pollen have been treated on several occasions and for different purposes. This work will not be discussed here.

1. Upadhya & Purohit erroneously reported that the method using tuber tissue plugs comes from Rappaport et al. (1965). As a matter of fact, Ferwerda (1965) reported on this method at a FAO/IAEA meeting in 1964. He adopted it several years earlier from the common practice in the Netherlands to use potato plugs in phytopathological tests, where it had already been applied for several decades.

Practically all mutation work has been performed with the common, tetraploid potato. In 1964 Ferwerda (cf. van Harten & Bouter, 1973) irradiated dihaploids and secondary dihaploids. Some other preliminary work with potato material at several levels of ploidy has been reported by Gomes Cuervo & Nelson Estrada (1972) and by Moreno & Nelson Estrada (1973). An experiment involving 150 X-irradiated, whole small-sized, dihaploid tubers of cv. Désirée was published by van Harten & Bouter (1973). During a period of 8 months all emerging sprouts were dissected ('milked') when about 3 cm and planted individually. Especially the latest developing sprouts are in majority of adventitious orgin and produce higher frequencies of non-chimeral mutants.

It is only a matter of time before shoots and plantlets, obtained from cultures in vitro of different kinds of potato material, are used in mutation programmes. The results of a co-operative programme between ITAL and the IvP will soon be published. Techniques have been described by Roest & Bokelmann (1976).

2.3.4 Mutagenic treatments

During the years many different mutagenic agents have been applied. In the beginning mostly X-rays, ^{32}P and, to a smaller extent, γ-rays were used in a wide range of doses, dose rates and concentrations. More recently neutrons have been administered and in a still increasing number of cases also different chemicals. Unfortunately data are often not exact, especially in early literature.

For X- and γ-rays, doses range from 400-10.000 R for vegetative parts, 2000-3000 R being the doses preferred by most workers. Usually 8000-10.000 R is lethal. The effect of dose rate has not often been studied, although it seems that the rate of survival, the mutation frequency and the amount of chimerism can be affected in this way (McCrory & Grun, 1969; van Harten et al. 1972; Mezentzev & Yashina, 1971, 1973). Considerable differences in response have been noted between different cultivars and different physiological stages. ^{60}Co or ^{137}Ce sources have been used for acute γ-treatments of potato (Heiken 1961; Benvenuti et al. 1963; Mezentzev, 1970; Kaneko, 1975). Essential differences in effect between X-rays and γ-rays have not been reported

Plants have been irradiated with fast and thermal neutrons, for example by Solomko (1965a), van Harten et al. (1972), Gomes Cuervo & Nelson Estrada (1972) and Miedema (1973a). Doses and dose rates used were very different, the outcome also depending on the reactor used. The relative biological effectiveness (R.B.E.) of neutrons normally is higher than 1. In one of our experiments, for example, (van Harten et al., 1972) a treatment of whole tubers of cv. Désirée in the BARN reactor of ITAL produced 24% of visible mutants after exposure to 810 R of fast neutrons. For a comparable effect at least 2000 R of X-rays would be required, i.e. the R.B.E. of fast neutrons is 2 or 3. Reports on other physical agents like high-energy protons, different radio-isotopes and ultraviolet rays are relatively scarce (Jauhar & Swaminathan, 1967; Tarasenko, 1977).

Much work with chemicals has been done in Russia, for example by Korableva (1961), Tarasenko (1965), Solomko (1965) and many others, but reports are hardly accessible. Ferwerda (1965) and Umaerus (1966) were the first to apply ethyl methanesulphonate (EMS) solutions, which were pipetted on the tuber eyes at concentrations of for example 1.5%. EMS has remained the most widely used chemical agent although several others like ethylene imine (EI), diethyl methanesulphonate (DES), N-nitroso-methyl urethane

(NMU) etc., have been mentioned (Nayar & Chauhan, 1968; Jauhar, 1969a,b; Pavek, 1972; Upadhya & Purohit, 1973; Upadhya et al., 1974a,b). Again concentration, duration of treatments, temperature, buffer etc. were different. As up to now results have been rather inconclusive, it seems premature to discuss experimental conditions in detail.

Finally it has to be kept in mind that because of physiological effects in vM_1, no conclusion can be made about mutation rates after analysis of vM_1 only. Kukimura & Takemata (1975) recently presented evidence that radiation damage, after treatment with γ-rays, was also transmitted to vM_2.

2.3.5 Types of induced mutation

Most cases in which the induction of mutation has been proved beyond doubt, concern genetic changes of morphological characters, such as size, shape and colour of the foliage or the subterranean parts.

The work of Heiken (1960) has confirmed that most mutations manifest themselves as periclinal chimeras and, in many cases, apparently show pleiotropic effects. (N.B. In practice it is difficult to distinguish between pleiotropic effects and close linkages of genes.) Most induced mutations have a negative effect ('loss' mutations). A first example of something being 'gained' is the induction of increased pigmentation in the corolla, again reported by Heiken (1960). The same author also made valuable observations and remarks about the practical application of irradiation in potato breeding. He further found that even drastic morphological mutations do not necessarily lead to inferior plants from a breeding point of view. Heiken did not observe differences between the spectra of spontaneous and induced mutations. Unfortunately his work did not yield mutations of direct practical value.

The induction of mutations for earliness, increased resistance to different diseases and increased starch content of the tubers has been reported by Solomko (1962, 1965a). Kishore et al. (1963) found that after X-irradiation 19 out of 55 plants were more resistant to *Phytophthora infestans*. However this material was tested only during one vegetative cycle. Some induced restistance against leafroll-virus was reported by Rudorf & Wöhrmann (1963) in vM_3, which authors did not think much of mutation breeding as a method of practical value. Induction of earliness and increased starch production was reported by Tarasenko (1965) and Kukimura & Takemata (1975). Umaerus (1966) found tubers which were less discoloured after cooking. A promising mutant with shorter stolons was obtained by Roer (1967). This mutant could not be further utilized because this positive change was accompanied by cracks in the tubers.

Jauhar & Swaminathan (1967) induced shallow eyes and an improved tuber-skin colour in two important Indian cultivars. According to Upadhya (1976, pers. commun.) those clones unfortunately were lost. So-called 'hooded eyes' were found by Udai Singh (1970). Upadhya et al. (1974a) obtained day-neutrality in several Indian potato clones. Their efforts to induce resistance against bacterial wilt yielded no results. The same work has been discussed by Kishore et al. (1975).

Ferwerda, whose work unfortunately has been buried mainly in internal reports only (see van Harten, 1970 and later), demonstrated for several important Netherlands cultivars like Bintje, Désirée and Burmania that induced mutations may affect practically any plant character and may work in opposite directions, for example towards increased

pigmentation of the tuber skin as well as towards paler types. Several characters of economic importance like cooking quality, tuberflesh colour, eye-depth, underwater weight, stolon length, resistance against *Phytophthora* and leafroll-virus could be improved via mutagenic treatment. The experiments at the IvP were performed only to demonstrate the feasibility of the method and not to produce new cultivars. Resistance to leafroll-virus was induced in a few clones of cv. Bintje. They remained significantly more resistant during 7 successive years of testing in infected field plots, without showing any aberration of the Bintje phenotype. Thus desirable mutations are not always accompanied by pleiotropic effects or such effects can be eliminated by further selection without losing the desired genetic change itself.

At IvP some promising results were found, especially with radiation-induced higher levels of resistance to *Phytophthora*. To find definite proof for such effects is very tedious. Many people do not realize that the effect of soil and climatic conditions on field trials makes it absolutely necessary to repeat such experiments for 5 or more successive years. The same holds for yield trials, where in different years contradictory results may be obtained. So-called positive results from a study of only one or two vegetative generations are without value. A too short period of study may also account for the contradictory results of the early reports by, for example, Johnson (1928, 1937) and Sprague & Lenz (1929).

2.3.6 The frequency of induced mutations

In the first publication in which frequencies of induced mutations are mentioned, Asseyeva & Blagovidova (1935) described how they obtained 10 morphological mutations after X-irradiation of 390 tubers of cv. Wohltmann. Another cultivar, Epicure, yielded no visible mutations at all. At present the effect of the genetic constitution of the starting material on the mutation frequency seems to be sufficiently demonstrated.

An aspect that is normally neglected is the difficulty of comparing results of different authors, because the number of characters for which (assumedly) genetic changes are scored differs from author to author. At a dose of 2000-3000 R of X-rays, which usually yields the best results, Heiken (1960) found up to 37% of mutated plants in his most successful series. He considered the mutation frequencies induced in general high enough to justify further investigations. In Italy (Anonymous, 1963) mutation rates of up to 20% were reported after γ-irradiation. Further details are not available.

Very optimistic are the reports of Solomko (1962, 1965a,b) who claimed that only 4-8% of the induced changes are of an adverse nature. In some well-known Russian cultivars like Early Rose, Epron and Sedov even 17% were reported to be useful mutants. Some of them, according to Solomko, were suitable for direct practical use. Rudorf & Wöhrmann (1963), on the other hand, stated that the frequency of induced mutations is too low for practical purposes. This could be caused by diplontic selection, i.e. competition between mutated and non-mutated cells (see Gaul, 1959).

Asseyeva & Yashina (1968) concluded that 6 out of 200 induced mutations investigated had some practical significance. Only one of those 6 mutations was found in a cultivar of commercial value. The authors were rather pessimistic about their findings and added that the spectrum of artificially induced mutations is inferior to that of spontaneous ones. Whether one agrees with the Russian views or not, one thing is certain: if

mutation breeding in potato is to be applied in practice, many different mutations are necessary. If these can be obtained, the mutation method seems at least as promising as starting from many thousands of potato seedlings from which often only one or even no new cultivar at all is derived.

Efforts to induce high mutation frequencies have been described by Nayar (1969) and Nayar & Dayal (1970). By periodically removing and planting separately the sprouts which emerge from irradiated tuber halves, the frequency of vM_2 plants carrying mutations could be increased up to 50%. This is 16 times higher than the frequency found by Heiken (1960) and 3 times higher than that reported by Nayar et al. (1965). Kukimura (1972) reported on γ-irradiation of tetraploid true seed of potato with special reference to (monogenically inherited) stem colour. At a dose of 32 krad, Kukimura calculated for 4 characters a mutation frequency of 2.0-10.9 x 10^{-6} per rad. In an experiment with fast neutrons, van Harten et al. (1972) obtained up to 28% of visible mutants in vM_2. In another experiment with dihaploid potatoes (van Harten & Bouter, 1973) even up to 38% of visible mutants was scored after treating whole tubers with 3000 rad of X-rays at a dose rate of 1000 rad/min. From the above results one can conclude that the frequency of mutations that can be induced is not really the limiting factor with respect to the practical application of the mutation technique in potato breeding.

2.3.7 Chimeric structures and histogenic effects after irradiation

Vegetatively propagated plants, that carry mutations usually manifest themselves as rather stable periclinal chimeras after some cycles of vegetative propagation. For potato, the periclinal nature of both spontaneous mutants (Asseyeva, 1927) and induced ones (Heiken, 1960) has been established.

For an efficient use of mutations in breeding programmes the mutants should be in a solid (chimera-free) state. A method to produce high frequencies of solid mutants has been worked out (van Harten et al., 1972). In brief the method employs tuber-eye plugs from which the main eyes have been removed before irradiation with high doses and dose rates. The sprouts which develop from the eye-region after irradiation, are dissected (milked) and individually planted out. Especially the sprouts which develop about 3 months after irradiation, and which are mostly of adventitious origin, produce mutants which are practically 100% solid.

If a plant, for example a periclinal chimera for tuber-skin colour, is irradiated, cells of one of the histogenic layers may be destroyed and replaced by cells either from the same layer or from another one. In the latter case the replacement cells are genetically different and therefore the tuber skin may have a different, often sectorial appearance. If existing periclinal chimeras are used as starting material, these so-called histogenic effects after irradiation can be easily demonstrated. Such effects can be recognized already in vM_1.

The first example of visible histogenic effects, induced via X-rays in potato has come from Asseyeva (1931). She referred to work of Gusseva & Lopatin. Irradiation of so-called monochlamydous tubers (L-I mutated, L-II + L-III non-mutated) led to dichlamydous types (L-I + L-II mutated, L-III non-mutated). The author reported that similar effects were obtained after mechanical damage, although at much lower frequencies. The method can be used to detect the possible periclinal constitution of plants. Heiken X-irradiated 50 sprouting tuber halves of a spontaneous subdivided-leaf mutant with 4000

rad (Heiken & Ewertson, 1963; Heiken et al., 1963). In the vM_1, 5 plants showed one or more normal shoots. The high frequency of normal shoots, together with the fact that they were observed already in vM_1, indicate that the change was caused by histological effects. In practice the method of eye-excision of potato tubers is mostly used to detect chimerism.

Additional studies are necessary to determine the importance of these histogenic effects for different types of irradiation and to clarify relationships between mutagenic and histogenic effects of irradiation.

2.3.8 Concluding remarks and suggestions for further research

After the important contributions of Heiken and others around the sixties, new potato cultivars, obtained via the application of mutation techniques, seemed only a question of time. However, at present, more than 15 years later, only one cultivar, obtained in this way, has been released (cv. Konkei 45 with improved tuber-skin colour in 1973 in Japan). In addition Aleksahin (Anonymous, 1973) has reported that in the USSR some potato cultivars have been approved for use by farmers, e.g. one cultivar, obtained by Skarnikov after irradiating seedlings with 800 R of γ-rays, showing better resistance against *Phytophthora*. Technical problems often are said to account for the lack of success. Nevertheless the literature review shows, that high frequencies of mutations can be produced, which, if experimental conditions are properly chosen, can be obtained practically chimera-free. Up to now this task has been rather laborious and it seemed necessary to look for better and quicker methods. Additional data on the fate of a mutated cell in the apical area or at other places where regeneration can occur have to be collected. The behaviour of the irradiated apex itself also deserves further attention. Such investigations may also increase our insight into histogenic effects.

For practice it would be very useful if whole plants could be grown from single mutated cells, not only in order to overcome chimerism, but also to make a better use of the mutations which are induced in different parts or layers of the plant. The adventitious bud method, with buds of which the apex often arises from a single epidermal cell, has given positive results with several ornamentals. If this method could be developed for potato, it would be a simple rapid way to produce large numbers of solid mutants. It is also of considerable practical importance to know whether all cells of a shoot apex have an equal chance to mutate.

The tetraploid nature of the common potato leads to many complications from a breeding point of view. For general breeding work as well as for mutation breeding work, the use of di(ha)ploid clones would considerably speed up both fundamental and practical work. For mutation studies heterozygous test clones at the diploid level would be of much value.

Finally it seems nowadays that the effect of pleiotropism is less important than was anticipated in the past. Provided that high quantities of mutations can be induced, types without negative side-effects undoubtedly can be found.

In addition to technical problems there are, as was stated in the introductory chapter, a number of other reasons why mutation breeding in potato has not been successful up to now. First much of the research on mutations has never passed the stage of preliminary trials. Many experiments were conducted on a limited scale and only for a few vegetative

generations. It is indeed remarkable that plant breeders at scientific institutes as well as at private stations begin conventional potato breeding programmes with many thousands of crosses, whereas they expect miracles from the irradiation of, let us say, 200 potato tubers or eye-plugs.

For successful mutation breeding work it is essential to be realistic about objectives as well as the practical approach. As long as sufficient genetic variation can be obtained more easily or more quickly by other methods, the mutation technique should not be applied. But the failure in, for example, the Netherlands to produce a suitable substitute for the famous cv. Bintje for over 60 years, clearly demonstrates that masses of conventional work does not always lead to success. Mutation breeding should not replace initial potato crossings. The most promising approach is to apply the method in advanced stages of breeding programmes to cure certain, well-defined 'defects' in promising breeding material and in commonly accepted cultivars.

3 Production of a dihaploid potato clone with genetic markers

3.1 Introduction

In 1970 a programme for the creation of a dihaploid potato tester clone with genetic markers was initiated. The clone was to serve as basic material for all further mutation studies, such as the determination of mutation frequencies after different treatments, the study of differences in mutability per histogenic layer and the investigation of adventitious shoot production.

In early mutation experiments, for example with diploid self-fertilizing crops like barley or pea, (recessive) mutations (e.g. AA → Aa) were normally detected by selfing the M_1 and studying the successive segregating generation. The use of tester stocks, heterozygous for one or more genes, made it possible to determine the genetic effect of mutagenic treatments directly in the treated generation. Often colour genes were used. Early reports about the use of marker genes in mutation studies are from, for instance, Sparrow & Pond (1965) and Cuany et al. (1958a,b) for *Anthirrhinum majus*, Stein & Steffensen (1959b) for *Zea mays* and Gröber (1962) for *Lycopersicon esculentum*. At present many fundamental mutation studies are performed on stamen hairs of diploid clones of *Tradescantia* species, which are heterozygous for colour (see Mericle & Mericle, 1967; Ichikawa & Sparrow, 1968 and Ichikawa et al., 1969).

The common cultivated potato is tetraploid (2n = 4x = 48), so that comparable mutation studies can only be done if one could work with genetic characters which are present in simplex condition (e.g. Aaaa). Since these simplex characters occur at relatively low frequencies and, moreover, are difficult to identify as such, I decided to try and produce a potato clone at the diploid level by combining different dihaploids. Parallel with the definition of haploids, which are sporophytes with the gametic chromosome number, dihaploids are haploids which are derived from tetraploids (Kimber & Riley, 1963; Riley, 1974.) A suitable clone (2n = 2x = 24) should contain a number of well-known genetic markers in heterozygous condition. In addition, the clone should be vital, male as well as female fertile, healthy and easy to handle in the greenhouse.

Practically all potato dihaploids nowadays are produced from crosses between tetraploid *S. tuberosum* L. (the common potato) as female parent and the cultivated *S. tuberosum* Group Phureja (Dodds, 1962) or *S. phureja* (Hawkes, 1956, 1963) as pollinator, according to the method suggested by Hougas et al. (1958). Haploid embryos develop by parthenogenesis. The frequency of haploid production is affected by the female parent as well as by the pollinator (Hougas et al., 1964 and many others afterwards). At present monohaploids (n = x = 12) can be obtained by successive cycles of parthenogenesis (Van Breukelen et al., 1975). Anther culture, another method to produce haploids, has been rather successful for some wild *Solanum* species (Irikura, 1975;

Irikura & Sakaguchi, 1972), but much less so for *S. tuberosum* (Dunwell & Sunderland, 1973).

3.1.1 Genetic markers in potato

Our knowledge of potato genetics, especially at the tetraploid level, is still limited and only a few suitable, well-defined genetic markers are available (De Jong, 1971). Although up to now 75 potential genetic markers have been reported for potato (Kessel, 1972), most of them are not suitable for genetic or mutation studies for different reasons. A practical complication is the absence of a standard nomenclature for potato genes (Howard, 1970a). The inheritance of tuber, sprout and flower colour in tetraploid potatoes was studied by Lunden (1937, 1960, 1974). He described a number of genetic factors.

Van Harten et al. (1973) analysed the nature of an induced mutation for leaf shape, indicated as 'ivy leaf' (*Il*), in cv. Burmania. The *Il* clone, obtained in 1961 after X-irradiation of tuber pieces with 2 krad, was studied for many years. The *Il* character, which is present in all three histogenic layers of this (tetraploid) clone, must be caused by a (rare) mutation towards dominant. (N.B. Another explanation: the occurrence of a recessive mutation for an inhibitor gene, present in heterozygous condition, must be rejected, as many selfings in normal-leaved cv. Burmania never produced any *Il* plant). Deviations were observed from normal Mendelian segregation ratios. If there is no chromatid assortment ($\alpha = 0$), a system with five complementary genes is needed to explain the observed deviations. For one of these genes a mutation from nulliplex towards simplex must have occurred. If α reaches very high values (e.g. 1/8), only one gene suffices to explain these deviations.

In recent years an increasing number of clones at the diploid level (dihaploids, cultivated diploids, wild diploids and different crosses) have become available, which has led to a considerable speeding up of the collection of genetic data for potato. Nevertheless, only a few linkage groups are recognized at present and most of the known genes cannot yet be assigned to specific chromosomes.

Hermsen et al. (1973), using trisomic analyses, localized a single recessive mutant gene (*v*) for light green plant colour on chromosome 12 of dihaploid potato material. Dodds & Long (1955, 1956) studied the inheritance of colour in diploid cultivated potatoes, mainly *S. rybinii* (now *S. tuberosum* group Phureja). Gene *P* was found to control the synthesis of blue pigment and to act epistatically to gene *R* which controls the synthesis of red pigment. The authors further established a linkage group for the already known genes *B*, *I* and *F*. Gene *B* is a localization factor, gene *I* controls the distribution of pigment in tuber skin and stem and gene F controls pigmentation in flowers (additional data are given in Section 3.2).

Recessive genes for yellow margin and narrow leaflet in *S. rybinii* were studied by Dodds & Paxman (1962). Simmonds (1965) described 12 spontaneous mutants in cultivated diploid potatoes of the Groups Phureja and Stenotomum, such as yellow margin, narrow leaflet, leaf-roll type, curled leaf, crumpled leaf, droopy and shorty. The inheritance of most characters is unknown. Only four mutants reached the flowering stage and only 'droopy' could be used in crosses. Recently De Jong (De Jong, 1971; De Jong & Rowe, 1972) screened large populations for marker genes. These populations were ob-

tained via selfing of hybrids from crosses between diploid cultivated potatoes and dihaploids. Possible new markers were searched for and others described earlier were submitted to additional investigations. The gene *Ow* (controlling the pigmentation of the ovary wall) could be linked to the *B-I-F* linkage group. Another major gene that controlls tuber shape could be associated with the same group. Studies were also made of genes controlling the synthesis and distribution of anthocyanin, of other possible chemogenetic markers and of other genes involving morphological and physiological characters. Kessel (1972) and Kessel & Rowe (1974) described the development of interspecific and intraspecific aneuploids from triploid-diploid crosses for the localization of genes on chromosomes. The inheritance of some previously unknown traits was studied. The single dominant gene *Pw* (pigmented whorl) could be linked to the *F-Ow-I-B* linkage group. Another single dominant gene *Ul* (underleaf pigmentation) could not be linked to any known group.

Finally, in the diploid wild species *S. chacoense* a gene for albinism was reported by Lam & Erikson (1971).

3.1.2 Potato dihaploids in mutation breeding

Only very few examples of the use of potato dihaploids in mutation work are known. Acting on a suggestion by Hougas & Peloquin (1958), Dommergues (1962) referred to such a clone under investigation at his institute, but this work does not seem to have been continued.

In 1964, Ferwerda (see van Harten & Bouter, 1973) irradiated tubers of 125 different clones of (primary) dihaploids and secondary dihaploids (i.e. crosses between primary dihaploids) with 2 and 3 krad of X-rays. Rather surprisingly dihaploids recovered from direct physiological damage practically as quickly as normal tetraploids. The frequency of visible mutations, determined by studying six plants per irradiated clone, remained low (5.5% for the 2 krad series and 14% for the 3 krad series).

Van Harten & Bouter (1973) irradiated a dihaploid clone of cv. Désirée with 3 krad of X-rays. In this experiment 150 whole tubers were treated with a dose rate of either 50 or 1000 rad/min. Mutation frequency and percentage of uniform mutants were determined in vM_2. Each series consisted of more than 600 clones. Per clone three plants were studied. The 1000 rad/min series produced mutated plants at a frequency of 32% compared with 26% for the 50 rad/min series. The difference between both series proved to be statistically significant in a 2 x 2 contingency test ($\chi^2 = 5.23; P < 0.025$). Depending on when the emerging sprouts were dissected, up to 67% of uniform mutants were obtained.

3.2 Material and methods

In 1970, five different sources of diploid or dihaploid material were available. These were studied for their suitability as test material. Four sources, designated IvP48, 70R, 69G and Tester III, were obtained from Hermsen of IvP. A fifth source, DBK, consisted of a few dihaploid plants extracted from the ivy-leaf mutant in cv. Burmania (van Harten et al., 1973).

IvP48 is a Phureja clone derived from a sib-cross between F_1 plants from PI 225702-2

x PI 225682-22. This clone is rather vital and fertile, and homozygous for the loci *P*, *B* and *I*. 70R refers to a group of dihaploid plants extracted in 1970 from cv. Radosa. In this group the gene R_3, which determines monogenic resistance to late blight (*Phytophthora infestans*), is present in an heterozygous condition in 50% of the Radosa dihaploids. The genetics of other characters of this group such as leaf and stem colour are unknown. Some clone numbers like 70R110 had a lighter leaf colour than normal. 69G indicates a group of dihaploids from cv. Gineke, which, according to Hermsen (pers. commun.) very probably contain a single recessive gene for light green leaf in homozygous condition. Tester III plants were derived from a cross between a (diploid) plant of *S. tuberosum* group Stenotomum and a dihaploid clone introduced from the USA (code US-W4). Via sib-mating some colourless plants, probably recessive for colour genes, were isolated.

The different gene symbols used throughout this publication stand for the following characters:

P – A factor controlling the synthesis of blue (or purple) pigment. *P* is epistatic to another colour factor *R* that controls the synthesis of red pigment (N.B. Factor *R* has not been considered in this publication).

I – A factor complementary to *P*, controlling the distribution of pigment in tuber skin and stem, *ii* suppressing tuber pigmentation.

B – A multiple allelic factor controlling the localization of anthocyanin at the base of the cotyledons and of all plants parts which are homologous to cotyledons like leaves, leaflets, tuber scales and flowers. *B* is only expressed if the gene *P* (or *R*) is present. In our work only the highest allele B^d is used. Lower alleles of *B* display more restricted pleiotropic effects.

R_x – (Assumedly) a factor determining monogenic resistance to all races of late blight except race *x*. The resistance is based on a hypersensitivity reaction (N.B. Although the use of the symbol R_x for late blight resistance genes is strictly speaking incorrect, we have used it throughout the work).

V – A factor controlling the green colour of the plants. In recessive condition (*vv*) a light green colour can be observed already in the young seedling. The leaflets are undulate oval and the growth vigour is less than in normal green plants.

Y_m – A factor leading in recessive condition to leaflets with yellowish margins and to plants which often remain small.

To combine as many potential genetic markers as possible into a test clone for general use, in 1970 the double cross (IvP48 x DBK) x (70R x 69G) was planned. As the results show, this goal was not achieved. Therefore many other crosses were made, most of them being repeated several times. As soon as certain crossing products looked promising from the point of general growth, health, vitality, fertility, penetrance of marker genes etc., they were submitted to different tests. Special attention was paid to the capacity of selected clones to reproduce vegetatively from different plant parts. In a number of cases test crosses were made to find out whether the marker genes indeed were heterozygous. Promising clones were propagated and submitted to different X-irradiation treatments to establish radiosensitivity and mutability, the latter especially of the different marker genes present. Several other plants with possible markers were tested like a clone of cv. Spartaan with physiological curling (black veinal necrosis) of the leaves. They all had to be rejected for different reasons like e.g. very poor fertility, a high amount of anthocyanin, etc.

A short description is given of the test for late blight, developed by Toxopeus (1954) at IvP. In this test, referred to as the detached leaflet test, potato leaflets are selected. The leaflets are put upside down on a frame with nylon netting, placed in a tray with wet blotting paper at the bottom. The leaflets are wetted with distilled water and then sprayed with suspensions of zoöspores of the desired physiological race of *Phytophthora*. A suspension contains about 50 zoöspores mm^{-3}. After inoculation a plastic cover is put on top of each tray. The closed trays are wrapped in a plastic bag which is sealed and kept at 15°C. After five days all leaflets are scored for lesions and sporulations, using a scale from 0 (no sensitivity) to 4 (hypersensitivity). Normally a cultivar without *R* genes like cv. Bintje is used as control. In the present case dihaploids of cv. Radosa were scored for the presence of R_3 genes. Inoculations were done with two different physiological races, i.e. race 1,3,4 and race 1,4. Leaflets heavily affected by race 1,3,4 and unaffected by race 1,4 were selected.

3.3 Results and comments

In 1970, 576 crosses were performed between the five prospective parents. Some positive results were obtained from the single cross 70R110 x IvP48, from which among others one clone, 71A8, was obtained. Clone 70R110 was one of the few flowering dihaploid clones of cv. Radosa and contained almost no anthocyanin. Moreover, the leaves were relatively light green. Crosses between 70R110 and IvP48 yielded one berry with five seeds only.

In total, 117 independently produced dihaploids of cv. Radosa were tested in a detached leaflet test for late blight in 1970. Forty-nine plants were not affected at all (score 0) against 68 plants showing from very light sensitivity to severe symptoms (score 1 - 4). The data obtained do just not fit a 1 : 1 ratio.

3.3.1 Clone 71A8

Although from the start, we recognized that clone 71A8 had some unfavourable characteristics, this clone was studied, because few prospective parents were available for further crosses. Investigations showed that this clone (with a proven genetic constitution *Bb-Ii-Pp-*R_3r) was unsuitable because it had inherited some unfavourable traits from its parent IvP48, such as sensitivity to attacks from mildew and spidermite in the greenhouse. In addition, the clone showed rather poor growth, vegetatively as well as generatively. Efforts to produce adventitious shoots from tuber slices, stem internodes, leaf petioles, etc. were not successful. In 1972, some flowers could be obtained after grafting on tomato but all flowers were male and female sterile.

In the same year tubers of Clone 71A8 were exposed to a number of preliminary irradiation treatments with 1, 2 and 3 krad of X-rays at dose rates of 50 and 500 rad/min. For each treatment only 20 small tubers were available. Unexpectedly the clone was very radiosensitive and all tubers of the 2 and 3 krad series died. In none of the surviving plants were mutations for the marker genes observed. Moreover, the general mutation frequency remained low (< 5%).

Clone 71A8 was also subjected to the detached leaflet test for late blight. The results of this work, after several repetitions with increasing numbers of leaflets, remained com-

pletely incomprehensible and controversial. In the beginning it was thought that the results were caused by inadequate experimentation, but later investigations revealed that the Radosa material was completely unsuited to our purposes. According to Dutch *Phytophthora*-specialists, the effect of gene R_3 most likely was obscured by the presence of other *R*-genes (possibly R_{10}). Also, a certain amount of uniform (horizontal) resistance was said to affect the original black/white pattern observed in the Radosa-plants.
As it was our aim to investigate mutations in potato and not fundamental problems of disease resistance, we decided to terminate the study of this problem and to look for another tester to replace 71A8. The choice of new testers remained rather limited. For the ivy-leaf mutant, up to 1972 only 12 dihaploids could be produced. These plants seldom produced flowers and, if they did, the flowers aborted, so that it was impossible to use them for further crosses or for genetic analysis.

3.3.2 Clone 72F263

In 1972, Hermsen established by trisomic analysis (Hermsen et al., 1973) that the light green leaf colour of Tester III, one of the prospective parents in the 1970 programme, showed a monogenic recessive inheritance (designated *vv*). Tester III was found to be also recessive for all genetic markers mentioned before. Crosses were attempted between Tester III and IvP48. IvP48 was selected again, despite its sensitivity to spidermite and mildew, because of the marker genes that are present in this clone. In addition to the established factors, *P*, *B* and *I*, the gene *F* was studied as a possible marker in Clone IvP48. This gene, which governs the distribution of pigment in the flower corolla, produces blue flowers when dominant and flecked flowers in an homozygous recessive condition.

F_1 plants of the cross Tester III x IvP48 have the genetic constitution *Bb-Ii-Pp-.f-Vv*. As the result of a later irradiation experiment demonstrated, most probably the recessive character yellow margin, indicated as y_m (Dodds & Paxman, 1962; Simmonds, 1965), was also present in heterozygous condition. From this cross 400 seeds were obtained. From the resulting seedlings 120 could be grafted on tomato. Continuous selection for earliness, tuber production, flowering, seedset, penetrance of marker genes, etc. finally reduced the number of potential testers to only four, indicated as Clone 72F13, 72F39, 72F51 and 72F263.

Cuttings of those four clones were X-irradiated to check radiosensitivity and mutability. Cuttings were used, because it was thought that more uniform treatments could be given to cuttings than to tubers. This method has certain advantages above the irradiation of tuber eyes e.g. irradiation of cuttings can be done at any time of the year and large numbers of cuttings can be irradiated which is very useful if tuber set is low.

All four clones displayed a rather high radiosensitivity. Clone 72F13 and 72F263 showed the highest rates of survival. Clone 72F263 (from Tester III plant 4 x IvP48) was considered more suitable than Clone 72F13 because of the better penetrance of the marker genes, especially in the progenies of selfed plants. Tuber set was rather disappointing and mutation rates in general remained very low. For example, no mutations were found for the character embryo spot after exposing top cuttings and axillary cuttings of Clone 72F13 to 2.5 and 3.5 krad X-rays at a dose rate of 50 rad/min. Per series 80 cuttings were used. An additional disadvantage of Clones 72F13 and 72F263 was again

their susceptibility to spidermite and mildew, which attack especially under less favourable conditions. Another drawback was the abundant vegetative growth of these clones in the greenhouse.

In 1975 and 1976, mutability and radiosensitivity of Clone 72F263 was studied again. In this experiment, performed in co-operation with B.J. van der Knaap, former student of plant breeding, doses up to 4 krad of X-rays and dose rates up to 150 rad/min were applied. Stem cuttings with a dormant axillary eye were irradiated. During exposure the basal part of the cutting was inserted in a piece of lead to keep physiological damage as low as possible. The foliage part was also protected by a lead shield. Cuttings were either irradiated in 'dry' or in immersed conditions.

Of the 1427 plants grown by taking cuttings from 784 irradiated stem cuttings, 169 (i.e. 11.8%) were mutated; 73 (5.1%) were mutated in the foliage part. Ten types of foliage mutation were recognized. Mutations in the subterranean part, which mostly manifested themselves as chimeric stripes were found in 102 plants (7.3%). In total 2.9% of all tubers produced were mutated in one way or another. Practically no mutations were observed for the heterozygous marker genes. Only one mutation for yellow margin (gene Y_m) was obtained. As compared with other mutation experiments (see Chapter 2), the mutation frequency of this diploid clone remained very low. No explanation can be given for this result. Higher doses could not be applied because of the high radiosensitivity of this clone. It was found that immersion of the cuttings during irradiation leads to a mutation frequency that is somewhat lower than with normal 'dry' irradiation (about 12% versus about 15%). Immersed cuttings, on the other hand, gave a better rate of regeneration. In contradiction to earlier findings (Nayar & Dayal, 1979; van Harten et al., 1972), the sprouts which were dissected in a later stage had a lower mutation frequency than the ones developed earlier. Hence Clone 72F263 was not a very suitable candidate for further investigation.

3.3.3 Clone M1178

In 1972, the male sterile, dihaploid Clone M1178 of cv. Maritta, carrying the monogenically inherited factor R_1 for hypersensitivity resistance to all races of late blight except race R_1, was added to the test programme as a possible parent. This clone was free of anthocyanin and carried, as far as was known at that time, only recessive genes for embryo spot. In addition it seemed sufficiently resistant to mildew and spidermite and produced reasonable numbers of tubers.

In 1973, cuttings were X-irradiated with either 2.5 or 3.5 krad at dose rates of 50 and 300 rad/min, respectively. Eighty cuttings were used per series. In vM_2, screened in 1974, only two positive cases of mutations from $R_1 \rightarrow r$ were found after submitting leaflets to the detached leaflet test. In a second irradiation experiment in September 1973 with ten series of 50 cuttings, somewhat higher doses of X-rays were used (3 or 4 krad). Few plants survived (i.e. 33) for a culmination of reasons: adverse seasonal effects, a heavy attack of aphids and whitefly in the greenhouse, (and a subsequent heavy spray) and the high dose of irradiation. Only one plant of the 4 krad treatment was found to be mutated from $R_1 \rightarrow r$. In a third irradiation experiment, late in 1973, 2, 3 and 4 krad were administered to three series of 30 cuttings each. No mutants from $R_1 \rightarrow r$ were found at all in vM_1.

Additional crosses between dihaploid plants of cv. Maritta and other diploids and dihaploids revealed that the Maritta material most probably contains one or more complementary genes for colour inheritance.

3.3.4 73G and 74H series

In 1972, Clone M1178 and 72F263 were crossed. In this way we tried to increase radioresistance and resistance to several diseases by introducing tuberosum genes into Clone 72F263. From the seedling population obtained (indicated as 73G), plants with nodal band, dark-green leaf and the R_1 gene were selected. Unfortunately, all these plants had inherited male sterility and only a very low level of female fertility of Clone M1178. Therefore the selected 73G numbers were crossed again with plants of Tester III, which are rather fertile in both directions. From this progeny (indicated as 74H) again plants carrying nodal band, the R_1 gene, normal green leaves and coloured tubers were selected (assumed genetic constitution *Bb-Ii-Pp-V.-*R_1*r*).

Grafts were made on tomato to study fertility and vitality and for crossing. Crosses were made in spring 1975 but the adverse dry and hot weather and the necessity of spraying against whitefly, mildew and spidermite caused such a delay that the season was over before most plants had reached the flowering stage. Seeds were not produced. In the meantime the 74H material was tested for R_1 resistance. When results of five different series were pooled, 381 leaves were infected whereas 373 were resistant. Results were well in accordance with the expected 1 : 1 ratio. When considered separately, the results of some groups considerably deviated from the expected ratios, but these series were relatively small and have to be investigated again.

It seemed worthwhile to continue work on the 74H series. Among the selected numbers, one clone indicated as 74H-6-30 was grafted in 1975 and flowers were obtained. After this clone was crossed with 72F263, many seeds were produced. One promising clone, indicated as 75-34-20, was crossed in 1976 with six different test clones. The same crosses were made with Clone 74H-6-30. In March 1976, 420 cuttings were submitted to 2, 3 or 4 krad of X-rays and a dose rate of 50 and 500 rad/min. A check for late blight mutations in April inconclusive results. Mutations for genes determining embryo spot were not observed at all and the percentage of morphological leaf mutations remained below 1%.

3.4 Additional remarks

Despite considerable effort, the results of our programme to develop a suitable diploid tester clone in time for mutagenic studies in potato, have not been very positive. We realized in 1972 that our aims and expectations may have been too high. Therefore we decided not to wait any longer and to start the intended experiments on the behaviour of irradiated shoot apices, layer sensitivity, etc., with other suitable material. The search for a proper tester clone continued until 1977 although at a reduced intensity.

4 Formation of adventitious roots and shoots from potato leaves, leaf parts and stems in vivo

4.1 Introduction

4.1.1 Scope of investigations

As a part of our efforts to improve methods of mutation breeding in potato, we tried to develop a suitable method for adventitious shoot production in vivo. Such shoots (literature also speaks of buds and sprouts) by preference, should be of single cell origin. We decided to concentrate on methods in vivo because of the promising results obtained with several plant species, especially when leaf petioles were used. In addition little was known about the techniques in vitro when we started.

Studies by Howard (1964a) and Miedema (1967) had already revealed the multicellular (and endogenous) origin of adventitious shoots induced on potato roots. The ability of potato tuber tissue to regenerate adventitious shoots had been studied by Miedema (1973b) and others. Adventitious shoots from leaf petioles often are of epidermal origin and ultimately may arise from a single cell. Then mutants are (practically) chimerafree.

Many mutations of potential interest, which have been induced in plant cells, may remain unobserved. For example a mutation for increased yield has no practical significance if it is confined to cells of L-I only. If a complete plant could be grown from such a mutated cell, this mutation could manifest itself. This is an additional reason to investigate adventitious shoot formation from (epidermal cells of) leaf petioles and, possibly, from stems of potato.

Plants of epidermal origin can also be used for the investigation of suspected periclinal chimeras. Klopfer (1965a) demonstrated that the tissues of the potato plant can be traced back to three histogenic layers (L-I, L-II, L-III). Methods are available to isolate tissues derived from these layers.

For some characters, L-I constitution can be studied directly, for example when studying tuber-skin colour, chlorophyll mutations, chromosome numbers and the like. According to Howard (1964a, 1967), treatment with X-rays leads to replacement of L-II by L-I tissue in 5-10% of the potato plants studied. He claimed that radiation treatment is useful when both L-II and L-III are mutated and when the constitution of L-I is unknown. The method can not be used when L-III is normal and L-II is mutated. This method, which was also used by some other investigators like Péreau-Leroy (1975), was criticized by van Harten (1972), because several unnecessary sources of error are introduced in this way. In my opinion, if possible, inducing adventitious buds on L-I, in vivo or in vitro, is to be preferred.

The genetic constitution of L-II can be investigated by generative propagation, because both egg cells and pollen can be traced back to the sub-epidermal layer (L-II). This test,

indicated as the Baur test (Baur, 1910), is difficult to apply in potato because of flower biology and genetics.

Following the method demonstrated by Bateson (1916, 1921), Howard (1964a) and Miedema (1967) induced adventitious shoots on potato roots, which are of endogenous origin and therefore reveal the nature of L-III. The same information, although somewhat less reliable, can be obtained in potato via de-eying of tubers. This method is commonly referred to as the Asseyeva method (Asseyeva, 1927, 1931), although it was mentioned by Rechinger (1894). According to this method adventitious shoots develop at the cut surface of the tuber pieces, mostly on the vascular ring or in the eye-holes. The appearance of shoots is normally preceded by a callus phase.

Some findings from the literature on the formation of adventitious organs and the use of such shoots in mutation breeding are discussed in the two following sections of this introduction.

4.1.2 Formation of adventitious organs

The occurrence of adventitious organs is ascribed to disturbances of internal regulations (Brand & Venverloo, 1973), e.g. when normal buds are absent or non-functioning. Primordia of such organs can arise in the cambium, in wound callus or, without an intermediate callus phase, in differentiated tissues (Priestly & Swingle, 1929; MacDaniels, 1953). According to Torrey (1966), organogenesis starts in a group of undifferentiated cells, indicated as 'meristemoid'. Either roots, shoots or embryos arise, depending on the type of organogenic stimulation.

It has been known for a long time that differences in regeneration capacity (i.e. strictly speaking the capacity to produce new parts after removal or inactivation of the corresponding parts elsewhere in the plant) exist between different species and, within species, between different stages of development or different plant parts or tissues. Roots and shoots may originate from different plant tissues. In general roots are produced more easily than shoots. Root or shoot development within one species may be favoured by difference in age of the starting material. It is known in practice, for example, that young leaves of *Begonia* spp. produce more roots but less shoots than older ones.

Gorter (1968) distinguished the following stages: Dedifferentiation and meristem formation, determination and growth. A further division of the stage of determination, namely organization of the dedifferentiated cells into an organised meristem, followed by determination to either root or shoot primordium, is suggested. The cause of dedifferentiation (or initiation of divisions) must be the polarity of the cells involved and refers to the important role of the auxin content with respect to the different stages of root formation. Venverloo (1973) distinguished the following steps: initial cytological changes, first mitoses and cytokinesis, further divisions and formation of a group of meristematic cells, primordium formation and functioning of the apical meristem.

There are still many uncertainties about the different steps of the process. For a long time the idea prevailed that roots form before shoots (Hagemann, 1932; Went, 1938) and that adventitious shoots only develop from rooted plants or plant parts (see e.g. Lindemuth, 1903a,b). This may be true for some species like *Peperomia sandersii* (Harris & Hart, 1964), but is certainly not always the case. Dore (1955) found for roots of horseradish *(Armoracia rusticana)* that either a shoot or a root could arise from a meristem,

developed after dedifferentiation, but not very much evidence is available for other plant species.

Cultivars of several genera like *Begonia*, *Streptocarpus* and *Saintpaulia* very easily produce adventitious shoots, but other plants like potato, tomato or members of the fam. *Gramineae* are much more reluctant to do so. Many attempts have been made to promote adventitious shoot formation in different plant species and find a quick, practical way of vegetative propagation for crops of economical importance, especially the ornamentals. It is often difficult to prove that shoots are really of adventitious origin and not from overlooked buds in or near the axils of leaves.

Broertjes et al. (1968) listed over 350 plants that produce adventitious shoots and subsequently whole plantlets, on different parts of detached leaves. The authors said that this list is far from complete. Following Winkler (1903), they distinguished three types of 'new formation' according to position: (1) regeneration at the petiole base or cut end of the leaf blade, (2) regeneration on other leaf parts, (3) regeneration on roots which are formed from the detached leaf. The last group in fact are root buds. Group 1 is most common.

4.1.3 Adventitious shoots in mutation breeding

Adventitious shoots are very useful for mutation breeding (Sparrow et al., 1960; Broertjes, 1969, 1972a; Broertjes et al., 1968 and many others). First of all, mutations which occur in, for example, extra-apical cells and which would normally be lost, can be retained if there is a proper adventitious shoot method. Such mutated cells could then be transferred into solid mutants. A second advantage is that by using adventitious shoots in mutation breeding programmes, results of commercial value can be obtained in a remarkably short time, as Broertjes demonstrated with cultivars of *Streptocarpus*, *Achimenes* and *Kalanchoë* (Broertjes, 1969, 1972b; Broertjes & Leffring, 1972). Another advantage is the high mutation frequency obtained via irradiation of detached plant parts and subsequent application of the adventitious shoot technique. Broertjes (1969) reported that 30% of all adventitious shoots from irradiated *Streptocarpus* leaves were mutants. The author explained this high frequency by assuming that irradiation of adventitious shoots leads to considerably less diplontic selection than when shoot apices, which normally consist of more cells, are used. A further point in favour of working with adventitious shoots is that sometimes 100% of solid (i.e. non-chimeric) mutants can be obtained, probably of single-cell origin.

Naylor & Johnson (1937) demonstrated that adventitious shoots, initiated at the petiole base of cut leaves, are of single-cell descent. The same result was found for many other plant species. An additional advantage of epidermal cells of petioles is that such cells are roughly all in the same stage of mitosis (G-I). This synchronization facilitates the reproducibility of mutation experiments.

Not all shoots derived from epidermal cells trace back to one cell only, as for example Hülsmann (1936) concluded from experiments with leaves from *Begonia rex*. If more initial cells are involved, they are not necessarily identical and, as a consequence, chimeras may arise. This has been demonstrated very convincingly via the formation of adventitious shoots in (periclinally chimeric) graft hybrids of *Solanum* spp. (Winkler, 1907; Günther, 1957, 1962; Brabec, 1960). In addition Stewart & Dermen (1970a) showed

with periclinally chimeric plants of *Chrysanthemum morifolium* that adventitious shoots from stems originate frequently, if not exclusively, from two or more cells, and that these cells may derive from two or more different apical layers.

Besides adventitious shoot formation in vivo, also methods in vitro can be used, the sources being explants of leaves or flowers, anthers, embryos, calluses, etc. The essential difference between both methods is that for work in vitro aseptic conditions are created and maintained.

Methods in vitro are becoming increasingly popular in different fields of scientific work, including mutation breeding (Devreux, 1973). The detection of induced mutations in vitro remains a practical problem. Another drawback of many cultures in vitro is their genetic instability, probably because they lack the controlling mechanism, present in organized plants or plant parts (see e.g. Partanen, 1963; Melchers, 1965; Sacristán, 1971; Bennici, 1974; Heyn et al., 1974; Murashige, 1974; D'Amato, 1975; Irikura, 1975). Thus chimeric structures may arise. The best way to induce mutations is to treat single-cell cultures, although even this method does not completely exclude chimera formation (see e.g. Cooper et al., 1964, who worked with single-cell clones, isolated from crown gall callus of *Nicotiana tabacum*).

4.2 Factors affecting adventitious organ formation and further differentiation

Many factors are reported to influence adventitious organ formation. However, there are diverse opinions as to the relative importance of these factors. An explanation may be that not all plant parts, species and stages of the process have the same requirements. In addition unknown factors undoubtedly play a role. Much useful information has come from breeders and growers who vegetatively propagate e.g. cultivars of *Begonia, Azalea, Hibiscus* and several Gesneriads on a commercial scale. Another source of information is the work in vitro, where conditions can be well controlled. Then the effect of specific treatments can be distinguished more clearly.

Care must be taken when applying findings of work in vitro directly to systems in vivo. In general it is much more difficult to direct processes in vivo than in vitro, probably because the internal hormonal balance has more control in larger plant parts. Moreover, much younger plant parts can be used for work in vitro, which favours regeneration.

Three major groups of factors influencing the formation of adventitious organs, can be distinguished: (1) factors related to the mother material, (2) hormonal and nutritional conditions, (3) climatic conditions.

4.2.1 Mother plants and starting material

Genetic differences may strongly affect the capacity of plants or plant parts to regenerate, in nature as well as under artificial conditions (Hagemann, 1932; Broertjes & Leffring, 1972; Miedema, 1973b). Differences not only exist between plant families (e.g. *Gesneriaceae* versus *Gramineae*) but also between different clones or cultivars, probably because of the relative amounts of internal growth hormones present in different families or cultivars.

The age of mother plants and starting material also plays a role. Young, but full-grown leaves generally regenerate better than older ones, as Heide (1965b) showed for *Begonia*

leaves. Young leaves often quickly rot and then die off. Other points of importance are the general vigour and the nutritional condition of the mother plant. Conditions under which the mother plants have been cultivated may play a role. For example Appelgren & Heide (1972) found for *Streptocarpus* spp. that the formation of adventitious organs was stimulated by exposing the mother plants to relatively low temperatures.

4.2.2 Hormonal and nutritional conditions

The plant regulators auxins and cytokinins are the most important for regeneration processes and organogenesis. Literature has been reviewed by, among others, Van Overbeek (1966), Helgeson (1968), Galston & Davies (1969), Heide (1972) and Weaver (1972). Reliable information on the endogenous contents of growth hormones in plants and plant parts is scarce and difficult to collect. They differ between species, plant parts, external conditions such as temperature (Heide, 1964, 1965b) etc. It seems logical to assume that the hormonal requirements of the different steps of organogenesis of adventitious organs differ as well (Reinert, 1972).

Auxins are for instance required for cell elongation and for cell proliferation. Cytokinins can induce or enhance cell divisions, callus formation and further differentiation of organs. They often counteract effects of auxins (Miller et al., 1955; Skoog & Miller, 1957). In general high auxin concentrations favour root formation and inhibit shoot formation. On the other hand a small addition of auxin may strengthen the stimulating effect of kinetin on shoot formation (Heide, 1965b). The stage of development may affect the role of growth regulators. Wickson & Thimann (1958) and Sachs & Thimann (1967) found that application of kinetin to lateral buds released the inhibitory effect of auxin on their development. Once these buds had started to grow, auxin also stimulated growth.

The ratio auxin: cytokinin has been reported to determine the type of differentiation (Skoog & Miller, 1957). Mostly a high ratio of auxin: cytokinin stimulates root regeneration and inhibits shoot formation, whereas a low ratio causes the opposite effect (see e.g. Heide (1965b) for *Begonia* x *cheimantha*). In other cases, however, auxins may be required for shoot formation (Harris & Hart (1964) for *Peperomia sandersii*; Appelgren & Heide (1972) for *Streptocarpus*). Sometimes auxin stimulates shoot formation when cytokinin is present (Nitsch (1968) for *Plumbago indica*). Miedema (1973b) was unable to correlate varietal differences in shoot formation capacity of rooted tuber slices of potato with various levels of cytokinin activity in root extracts.

The role of other regulators like gibberellins, abscisins or ethylene in regeneration and organogenesis is not completely understood. Mostly they inhibit root formation and stimulate shoot development, but the opposite may also occur, depending on the concentrations. Ethylene sometimes depletes the endogenous auxin level and increases bud formation.

It is a well-known fact that the percentage of rooted cuttings is strongly affected by, for example, the composition and pH of the rooting medium, but detailed information is lacking. The effect of the nutritive condition on adventitious shoot formation under conditions in vivo has hardly been investigated. Again Miedema (1973b) showed the necessity of mineral nutrients (especially potassium and nitrogen) in the rooting medium for shoot formation from tuber slices of potato.

4.2.3 Environmental conditions

Information on the effects of environmental conditions is scarce and sometimes controversial. For leaves of *Begonia* spp., Rünger (1959), Heide (1964) and Hilding (1974) found that a relatively low temperature favours adventitious shoot formation, whereas root formation is favoured by higher temperatures. This result was confirmed for cultivars of *Streptocarpus* by Appelgren & Heide (1972). Short days had a positive effect on shoot formation from leaves of *Begonia* x *cheimantha* (Heide, 1965a). This method is used on a commercial scale to increase the multiplication rate of such leaves. A change in climatic conditions, e.g. higher temperatures, may affect endogenous auxin/kinetin balances (Heide, 1964).

Dark treatment of detached leaves of *Begonia* spp. for 2-10 days strongly counteracts the inhibitory effect of high temperature on shoot formation. Rooting capacity decreases under such conditions, very probably because of a lower auxin level (Heide, 1968). Not only the daylength, but also the light intensity may be of importance.

In practice, certain periods of the year are known to be more favourable for the production of adventitious shoots than others. The physiological background of this effect is not exactly known. Undoubtedly there are relations between the effect of climate, the stage of development of the plant and the actual stage of regeneration.

Finally, it has been known for many years from experiments with low doses of radiation (e.g. X-rays), that such doses can influence the physiological state of the treated material, for instance, by promoting growth (see e.g. Sax (1963) for a review). In some cases the low-dose effect was shown to be caused by interference with the synthesis of growth hormones. Especially in Eastern European countries much work is done in this field.

4.3 Formation of adventitious organs in potato

4.3.1 Literature on experiments in vivo

Literature on this subject was carefully reviewed by Miedema (1973b). Only some important findings of earlier date, together with a few new references, are summarized here. First Rechinger (1894), and subsequently many authors reported that adventitious shoots can be produced from de-eyed tubers or tuber pieces. Normally this process is preceded by a callus phase. The (relatively few) adventitious shoots predominantly develop at the vascular ring of tuber slices or in eye-holes. Differences in regeneration capacity between cultivars exist, as Miedema (1973b) found when testing tuber slices of 40 cultivars for auxin-induced rooting capacity. Rooted tuber slices easily produce adventitious shoots in moist soil (Miedema, 1967), but the stimulatory effect of roots on shoot formation could not be explained.

Of special interest are the results obtained with stems, stem parts, leaves and leaf parts. Kupfer (1907) referred to early work of Knight, performed in 1816, with potato leaf cuttings planted in soil. Knight only observed some swellings of the petiole bases whereas Kupfer found swellings and roots. Moreover, Kupfer once obtained a tuber from a shoot. As in this specific case not roots or swellings were present, it was thought that shoot formation and root formation are antagonistic processes.

Isbell (1931) repeated Kupfer's experiments with leaf cuttings. Within three weeks a number of cuttings had produced roots. In one case a tuber developed near the base of the petiole. This observation was explained by the presence of an overlooked axillary bud, as in further experiments, when all axillary buds were carefully removed, no tubers developed. Isbell suggested that sometimes tubers may develop adventitiously at the petiole base and also concluded that older leaf cuttings die more quickly than younger ones.

A different method was tried in the 1930s by C.A. Jørgensen (pers. commun. by G. Helms Jørgensen Jr.). Tubers were left to sprout. Rooted sprouts of 6-10 cm were detached from the tubers and placed on a stone or piece of wood on top of the soil, in which the roots were led. All axillary buds at the petiole base which started to develop after the sprout tip had been severed, were carefully removed. A whorl of shoots developed at the plant base which were said to be of adventitious origin. This conclusion was unjustified as will be demonstrated in Section 4.4.3.2.

Krenke (1933a,b) induced adventitious shoots at the apical end of decapitated tuber sprouts after inoculation with *Bacterium tumefaciens*. Production of shoots on callus formed on the cut surfaces of stems was studied by Lauer & Krantz (1957). After all buds present were removed, callus developed mainly from the vascular ring at the basipetally situated cut surface. In a later publication Lauer (1963) reported that a high level of nitrogen in the soil and, to a lower extent, also a high level of potassium stimulated adventitious shoot formation. In 1967, he reported that cultivar choice, photoperiod, temperature, location and type of incision and phosporus content significantly affected the adventitious shoot producing capacity (Lauer, 1967). Almost 950 potato clones were used.

From callus Klopfer (1965a) obtained adventitious shoots on eye-less, etiolated internodes, planted in soil. These shoots appeared after about six weeks, mainly at the basal part but also at the top. A new successful technique, the Dionne method (Ross et al., 1967) using decapitated potato stems grafted onto tomato, was developed for experiments to produce polyploids. Adventitious shoots developed from where the axillary buds were excised. Frandsen (1967a) and many others after him also obtained excellent results with this technique.

Simmonds (1964) using leaf-cuttings, got 50% of rooting but no shoots. Klopfer (1965a), in similar experiments, also failed to obtain shoots. Simmonds mainly considered obtaining shoots from endogenous tissue, via callus, whereas nowadays often the use of petioles is favoured.

Miedema (1973b) postulated differences in auxin-sensitivity between various tissues when trying to explain why most adventitious shoots from potato leaf cuttings are of epidermal origin, whereas adventitious roots develop endogenously. He further showed that genetic factors may determine whether adventitious organs can be formed in potato.

Before 1969, some preliminary experiments on adventitious shoot formation were done at IvP by Ferwerda (unpublished) applying the method of Kupfer (1907) and Isbell (1931). This work was unsuccessful as were attempts to produce shoots from leaf discs by methods commonly used for propagating cultivars of *Streptocarpus* and *Begonia*.

4.3.2 Literature on experiments in vitro

Recently some success has been recorded with production of shoots in vitro from different plant parts and tissues of potato. Adventitious shoots from tuber explants have been obtained for example, by Wurm (1960), Fellenberg (1963), Miedema (1973b) and Lam (1975). In some cases embryoids (e.g. Dunwell & Sunderland, 1973) and even plantlets (Irikura, 1975) were produced from anther cultures. Up to now no shoots have been obtained from culturing root tips (Bajaj & Dionne, 1968). Some other successful attempts were mentioned recently by Behnke (1975, 1976) and by Roest & Bokelmann (1976). Callus growth was reported to be easily induced and in one experiment (Behnke, 1975) callus differentiated into plants frequently. Behnke (1976) mentioned the production of single-cell cultures from dihaploid calli of potato.

Starting from compound potato leaves, Roest & Bokelmann (1976) obtained numerous adventitious shoots on small rachis parts, split lenghtwise. These were transferred to another medium where roots initiated. The plantlets could be transplanted into soil and developed into full-grown plants. This work is very promising and at the moment Roest, van Harten and their coworkers are checking whether mutants, obtained after irradiation of this material in vitro, are chimera-free, i.e. ultimately arose from single cells.

4.4 Experiments

4.4.1 Preliminary remarks

During the period 1969-1975, more than 100.000 leaves or leaflets of different potato cultivars have been used in experiments to find a feasible method for inducing adventitious shoot formation. Initially it was assumed that the most promising approach in vivo was to start from rooted material. One reason was that rooted leaves or leaflets can be kept alive for several months, depending on cultivar, environmental conditions, etc. Then shoot promoting substances like kinetins can be applied at different times. As adventitious shoot formation from dissected potato leaves is very exceptional in nature, we expected that the application of such substances would be favourable. Moreover Miedema (1967) and others found that adventitious buds occur very easily on potato tuber pieces if these pieces have rooted previously. Finally kinetins, which may stimulate the formation of adventitious shoots, are taken up much better via roots than via unrooted petioles or petiolules.

Before 1968 the cultivars Bintje, Multa and Désirée had been studied at IvP for mutation breeding purposes. After an initial rooting experiment, cv. Bintje was selected for further work because of its superior rooting properties (even without addition of growth substances) and its vitality. Material of this cultivar was studied for more than four years (see Fig.2). We intended to replace cv. Bintje by other clones after the most suitable conditions for adventitious root and shoot formation had been established. There were two lines of thought: first to use a diploid tester clone, after such a clone had been developed, (see Chapter 3) for all future work on mutations and related fields; second, to test the possible L-I origin of adventitious shoots by using a clone with a periclinally chimeric structure.

In 1971 mutant Clone B 165 of cv. Burmania was added to the programme, and in

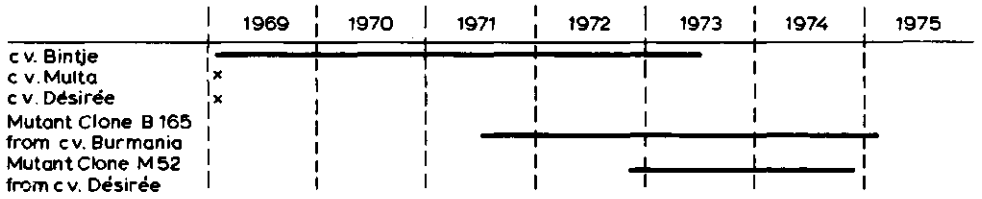

Fig. 2. Potato cultivars and mutant clones used for experiments on adventitious organ formation in vivo.

1972 the more vigorous Clone M 52 of cv. Désirée was included. Both clones are periclinally chimeric for tuber-skin colour (genetic constitution: L-I yellow, L-II + L-III red). The tubers produced have a yellow/red-splashed appearance. With these mutant clones, it is possible to determine, immediately after tuber formation, whether the shoots from which the tubers arise are of L-I origin, because then tubers have a completely yellow skin. Similarly shoots that originate adventitiously can be distinguished from those from axillary buds, because axillary buds reproduce the chimeric structure of the parent (see further van Harten, 1972).

From 1971 onwards, limited use could be made of growth chambers at the Department of Arable Crops (DAC) of the Agricultural University, so that conditions could be better controlled than in the greenhouses of IvP.

A practical problem in the beginning was that individual treatment of these leaves or leaflets, e.g. in glass tubes was very time-consuming. Rooting in solid media like soil, was soon found to be impractical, as the soil had to be washed away to study rooting capacity, etc. Moreover, in solid media it was difficult to reproduce exactly the same conditions for all leaves. Several alternative methods were tested (Section 4.4.2.5) and, after a number of failures, an excellent system with small plastic tubes in trays, was developed. Additional data are presented in Section 4.4.2.1. Finally it must be emphasized that it was not our primary aim to study the physiology of adventitious organ formation so that many details have been omitted. Root formation initially was regarded as an intermediate step to producing sufficiently large numbers of adventitious shoots from potato leaves or leaflets for mutation breeding purposes under conditions in vivo. This goal unfortunately, was not achieved.

4.4.2 Adventitious root formation and longevity

4.4.2.1 Effect of cultivar on rooting capacity and longevity of leaves and leaflets

The rooting capacity in sand of cvs Bintje, Désirée and Multa was compared in an initial experiment (Exp. 69.1[2]). In February 1969, 120 leaflets per cultivar were collected 40 days after tuber planting in a greenhouse at IvP. During plant growth additional

2. Although not all experiments are mentioned, for practical reasons the original numbers of the different experiments have been used throughout this publication.

thermoluminescent light (normally Philips, type TL 33) was supplied, as is common when rearing a winter generation of potatoes. In general potatoes can be reared in greenhouses even without additional light except in December and January. With each day a light intensity of about 20 W m^{-2} (or 5000 lx) for (for example) 12-14 h flowering plants are obtained.

Temperature in the greenhouse varied between 18 and 23°C throughout the experiment. In half of the treatments indoleacetic acid (IAA) or indolebutyric acid (IBA) was applied by dipping the petioles in a mixture of 2 g IAA or 1 g IBA in 98 and 99 g talc, respectively. The doses of auxins were based on findings in literature and on preliminary experiments, which revealed that IBA was more effective in stimulating rooting than IAA. Sometimes Chrysal (a commercial disinfectant made by Bendien, Naarden) was added to a concentration of about 12 g/l. All leaflets were planted in humid sand under extra glass and protected from direct sunlight by cheesecloth. During the first week the leaflets were sprayed with tap water twice a day to prevent drying out. Natural light was not supplemented. After 6 weeks the sand was washed away and rooting percentage and rate of survival per treatment were determined.

Results presented in Table 1, demonstrate the superior rooting capacity of cv. Bintje under all conditions. The average rooting percentages were: 67% for cv. Bintje, 46% for cv. Désirée and 35% for cv. Multa. Values for cv. Bintje differed significantly (χ^2 test, $P < 0.05$) from those for the other two cultivars. Compared with the control IAA gave more rooted leaflets in cvs Bintje and Désirée, but not in cv. Multa. No explanation can be given for the aberrant behaviour of cv. Multa. With IBA rooting percentages were lower in all three cultivars. The addition of Chrysal gave only slightly better results.

In all treatments cv. Multa produced the lowest number of roots per leaflet. The other two cultivars produced similar numbers of roots. The application of auxins in general led to more roots per leaflet (IAA approx. 2x control, IBA approx. 5x control). These roots on the average were shorter than when no auxin was used.

First symptoms of decline of the leaflets (yellowing of the leaf discs and a flaccid appearance) were observed after three weeks, but surprisingly rooted (and sometimes even unrooted) leaflets were still alive after three months. Cv. Bintje on the average was more vigorous than the other two cultivars. As after three months still no adventitious shoots were found, the surviving leaflets were discarded.

In 1971, the rooting capacity of cv. Bintje was first compared with that of the newly introduced mutant Clone B 165 from cv. Burmania (Exp. 71.15). In November 1971, from each cultivar 40 leaflets and 40 small compound leaves, about 40 days old, were

Table 1. (Exp. 69.1). Effect of cultivar on proportion (in %)of leaflets rooting with different treatments after 6 weeks in sand. Each value is the average of 20 leaflets.

Treatment	Cv. Bintje	Cv. Désirée	Cv. Multa
1 g IBA in 99 g talc	30	15	15
1 g IBA in 99 g talc + Chrysal	35	20	10
2 g IAA in 98 g talc	90	65	25
2 g IAA in 98 g talc + Chrysal	95	70	30
Control	75	50	60
Control + Chrysal	75	55	70

Table 2. (Exp. 71.15). Effect of cultivar on proportion (in %) of leaves and leaflets rooting after 6 weeks in test tubes containing different solutions. Each value is the average of 16 leaves and leaflets.

Treatment	Cv. Bintje	Mutant Clone B 165
KIN 2 mg l^{-1}	38	–
KIN 2 mg l^{-1} + IBA 0.02 mg l^{-1}	19	–
KIN 2 mg l^{-1} + IBA 2 mg l^{-1}	44	–
IBA 2 mg l^{-1}	87	44
Control (H_2O)	69	19

collected and put in test tubes (diameter 1 cm), containing different concentrations of kinetin (KIN) either with, or without IBA (exact concentrations are given in Table 2). Two or three drops of Chlorix (a commercial disinfectant made by Fenix, Zwolle) were added to the control (tapwater). The test tubes were placed in a greenhouse under a piece of cheesecloth at a temperature varying between 18 and 23°C. One row of thermo-luminescent lights, at about 1 m above the plant material supplemented the natural light for 14 h each day. Rooting percentages after six weeks are shown in Table 2. The values for rooting in cv. Bintje again differed significantly from those for mutant Clone B 165 ($P<0.01$). The rate of survival in cv. Bintje was also better. Although auxin normally stimulates root formation, it seems to have had a negative effect on cv. Bintje when

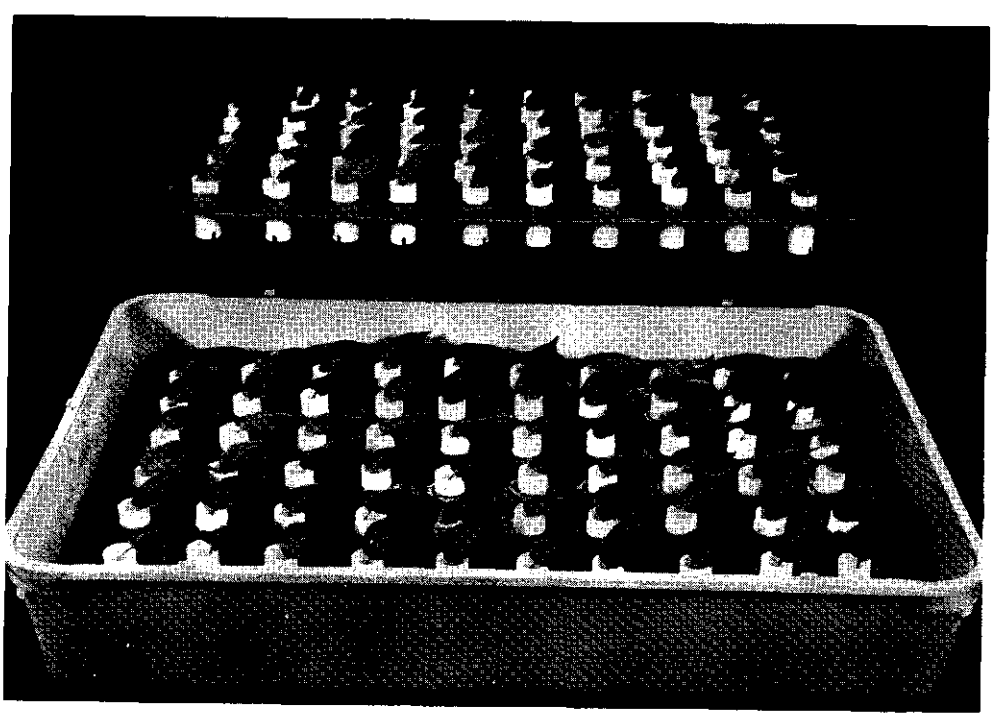

Fig. 3. Plastic tray (about 45 x 30 x 8 cm) with potato leaflets, inserted in plastic pipes (diameter 1 cm).

Table 3. (Exp. 72.10). Effect of cultivar and daylength (14 h and 10 h) of mother plants on proportion (in %) of leaves rooting after 7 weeks in trays containing different solutions. Values for 2,4-D treatments are the average of 60 leaves. Values for IBA 1 mg/l and control are the average of 30 leaves.

Treatment	Cv. Bintje		Mutant Clone B_165	
	14 h	10 h	14 h	10 h
2,4-D 0.1 mg l^{-1}	90	67	–	–
2,4-D 0.1 mg l^{-1} + IBA 1 mg l^{-1}	92	77	–	18
IBA 1 mg l^{-1}	100	100	–	47
Control (H_2O)	90	100	–	3

applied at 0.02 mg/l with 2 mg/l KIN. No proper explanation for this odd result can be given. As no adventitious shoots were formed, all material was discarded after 2.5 months.

Rooting capacity of leaves of cv. Bintje and mutant Clone B 165 was also studied (Exp. 72.10) in growth chambers at the DAC. Leaves of 9-10 weeks old, 720 in total, were collected in June 1972 and placed in short plastic pipes (diameter 1 cm), which were inserted in a sheet of plastic. The sheet fitted a tray to which different solutions can be added. Each tray contained 30-60 leaves or leaflets as is shown in Fig. 3. The advantage of this system is that all material can be treated exactly the same without much labour.

In Exp. 72.10 two photoperiods: 14 h and 10 h were chosen for the mother plants. Leaf material was placed in a growth chamber at 10 h daylength. Two rows of thermoluminescent lights were installed at about 1 m above the trays. Depending on the type of growth chamber, on the distance between light source and leaves and on the age of the fluorescent tubes the average light energy in growth chambers was 14 W m^{-2} which is sufficient for good vegetative growth. A low light intensity was preferred as it is said to be favourable for shoot formation (see Section 4.2.3). The air temperature in the growth chambers was kept at 18°C and the relative humidity of the air at 80%. Different treatments with 2,4-D (2,4-dichlorophenoxyacetic acid) 0.1 mg/l (sprayed repeatedly) or IBA 1 mg/l were given.

The results, presented in Table 3 again demonstrate the superior rooting capacity of cv. Bintje leaves under various conditions. Rooting of leaves of mutant Clone B 165 seems to be stimulated by the short day treatment of the mother plants. The rate of survival after two months for both cultivars under both daylengths, with and without IBA 1 mg/l is presented in Table 4. (Treatments with and without 2,4-D are pooled as this treatment

Table 4. (Exp. 72.10). Proportion (in %) of leaves of cv. Bintje and of mutant Clone B 165 (cv. Burmania) surviving after 9 weeks. Each value is the average of 90 leaves (30 leaves per tray).

Treatment	Cv. Bintje		Mutant Clone B 165	
	14 h daylength	10 h daylength	14 h daylength	10 h daylength
IBA 1 mg l^{-1}	47	86	13	68
Control (H_2O)	81	73	5	61

does not affect survival). The results for cv. Bintje are in accordance with the expectations except for the low survival with IBA 1 mg/l at 14 h daylength, but this result may be due to contamination of the water of two trays. 2,4-D was found to have practically no effect on survival. In this experiment some shoots originated. This finding is discussed in Section 4.4.3.1.

The superior rooting capacity of leaflets of mutant Clone M 52 from cv. Désirée, compared with that of mutant Clone B 165 from cv. Burmania, is shown in Exp. 73.6. In this experiment different concentrations of KNO_3 were applied after one week. Fig. 4 shows that after 11 weeks rooting capacity of Clone B 165 was significantly lower than that of Clone M 52 ($P < 0.01$). Application of KNO_3 had no significant effect on rooting. No significant differences in vitality were observed between both clones.

The results clearly demonstrate that the choice of cultivar considerably affects the outcome of rooting experiments with leaves or leaflets. Of the cultivars studied, cv. Bintje produced the best results and cv. Burmania the worst. The experimental results further showed that different cultivars respond in a different way to various treatments and applications. By choosing suitable conditions also those cultivars, which normally produce only low percentages of rooted leaves, can be stimulated to give better results. The observed differences in rooting capacity etc. between cultivars, most probably are due to the existence of differential physiological activity between the cultivars and differences between endogenous hormone balances.

All experiments have demonstrated that high percentages of leaves or leaflets can be kept alive for three months or longer, even if they did not produce roots. However, another point is whether such material is still able to produce adventitious shoots.

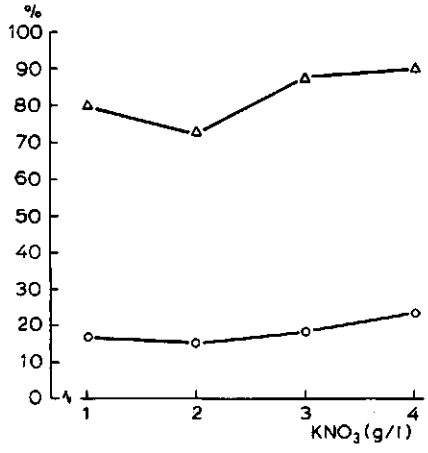

Fig.4. (Exp. 73.6) Effect of cultivar on proportion in % of leaflets rooting after 11 weeks in trays containing different concentrations of KNO_3, applied after 1 week. Each point represents 24 leaflets. ○ = mutant Clone B 165 from cv. Burmania, Δ = mutant Clone M 52 from cv. Désirée.

4.4.2.2 Effect of physiological age of leaves and leaflets on rooting capacity and longevity

Although over the years material of different physiological age has been used, few experiments were designed especially to compare the rooting frequency of different age groups under similar conditions because the age of leaves or leaflets generally does not seem to limit rooting. On the other hand it is logical to assume that the physiological age of the material may affect its ability to produce adventitious shoots. In early experiments mother plants were raised in the greenhouse. In later years use was made of growth chambers at the DAC where conditions could be better controlled.

In Exp. 69.5, 960 terminal leaflets of cv. Bintje were collected on 21 October 1969 from tuber plants which had been planted on 1, 11 and 21 September and on 1 October. Within each group of 240 leaflets 120 were treated with auxin, by placing the basal ends for $1\frac{1}{2}$ h in Petridishes in a solution of IBA 10 mg/l. Then the leaflets were planted in humid sand for propagation in a glass box, covered with cheesecloth and placed in the greenhouse. Temperature was kept at about 20°C. Two different photoperiods (8 h and 16 h) were applied. Two rows of thermoluminescent lights were installed 1 m above the trays.

As shown in Table 5 rooting in all cases was excellent. Pooled per planting date 96%, 87%, 71% and 77% of rooted leaflets were counted three weeks after the start of the experiment in the groups of leaflets collected 10, 20, 30 and 40 days after emergence of the parent plants respectively. The rooting in the 10 and 20 day groups was significantly higher than in the 30 and 40 days groups ($P < 0.01$). In general IBA gave slightly more rooting. No significant differences in rooting were observed between daylength regimes.

Use of young leaflets, application of IBA and 16 h light increased the length of the roots. For example the roots of leaflets, exposed to 16 h light, were on the average 2.5 cm longer than those after 8 h light. Usually the youngest leaves remained vital for a longer period. They also produced more callus at the petiole ends.

Experiment 69.5 was repeated in a slightly modified way in 1970 (Exp. 70.1). Terminal leaflets of cv. Bintje were collected from mother plants, grown in a greenhouse, 20, 30, 40 and 50 days after emergence. Different media (sand, peat, potting compost, some mixtures and perlite, an artificial rooting medium) were used. Only one photoperiod of 16 h was applied. The daylight was again supplemented by two rows of thermoluminescent light, installed about 1 m above the leaflets. Temperature in the greenhouse varied from

Table 5. (Exp. 69.5). Effect of physiological age on proportion (in %) of leaflets of cv. Bintje rooting after 3 weeks in sand. Leaflets were collected 10, 20, 30 and 40 days after emergence of the parent plants. Each value is the average of 60 leaflets.

Treatment	Days after emergence			
	10	20	30	40
16 h daylength	90	84	56	70
16 h daylength, IBA 10 mg l^{-1}	97	86	78	80
8 h daylength	100	86	72	86
8 h daylength, IBA 10 mg l^{-1}	98	94	79	72

Table 6. (Exp. 70.1). Effect of physiological age on proportion (in %) of terminal leaflets of cv. Bintje rooting after 3 weeks in different media. Leaflets were collected 20, 30, 40 and 50 days after emergence of the parent plants. Each value is the average of 20 leaflets.

Media	Days after emergence			
	20	30	40	50
Peat	40	65	45	65
Potting compost	45	45	80	65
Compost/soil mixture	40	65	85	70
Sand	65	70	60	65
Perlite	25	40	25	25

18-23°C. Rather unexpectedly the youngest leaflets (20-day group) showed the lowest percentage of rooting: 43% compared with 57%, 59% and 58% for the other three groups (see Table 6). The observed differences were statistically not significant. As Table 6 shows, only small differences in rooting per age group are found in sand and perlite. Rooting in sand on the average was about 20% lower than in Exp. 69.5. Differences most probably are explained by effects of the season.

In conclusion it seems that the physiological age of leaves and leaflets does have a certain effect on rooting, but results are controversial. For further experiments the use of young, but full-grown leaflets is advocated, as such leaflets root easily and remain vital for considerable periods.

4.4.2.3 Effect of growth of the parent material on rooting capacity

As this subject has not been studied in detail, only a few general remarks and conclusions are given. Moreover conditions under which parent plants were raised, especially at the start, could not be controlled adequately in greenhouses.

It was known that for potato, as for most other plant species, prolonged dark periods or short-day conditions often yield weak plants with flaccid leaflets.

In Exp. 71.12 with 1200 leaves of cv. Bintje, the parent plants were raised under two different regimes: a photoperiod of 8 h and 15°C and a photoperiod of 12 h in combination with 20°C. After 4 weeks the proportion of non-yellowing leaves in 12 h light was always about 20% higher than in 8 h light, irrespective of further treatments. In Exp. 71.13, done in a similar way to Exp. 71.12, the positive effect of the longer photoperiod on cv. Bintje was again indicated.

In Exp. 72.10 the effect of short-day conditions on mother plants of cv. Bintje and of mutant Clone B 165 from cv. Burmania was compared. For experimental details and results on rooting see the end of Section 4.4.2.1 and Table 3. No significant differences for cv. Bintje were found between both regimes of the parent plants. For both photoperiods more than 85% of the leaves had rooted after 7 weeks. The results with and without auxin did not differ significantly. For mutant Clone B 165 the results were very different. No rooted leaves at all were obtained from mother plants grown under the 14 h regime. Under short-day conditions 47% of the leaves, treated with IBA 1 mg/l had rooted compared with only 3% without IBA. The positive effect of the short photoperiod regime

of the parent plants in addition to that of IBA on rooting of leaves from (clones derived from) cv. Burmania fully confirms earlier findings (e.g. Exp. 72.7 in which mother plants of cv. Burmania were submitted to various photoperiods).

Exp. 73.4 indicated that a shorter photoperiod treatment of the parent plants also improved rooting in mutant Clone M 52 from cv. Désirée. Because of technical difficulties not only the length of photoperiod but also the temperature and relative humidity varied considerably during this experiment and therefore the results are not further discussed here.

4.4.2.4 Properties of compound leaves, leaflets and leaf parts in relation to rooting capacity and longevity

In most experiments either single leaflets (terminal or lateral ones) or compound leaves of potato were used. This material was detached from the mother plants by cutting or by breaking. Rooting normally occurs in the basal portion of the petiole or petiolule.

For nearly all cultivars or clones single leaflets showed a better rooting capacity than compound leaves, although for cv. Bintje differences in most experiments where this was tested, were not significant. For mutant Clone B 165 from cv. Burmania differences were much more pronounced as can be concluded from Exps. 72.6a and 72.6b, which were performed with in total 1440 leaves and leaflets of 6 weeks old in trays. Mother plants were raised in a growth chamber. Leaves and leaflets were also grown in a growth chamber at 18°C, a photoperiod of 10 h and a relative humidity of 80%.

Fig. 5 shows proportion of leaves and leaflets rooting after different treatments with 2,4-D together with IBA 1 mg/1. The differences observed between both groups are statistically highly significant ($P < 0.01$). No proper explanation can be given for the fact that the points for leaves and leaflets coincide at 2,4-D 0.1 mg/1 + IBA 1 mg/1. Even

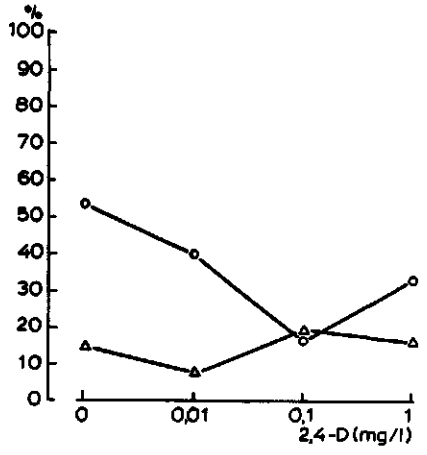

Fig. 5. (Exp. 72.6a). Proportion (in %) of compound leaves and single leaflets rooting after 6 weeks. Mutant Clone B 165 from cv. Burmania was treated with different concentrations of 2,4-D in trays. (IBA 1 mg/l was added in all treatments). Each point represents 81 leaves or leaflets. o = single leaflets, Δ = compound leaves.

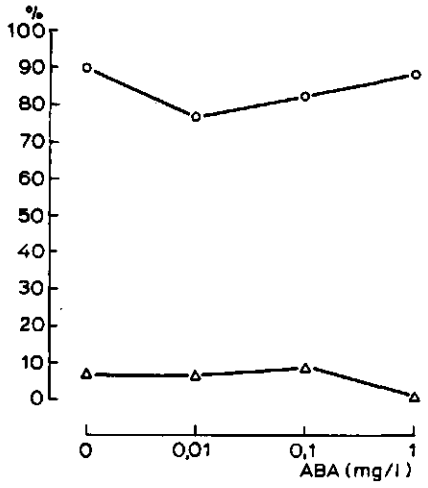

Fig. 6. (Exp. 72.6b). Proportion (in %) of compound leaves and single leaflets rooting after 6 weeks. Mutant Clone B 165 from cv. Burmania was treated with different concentrations of abscisic acid (ABA) in trays. (IBA 1 mg/l was added in all treatments). Each point represents 81 leaves or leaflets. o = single leaflets, Δ = compound leaves.

more striking differences were observed in Exp. 72.6b (Fig. 6) after treating different kinds of starting material of mutant Clone B 165 with abscisic acid (ABA) in various concentrations, together with IBA 1 mg/1.

In Exp. 71.17 it was observed that also parts of leaflets are able to produce roots frequently. When leaflets of (clones derived from) cv. Burmania were cut into two parts along the main vein, up to 30% of the parts rooted. Good results were obtained also with basal halves of leaflets, but those parts which had not rooted, quickly showed symptoms of rot and decline and, died.

The results of practically all experiments indicate that in general single leaflets root best. Over the years differences in rooting between lateral and terminal leaflets have not been found.

The longevity of leaflets in solid media as well as in trays containing solutions was in general also much better than that of whole leaves. If, for some reason, very young plant material must be used, it may be better to use young compound leaves, because in general such leaves remain vigorous for a longer period.

4.4.2.5 Rooting in different media

Before in 1972 a final decision was made to treat leaves or leaflets in trays, the suitability of several other rooting media was tested. The most important findings are briefly mentioned here.

Section 4.4.2.2 described how in Exp. 70.1 leaflets of different age of cv. Bintje root in various solid media like peat, sand, potting compost and perlite (see Table 6). Except for perlite, where rooting was very low, the media gave about similar rooting results. Solid media have disadvantages: growing conditions are not exactly the same for each leaf or

leaflet, proper application of solutions to the medium is difficult and laborious, and root formation can be studied only by digging up each individual. An advantage of perlite is that the effect of applying different solutions can be studied much better in this neutral medium than in other media like peat or compost. Moreover perlite and, to a lesser extent, sand provide relatively sterile conditions, which diminishes rot and deterioration and increases the lifetime of the material, even when no roots are formed.

To a certain extent the use of glass tubes with liquid media is an improvement when compared with solid media. A drawback of this method is that small leaflets easily become immersed in the solution and than quickly deteriorate because of moisture oedemia. Moreover the method remains laborious and, unless growth substances are added, rooting is lower than in natural solid media.

After some unsuccesful attempts with potato leaves, inserted in Tempex sheets floating in trays with solutions or inserted in Baystrat (a soft foam of polyurethane), the tray method (see Section 4.4.2.1) was developed.

The use of demineralized water and well water leads to significantly higher rooting frequencies and longer roots than with normal tap water. Therefore for all experiments after 1972 tap water was no longer used. The better rooting in well water and demineralized water most probably can be explained by the detremental effect of fluoride in tap water.

4.4.2.6 Effect of different growth substances on rooting

Potato cultivars which are reluctant to produce roots from petioles or petiolules can be stimulated to do so by the application of small amounts of auxins. As our ultimate goal was to produce adventitious shoots and because auxins are known to counteract shoot formation, auxin concentrations were kept as low as possible. In most experiments the effects of auxin application were studied only in combination with effects of other treatments. IBA, and sometimes NAA, was preferred to IAA, as auxins were mostly applied via aqueous solutions and IBA or NAA are more stable than IAA.

Over the years a considerable number of growth substances like IAA, IBA, NAA, kinetin, 2,4-D, BA and GA_3 have been applied, separately or in combination with each other, in various concentrations and in different ways (in aqueous solutions, sprayed on leaf discs, in talc, etc.). The applications were either made simultaneously or at different moments, once or repeatedly. Most experiments were repeated several times.

In early experiments on solid media, KIN 14 mg/1, sprayed on leaf discs (in the presence of a wetting agent: Tween 20) inhibited root formation but increased the life-time of leaflets which had not rooted yet. In most later experiments on liquid media, some positive effect of kinetin on growth vigour and rate of survival was observed, but mostly differences were not significant. Kinetin sprayed on leaf discs inhibited root formation less than when petioles were dipped in solutions of the same concentration.

Auxin (e.g. IBA 1 or 0.1 mg/1) counteracts the inhibiting effect of kinetin on rooting. Sometimes IBA lengthened the life of unrooted leaves. Leaflets treated with IBA only, produced more, but initially shorter roots than the control.

In Exp. 71.15, (see also Table 2) 80 leaves and leaflets of cv. Bintje (16 per treatment) were placed in test tubes, which contained solutions of IBA (0.02 and 2 mg/1) with or

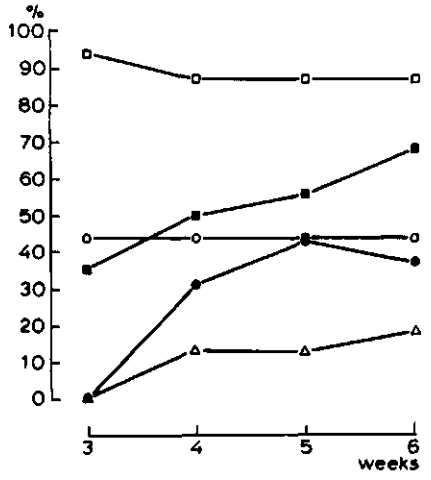

Fig. 7. (Exp. 71.15). Effect of different treatments with IBA and KIN on proportion (in %) of compound leaves and single leaflets of cv. Bintje, rooting in glass tubes in relation to time. Each point represents 24 leaves and leaflets. ■ = Control (H_2O), □ = IBA 2 mg/l, ● = KIN 2 mg/l, ○ = KIN 2 mg/l + IBA 2 mg/l, Δ = KIN 2 mg/l + IBA 0.02 mg/l.

without KIN (2 mg/1). The percentages of rooting after 3, 4, 5 and 6 weeks are given in Fig. 7. Application of IBA 2 mg/1 significantly increased the amount of rooting ($P < 0.01$) and also led to much earlier rooting. All treatmants with kinetin produced after 6 weeks significantly lower proportions of rooting than the control, even if IBA 2 mg/l was added. However, as the figure shows, application of IBA 2 mg/1 with KIN 2 mg/1, led to earlier rooting. This was also observed after application of IBA 2 mg/1 only.

In a number of experiments GA_3, either with or without IBA 1 mg/1, was tested for any effect on adventitious shoot formation. Experiments were done in greenhouses and growth chambers, mostly with cv. Bintje and mutant Clone B 165 from cv. Burmania. GA_3 had a considerable effect on root formation as shown by the results of Exp. 72.1. Leaf parts of cv. Bintje and mutant Clone B 165, consisting of terminal leaflets and the first pair of lateral leaflets were placed in trays, containing solutions of GA_3 in water with concentrations ranging from 0.01 to 100 mg/1 with or without IBA 1 mg/1. Each tray contained 30 leaves collected from 7 weeks old plants. Trays with tap water, with and without IBA 1 mg/1 were added as control. The experiment, started in January 1972, was done in a greenhouse at IvP. Temperature was kept between 18 and 21°C and supplementary thermoluminescent light (two rows TL33 at about 1 m above the trays) was given for 12 h daily.

In Fig. 8 only the rooting percentages of cv. Bintje are presented as in mutant Clone B 165 rooting did not occur in most treatments and remained lower than 5% in the control (IBA 1 mg/1) and in the treatment GA_3 0.01 mg/1 + IBA 1 mg/1. The figure shows that IBA 1 mg/1 in all treatments gave higher rooting percentages. No explanation can be given for the relatively high percentage of rooted leaves with GA_3 50 mg/1 + IBA 1 mg/1. The experiment yielded several shoots.

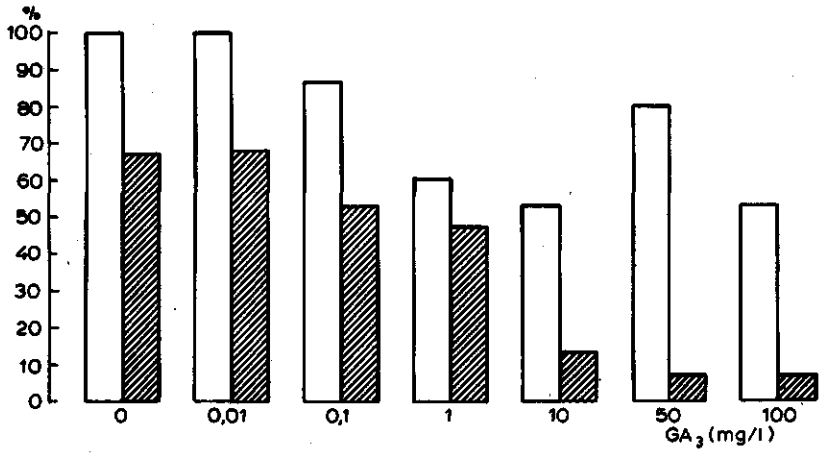

Fig. 8. (Exp. 72.1). Effect of different treatments with GA_3 on proportion of compound leaves of cv. Bintje. rooting after 6 weeks in trays. Leaf age: 7 weeks. Each point represents 30 leaves. White columns = with IBA 1 mg/l, hatched columns = without IBA.

In Exps. 72.4 and 72.7 we tried to repeat the experimental conditions of Exp. 72.1 which gave adventitious shoots. This time well water was used which resulted in slightly higher rooting percentages for cv. Bintje. For mutant Clone B 165 the overall percentage of rooted leaves increased from 4% to 27%. From the point of view of adventitious shoot formation the repeated experiments were not succesful.

Since 1972 the effect of 2,4-D has been investigated in experiments involving more than 10.000 leaves and leaflets of different cultivars or clones. The high numbers of leaves or leaflets were studied because shoots were obtained in several experiments. Mostly 2,4-D was sprayed once or repeatedly on the leaf discs and a range of concentrations was applied. For the cvs Bintje, Burmania (mutant Clone B 165) and Désirée (mutant Clone M 52), the proportion of rooting and survival was not significantly affected within the range of application of 0-1 mg/l.

Because of positive results with 6-benzylaminopurine (BA) as a stimulator of shoot formation in recent experiments in vitro, the range of action of this cytokinin was also tested for potato leaves and leaflets with commonly used cultivars and clones. A wide range of concentrations was applied. The application of BA in all experiments, even at low concentrations of 0.1 mg/1, led to significantly less rooting, which was in accordance with the findings in the literature. After BA application the proportion of leaves or leaflets surviving also decreased. In some experiments indeed some adventitious shoots were obtained.

The effect of abscisic acid (ABA), solved in H_2O at 50°C in concentrations 0.01, 0.1 and 1 mg/1, on rooting was studied only in Exp. 72.6b with leaves and leaflets of mutant Clone B 165 from cv. Burmania. With IBA no significant effect of ABA on rooting was observed (see also Fig. 6). ABA application drastically improved the vigour of the material, but as no indications for adventitious shoot stimulation were found, the work with this growth substance was not continued.

Finally some experiments, involving in total 1350 leaves and leaflets were performed to study the effect of citric acid (Exps. 73.15 and 73.16). Citric acid is known from work in vitro to counteract the effect of auxins and to stimulate shoot formation. However, no shoots were obtained and the experiments were not continued. Citric acid in doses of 10-100 mg/1 gave slightly, but not significantly better rooting.

4.4.2.7 Effects of light, photoperiod and temperature on rooting

Results presented in this section should be treated with some caution. Even after 1971, when growth chambers became available, not all experimental conditions could be kept completely under control. For example only exceptionally was sufficient room there to raise mother plants in growth chambers. Moreover, normally only one growth chamber was available at a time, so that it was impossible to compare identical potato material, e.g. of one mother clone under two regimes of light.

In Exp. 69.5, 960 terminal leaflets of cv. Bintje were exposed to photoperiods of either 16 h or 8 h. Leaflets of different physiological age were used (for experimental details see Section 4.4.2.2). For simplicity the results of the different age groups have been pooled here. After three weeks, 80% of the leaflets had rooted in 16 h light and 73% in 8 h light. The results of this experiment indicate a positive effect of the longer photoperiod on rooting ($P < 0.01$). This is in accordance with the outcome of most later experiments.

In general it seems that for most potato cultivars used here the length of the daily photoperiod, under which the leaves are grown is not of major importance for rooting. The same can be said for the normal range of temperature. As it was thought that starting material and growth substances would be the most important factors for rooting (as well as for adventitious shoot formation), we decided to standardize other conditions as much as possible. Most experiments were at about 21°C, a photoperiod of 12-14 h and a relative humidity of 80-90%. In practically all experiments thermoluminescent light was used, at 1 m above the leaves or leaflets. The light intensity in growth chambers was about 14 W m^{-2} and up to 5 times as high in greenhouses, depending on seasonal effects and on the amount of supplementary light. Provided that enough light is given to enable vegetative growth, different levels of light intensity have no effect on rooting.

Different treatments may interact, for example the length of the photoperiod and the application of growth substances. In Exp. 69.5 with leaflets of cv. Bintje a high concentration of IBA (10 mg/1) in solution always stimulated rooting if the photoperiod (16 h) was long. Then the proportion of leaflets with roots increased from about 75% to 85%. (N.B. the differences observed, however, were not significant). Under a short-day regime (8 h light) results were less clear (see Table 5). In Exp. 72.10 it was observed that SD conditions (10 h daylight), in combination with application of IBA 1 mg/1, may stimulate rooting in (clones derived from) cv. Burmania, but not in cv. Bintje (see Table 3). In Exp. 71.11 with leaves of cv. Burmania, the addition of IBA 3 mg/1 in solution even completely inhibited root formation if the leaves had been previously in the dark for four days or longer, whereas in the control some rooting was found.

The effect of dark periods on rooting (and on adventitious shoot formation) was studied too. In April 1969 leaflets of cv. Bintje, collected from 4 weeks old plants, were placed in glass test tubes containing solutions of kinetin or IBA (Exp. 69.3). 70 leaflets

were put in a dark growth cabinet at 15°C, and 66 leaves in a laboratory room at 20°C, to which room the first 70 leaflets were transferred after one week. The laboratory room had windows only on the north side. The leaflets were placed in front of these windows. No supplementary light was given. Rooting capacity after different applications of growth substances (10 leaflets per treatment) for both groups of leaflets are shown in Table 7. The dark period and the initial low temperature, as expected, resulted in considerably lower rates of rooting, irrespective of the concentration of growth substances. Under the same conditions auxin production is inhibited, which could be favourable for adventitious shoot formation. Of the 66 control leaflets 79% were rooted after 10 weeks compared with only 17% in the groups of 70 leaflets that had been put in the dark. Differences between both groups, of course, were highly significant.

Because several (adventitious?) shoots were produced in this experiment, an almost identical experiment with leaflets of cv. Bintje, was started in July 1970 (Exp. 70.4). This time no rooting was observed after a dark period of one week. Adventitious shoots were not produced at all.

The effect of length of dark period was studied in Exp. 71.11 with 1200 leaves of mutant Clone B 165 from cv. Burmania. Four dark periods (0, 2, 4 and 8 days) were given. Without auxin, the proportion of leaves rooting was only 0-5%, irrespective of the length of the dark period. When IBA (1 g in 99 g talc) was added, the proportion of rooting was 68%, 48%, 35% and 0% after a dark period of 0, 2, 4 and 8 days, respectively. The observed differences were significant at the level $P < 0.01$.

A dark period also negatively affects the vitality of the leaflets. Decline and rotting of leaflets is accelerated by a toxic effect of the applied kinetins or auxins (concentrations 0-3 mg/l), if the dark period is four or more days. When dark periods are shorter, the leaflets are able to recuperate, especially when IBA has been applied previously. Even though all cultivars studied responded negatively to dark periods, varietal differences were observed. Cv. Burmania seems to be less sensitive to adverse effects of lack of light than cv. Bintje. Few experiments were performed to study the effect of temperature only. In most cases a tendency towards less rooting at lower temperatures was observed.

In general it can be concluded that for cv. Bintje rooting capacity and vitality of potato leaves and leaflets are negatively affected by exposing them to dark periods and to a lesser extent by shorter photoperiods and by lower temperatures. For clones of other cultivars like cv. Burmania and cv. Désirée, these effects are less clear-cut.

Table 7. (Exp. 69.3). Effect of one week of darkness followed by LD conditions on proportion (in %) of single leaflets of cv. Bintje rooting after 10 weeks in test tubes containing different solutions. Each value is the average of 10 leaflets, except for the value of the untreated leaflets in the control, which consisted of 6 leaflets only.

Treatment	1 week of darkness	Control
KIN 2 mg l^{-1}	20	100
KIN 2 mg l^{-1} + IAA 0.01 mg l^{-1}	40	80
KIN 2 mg l^{-1} + IAA 0.02 mg l^{-1}	30	80
KIN 3 mg l^{-1}	20	60
KIN 3 mg l^{-1} + IAA 0.01 mg l^{-1}	10	70
KIN 3 mg l^{-1} + IAA 0.02 mg l^{-1}	–	90
Control (H_2O)	–	67

4.4.2.8 Concluding remarks on rooting

In the previous sections some effects have been described, which together mainly determine the rooting capacity of potato leaves and leaflets. The effect of the cultivar used was considerable. The effect of the physiological age of leaves and leaflets on root production in general was rather limited and results were controversial. The use of young but full-grown leaves or leaflets had advantages over older ones, especially because of superior growth vigour. We could not determine exactly what influence the conditions under which the parent plants were grown had on rooting.

Rooting and longevity of single leaflets in solid as well as in liquid media was much better than of compound leaves. In liquid media it is easier to apply growth substances and root development can be followed better than in solid media. In addition solid media are undefinable, except for artificial ones like perlite.

As expected, root formation was stimulated and speeded up by the application of auxins, notably of IAA and IBA. Kinetin in general was found to counteract rooting but to prolong the life-time of, especially unrooted, leaves or leaflets. Kinetin directly sprayed on the leaf discs inhibited rooting less than when applied directly to the root area. The application of combinations of growth substances in different concentrations often led to controversial and inexplicable results. Strong interactions, e.g. with cultivar effects, were found, which may be partly explained by differences in endogenous hormone balances between different cultivars. GA_3 (in high concentrations) and BA were found to inhibit root formation. Different concentrations of 2,4-D did not significantly affect rooting capacity.

Results on the effect of light, length of photoperiod and temperature sometimes were contradictory and cultivar-dependent, although mostly dark periods, short days and low temperatures had a negative effect on rooting and on proportion of leaves and leaflets that survived.

4.4.3 Adventitious shoot formation

4.4.3.1 Leaves and leaflets

Between 1969 and 1975, only about 120 shoots were obtained from many thousands of leaves and leaflets of different cultivars. Moreover, there is no definite proof that all these shoots were of adventitious origin. Data on experiments in which more than five shoots were obtained, are given in Tabel 8.

In Exp. 69.3 (see also Section 4.4.2.7) leaflets of cv. Bintje were grown in test tubes containing different solutions. The leaflets were cut from the compound leaf in a special way in order to prevent complete immersion of the leaf discs in the test tubes. If terminal leaflets were used, a part of the central rachis, from which lateral leaflets had been removed, was maintained. When lateral leaflets were used, sometimes a part of the rachis was split lengthwise. One group of test tubes was placed in a laboratory room. The other test tubes were placed for one week in a completely dark growth cabinet and afterwards transferred to the same laboratory room.

In one of the tubes placed directly in the laboratory room, a small shoot of 1 cm was observed at the rooted basal end of a leaflet, 20 days after initiation of the experiment.

Table 8. The formation of adventitious shoots from leaves or leaflets of different potato cultivars.

Exp.	Resulting leaf shoots			Starting material			Shoot number	Additional data
	number	%	position	cv./mutant clone	type	age		
69.3	8	6	cut basal part petiolule	Bintje	leaflets	4 weeks	136	7 shoots after 1 week complete darkness at 15°C, followed by laboratory conditions (Normal photoperiod and about 20°C). 1 shoot in control. Shoot formation irrespective of degree of rooting, with and without IAA or KIN between 20 and 75 days initiation.
72.1	16	2	cut basal part petiole	Bintje and mutant Clone B 165	compound leaf parts	7 weeks	840	Greenhouse at between 18 and 21°C and 12 h photoperiod. 1 shoot in cv. Bintje with IBA 1 mg/l. 15 shoots in mutant Clone B 165 with and without IBA 1 mg/l and GA_3 (various concentrations). Shoots of mutant Clone B 165 mainly on unrooted leaves. Tubers always yellow/red-splashed.
72.7	10	1	cut or broken basal part petiole or petiolule	Bintje and mutant Clone B 165	compound leaves and leaflets	6 weeks	780	Growth chamber at 18°C and 10 h photoperiod. 9 shoots from cv. Bintje with and without IBA, GA_3 or BA (various concentrations). 1 shoot from mutant Clone B 165 with IBA. Shoots on rooted as well as on non-rooted leaves or leaflets. Sometimes immediate tuber formation at adventitious shoots.
72.8	36	5	broken basal part petiole	mutant Clone B 165	compound leaves	10 weeks	720	Greenhouse at about 21°C and 14 h photoperiod or growth chamber at 18°C and 10 h photoperiod. Shoots with and without IBA, 2,4-D or GA_3 and irrespective of photoperiod. Adventitious origin of shoots doubtful. Tubers yellow/red-splashed.
72.10	6	1	broken basal part petiole (Bintje) and higher on petiole (B 165)	Bintje and mutant Clone B 165	compound leaves	9 weeks	720	Parent plants raised at 10 or 14 h photoperiod. Detached leaves in growth chamber at 18°C and 14 h daylength and sprayed with 2,4-D 0.1 mg/l. Shoots irrespective of rooting; with and without IBA application. 1 shoot in cv. Bintje and 5 in mutant Clone B 165.
72.11	10	3	broken basal part petiole	mutant Clone B 165	compound leaves	9 weeks	360	See Exp. 72.10. Also with 2,4-D (various concentrations). All tubers yellow/red-splashed.
73.3	9	2	cut basal part petiolule	mutant Clone M 52	leaflets	9 weeks	540	Growth chamber at 18°C and photoperiod of 4 h or 10 h. Shoots mostly on rooted leaflets with and without application of 2,4-D or KNO_3 (various concentrations). All tubers yellow/red-splashed.
73.5	19	3	cut basal part petiole or axils lateral leaflets	mutant Clone B 165	compound leaves	6 weeks	720	Mother plants grown under short day conditions. Leaves in growth chamber at 18°C and 10 h photoperiod. Callus formation, especially in axils, preceded shoots. Shoot formation irrespective of GA_3 or 2,4-D application. All (8) tubers yellow/red-splashed. Generally poor rooting.

The test tube contained a solution of KIN 2 mg/1 + IAA 0.01 mg/1. Seven shoots were obtained from the 'dark' series, of which three were found in test tubes with H_2O only at the cut base of the petiolules of terminal leaflets. In five test tubes the leaflets had formed roots. All plant parts from which the shoots arose showed callus-like swellings. From later experiments it was concluded that the use of young leaflets, application of kinetin and 2,4-D, dark conditions and liquid media stimulated callus formation at the base of the petiole or petiolule.

The position of the shoots obtained suggests that they may have originated in, or close to, the original axillary regions of the leaflets of the compound leaves. Small remnants of meristematic tissue could be present in those areas. But regeneration of potato shoots from such regions in nature seems highly exceptional and was only observed in the later Exp. 73.5 (see the end of Section 4.4.3.1). Because of the type of starting material and as only a few shoots were obtained, no definite conclusions can be made about the axillary or adventitious origin of the shoots. The observations that the petioles or petiolules had calluslike swellings at their base and that it took three or more weeks for the shoots to develop could indicate adventitious origin. Also no conclusions can be made about the number of initial cells from which these shoots arose.

We tried to reproduce exactly the conditions of the rather promising Exp. 69.3 in Exp. 70.4, but this time not a single shoot was obtained! This negative result indicates that other, still unknown factors determine adventitious shoot formation.

Shoots at the cut surface of leaf stalks were observed only two more times in 1969 and not at all in 1970. In Exp. 69.5, a terminal leaflet of cv. Bintje of about 30 days old directly produced a small tuber of about 2 mm at the cut surface of the petiole in the control which was exposed to natural light, supplemented by 16 h thermoluminescent light in a greenhouse at about 20°C. The tuber developed without a period of rest into a complete plantlet. The original leaflet had not developed roots.

In Exp. 69.6a under almost identical conditions, one shoot was produced from a terminal leaflet of cv. Bintje. This time roots were about 5 cm long. In both experiments (69.5 and 69.6a), the leaflets were grown in humid sand. The basal parts from which the shoots had arisen, were swollen and calluslike.

In 1971, despite experiments with 20.000 leaves and leaflets grown under reasonably well controlled conditions (trays, growth chambers), only one (!) adventitious stolon with a small tuber was obtained. Callus formed in practically all experiments but no organized buds were found. At the base of petioles more callus was formed than at the base of petiolules. Sometimes after a number of weeks small white swellings occured on older, brownish callus, but they were found to have a loose, unorganized callus-like structure.

Experiments in 1972 with cv. Bintje or mutant Clone B 165 from cv. Burmania, were somewhat more promising than those of previous years. In total about 80 shoots or tubers were produced from 10.000 leaves and leaflets. In Exp. 72.1, the effect of various concentrations of GA_3 in solution was studied with 420 leaf parts of both cultivars (for experimental details see Section 4.4.2.6). Relatively high concentrations of GA_3 (e.g. 10-100 mg/1) strongly stimulated callus production in cv. Bintje. Within two weeks after application of GA_3, some shoots were observed. In cv. Bintje one shoot was found in the control series (with IBA 1 mg/1). This shoot could be detached and produced a plantlet from which later normal tubers were obtained. The leaves from mutant Clone B 165

together produced 15 shoots in different treatments. All shoots were situated at the cut base of the petiole and most shoots developed from unrooted leaves.

Somewhat unexpectedly all tubers produced from the detached shoots of this periclinal clone from cv. Burmania, were found to have yellow/red-splashed tubers. Thus the shoots were not of L-I origin only.

We tried to repeat this promising experiment, but in Exps. 72.2 and 72.3 which were practically identical, no shoot formation was observed. Again no shoots were obtained in Exp. 72.5 after BA application to 540 leaves of mutant Clone B 165 from cv. Burmania. Higher concentrations of BA were found to increase callus production

Two shoots were found after treating 720 leaves and leaflets of mutant Clone B 165 with 2,4-D 0.01 mg/l + IBA 1 mg/l, but both shoots died before tubers were produced (Exp. 72.6a). One yellow/red-splashed tuber was obtained from a shoot in the control of Exp. 72.6b. This shoot had developed at the petiole base of a leaf of mutant Clone B 165 in a tray which contained well water with IBA 1 mg/l only.

In Exp. 72.7 with cv. Bintje and mutant Clone B 165 from cv. Burmania 10 shoots or tubers appeared at the cut surface of the petioles or petiolules with and without IBA 1 mg/l, GA_3 0.1 mg/l or BA 0.1 mg/l. Except for one shoot from mutant Clone B 165 all others arose in cv. Bintje. Temperature in the growth chamber was kept at 18°C and the length of the photoperiod was 10 h. The shoots were observed on rooted as well as on unrooted leaves and leaflets.

Relatively succesful was Exp. 72.8 which started in May 1972 with 720 compound leaves of mutant Clone B 165 and cv. Bintje. The leaves, collected 70 days after planting the tubers, were treated with 2,4-D 0.1 mg/l or GA_3 0.1 mg/l. These substances were sprayed on the leaf discs either once or repeatedly. Two photoperiods: 10 and 14 h of thermoluminescent light were given. Within three weeks in total 36 shoots (i.e. 5%) were produced (see Fig. 9) in the treated as well as in the control series, irrespective of photoperiod. Five tubers of mutant Clone B 165 were obtained from the shoots. Those tubers, which occurred in the 10 h photoperiod, without exception were yellow/red-splashed. An axillary origin of some of the shoots again cannot be completely excluded. In this experiment sometimes roots and shoots were present at the same time.

One shoot was obtained from cv. Bintje and five from mutant Clone B 165 in Exp. 72.10 containing 720 compound leaves. In this experiment the leaf discs were sprayed with 2,4-D 0.1 mg/l 1 to 5 times. (For experimental details see Section 4.4.2.3). The shoots were found scattered over all treatments. Three shoots were situated at a distance of 0.5-1 cm from the cut surface. This position suggests an adventitious origin but in mutant Clone B 165 only yellow/red-splashed tubers were produced, so that the tubers can not be derived from L-I cells only.

The previous experiment was repeated (Exp. 72.11) with 360 compound leaves (aged 9 weeks) of mutant Clone B 165, to check whether a slight damage of the petioles (e.g. by bending or by making a small incision with a razor blade) would stimulate the formation of adventitious shoots as was done with roots developed from potato tuber slices (Miedema, 1967). In Exp. 72.11, 10 shoots developed, 7 in the 14 h photoperiod. All shoots were found within three weeks after starting the experiment. They were all situated at the cut base of the petioles and, again, only produced yellow/red-splashed tubers. One shoot of (possible) adventitious origin was obtained in 1972 in an experiment with 480 compound leaves of the diploid test plant Tester III x IvP 48 (see Chapter 3). The

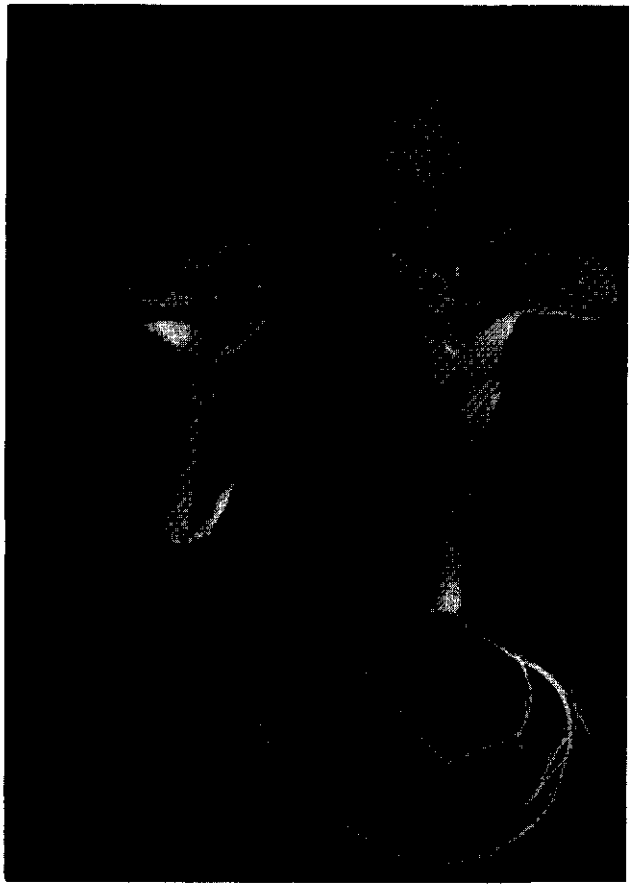

Fig. 9. (Exp. 72.8). Single leaflets of mutant Clone B 165 from cv. Burmania showing an adventitious shoot and root respectively at the basal part of the petiolules after treatment with 2,4-D 0.1 mg/l.

shoot was found in a tray with IBA 1 mg/1, after exposure to 10 h thermoluminescent light and 18°C.

In 1973 about 9000 leaves and leaflets were used, which together produced only about 30 shoots. In Exp. 73.3, 9 shoots were produced within three weeks after submitting 540 leaflets of mutant Clone M 52 from cv. Désirée to two photoperiods (4 h or 10 h).

Interesting results were also obtained in Exp. 73.5 with 720 compound leaves of mutant Clone B 165 from cv. Burmania. Spread over almost three months 19 shoots were obtained in practically all treatments (see Table 8).

Curiously enough this time most shoots did not occur at the petiole base, but in the axils of the lateral leaflets. The emergence of shoots in compound leaves from the axils of lateral leaflets is very remarkable and has not been observed before. Apparently axillary regions can be induced under certain conditions to display more meristematic activity than other parts of the petiolules. It is not known whether there are meristematic rem-

nants in those regions. Another explanation could be the disturbance of the internal hormonal balance after application of 2,4-D. Additional microscopic work was done, but in none of the sections was there any sign of clusters of meristematic cells in the axillary regions. For the first time, we saw that the axils of the lateral leaflets had thickened and made reddish callus. In later experiments, this same phenomenon was found also at the basal part of leaf discs of single leaflets. However microscopic work revealed that such callus swellings never developed into an organized apex and subsequently into an adventitious shoot. In Exp. 73.5 eight tubers were produced. They all were yellow/red-splashed, irrespective of the position of the shoots.

In 1973 in a few other experiments some shoots were obtained under various conditions, but by the end of the year it had become clear that regular production of adventitious shoots from leaves and leaflets of potato under conditions in vivo was not feasible. Moreover, in the opinion of researchworkers like Broertjes (pers. commun.), the presence of callus, as was observed in most experiments, makes direct regeneration of adventitious shoots from single epidermal cells very unlikely. Therefore, after some final efforts in 1974, we decided to abandon the search for a suitable method to stimulate adventitious shoot formation in vivo.

4.4.3.2 Stems and stem parts

Experiments with eye-less pieces of internodes Klopfer (1965a) obtained some adventitious shoots from potato callus, formed at the basal and, sometimes at the apical end of the eye-less pieces of internodes. These internodes were taken from elongated shoots that had developed from sprouting tubers under dark conditions in the spring. Although it was realized that such shoots originate from a callus which may contain all three histogenic layers, we tried in some small experiments to find out whether this method might be useful for mutation breeding.

In 1970 the method of Klopfer was tested with internodes of about 1.5 cm from a number of cultivars. The internodes, 90 in total, were planted in humid sand in a propagating box. The first callus formation was observed after three weeks and one week later some shoots were observed. 26 shoots were obtained, mostly at the basal end of the internodes. For some cultivars, up to 90% of the internodes produced shoots. After eight weeks no more shoots were formed. In this experiment we did not try to produce tubers from the shoots obtained.

The experiment was repeated in autumn 1971 with 75 internodes of cv. Bintje in a mixture of fertile garden soil and perlite in a propagating box in the greenhouse. A part of the internodes was dipped in IBA (in talc). This time the internodes were taken from green aerial stems. After eight weeks some internodes had produced roots, mainly at the basal part, but there were hardly any callus swellings and shoots were not obtained at all.

In another experiment with internodes of Clone 71A8 (Clone 70R100 x IvP48, see Chapter 3), callus formation at the basal and apical end was obtained but no shoot formation was observed. In 1974, internodes of another three clones, treated with IBA (in talc) were studied. Some roots and callus swellings occured but no shoots were produced.

As the method was thought to have no practical value for mutation work, we decided to stop work on internodes and to try another approach: induction of shoots from stems according to the Jørgensen method.

The Jørgensen method The results obtained via this method are summarized here, as a more extensive publication has been written already (van Harten & Bouter, 1974). Our main aim was to investigate whether young, rooted shoots, after being detached from mother tubers, did produce adventitious shoots from L-I origin at the basal part. The treatment was done, according to the advice of G. Helms Jørgensen, son of the late C.A. Jørgensen, who developed this (unpublished) method about 40 years ago.

In four experiments, 122 single-eyed tuber pieces of mutant Clone B 165 and mutant Clone M 52, (periclinal mutants from cv. Burmania and cv. Désirée) were grown in a greenhouse at IvP (Fig. 10, stage A). After three weeks the main shoots were about 8 cm long and had produced roots at the basal part of the shoots while still being attached to the tuber pieces (Stage B). Then the plantlets were nipped off, just above the tuber piece. All visible axillary buds were carefully removed and the apical tips were severed. The plantlets were then placed on a piece of wood with the roots led into the soil (Stage C).

Within three weeks, 3-5 shoots had developed near or at the edge of the basal stem parts (Stage D). After approximately two weeks those shoots could be severed again, planted apart and grown till tuber formation, which was observed on the average after 10 weeks. All tubers were screened for their tuber-skin colour to determine the histogenic origin of the shoots from which the tubers arise. As Table 9 demonstrates, yellow tubers were not obtained at all. The fact that red as well as yellow/red-splashed tubers were found, suggests that these tubers were not of adventitious but of axillary origin. Tubers with red skin could be of adventitious origin, but they are certainly not derived from L-I.

One may conclude that with the Jørgensen method adventitious plantlets cannot be

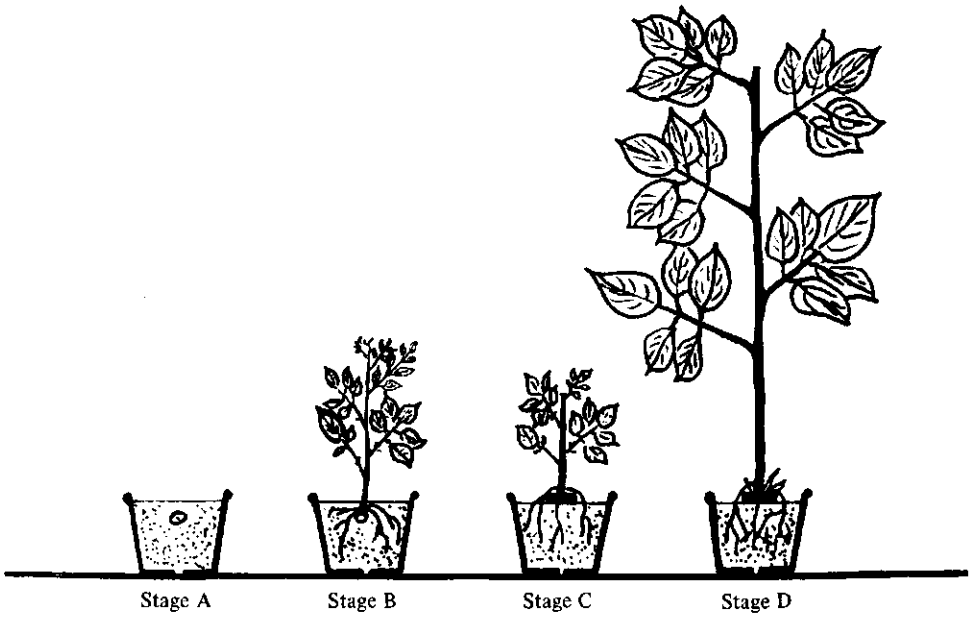

Fig. 10. The different stages of the Jørgensen method of shoot formation. Stage A: Single-eye tuber piece; Stage B: Sprout and root development after 3 weeks; Stage C: Plantlets with apical tip severed and roots nipped off, place on piece of wood. Axillary buds in the basal part removed; Stage D: Adventitious (?) shoot formation around basal part, 6 weeks after starting the experiment.

Table 9. Number of plantlets studied, developed shoots, produced tubers and variation in tuber-skin colour in plantlets obtained via the Jørgensen method (from: van Harten & Bouter, 1974).

No of Series	Clone	Plantlets	Shoots		Tubers	Tuber-skin colour		
			total number	average per plantlet		yellow/ red-splashed	red	yellow
1	B 165	38	311	8.2	77	66	11	0
2	B 165	26	118	4.5	14	6	8	0
3	M 52	24	90	5.1	35	10	25	0
4	B 165	24	51	2.1	5	1	4	0

produced from L-I. The method is also not of much interest as a general method of vegetative propagation, because of the amount of work involved.

4.4.3.3 Concluding remarks on adventitious shoot formation

In Section 4.4.3.1 and 4.4.3.2 it was shown that regeneration of shoots from potato leaves, leaflets, stems and stem parts *in vivo* is possible, but that the incidence of such regeneration is very low, especially for leaves and leaflets. These results are in accordance with findings in literature. Up to now no definite proof could be given as to the adventitious origin of the shoots obtained from leaves and leaflets. Moreover, it is most unlikely that the shoots obtained in our experiments originated from L-I.

Unfortunately the experiments did not provide sufficient new information about the different organogenic processes in potato to enable us to explain adequately why we did not attain our most important objective: the large-scale production of adventitious shoots, preferably from L-I origin only. Most probably there are internal barriers, evidently of a genetic nature, in the potato material itself. We are unable to specify them exactly but it is very likely that endogenous hormone balances play an important role, at least under conditions in vivo. The physiological condition of starting material and parent plants, either directly or via such balances, certainly also affects adventitious root and shoot formation.

In the beginning years (1969-1972), results were disappointing and often contradictory but we still hoped that better controlled experimental conditions would improve results. However even when growth chambers became available and when use was made of tuber material that had been carefully selected, results remained unpredictable and often irreproducible.

After we realized that further experiments along the same line were unlikely to offer new prospects, we looked for new ways to obtain adventitious shoots in potato. In 1974, we decided to start a joint project with C. Broertjes and S. Roest of ITAL, who had considerable experience in producing large quantities of adventitious shoots from small explants of other plant species. They found that explants of *Chrysanthemum morifolium*, irradiated before multiplication under vitro conditions, practically always produced either solid mutants or non-mutated adventitious plants. This result strongly suggests a single-

cell origin of these adventitious shoots (Broertjes et al., 1976) and therefore was promising for our purpose.

To check the possible L-I origin of potato shoots, we used mutant Clone M 52 from cv. Désirée for this work. Segments of the potato rachis of about 0.5 cm were split longitudinally and grown in vitro on a modified Murashige/Skoog medium (Roest & Bokelmann, 1976). This material gave the best regeneration in vitro when compared with explants of tubers, peduncles, pedicels, petioles and leaf discs. Addition of GA_3 to the basic medium was essential for adventitious shoot development. Per shoot forming explant, in the presence of GA_3, up to 50 adventitious shoots were obtained.

With the (periclinally chimeric) mutant Clone M 52 it was possible to check the histogenic origin of the shoots obtained by studying the skin colour of the tubers produced. Results, still based on relatively low numbers of observations, were both surprising and promising. In three series of experiments with different kinds of starting material (pieces of rachis, stripped epidermis from potato stems (L-I only) and subepidermal stem tissue) either solid yellow or solid red tubers were produced. In all series solid yellow tubers were the majority. The absence of periclinal chimeras (which should have produced yellow/red-splashed tubers) and of sectorial chimeras, strongly suggests a one-cell origin of the shoots obtained in vitro.

The results obtained up to now are very promising and agree with those of Broertjes et al. (1976) with irradiated explants of *Chrysanthemum morifolium*. Results will be published after some additional experiments have been performed. An experiment combining irradiation and in vitro techniques for potato in cooperation with ITAL is also under way. Here a clone of cv. Désirée with solid red tubers is used.

Why can one succesful produce adventitious shoots in vitro but not in vivo? In Section 4.2 it was mentioned that with small selected plant parts external regulation is easier and information on the effect of different treatments is more reliable. Moreover it is possible to use very homogeneous starting material which can be raised under sterile conditions and is often younger than for work in vivo. The sterile conditions are favourable to avoid infections during prolonged periods. Nevertheless these considerations do not completely explain the success obtained so far with in vitro techniques in potato, because small plant parts, different age groups, etc. can also be used in vivo.

Another possible answer is that in all experiments in vitro with potato firstly shoots were induced, followed by rooting. In the few successful experiments in vivo, adventitious shoot formation was observed, irrespective of the presence of roots. Moreover in all experiments in vitro adventitious shoots developed after a callus phase, whereas in the experiments in vivo we tried to avoid callus formation as much as possible because the presence of an intermediate callus phase often decreases the chance of any shoots produced being of single-cell origin.

It is possible that adventitious shoots in potato under in vivo conditions can arise only from callus, although in some experiments shoots arose from a petiole base without visible callus formation. We further observed in Exp. 73.5, that the shoots in the axils of lateral leaflets also came from callus. Formation of different types of callus on leaf discs and in several other plant parts has also been observed many times but no shoots were obtained from leaf parts except in Exp. 73.5.

For regeneration via callus which had developed on stem parts in vivo, the situation was different, because many adventitious shoots could be obtained. Finally, the work in vitro has not given us any clues for a suitable in vivo method to induce adventitious shoots (preferably from single cell-origin) from potato leaves and leaflets. The in vitro approach itself however seems very promising.

5 Literature on organization, post-irradiation behaviour and histogenic effects in shoot apices

5.1 Organization of the shoot apex

Different words are often used to indicate the apical area of a shoot. Dermen (1960) mentioned growing point, shoot tip, shoot apex, apical meristem and apical dome. Unless stated otherwise, shoot apex is used in this book to indicate, in a rather general and topological sense, the area including the apical meristem, i.e. the part of the stem laying distal to the youngest leaf primordium (Cutter, 1965), and a few lower leaf primordia.

Several good reviews on shoot apices have been published during the last decades, for example, by Gifford (1954), Von Guttenberg (1960), Clowes (1961), Romberger (1963), Cutter (1965), Newman (1965), Nougarède (1965, 1967), Balkema (1971), Gifford & Corson (1971) and Hara (1973).

5.1.1 Initial cells

Since Wolff in 1759 recognized the apex as the zone from which the rest of the plant originates, the existence of a single, so-called initial cell from which all later cells are supposed to originate, became an important subject of investigation. After such a cell was found in cryptogams, Hofmeister (1852) believed that he was also able to demonstrate the presence of such a cell in phanerogams.

In a strict sense, an initial cell should be a cell that divides into two daughter or sister cells, of which one remains in the fixed (apical) position and the other is added to the meristematic tissue that eventually differentiates further (Esau, 1965, p. 90). Microscopic work soon revealed that it is very difficult, if not impossible, for phanerogams to indicate a specific cell as the initial cell, because a number of similar cells are situated at the apical summit. Nevertheless, the concept of a single initial cell has received support throughout the years from many investigators and discussions on this subject still continue.

Only one initial cell per layer was reported, for example, by Haberlandt (1881) in *Ceratophyllum demersum*, but several other authors (e.g. Bergann & Bergann, 1962) were not convinced by this finding. Indeed it is arbitrary to designate a cell that does not differ from its neighbouring cells except for its position at the apical tip, as 'the' initial cell.

5.1.2 The Histogen theory

Hanstein (1868) described a kind of stratification within shoot apices of angiosperms. He called this phenomenon the Histogen theory. According to this theory a central core of irregularly arranged cells is covered by a number of regular, mantlelike layers of cells. Each layer and the core are said to be derived from initial cells, which are found in a

vertically superimposed position at the ultimate tip of the apical dome. Thus Hanstein abandoned the idea of a single initial cell for the whole plant, but it is not clear whether he and his co-workers believed in the existence of either one initial cell or a small group of initials for each layer.

There is no doubt that Hanstein himself attached more importance to the general distribution of growth in the apex than to the behaviour of the individual cells. When speculating about numbers of initial cells, one should keep in mind that the number of such cells depends on how far one goes back in the ontogeny of the plant as was pointed out correctly by Balkema (1971). In fact zygotes are unicellular and in several cases vegetatively propagated plants can be grown from one single cell.

The ideas, expressed by Hanstein on a kind of stratification in shoot apices are at present generally accepted. Another important feature of the Histogen theory is the strongly predestined role claimed for the different growth layers on histogens and their initials. The outermost layer, called dermatogen, was said to produce only the epidermis, whereas the underlying layer (sometimes layers) or periblem forms the cortex tissue and the central part or plerome the procambium and pith.

This aspect of Hanstein's theory has been strongly criticized. Several authors' observations did not fit his theory, for example Cross & Johnson (1941) who referred to a three-layered tunica in *Vinca rosea*. Foster (1939) even concluded that the Histogen theory had no general validity for seed plants. On the other hand Newman (1965), more recently, pointed out that Hanstein's concept was not as rigid as criticizers believed it to be.

5.1.3 The Tunica-Corpus concept

A new view on shoot organization of angiosperms, which is more flexible about the origin of different plant tissues was developed by Schmidt (1924) and his teacher Buder (1928). This so-called Tunica-Corpus concept only distinguishes between the tunica, which consists of one or more peripheral layers of the apex, and the central mass of cells or corpus. The tunica layers are characterized by anticlinal mitotic divisions, which leads to a certain independence of the different layers. In the corpus, divisions occur in all directions.

According to the flexible interpretation of for example Jentsch (1957) and Bergann (1957a), the Tunica-Corpus theory implies a general, topological zonation rather than a rigid predestined system of cell layers. Especially during initiation of leaves and buds, the discrete character is sometimes disturbed because of the occurence of some periclinal divisions in tunica layers.

During the years several adaptations and modifications of this theory, e.g. the Mantle-Core concept of Popham & Chan (1950) and the Histogenic-Layer concept of Dermen (1945, 1947a), have been proposed and partly accepted. Nevertheless, in general the Tunica-Corpus concept is still considered valid by a vast majority of authors. Others, like Von Guttenberg (1960), rejected the theory entirely because 'it does not relate the apical activity to the origin of tissues'. (Esau, 1965, p. 94). Evidence for the general correctness of the views of Buder and Schmidt was supplied initially from histological work and later on mainly from observing the behaviour of chimeras, notably the (periclinal) ploidy-chimeras, (Gifford & Corson, 1971). (The term ploidy-chimera comes from Brabec (1965)

and is preferable to the more general word cytochimera (Dermen, 1945)).

Satina et al. (1940) and Satina & Blakeslee (1941), although agreeing with the Tunica-Corpus concept, preferred the term germ layers to tunica and corpus layers. From their work with ploidy-chimeras of *Datura stramonium* they concluded that three independent germ layers, indicated als L-I, L-II and L-III (Satina & Blakeslee, 1941) exist in both floral and vegetative shoot apex. One should realize that the central core or plerome in the sense of Hanstein (1868) does not correspond to L-III, but to the cells underneath L-III, which, in the opinion of the authors, are derived from L-III. Thus Hanstein's periblem corresponds to L-II and L-III. No distinctive initial cells were observed in *Datura stramonium*.

Dermen, who also worked with ploidy-chimeras e.g. of cranberry *(Vaccinium macrocarpon)* and later of many other plant species, raised some objections to the Tunica-Corpus theory and referred to the outermost three layers of the apex as primary histogenic layers, histogenic layers, apical layers or simply histogens (Dermen, 1945, 1947a, 1947b and later). He believed that probably three histogens are present in the shoot apices of all angiosperms (Dermen, 1951). Sometimes ploidy-chimeras involving more layers, e.g. of the 2,2,2,2,4 type, were found (Dermen, 1967) but he believed that they were derived originally from simple 2,2,4-types.

5.1.4 The 'anneau initial' concept

Besides the ideas on initial cells and layers, another division within apices was proposed. According to Plantefol (1947) leaves are disposed along several foliar helices which end in a meristematic tissue at some distance from the apical tip. He thought that the cells of this tissue which together are called the 'anneau initial', should be assigned the role of the apical initial cells of earlier theories. No true initials were thought to be present at the summit of the axis during the vegetative phase of development of dicotyledonous plants.

Buvat (1952), following Bersillon (1951), called this second group of differentiated cells the 'méristème d'attente', because he thought that no divisions occured here during the vegetative phase. After transition into the generative phase the 'méristème d'attente' starts to divide and takes over the activity of the 'anneau initial'. A prerequisite for this is, that already differentiated cells at the apex have to dedifferentiate first. Evidence for these opinions was claimed to come from cytological observations.

Some years later Buvat (1955) modified his stand point in so far that he reported that summital cells may show some division. Buvat also admitted that these cells were in a sense mother cells because of their position. However he maintained his opinion that such cells have no specific organogenic properties.

This French concept of zonation was not entirely new. Differences in zonal structure in the apical meristems of angiosperms in relation to the stage of development, particularly between the vegetative and the floral shoot, had already been reported, for example by Gregoire (1938). Foster (1938, 1939, 1941), although mainly referring to gymnosperms, stated that in the apical area more or less well defined tissues zones can be distinguished which may show differences in cell size, nuclear size, planes of divisions, relative frequency of mitosis, stainability and thickness of cell walls. In this work no anatomic evidence for the existence of initial cells was found.

Differences within shoot apices were reported e.g. also by Majumdar (1942), who

demonstrated cyto-histological zonation in an angiospermous plant, *Heracleum sphondylum*. This zonation is superimposed on the division in tunica layers and corpus. According to Majumdar there is a self-perpetuating group of central initial cells at the summit, surrounded by a cylinder of more active flank meristem from which the primordia initiate.

The original, rather rigid concept of Buvat (1952), which was strongly criticized by most scientists in this field, for example by Gifford (1954), stimulated a revival of shoot apex investigations.

5.1.5 Present views and conclusions

In recent years it has become more and more clear that the often cited controversy between mainly the Anglo-Saxon and French school must be attributed at least partly to misinterpretations of each other points of view. Undoubtedly linguistic problems played a role. Due to recent cytological work, studies of chimeras and of marked cells, the opinion of the two schools are no longer so extreme.

Nowadays it is generally accepted that cells at the summit of vegetative apices divide, although at much lower rates than along the flanks. Many factors may influence the mitotic activity in different cells or plant parts and at different stages of plant development (Balkema, 1971).

A problem which still remains is the possible presence, role, number and position of initial cells or cell groups. Foster (1939, 1941) doubted whether such cells with a constant number, form and sequence of division could be found. Dermen (1945) stated: 'It appears to be purely a matter of chance whether one cell, two cells or three cells have a central position in each histogenic layer'. Thus the size of a mutated 'sector', derived from a mutated cell in the apical summit, depends only on the number of cells present at that moment in apical position (provided the mutation has not affected the fitness of the mutated cell).

Bergann (1954 and later) denied the existence of a 'Scheitelzelle' in a shoot apex and rejected the possibility that each histogenic layer could be traced back to one initial cell. Soma & Ball (1964) and Ball (1972) concluded from their experiments with *Lupinus albus* cells, which were marked by puncturing a single cell or by applying a very small amount of powdered carbon to the apical tip, that cells from the apical summit shift to the flanks during shoot growth. Therefore they did not believe in initial cells with a stable position. There is, of course, no definite proof that the authors indeed were able to mark the 'real' initial cell or cells (if such cells do exist). In this respect Catesson (1953) & Steffensen (1968) referred to a rotating movement of the apical dome, e.g. in relation to the place of initiation of the youngest leaf.

Other objections to the ideas on initial cells have come e.g. from Thielke (1951), Bergann (1965) and several others. Some of these authors even denied the permanent character of a group of apical initial cells as do many French scientists like Dulieu (1970), who stated that permanent cells (i.e. initial cells in a strict sense) do not exist, neither at the apical summit nor along the flanks, not even in the sense of a cell lineage with a permanent axial position for one of its elements.

Dulieu (1970; pers. commun., 1972) considered that his ideas were in line with those expressed before by Soma & Ball (1964) and by Nougarède (1965). He thought of initial

cells as a group of cells, left as a residue at the summit if regeneration occurs from the flanks which is normally the case. According to Dulieu the dimension of this residue determines its stability, and therefore the stability of cell lineages of a possible mutation in one of the residual cells.

Stewart & Dermen (1970b) who studied narrow and wide mutated 'sectors', caused by mericlinal stripes in periclinal chimeras, found that narrow sectors were always short. Wide sectors on the other hand were remarkably stable. This last fact indicates that such sectors can be traced back to a kind of initial cell, which must be situated at the summit of the apical dome. Only if such initials do retain their position for a longer period, a long sector can be formed. If an initial cell divides, the authors consider the most distal daughter as the new initial, or as the continuation of the old one.

The size of the observed wide sectors provides evidence that two or three initial cells per layer do exist. Stewart & Dermen calculated that an initial cell of *Ligustrum ovalifolium* divides only once in 12 days. According to these authors, this low rate of division suffices to make these initial cells the ultimate source of all growth. It also explains why several authors found no divisions at the apical summit and introduced concepts like that of a nondividing 'méristème d'attente'.

At present most workers believe that the apical meristem is made up of a dynamic, changing population of dividing cells. Romberger (1963) stated that 'cells behave as they do because they are where they are' or, in other words, so-called initial cells are initials because of their particular position in the apex. Recently, Soma (1973) said that apical meristems seem to be selforganizing, the growth and morphogenesis of which may be determined by the apical meristem itself. (N.B. 40 years earlier Thoday (1939) almost used the same terms: 'It will probably be admitted that the shoot apex is a selfdetermining and dominant centre of development').

In conclusion it is clear that, whatever views exist, some kind of ultimate 'source' of primary growth, or a 'continuing meristematic residue' in the terms of Newman (1965), must be situated in the distal part of the stem. The nature of this source remains the topic of many investigations and discussions.

With respect to the organization within a shoot apex, for most phanerogams the presence of a number of rather stable cell-layers has been established. Normally one or two, mainly anticlinally dividing (tunica) layers are present, but sometimes, e.g. in the genus *Saccharum*, only corpus tissue is found (Thielke, 1951). Each layer has a limited number of so-called initial cells (in a non-deterministic sense), situated at the distal end of the apical dome. Those cells are probably of a non-permanent nature. Division at a very low rate is sufficient to maintain their function for a certain time as the ultimate source of all cells and tissues of the growing shoot.

5.2 Axillary and adventitious buds

In many respects axillary and adventitious buds are similar to apical shoots parts. Nevertheless in some aspects they do differ mutually as well as compared with terminal shoot apices. Differences between axillary and adventitious buds, according to most authors, are found especially in their place of origination and in the rate of differentiation of the tissues from which they arise.

Axillary buds are formed on the stem, apparently in close association with the leaves,

somewhat later than the leaves beneath them (Sussex, 1955). According to Esau (1965, p. 109) the term 'axillary' is somewhat inaccurate, as there is no direct developmental relation between the bud and the subtending leaf. This is particularly clear in, for example, grasses where the bud initiates close to the leaf above it. Most authors, however, still refer to axillary buds in relation to the subtending leaf. Axillary buds are separated from the top meristem by one or more leaves. Sometimes axillary buds can be recognized as such by their connection with the primary vascular tissue via a so-called branch trace, e.g. in apple (MacDaniels, 1953).

Initiation of axillary buds is marked by cell divisions in all directions in the deeper layers of the stem parts above the base, together with or before anticlinal divisions in one or more superficial layers. Some time after initiation, they acquire the organization of a normal apical bud (Garrison, 1955). A point of some controversy is whether axillary buds reproduce the layered structure of the apical area from which they arise. For example in potato, Howard et al. (1963) suggested that axillary buds are often derived from L-I and L-II of the shoot apex only, because of a tendency of cells from L-II to replace L-III. Periclinal divisions, occasionally occuring in L-II, may be responsible, Klopfer (1965b) on the other hand presented evidence for an identical reproduction of the three-layered structure of the potato shoot apex when axillary buds are formed.

Schmidt (1924) mentioned that the relative contribution of the different layers does not necessarily have to be the same for bud and leaf initiation in a certain plant species. According to Von Guttenberg (1960), axillary buds more often originate from deeper layers than the leaf buds. Working with plants of the genus *Linaria*, Champagnat (1961) on the other hand reported that for this species axillary buds are of epidermal origin.

Axillary buds develop either directly from the apical meristem or from a more or less differentiated meristematic residue, separated from the apical meristem. Apparently this residue is mostly of apical origin. According to some authors (see e.g. Dormer, 1972) sometimes there is no remnant of an apical meristem. The axillary (?) meristem is then supposed to differentiate as part of the ordinary surface of the stem. Although again considerable variations may exist between species (e.g. Garrison, 1955), usually the youngest leaf primordium in the shoot apex does not have an axillary bud.

At a certain moment axillary buds go into dormancy under the influence of some action of the apical bud. There are at least two main hypotheses to explain how axillary buds are arrested (Philips, 1969; Guern & Usciati, 1972). One postulates inhibition by nutrient deprivation and the other hormonal inhibition. The dormancy may terminate in a natural way, e.g. by seasonal influence, or can be broken artificially.

Most details from literature on adventitious buds have been given in Chapter 4. Here it is sufficient to recall that such buds arise outside primary meristematic regions or meristematic remnants of apical origin. They can occur in complete plants or in explants, and may be of exogenous or endogenous origin. Depending on many factors, they may be derived from one single cell or from more cells. When more cells are involved also more than one (histogenic) layer may be represented in the new bud.

5.3 Mutations and the consequences of their position of origin within the plant

Mutations, either of spontaneous origin or artificially induced, may occur in any part of the plant and in all kinds of tissue. However, a single mutational event is restricted to

one cell only. The further fate of this mutated cell is strongly affected by its position within the plant (Dommergues, 1964). Bergann (1968) in this respect distinguished between intra-apical and extra-apical mutations. Intra-apical mutations are those occuring both in shoot apices and in axillary meristems. All mutations outside these regions are indicated as extra-apical.

As was mentioned in Sections 5.1.1 and 5.1.5 a relatively limited number of 'initial' cells exists within shoot apices. If a non-lethal mutation is induced in such a cell, a mutated cell lineage may be produced. Its dimensions depend on the original number of 'initial' cells, the number of cells surviving and actively dividing after mutagenic treatment, on the position of the mutated cell within the apex and on the fitness of this cell and its derivatives to compete with (apparently) non-mutated cells (diplontic selection).

Renner (1936), Thielke (1951), Dermen (1951) and many others demonstrated that mutations may occur in any layer of an apical meristem. Bateson (1916) raised the question whether spontaneous mutations occur equally often in each layer. The same question, of course, can be asked about artificially induced mutations. (N.B. Calculations must be made on the basis of mutation rate per cell.)

According to Dommergues (1964) it is logical that, after mutagenic treatment, all cells of an embryo or of a bud have an equal chance of being mutated. In the light of present knowledge, the correctness of this statement is doubtful. Shoot apices are not as homogeneous as was believed in the past. Cells within the apex may act quite differently, physiologically speaking. In addition it seems logical to assume that differences in physiological activity between apical cells leads to various reactions of these cells to irradiation.

There is no common opinion about whether mutations in the main shoot apex are more important than those in the axillary meristems for the plant breeder. Lapins et al. (1969) believed that 'the apical meristem rather than axillary meristems should be the center of interest in inducing mutations', because this is the area with the highest meristematic activity. On the other hand it should be remembered that fewer cells are present in axillary buds so that the chance of obtaining large mutated zones or even solid mutants is increased.

It has been demonstrated that the stage of development of the buds may affect the result of irradiation with regard to induction of mutation. For example, older shoots often produce more mutations than younger stages as was shown for apple and pear by Lapins et al. (1969) and for *Parthenocissus tricuspidata* by Langenauer et al. (1972, 1973).

Finally, Bergann (1968) thought that extra-apical mutations are of no value to the plant breeder, no matter whether they occur in the common plant tissues or in meristems. This view is incorrect. In a large and still increasing number of plant species, formation of adventitious shoots can now be provoked from different parts of the plant outside the apical regions (Broertjes et al. 1968, and many later authors). Thus also extra-apical mutations can be put to practical use. When adventitious shoots arise from single mutated cells, a solid mutant can be obtained immediately.

5.4 Chimerism

A plant consisting of two or more genetically different somatic tissues is called a chimera. The word chimera was originally proposed by Winkler (1907) for organisms like

graft-hybrids, where a stable individual results from a combination of tissues of different plant species without cell fusion. Baur (1909) extended this definition to include intra-specific organisms consisting of (somatic) cells that are genetically dissimilar. Chimerism may follow when more than one cell is present at the moment of a mutation and exceptionally under some other conditions e.g. when somatic crossing-over occurs (Vig & Paddock, 1970).

Originally, two categories of chimeras were recognized, namely sectorial chimeras (Winkler, 1907) and periclinal chimeras (Baur, 1909). A third group, indicated as mericlinal chimeras, was added by Jørgensen & Crane (1927). In vegetatively propagated phanerogams with layered apices, after one or a few cycles of propagation, periclinal chimerism is the common situation for plants carrying a mutation (Dermen, 1974b; Thielke, 1951; Tilney-Bassett, 1963). Most so-called mutated 'sectors' in literature are, in fact, mericlinal chimeras since the mutation is confined to a part of one histogenic layer and its derivatives. In all three types of chimera, the mutated cells remain united and occupy a certain area of the plant. If the mutated cells are dispersed throughout the plant, chimerism is of a mosaic nature.

In some publications the word chimeroid is used for an extra-apical heterohistic mutant (Bergann, 1967). A distinction between chimeras and chimeroids seems meaningless.

Several ways of further classifying periclinal chimeras exist. Some of them are briefly described here because many words are used wrongly and inconsistently in the literature. Asseyeva (1931) who worked with potato, distinguished three categories of periclinal chimeras according to the layer in which a mutation occurred: the epidermal type, the subepidermal type and the dichlamydeous type in which both outer layers of the apical meristem are mutated. The epidermal type and the subepidermal type are both indicated as monochlamydeous types (the words 'monochlamydal' and 'monochlamydous' are used also in literature). If there are three histogenic layers, these categories do not cover all possibilities, e.g. a mutated L-III or a structure with L-II + L-III mutated cannot be properly indicated.

Bergann (1954) proposed another system of classification, which is summarized in Table 10. This table gives the six situations which are possible if only two genetically

Table 10. Classification of types of periclinal chimeras. Adapted from Bergann, 1954.

Type of plant	Type of chimera	Mutated layer(s)		
		L-I	L-II	L-III
Heterohistic (periclinal chimera)	1. Monecto-chimera	x		
	2. Monecto-chimera (invers)		x	x
	3. Diecto-chimera	x	x	
	4. Diecto-chimera (invers)			x
	5. Meso-chimera		x	
	6. Meso-chimera (invers)	x		x
Homohistic	Non mutant			
	Solid mutant	x	x	x

different components are present. The normal starting point is a mutation in a single layer. A mutation, in L-I for example, may be duplicated and subsequently produce a diecto-chimera. By adding the word 'invers', Bergann classified all existing types of periclinal chimeras in which only one mutation is involved. The 6th possibility (meso-chimera invers) has been added here because Bergann makes no distinction between both types of meso-chimera. Bergann's system is useful, although in some cases it may be confusing.

Sometimes three genetically different components are present. Bergann (1954, 1955, 1967) who referred to this last category as trichimeras, thought such types occur rather frequently in plants that have been propagated vegetatively for a long time. This opinion is rather doubtful, especially as L-II and L-III often show a tendency to become genetically identical. This was demonstrated, for example, by Péreau-Leroy (1975) in carnation (*Dianthus caryophyllus*).

5.5 Rearrangements of cell layers

As was mentioned in Section 5.1.5, the layered structure of shoot apices is partially unstable. According to Clowes (1957), different plant species may show various degrees of instability. The inner apical layers (i.e. L-II and L-III) are always less stable, i.e. they lose their discrete character more easily. Mostly this instability is caused by periclinal divisions which often occur after inactivation or death of adjoining cells. Such divisions may lead to penetration of cells from one histogenic layer into another (Bergann, 1957a).

The phenomenon is, from a breeder's point of view, of special interest in vegetatively propagated plants but also, be it to a lesser degree, in seed propagated plants. Normally, mutations in L-I or L-III are lost during generative propagation as pollen and egg-cells originate from the (subepidermal) L-II layer. If mutated cells from L-I or L-III penetrate L-II, they have a chance of being transmitted via the seed to the next generation.

Effects of rearrangements can be studied by phenotypic observations if use is made of plant material in which the layers differ genetically in one or more visible characters, i.e. of periclinal chimeras. Recently Stewart et al. (1974) have added interesting information concerning competition between apical layers.

One of the very few publications giving rates of cell exchange between layers is by Stewart & Burk (1970). Their values are based on calculations of dark green spots on the surface of white tobacco leaves with the original constitution L-I green, L-II + L-III white. The dark green spots occur when green L-I tissue replaces L-II cells. They estimate the frequency of periclinal divisions in L-I at 1 : 3100. Another calculation, based on a survey of young leaves, gave a ratio of one periclinal division to about 1700 anticlinal divisions.

The results clearly demonstrate the low frequency of disturbances spontaneously occurring in the layered structure of shoot apices. Nevertheless, according to Bergann (1957b, 1967), the frequency in nature is high enough to be of practical importance for plant breeding. Irradiation may considerably increase the frequency of such events. Especially in the DDR (Bergann, Klopfer, Pötsch) and in the USA (Dermen, Popham, Stewart) attention has been paid to both spontaneous and radiation-induced histogenic effects.

A number of different processes or types of rearrangements can be distinguished. Bergann & Bergann (1959, 1962) recognized the following types:

a. Perforation, when cells of the outer layer(s) are replaced by cells of L-II or L-III;

b. Reduplication, when the outer layer or layers partly or completely duplicate via periclinal division and replace L-II or L-III;
c. Combination of a and b;
d. Transgression or lateral replacement, a process within one (genetically heterogeneous) layer whereby one component shifts the other one sideways.

Bergann & Bergann (1962) referred to perforation and reduplication as vertical processes, i.e. events between layers; transgression is called a horizontal process. A combination of perforation or reduplication and transgression according to Bergann & Bergann can lead to so-called 'translocation', i.e. complete layer-reversion. The authors gave a few examples dealing with types derived from *Pelargonium zonale*.

Some comments must be made on this work. The choice of the words translocation and transgression is rather unfortunate, since they have an older established meaning in cytogenetics and general genetics. Even if these words are used in a histogenic context, it is difficult to adopt them. The consistent use of 'layer-inversion' and 'lateral layer replacement' is perhaps preferable. Another disadvantage of the East-German work is that exact data for the frequencies of the described events are never given and these are necessary if one wants to distinguish between 'real' mutagenic effects and histological effects of mutagenic treatments.

Dermen (1960) proposed the words 'replacement' and 'displacement' to describe certain histogenic events. Replacement is used for events in which cells from outer layers penetrate the inner tissue and replace cells there. Displacement is used to indicate the opposite when internally situated cells replace those in outer layers.

Finally care must be taken when drawing conclusions about histogenic effects in plant material where competition (diplontic selection) occurs (Stewart et al., 1974). Thus in periclinal chimeras competition is between mutated and non-mutated layers and their derivatives. This phenomenon certainly plays a more important role in ploidy-chimeras, with cells of different size and maybe, different vitality, than in periclinal chimeras in which histogenic layers only differ for example in a gene for flower colour.

5.6 Non-genetic effects of radiation upon shoot apices

5.6.1 General remarks

After irradiation generally two groups of effects are distinguished: primary injury (often but not quite correctly indicated as physiological damage) and genetic changes or mutations (Sax & Swanson, 1941; Anonymous, 1970, p. 85). Only effects of the first group are discussed here.

This group includes a large number of very different, non-genetic effects, which may be of physiological, morphological or anatomical nature. Cells may die, collapse, show an increased vacuolation, or look 'empty'. Cell divisions may be inhibited or retarded. Sometimes an excessive formation of idioblasts is observed. The whole apex may become disorganized and show disturbance of the layered pattern and replacement of damaged cells. Often a new apex is formed from a few cells which survived irradiation or the whole apical function is taken over by axillary or adventitious buds. (Sparrow, 1961; Lea, 1962; Haccius & Reichert, 1963; Pratt, 1963; Iqbal, 1970; Balkema, 1971).

Several investigators have tried to develop a system for classifying different degrees or types of damage within shoot apices. Use is made of several methods and techniques. Sometimes numbers of damaged and undamaged cells are scored, e.g. per layer (Iqbal, 1970) In other cases autoradiographic techniques are used (Partanen & Gifford, 1958; Gifford et al., 1963; Brown et al., 1964) or enzymatical changes are scored (Fosket & Miksche, 1966). Sometimes different patterns of damage and recovery can be observed, depending on the dose of irradiation applied (Pratt, 1963).

Not all cells within shoot apices display the same physiological activity. In addition, at a given moment, not all cells are in exactly the same stage of the mitotic cycle. Such differences lead to various reactions of the cells upon irradiation treatments. Many studies have been done on differential radiosensitivity of cells, plant parts, various stages of development and plant species (Section 5.6.2).

In Section 5.6.3 attention is given to one specific category of non-genetic effects of irradiation, i.e. histogenic effects. As stated before such effects also occur without irradiation in nature, but their frequency can be considerably increased by radiation treatments. The breeder may benefit from histogenic effects or they may be considered as a stumbling block.

Sometimes genetic and histogenic effects are difficult to separate, so that occasionally results are wrongly interpreted. Pötsch (1966a), for example, referred to the work of Jank (1957a,b) with *Chrysanthemum indicum*, where undoubtedly histogenic effects were interpreted as real mutations. The same may be true for the report of Richter & Singleton (1955) on very high frequencies of flower colour changes after chronic gamma irradiation of different clones of carnation (*Dianthus caryophyllus*).

Finally the interesting question can be raised whether radiation-induced histogenic effects could be manipulated, for example by increasing the frequency of displacements at the cost of replacements or vice versa.

5.6.2 Radiosensitivity

Radiosensitivity (sometimes referred to as radiation sensitivity) is a complex phenomenon. In general authors mean the response of plants, plant parts, certain developmental stages or of a specific molecular function of a plant or cell to irradiation (Dertinger & Jung, 1970).

Henshaw & Francis (1935) mentioned 4 groups of factors that may be associated with radiosensitivity in tissues and cells: the state of activity (metabolic rate, growth rate), the stage in life cycle (age, degree of differentiation, phase in mitosis), physical conditions (temperature, water content, permeability) and protoplasmic constitution. Yamakawa & Sekiguchi (1968), Blixt (1970) and many others after them, concluded that resistance to radiation depends primarily on the repair capacity rather than on the original damage.

Both primary injury and mutagenic events are used to determine radiosensitivity. Blixt mentioned a number of criteria: measurement of germination inhibition, inhibition of growth or cell division, frequency of (different kinds of) chromosome aberrations, number of cells with micronuclei, flowering inhibition, seed fertility, pollen tube growth, survival of mature plants, cotyledon length, plant weight, staminal hair growth, etc.

Sparrow et al. (1953, 1961, 1965), Ichikawa & Sparrow (1967) and many others explained differences in radiosensitivity between distinct plant species or between diploid

and polyploid plants of one species mainly on the basis of differences in nuclear size and average interphase chromosome volume (ICV). Recent Russian research on *Vicia faba* and *Triticum* species suggests that radio-resistance is directly correlated with the sulphydryl content (Semerdzhyan & Nor-Arevyan, 1971). Most investigators agree that other internal factors and external conditions also may play a considerable role. Authors disagree about the relative effect of the different factors. Strangely enough the effects of external conditions on radiosensitivity have been seldom studied, whereas it seems that they may have a considerable effect.

An important point of discussion is whether, and to what extent, radiosensitivity, or certain components of this phenomenon, are controlled by the genetic constitution of the plant. Early examples of genetic control of radiosensitivity, reviewed by Blixt (1970, 1972) referred especially to bacteria (*Escherichia coli*) and animals (*Drosophila*, mice). Smith (1942) reported the first example for higher plants after irradiation of *Triticum monococcum*. More recent examples were described by Yamagata et al. (1969) for non-tuberbearing *Solanum* species and by Blixt (1970, 1972) for γ-sensitivity in *Pisum sativum*.

Differences in radiosensitivity are not only found between species, but also between different cultivars, subspecies and even between isogenic lines, as has been demonstrated by Johnson (1933) for *Atriplex hortensis* and by Walther (1969) for barley. Within one plant species differential radiosensitivity may occur between different parts, tissues or cells, between different stages of development (Gunckel & Sparrow, 1954) or different stages in the mitotic cycle of one cell (Sax & Swanson, 1941; Bishop, 1950). Clowes & Hall (1966) thought that differences in radiosensitivity of continuously irradiated cells of root meristems from *Zea mays* and *Vicia faba* plants must be explained by differences in length of mitotic cycles of the cells.

According to (part of) the old 'law' of Bergonie & Tribondeau (1906) actively dividing cells are more sensitive to irradiation. Nowadays broadly speaking this is still considered valid (Sparrow, 1951; Pratt, 1968). The well-known fact that in whole plants meristems are more sensitive than further differentiated parts, also complies with this law. Young, active cells have thinner cell walls, smaller vacuoles and contain relatively more protoplasma, so that more ionizing radiation is absorbed by the cell contents (Levitt, 1972).

Although an impressive amount of research has been done in this field, much has yet to be explained. Gunckel (1957) for example, stated that not all dividing cells are (equally) radiosensitive. Lapins & Hough (1970) concluded that the most actively dividing cells are not necessarily the most radiosensitive ones. These findings are of importance in relation to irradiation of growing points.

To study the radiosensitivity of single cells or certain cell stages, entire plants or organized plant parts are not the ideal material. On the other hand, very little is known of the behaviour of cell suspensions or callus after irradiation. From a γ-irradiation experiment, Bajaj (1971) concluded that callus tissue of different plant species has a lower radiosensitivity than seedlings, very probably because cells of callus cultures are less organized and have a low degree of differentiation. High relative radioresistance of callus tissues was also reported by Melchers & Bergmann (1959).

After irradiation, sometimes axillary apices take over the function of the main apex because they are more radioresistant due to the low level of activity caused by the apical dominance of the main apex. After irradiation the main apex is the first area to be

inactivated. This process has been indicated as 'internal disbudding' (Yamakawa & Sekiguchi, 1968).

5.6.3 Patterns of radiation-induced morphological/histological damage and recovery

Normally the structure of axillary buds is a true reproduction of the layered structure of the main apex, although sometimes 'failures' occur in nature. Radiation is known to increase the frequency of such phenomena. Literature was scrutinized to find out the types of radiation damage and recovery, how often such events occur, and whether type, dose and dose rate of the irradiation treatment has an effect on them.

5.6.3.1 Systems of classifying damage and recovery in shoot apices

At present most authors classify morphological damage in terms of 'localized', 'scattered' (or 'random') or 'uniform' damage patterns.

The first examples of localized damage were described by Sagawa & Mehlquist (1957) and by Miksche et al. (1962). Sagawa & Mehlquist X-irradiated three clones of carnation (*Dianthus caryophyllus*): the solid red-flowered cv. William Sim and two clones (pink and white respectively) with an aberrant L-I; L-II + L-III being genetically red. The high frequency of changes (50-90% of the white or pink flowers became red!) made it very unlikely that these were due to mutations. Anatomical investigations revealed that radiation destroyed the outer layers and caused subsequent regeneration of the new epidermis from the deeper 'red' cells. Changes from red to white or pink, on the other hand, were observed only at a frequency of 4% for the highest dose (5 kR). These changes most probably were due to real mutations.

Miksche et al. (1962) working with the gymnosperm *Taxus media*, claimed that in shoot apices an 'outer to inner pattern of decreasing radiosensitivity' may occur. In other words, the outer layer should be most radiosensitive. This work aroused much interest and has been often quoted, although not always correctly. Hildering & van der Veen (1966), for example, referred to the work of Kuehnert (1962) on tomato and considered his results as an example of the 'outer to inner' pattern. However, this deduction is not correct if one looks more carefully at these results. Moreover the model of Miksche et al. was not designed to indicate differences between histogenic layers, but between different areas of the shoot apex such as the initial layer group, the central mother zone and the pith rib meristem.

Often the frequency of periclinal divisions in a specific layer is used to indicate its radiosensitivity. However, such divisions could be considered as an effect of recovery, e.g. when another layer is disorganized and when cells of the layer in question have to replace the damaged cells.

As a second type the 'uniform' or 'all or none' damage was reported for example by Stein & Steffensen (1959a) for *Zea mays*, Foard & Haber (1961) for *Triticum vulgare*, Haccius & Reichert (1963) for *Nicotiana* sp., Fosket & Miksche (1966) for *Pinus lambertiana* and Mergen & Thielges (1966) for *Pinus rigida*. As the terms indicate, these authors found either completely inactivated apices or no damage at all. It seems that one should be careful in classifying apices in this way, as additional observations might lead to other conclusions.

A third type of damage was observed by Haccius & Reichert (1963) in *Arabidopsis thaliana* and by Pratt (1968) in *Prunus avium* (sweet cherry). This type is the so-called 'scattered' or 'random' damage and is clearly distinct from the uniform and localized (e.g. in one layer) damage types.

Pratt (1968) checked how often the different types occured. Out of 14 different plant species, 7 showed localized damage, L-I normally showing the lowest level of damage. The other 7 species showed uniform or scattered damage.

Another worker, Iqbal (1970), scored damaged and undamaged cells in different layers and regions of shoot tips. By calculating the amount of damage within single cells, he distinguished 4 degrees of damage. In a more recent publication, Iqbal (1972) also included nuclear and interphase chromosome volume and survival of seedlings.

Pratt (1963), working with ploidy-chimeras of apple, distinguished three types of recovery which sometimes overlap. These types are:

a. Direct healing of the apical meristem, leading e.g. to compression of a nonfunctioning, damaged zone between L-I and the inner layers.
b. Formation of substitute meristems (in cells that lie laterally to the damaged zone).
c. Development of axillary meristems, separated from the apical meristem by at least one leaf.

5.6.3.2 Examples and comments

The first example of a radiation-induced histogenic effect was reported by Asseyeva (1931) who mentioned that Gusseva & Lopatin in the USSR X-irradiated a potato with a monecto-chimeral structure for tuber-skin colour and obtained a diecto-chimera. This work was successfully repeated by Howard (1958). No information is available about the frequency of this event.

Crocket (1957) studied differences between irradiated and normal stem apices of actively growing plants of *Nicotiana tabacum* and interpreted his results in terms of the 'anneau initial' / 'méristème d'attente' theory. After 14 days of γ-irradiation (80 rad/day, 20 hours daily) periclinal divisions were found in the L-I of the 'méristème d'attente' area, but not in the corresponding L-I of the 'anneau initial' area, nor in tunica layers of unirradiated tips. In irradiated apices L-II becomes disorganized and loses its discreteness over the whole of the irradiated apex. Cells from L-I, which layer had not been damaged, replace the heavily disturbed L-II.

Crocket suggested a difference in radiosensitivity between L-I and L-II. He inferred that the 'anneau initial' (except for L-II) was probably more radioresistant than the 'méristème d'attente', which is in contrast to the common idea that resting cells generally are more radioresistant. This early work demonstrates that differences in radiosensitivity may exist between different areas within a shoot apex (viz. summital area and flank regions) and also between different histogenic layers.

One would expect a higher radiosensitivity of the cells along the flanks in vegetative apices as these cells normally show a higher mitotic activity because of leaf initiation in this area. This was not found in Crockett's work. Many conditions or circumstances can account for this, such as the type and period of irradiation, the length of the mitotic cycle in different apical regions and the stage of plastochron. As flanks and summit show a different reaction to radiation, it seems advisible to make different calculations for

these regions, e.g. when frequencies of damaged cells are determined.

From a practical point of view, it does not matter much from which region (summit or flanks) within the apex, organogenesis takes place as long as the relative role of the different histogenic layers in organogenesis remains the same. The situation becomes different when, as in Crockett's work, one layer is inactivated. Especially when mericlinal and periclinal chimeras are used as starting material for radiation treatments, differential radiosensitivity between layers may drastically affect the final result. Up to now, one can only speculate about the direction in which the different effects work. Moreover, does a high radiosensitivity of a layer also mean a high mutability?

Pratt (1959), working with the Concord grape, found that first (microscopically observable) radiation-induced damage of rooted cuttings occurs in L-II, which probably could be explained by a higher radiosensitivity of L-II. According to the author this seems to contradict the results of Sagawa & Mehlquist (1957) with carnation, but these authors were only distinguishing between a number of outer layers at one side and the inner tissue at the other side. Perhaps regeneration did not take place at all in the real apical meristem but from below this region.

From another experiment with chronic γ-irradiation of apple, Pratt et al. (1959) concluded that L-I has a lower radiosensitivity than L-II. They explained this conclusion by the lower number of divisions which occur in L-I. In a later experiment with apple (Pratt, 1960), L-I again appears to be more stable after irradiation with semi-lethal doses. In the last case ploidy-chimeras (L-I diploid, inner layers tetraploid) were used, which makes a comparison difficult. Pratt believed that layer sensitivity 'seems to be related with the position of the cells in the meristem rather than to the ploidy of those cells', but added that there might be a threshold dose at which differences in response could be found.

Dommergues (1961), exposing shoots of cv. Max Red Barlett pears to γ-irradiation, considered his results to agree with the conclusions of Sagawa & Mehlquist (1957) that the outermost layers are most sensitive to radiation. In our opinion the work of Dommergues does not provide sufficient particulars to allow such detailed conclusions. Lapins & Hough (1970) went even further and interpreted the results of Dommergues as indications of a relatively high radiosensitivity of L-II.

Gunckel & Sparrow (1961) reported that after irradiation of floral apices of haploid tomatoes (*Lycopersicon esculentum*), the outer layer of the apex shows damage first, which seems to be rather exceptional. Neutron irradiation of *Lycopersicon esculentum* and *L. pimpinellifolium* seedlings gave periclinal divisions in the central part of L-I and L-II (Kuehnert, 1962). L-II became disorganized, the central cells of it showing lysis. Via periclinal divisions in L-I, cells may be produced which penetrate L-II and take over the function of L-II which is said to be more radiosensitive than L-I.

Weaver (1963) mentioned a relatively high radiosensitivity for L-II of *Chrysanthemum morifolium* and made the important remark that 'since this tissue is gametogenic, such effects may contribute to an unfavourable environment for the selection of useful mutations in seed propagated plants'. This consideration brings us back to the question whether a high radiosensitivity also means a high mutability for the same area or layer. An answer cannot be given yet.

Weaver (1963) as well as Pratt et al. (1959) referred to different rates of mitotic divisions in the different histogenic layers as a possible explanation of the observed

'preferential destruction'. Clowes & Hall (1966) even concluded that such mitotic differences provide the only explanation for observed differences in radiosensitivity. Their work was on root tips which were continuously irradiated. For acute treatments or for shoot apices the situation may be quite different.

Conflicting results in successive years for γ-induced damage in carnation (*Dianthus caryophyllus*) were reported by Dommergues & Gillot (1965), which they explained by assuming differences in physiological condition of the plants in different years. Gunckel (1965) reported maximum damage in the outer layers of *Linum perenne* seedlings, treated for 8 days with γ-rays. Pratt (1967), irradiating apple and pear with γ-rays or thermal neutrons, observed most damage in the inner tunica layers and the corpus.

Crocket (1968) found in vegetative apices of *Coleus blumei* after 28 days of γ-irradiation, that most damage occured in L-II. He believed the majority of the plants to have a lower sensitivity in the outer layers. De Loose (1970), working with periclinal chimeras of *Rhododendron simsii*, reported that L-I is more radiosensitive than L-II. A second irradiation was used to transfer possible mutations, induced during the previous irradiation, to other histogenic layers.

Detailed studies were also performed by Lapins & Hough (1970) with apple and pear. Within the promeristem of the main apex and axillary buds, L-II was shown to be the most sensitive and L-I the most resistant to irradiation. However according to the authors, L-I does not participate in regenerative actions within the damaged meristems as no periclinal divisions in this layer were observed.

Iqbal (1970) very extensively studied the response of 850 (acutely) γ-irradiated shoot apices of *Capsicum annuum* at different levels of irradiation. The author stated that one of the main problems in radiobiology is whether restorative processes in plants (and animals) can be influenced after irradiation. He concluded that a certain minimum number of undamaged cells must remain in the apical meristem to make direct regeneration of the apex possible, otherwise axillary or adventitious buds will take over.

Although in this publication, no details about the relative radiosensitivity of the different histogenic layers were presented, he referred to his earlier work (not consulted) in which he reported that tunica layers showed more damage than corpus tissue. From his interesting observations that no regeneration took place when tunica layer-1 (L-I) was completely destroyed, Iqbal concluded that L-I may have a controlling influence on the organization of the apical meristem. More recently, Iqbal (1972) reported for the same plant species that in vegetative shoot apices the central zone was more radiosensitive than the flanks. L-II was more sensitive than L-I.

Maximum damage of L-II was observed by Katagiri (1973) in mulberry (*Morus* sp.), the axillary buds being more affected in L-II than in the main shoot apex. Chauhan & Singh (1975) observed some periclinal divisions in L-I of vegetative shoot apices of safflower (*Carthamus tinctorius*) after treatment with 10 and 20 kR of X-rays, in combination with 100 mg/l 2,4-D. They speculated about the role of L-I in regeneration.

From all these examples we can conclude that depending on species, stage of development and treatment, morphological reactions to radiation can be very different. Although it is difficult to make generalizations, it seems that most plants that have been investigated up to now, showed localized damage. L-II was found to be the most radiosensitive layer more often than L-I. The 'outer to inner' pattern, described by Miksche et al. (1962) does not seem to be the most common type of localized damage.

5.6.3.3 The effect of dose and dose rate on the frequency of histogenic effects

Information on this subject is very scarce as most authors did not publish exact data about the frequency of perforations, reduplications and the like. Moreover such data can be obtained only if periclinal chimeras with visibly different layers are used.

Pötsch (1966a,b; 1967; 1969), part of whose work has been discussed before, reported for *Euphorbia pulcherrima*, *Abutilon hybridum* and *Pelargonium zonale* that the relative frequency of different kinds of rearrangement effects may vary with dose. In an experiment on the bract colour of the monecto-chimeral clone cv. Eckes Rosa of *Euphorbia pulcherrima* (genetic constitution: white-red-red, or WRR), the ratio perforations (RRR): reduplications (WWR + WWW) was 1 : 1.5 in the control; 1 : 3.2 after 1 kR of X-rays and 1 : 1.6 after 3 kR. The percentage of changed plants, of course, increased with increasing dose of radiation (Pötsch, 1966a).

After irradiation of a meso-chimera of *Abutilon hybridum* (green-white-green for leaf colour) mostly solid green (GGG) plants were obtained, except for the lowest treatments (0.5 and 1 kR). The frequency increased with increasing dose. Apparently the majority of these solid plants originated from L-III tissue (Pötsch, 1966b).

In experiments with a diecto-chimeral clone of *Pelargonium zonale*, indicated as 'Salleron BBC', top cuttings (50 per treatment) were irradiated with 1, 2 and 3 kR of X-rays. When the two types of resulting perforations (BCC and CCC) were considered together, there was a dramatic increase in the frequency of perforations with an increasing dose, up to 84% of all shoots present at 3 kR. The percentage of shoots with reduplications (BBB) increased from about 2% in the control to 12% for 1 kR and 24% for 2 kR, but decreased again to 13% for 3 kR (Pötsch, 1969).

The results of Pötsch are interesting, not only because exact data are given, but also because they suggest a way to influence the frequency of different histogenic events on purpose by changing the dose and, probably also, the dose rate. Of course additional work is needed before definite proof of the feasibility of this approach can be given.

Some complications, which were not pointed out by Pötsch must be mentioned. Regeneration may also take place from plant parts outside the apical meristem, so that it is difficult to determine frequencies of perforations and reduplications per apex. Moreover a certain number of damaged cells may lead to complete destruction of L-I and L-II, but not of the reservoir of corpus cells, derived from L-III. Then cells tracing back to L-III are the only source of regrowth.

Péreau-Leroy (1969, 1975) studied orange/red-striped periclinal chimeras of carnation to find out whether differences in dose rate influenced frequency of different histogenic events. Starting from the (assumed) genetic constitution: L-I yellow-L-II red-L-III (probably) red, he found that higher dose rates gave more solid yellow and less solid red plants after exposure to 5 and 7 kR of γ-rays. Péreau-Leroy explained this result by saying that higher dose rates caused more damage to inner layers.

Another interesting point of this work is that Péreau-Leroy found differences in the frequency of the regeneration per layer between main apical meristem and axillary meristems after irradiation. Different physiological conditions of apical and axillary meristems at the moment of irradiation might explain this. Finally, the number of plants observed (380) allowed to draw reliable conclusions.

5.6.4 Concluding remarks

How can one explain the different reactions of (cells of) histogenic layers to irradiation? First as only a few experiments have been done carefully up to now, it may well be that certain generally accepted parameters e.g. to calculate degrees of radiation damage, are not at all suitable for that purpose. We found, for example, in literature that periclinal divisions in shoot apices did not necessarily occur in the layers that were most heavily damaged. According to Crockett, (1968), the observed 'change in polarity of some of the cells of L-I may be due less to the direct effect of ionizing radiation than to the broadening of the meristem proper'. Crockett also thought that experiments hitherto have not been sufficiently exact to relate more precisely morphological damage in shoot apices to radiation damage.

A second remark is that most investigators did not take into account environmental factors (concentration of oxygen, water content, temperature), which certainly affect the reaction of plant tissues to irradiation. Further, even cells of apparently homogeneous tissues may react very differently. Clowes & Hall (1966) believed that differences in cell stage or length of the mitotic cycle of individual cells were solely responsible for this different reaction but they only studied chronically irradiated root apices of *Zea mays* and some other species.

It seems logical to accept the existence of differences in physiological activity (and therefore in radiosensitivity) between cells of different layers or zones as the distinct layers have different functions with respect to organogenesis. One could even go further and assume that in each layer of the apex a different set of genes is switched on. In this way possibly a link could be laid with genetically determined radiosensitivity.

Another explanation for the differences observed between the apical layers could be sought in the characteristics of irradiation reactions. If for example X-rays from the air penetrate material with a different density, a so-called border reaction takes place. In the outer layers of the irradiated material, the energy level builds up to a certain equilibrum after which it slowly decreases again. (Back-scattering effects are not taken into account here).

For example Clark (1955) presented a graph which showed that for an X-ray apparatus, operating at 200 kV, the level of energy within irradiated material increases by about 20% over a distance of 1 cm tissue. As for example L-I cells of a potato shoot apex have an average diameter of 15-20 μm only, this increase of energy does not seem to be sufficient to explain differences observed in radiosensitivity between for example L-I and L-II, because it would mean a difference of energy administered to L-I and L-II of only about 0.35%. Because of the protective effect by the leaf primordia which normally surround the apical meristem during irradiation, in reality the differences are even smaller. It therefore seems unlikely that the variation in energy distribution within the shoot apex can explain the difference in radiosensitivity between histogenic layers.

Hence it is seems justified to conclude that within genetically homogeneous plants, differences in physiological or mitotic activity very probably are the main cause of the observed differences in radiosensitivity.

6 Radiation-induced damage and recovery of potato tuber eyes

6.1 Introduction

As was pointed out in Chapter 5 radiation damage and recovery in plants, notably in the apical parts, may be manifested in many ways. To gain insight in these processes, irradiated apices of several plant species were studied by microscopy, supplemented by observations of whole irradiated plants and plant parts.

Information on potato is very scarce. Our work in this field was aimed at filling this gap. Our main interest is the pattern of damage within the apical region after irradiation and the process of recovery and regeneration. In other words: which part of the apex shows most damage? In how far does this depend on radiation type, dose and dose rate? When and where does regeneration take place? Do all three histogenic layers participate to the same extent in regeneration from the apical area itself? As stated before (see also Chapter 5), information about such questions can be derived from microscopy of apices as well as from observations of whole plants or plant parts. In both situations the use of periclinal chimeras can be of advantage.

In our studies normal and irradiated shoot apices were investigated and results were compared with data from literature. We tried to relate the results of microscopic work with those obtained by scoring morphological aberrations in plants and tubers. In Section 6.2 the relevant literature on potato tuber eyes has been reviewed. Material and methods of all three experiments have been described in Section 6.3. The results are presented for each experiment separately in Section 6.4. The chapter is completed by a discussion (Section 6.5).

6.2 Literature

6.2.1 Morphology of the potato tuber eye

Morphologically the potato eye is the swollen end of a lengthwise compressed, subterranean axillary shoot or stolon (Artschwager, 1918, 1924; Sussex, 1955). The development of such axillary shoots is controlled by apical dominance (Kumar & Wareing, 1972). The physiological aspects of tuber development have been reviewed for example by Jolivet (1969).

Each tuber carries a number of reduced scaly leaves or leaf scars. A leaf scar with its subtended axil is indicated as a potato eye; the scar itself is called the eyewall (Artschwager, 1918, 1924). The central eye at the apical or rose end of a tuber is often referred to as the main or terminal eye. According to Melikyan & Tsovyan (1969), this terminal eye of a tuber is not the terminal bud of the stolon from which the tuber originated. They stated that the terminal bud dies during tuber formation and is replaced by another,

axillary bud of the swollen stolon end. Under the influence of the apical dominance of the terminal eye or group of eyes closest to the apical end, the other eyes of the tuber remain dormant for some time. This dormancy can be terminated either by seasonal influence or by artificial treatments.

Every eye contains several buds of about 0.5 mm length each. A superficial inspection of a dormant eye (e.g. under European conditions in December) normally reveals a suppressed central (terminal, main) bud and two lateral buds, some distance apart. Closer observation shows that central and lateral buds are surrounded by a number of leaf primordia which carry axillary buds themselves.

De Vries (1878a, b) made botanical studies of potato tubers and tuber eyes. The morphology of the potato eye at different stages of development of the tubers was studied in great detail by Krythe (1946, 1962). She demonstrated that the lateral buds, at first sight independent, actually develop in the axils of lower situated leaves of the central bud. The lateral buds grow until further development is arrested by the action of the central bud of the eye. Evidence for this arrest is that after damage of the central bud either one or both lateral buds resume growth.

Krythe showed for cv. Bintje that one month after harvest the central bud carries approximately 8 leaf primordia, with buds of second order in the lower axils of these primordia. The lateral buds also contain on average eight leaf primordia, with buds in some of the lower axils (third order buds). All axillary buds may hold a few primordia.

The buds of second and lower order and closest to the tuber surface, produce the stolons and then the tubers of the next generation. These buds are of special interest for mutation research. The higher situated axils produce the aerial plant part.

6.2.2 The apical meristem of subterranean potato shoots

Apart from the large size, the apical region of subterranean stems shows no fundamental differences when compared with the primary shoot of potato seedlings. The average size of the meristematic region of a central eye of a dormant potato tuber may be 150-200 μm wide and up to 100 μm high, but much variation exists.

Asseyeva (1931) concluded from studies with periclinal chimeras that most cultivars possess a two-layered tunica, but that sometimes a higher number of stratified apical layers occur. Baker (1943), using polyploid periclinal chimeras, distinguished only two independent cell layers. Steinberg (1950) performed anatomical studies and, from the occurence of only anticlinal divisions inferred that two tunica layers surround the corpus tissue. Sussex (1955) found three stratified cell layers with some periclinal divisions in the corpus at the end of a plastochron.

Howard et al. (1963) studied a colchicine-treated triploid (2n = 3x = 36) potato, *S.* x *juzepczukii* in this respect and expressed their results in terms of L-I, L-II and L-III. They agreed about the existence of one or more distinct layers internal to L-II, but believed that shoot apices of the axillary buds (which produce the stolons and tubers) often derive only from L-I and L-II and that stratified layers below L-II, can be traced back to L-II and not to L-III of the main stem. Frandsen (1967a) also suggested that L-III perhaps is not a stable layer. On an earlier occasion Howard (1959) reported that interchange between L-I and L-II is extremely rare or even absent.

Most authors agree that L-I has a higher degree of independence than L-II and L-III.

This is demonstrated, for example, by the relatively high stability of so-called L-I mutants such as Red King (Howard, 1970a) and our mutant Clones M 52 from cv. Désirée and B 165 from cv. Burmania. There are, however, also a few examples known of stable mutants with only L-II mutated, e.g. Holly Leaf (Howard, 1970b), or with only an aberrant L-III (Howard, 1972).

Klopfer (1965a, b, c; 1967) strongly advocated the existence of three stable and independent histogenic layers, not only in the main stem but also when sprouts of axillary origin are formed. He derived his evidence both from anatomical work and from morphological studies with plants that were periclinally chimeric for certain characters such as tuber-skin colour and leaf shape. Klopfer (1965b, 1967) made a restriction on the number of independent layers saying that in very young seedlings only two such layers (L-I and L-II) may exist. All older seedlings, tubers or shoots, in his opinion, possess three (practically) independent layers. This point of view is now generally accepted.

Additional evidence has been derived from investigations with colchiploids, which are mostly obtained via the so-called Dionne-method. In this method, the nodal subaxillary meristems of potato grafts on tomato are treated (Ross et al., 1967; Frandsen, 1967a; Langton, 1974). Colchiploids can also be obtained from less laborious seed treatments (Frandsen, 1967b; Hermsen & de Boer, 1971).

Most authors have found that after one tuber generation a considerable, although not an absolute, degree of equality in ploidy level in L-II and L-III occurs, irrespective of the L-I constitution. Howard et al. (1963) attributed this to a frequent replacement of L-III cells by L-II cells during the formation of axillary buds and to the occurrence of periclinal divisions in L-II. The opposite: L-III replacing L-II, may also occur.

Differences in ploidy level between layers may lead to differences in exchange rate between these layers, due to a difference in competitive ability. This competition makes ploidy-chimeras less suitable for investigations on the relative stability of histogenic layers in apices of normal plants. (It must be remembered, however, that competition may also occur between layers which are genetically different for only a single gene, for example when chloroplasts are mutated.)

Finally, within this section reference must be made to some opinions about so-called initial cells in the potato shoot apex. Evidence about the number of such cells per apical layer is mainly drawn from the size of mutated sectors, e.g. on tubers or leaves. Both Howard (1961a) and Klopfer (1965b, 1967) decided on six initial cells for L-I. However for L-II Howard (1961b, 1966) and Klopfer suggested the presence of two and six initial cells, respectively. No data are known for L-III. Nayar & Dayal (1970) finally mentioned the presence of about three initial cells in potato tuber eyes, but do not give further particulars, e.g. with respect to the various layers, nor do they provide any acceptable explanation for this assumption.

6.2.3 Irradiation of potato tuber eyes

For all scientific work the choice of proper experimental methods and conditions, leading to reproducible results is important. To produce mutations in potato, the best method available up to now is to irradiate single eye-pieces (see also Section 2.3.3). Even irradiation of tuber pieces with only one eye in fact means treating a large number of

buds or growing points in different stages of development. The buds from which for example the stolons arise are already present at the time of harvest.

One can expect differences in radiosensitivity between the buds within one single eye because of their various stages of development and because of the effect of apical dominance, which keeps the physiological activity of the lower situated sprouts down. Irradiation can disturb the main bud after which the lower buds are released from the apical dominance and start to sprout (Grodzinskii & Gudkov, 1969). Very soon after the lateral buds are released from apical dominance, they show a considerably increased radiosensitivity. The sensitive period of the whole eye lasts till a new centre of apical dominance has been established, after which the other buds may return to a stage of (relative) rest, depending on the period of the year. In a way apical dominance acts as a protective mechanism against radiation damage in resting plant parts.

6.2.4 Radiation-induced damage and recovery

The first anatomical and cytological observations on irradiated potato shoot apices as far as known were made by Korableva (1961). Irradiation with 500-2000 R of X-rays was reported to give temporary inactivation. Individual cells were elongated and showed abnormal nuclei. The amount of nucleic acids was lower than normal. No details about the reaction of cells in different apical parts were presented.

In 1964 and 1965, Montezuma de Carvalho (unpublished), who worked at IvP, made some histological observations on dormant and non-dormant excised buds of cv. Bintje, irradiated with 3500 R of X-rays. He observed that approximately three weeks after irradiation, the typical arrangement of meristem cells became more and more disturbed, whereas the activity of lateral buds increased. Non-dormant irradiated eyes showed complete arrest of mitoses for 24-48 h, with a maximum number of damaged cells after five days and a highly decreased mitotic activity at later dates. For irradiated dormant eyes the maximum number of damaged cells was found after seven days and mitotic activity was only slightly lower than normal. It was found that dormant cells showed 74.3% of abnormal anaphases compared with 81.1% for non-dormant eyes, which is in agreement with the expected lower radiosensitivity of the dormant apices. Again no comparison was made between different apical areas.

During the period 1967-1969 Ghatnekar, also a guestworker at IvP, studied some other aspects of this work. His results, which were published as internal reports only, are given here in some detail. Based on discussions with F.P. Ferwerda, Ghatnekar started from the point of view that differential radiosensitivity may exist between different histogenic layers within a shoot apex. Ghatnekar applied the method of radioactive isotope labelling to determine the metabolic stage of different cells under normal conditions and after irradiation, to compare sensitivity of different cells to X-rays and to study the recovery of cells in apical, lateral or axillary buds after irradiation.

Tuber eye-pieces of the cultivars Bintje, Désirée and Multa were partly treated with 3 kR of X-rays, applied at a dose rate of 160 R/min and partly used as control. Immediately after irradiation, H^3-TdR (tritiated thymidine) was applied for 24 h to the eye-pieces by dropping 0.2 ml of a standard solution on the sprouts, which were 0.5-2.0 mm long. Afterwards the sprouts were fixed in 1 :4 actic acid/alcohol, taking four sprouts

each day for 16 days. The material was then processed through upgraded series of alcohol, a mixture of alcohol and tertiary butyl alcohol, and tertiary butyl alcohol only and embedded in paraffin wax at about 60°C. Sections were cut at 3 μm(?) thickness with the microtome, passed through ethanol-water series to bi-distilled water, hydrolysed in 1 N hydrochloric acid at 60°C and stained in Feulgen.

After this treatment, autoradiographs were made with Kodak autographic stripping film AR-10. More than six silver grains per nucleus were considered as labelling. In non-irradiated meristems most labelling was found on the flanks (leaf primordia!) and in the rib meristem zone. The central zone of the apex was only weakly labelled. In irradiated meristems a very different pattern was observed. The overall intensity of labelling was much lower, but relatively more labelling was found in the central zone than along the flanks. This pattern was observed especially 2-6 days after irradiation.

Although a considerable variation in reaction was found, it could be established that mostly where the main apical meristem remained active after irradiation, the adaxial cells on one of the flanks took over the regeneration process. Where the main shoot meristem was inactivated, a group of lower situated cells showed intense labelled nuclei due to a high DNA synthetic activity. It was further reported that substitute meristems may originate from histomorphologically different tissues, situated sometimes at a distance of even 1 mm from the apical meristem of the main shoot.

Ghatnekar was unable to find differences in radiosensitivity between layers within potato shoot apices. He concluded that repetition of experiments often gives conflicting results and explained these by the heterogeneity (notably with respect to the physiological state) of the experimental material.

Attempts were made to interpret published results of different experiments with irradiated potato plants, which are periclinal chimeras for certain leaf or tuber characters, in terms of possible differential radiosensitivity of the histogenic layers. However not all histogenic processes, such as layer replacement, can be followed. If, for example, in a chimeral plant with the constitution L-I normal - L-II mutated - L-III normal, the L-II changes towards normal, it is very difficult to determine whether penetration of cells from L-I or from L-III into L-II has caused this effect. This complication can be avoided if a chimera in which all three layers differ genetically is available. This approach was used for carnation by Pereau-Leroy (1975), who successfully combined a mutation for flower colour with different ploidy levels. For potato such chimeras unfortunately are not available. An additional complication is that in most publications only a part of the data obtained are given. Because of these restrictions only some preliminary conclusions can be drawn from the reports studied.

Replacement by L-I (L-I mutated → L-I + L-II mutated) was reported by Asseyeva (1931) and Howard (1958). In a later publication, Howard (1962) did not explain these results by assuming replacement by L-I, but he suggested the formation of a new apex from only one or from a few remaining cells of a lower situated stem part. Apical ends of three groups of tubers, periclinally chimeric for tuber-skin colour, were irradiated with about 3500 R of X-rays by Howard (1964b, 1970a). Of 190 irradiated apical tuber pieces of the clones Red King, Bonte Sport and Müller purple (apparently) 50% of the tubers were unchanged. In addition about 5% showed replacement (L-I mutated → L-I + L-II mutated). In about 45% of the tubers, L-I adopted the genetic constitution of L-II and L-III. Varietal differences are observed. The results could suggest that L-I is more radio-

sensitive than the inner layers, because it loses its discrete character as a layer more often than other layers.

Klopfer (1965a) obtained 22 plants with changed tuber-skin colour after irradiating 100 tubers of the chimeric clone Rote Holländische Erstling (constitution red-yellow-yellow) with 3000 R of X-rays. Out of a total of 200 tubers 65% remained unchanged (i.e. spotted monecto-chimeras), 25% became yellow-skinned (i.e. the red colour from L-I cells disappeared) and 10% became red (i.e. tubers are either diecto-chimeras or solid red). Apparently changes from spotted (red-yellow-yellow) to solid yellow occur more often than to red (49 against 21 cases). This result again could indicate a lower stability after irradiation for L-I.

In the same publication Klopfer reported on irradiated leaf-shape mutants of cv. Bintje. Assuming that Klopfer's hypotheses on the layer constitution of the different leaf types are correct, we may conclude that after irradiation of periclinal chimeras, for 35 out of 77 cases (i.e. 45%) recovery took place from inner layers, whereas in only 8 cases a solid mutant was obtained from the original diecto-chimera. These results suggest a greater radiosensitivity of L-I and L-II than of L-III. Here no distinction can be made between L-I and L-II. (N.B. Klopfer did not mention leaf types in which only L-II, only L-III, or L-II + L-III are mutated.)

It is doubtful whether the used method of calculation is justified. For example it has not been taken into account that, after irradiation, regeneration may either take place from cells within the irradiated apex or from lower axillary or adventitious buds.

If the results from irradiated periclinal chimeras are compared with those obtained from unirradiated ploidy-chimeras of potato, it seems that also doubling of layers (after colchicine treatment) does not occur completely at random. Working with seeds of *Solanum bulbocastanum* that had been treated with 0.3% colchicine, Hermsen & de Boer (1971) for example, obtained from a total of 113 plants studied 72 unchanged plants (2x-2x-2x), three plants with the constitution 4x-2x-2x, nine plants with 2x-4x-4x and 29 plants with 4x-4x-4x. The other four possible combinations were not found. The results suggest that the different histogenic layers of the apex, after colchicine treatment, do not react exactly in the same way.

Coming back to the reaction of apical layers of potato to irradiation, the results obtained up to now suggest that in potato L-I may be more radiosensitive than deeper layers. Whether this is true has to be further investigated. Another question that remains as yet unanswered is: does differential radiosensitivity of histogenic layers also lead to differential mutability of cells in those layers?

In this respect it is interesting to quote Heiken (1960, p. 78): 'It has been ascertained by Asseyeva [1931, English summary, p. 195 and p. 205] that somatic mutations most frequently take place in the dermatogen (or in the 'epidermal complex of tissues') and much more rarely in the deeper... However there seems to be no reason to presume that differences in mutability between the cells of the primordial meristems do exist'.

Heiken explained the differences mentioned by Asseyeva, by saying that: 'in most cases only mutations affecting the epidermis will be recognizable'. It seems, however, reasonable to assume that in a cell layer which is more radiosensitive than another layer, calculated per cell, more single mutational events can be induced. On the other hand, the radiation damage of the most sensitive layer may be so high, that this layer is inactivated or even completely suppressed and is replaced by cells of a more radioresistant layer

which, in keeping with the above line of reasoning, will also carry less mutations. The final output, calculated as mutation rate per plant, then can be lower than the number of mutations which were originally induced. As said before it seems also important to know whether regeneration takes place from cells of the irradiated apex itself or via lower situated, existing or new buds.

Up to now we have insufficient information to answer the queries at the beginning of the chapter. Diplontic selection undoubtedly plays a role. Very probably the dose and the dose rate of irradiation also affect the final outcome (e.g. Pötsch, 1969).

6.3 Material and methods

Tubers of a mutant clone from cv. Désirée were obtained from the ZPC, a co-operative agricultural company in the Province of Friesland. This clone, which arose as a spontaneous bud sport, and was registered as M 52, is characterized by tubers with a yellow/red-splashed tuber skin, whereas tubers of cv. Désirée have an equal red skin. From a number of unpublished experiments (which are not discussed here) we concluded that the mutated clone is a periclinal chimera for tuber-skin colour with the genetic constitution L-I yellow, L-II + L-III red (normal tubers of cv. Désirée are genetically red in all three layers). The mutant has either white or pink flowers. When flowers are pink, all three histogenic layers are genetically pink. The white flower colour refers to a periclinal situation (L-I mutated) for this character. As far as is known, the mutant is completely identical to cv. Désirée in all other plant characters. The advantages of using a periclinal chimera were discussed before.

The general procedure was as follows: So-called eye-pieces were scooped from tubers by using a special spoon (Fig. 1). Depending on the physiological condition of the tubers either dormant eyes were scooped out or, when sprouting of tubers had started, these sprouts were removed by scraping. This was done three to six days before irradiation so that lower situated axillary sprouts could resume activity before irradiation.

After being scooped out the eye-pieces were replaced in their respective tuber holes for one day to prevent drying-out and to stimulate the production of a thin, protecting, corky layer on the wound surface of the tuber plugs. Eyes were collected as much as possible from the central parts of the tuber to avoid complications caused by developmental differences. Moreover, only the eyes that looked similar (originally after inspection with a hand lense and later after inspection under binoculars) were selected for further work.

Various numbers of tuber eye-pieces were irradiated in the different experiments: 400, 370 and 250 eye-pieces in three consecutive experiments (hereafter referred to as Exp. 1, Exp. 2 and Exp. 3, respectively). Irradiations were performed at ITAL with a Philips deep-therapy X-ray apparatus operating at 250 kV and 15 mA without additional filters. In the different experiments exposures ranged from 500 to 4000 rad at a dose rate of 240 or 245 rad/min. During irradiation the eye-pieces were placed on a rotating table.

After irradiation most eye-pieces were taken to a greenhouse and kept in moist river-sand, 2 cm below the surface untill further treatment. Eye-pieces, which were used for fixations during the first 24 h after irradiation, were kept in the laboratory. Eye-pieces set apart for morphological studies remained in sand untill about 50% of them had produced visible sprouts, at which time practically all eye-pieces had rooted. Afterwards the plant-

lets were transferred to fertile garden soil and, at a later stage, transplanted into pots of 12 cm diameter. Plants and, later, tubers were inspected regularly for visible abberrations.

For the microscopic work, tuber plugs of approximately 0.5 x 0.2 x 0.2 cm, each containing a single eye, were cut out with a razor blade. Fixations were made with CRAF V, applied for 24 h after which the fixed material was stored in 70% alcohol till further treatment. After two weeks all eye-pieces were dehydrated by passing through a tertiary-butyl alcohol series and, in the first experiment, embedded in paraffin wax (m.p. 55°C) (for details see Gerlach, 1969). Sections of 8-10 μm were made with a handmicrotome in such way that both the central bud (or what was left of it) and the two most prominent lateral buds were included. During the preparation of the sections it was necessary to keep the room temperature below 20°C, because of the relatively low melting-point of the paraffin wax. In later experiments paraffin wax was replaced by paraplast, which has a higher melting-point and is easier to handle.

Staining was done with safranin and fast green. Safranin mainly stains lignins, cutins and nucleoli, whereas fast green, which acts as a contrast to safranin, stains the cytoplasm. After preservation with Euparal, the microscopic slides were ready for further work.

To determine damage and recovery after irradiation, use was made of a number of parameters. Based on single cells, observations were made on: staining intensity in nuclei, cytoplasma and cell walls; size and degree of differentiation of the nucleus; degree of vacuolation in the cytoplasm; thickness of the cell walls; cell size; frequency of plane of divisions; occurrence of 'empty' cells, collapsed cells, necrotic cells and idioblasts. Attempts were made to distinguish different degrees of cellular damage according to the method of Iqbal (1970).

The distribution of radiation injury over the apical region was studied by determining how often these effects occured in different areas of apical layers. We tried to describe the effects observed according to the system of classification proposed by Pratt (1968), i.e. uniform, random or localized damage. Moreover we checked whether the different apical layers remained discrete or were invaded by cells from adjoining layers, e.g. in order to replace collapsed cells of the original layer.

Differences in the shape of the whole apex, e.g. flattening of the apical dome after treatment, were recorded. Attention was paid to the occurence of bifurcations, to the length of the period before recovery and to regeneration from axillary buds or from other tissues outside the original apical meristem.

6.4 Results and comments

6.4.1 Early radiation damage in scraped tuber eyes (Exp. 1)[3]

6.4.1.1 Experimental details

In Exp. 1, the microscopic work was meant as an orientation, supplementing observations on whole plants from irradiated tuber eyes. As in Exp. 2 and 3, the microscopy

3. This work was carried out in co-operation with H.A. Klunder, former student of plant breeding at the Agricultural University of Wageningen.

became more and more important, its results are given before those for whole plants (referred to as 'morphological' results) for reasons of uniformity.

Tuber eye-pieces from which the visible sprouts had been scraped six days earlier and which had started to grow again, were irradiated on 16 May, 1972. Scraping was thought necessary because unsprouted tubers could not be obtained at that time and unscraped tuber eyes would have been too heterogeneous as starting material.

Microscopic fixations were made 1, 3, 6, 12, 24 and 48 h after exposure to X-rays. Three doses: 750, 1500 and 3000 rad of X-rays were given and per dose 100 eyes were treated. A control of 100 eyes was added. For each fixation time and for each dose, six eyes were studied microscopically. In total 3600 sections were made.

6.4.1.2 Results and comments

General remarks A superficial microscopic inspection of the fixed material indicated that, even though the eye-pieces were checked visually before irradiation, the selected eyes showed considerable variation with respect to their stage of development. At the moment of irradiation some sprouts, for example, had six axillary buds, whereas others had none. Another complication was the distinction between radiation damage and damage caused by scraping the eyes before irradiation.

The results further showed that two days after exposure, the level of maximum damage had not been reached. Moreover, no signs of recovery could be observed either. Because of these restrictions, the microscopic results can only be considered as indicative.

Microscopic part Scraping, six days before irradiation, damaged the main sprout as well as one or both of the lateral (in fact axillary) sprouts. Sometimes other axillary buds were damaged. As the control plants were similarly damaged, this injury was not caused by irradiation. Most sections of control and irradiated eye-pieces, showed a number of newly-formed young apices with small, thinwalled cells. These apices must have regenerated in the period between severing and irradiation.

In the control, mostly the outerside of the leaf primordia was slightly damaged (N.B. by mechanical damage). No damaged cells were observed in the apices proper. In the 750 rad treatment, only a few scattered cells within the apices were damaged or necrotic. As the apices are protected by leaf primordia, this damage must be due to irradiation. Most damaged cells were found in the central part of the apical area. Results of the 1500 rad and 3000 rad treatments were inconsistent. In fixations made after twelve hours or more, several cases of hypertrophy in cells adjoining damaged cells were observed.

It took at least 12 h after irradiation, before damage could be observed microscopically in the 750 and 1500 rad treatment and only 3 h after treatment with 3000 rad. Mitotic divisions, as expected, were more arrested in the eye-pieces exposed to larger doses of X-rays. Divisions occurred more often along the flanks than in the central part of the dome in the control and 750 rad treatment. After larger doses the total number of dividing cells remained very low. Periclinal divisions were observed in L-I only in two sections of the 750 rad treatment. In L-II they were found more frequently in this treatment. No effect of the time of fixation was observed. In the 1500 and 3000 rad treatments periclinal divisions were practically absent. Often it was difficult to recognize L-II in the apex as a discrete layer.

Even though starting from homogeneous material clearly has advantages, scraping itself is not recommended in irradiation experiments for the collection of microscopic information.

Morphological results From a morphological point of view, especially the 750 rad treatment yielded some interesting results. Of the plants in this treatment, 35% showed a typical growth pattern, indicated as Type B-10 (after the first plant with this feature). Type B-10 was characterized by a main aerial shoot with two or three normal-looking, full-grown internodes. The lower internodes were very compressed, giving the plants a rosette-like appearance (Fig. 11). Some axillary sprouts in the lower stem parts usually developed when the main sprout died off. This pattern of plant growth was sometimes observed in the 1500 rad treatment, although less clearly. After exposure to 3000 rad, the B-10 type seemed absent. The first two or three internodes also remained suppressed and regeneration took place from axils situated near or below the soil level.

In Exp. 1 only tubers from white-flowering plants were used. After irradiation, a few pink flowers were observed in each treatment but also in the control. These (solid) pink flowers show that a certain percentage of cell displacement occurred, the proportion of which increased from 8% in the control to 20% after 3000 rad. The total number of observations, however, was too low to calculate the effect of irradiation on displacement.

Patterns of tuber-skin colour As was mentioned before, mutant Clone M 52 is characterized by a yellow/red-splashed tuber skin. In nature some aberrant types are found. They are often caused by damage to the tuber skin, followed by replacement of L-I by

Fig. 11. (Exp. 1). Potato plant of the so-called B-10 type after irradiation of tuber eye-pieces of mutant Clone M 52 from cv. Désirée with 750 rad of X-rays.

Table 11. (Exp. 1). Number and proportion (in %) of different types of tuber-skin colour after X-irradiation of desprouted tuber eye-pieces of the yellow/red-splashed mutant Clone M 52 from cv. Désirée.

Series	Control	750 rad	1500 rad	3000 rad
Total number of tubers	353 (100%)	407 (100%)	426 (100%)	454 (100%)
Number of yellow/red-splashed tubers	327 (93%)	361 (89%)	355 (83%)	218 (48%)
Number of solid red tubers	15 (4%)	30 (7%)	54 (13%)	189 (42%)
Number of tubers with red 'sectors'	11 (3%)	15 (4%)	15 (4%)	11 (2%)
Number of solid yellow tubers	– (–)	1 (< 1%)	2 (< 1%)	36 (8%)

cells of L-II or L-III. The number and proportion of normal, i.e. yellow/red-splashed tubers and of aberrant types, in the control as well as after X-irradiation, are presented in Table 11.

Aberrant tubers are classified into three groups: solid red, solid yellow and (yellow/red-splashed) tubers showing red 'sectors' in L-I. Such 'sectors' normally run from the stolon end to the apical end of the tuber and occupy a zone of the tuber of varying width. These so-called 'sectors' are not real sectors since they are confined to L-I only. They refer to mericlinal chimeras caused by effects of displacement or, in the terminology of Bergann & Bergann (1962), to perforation of part of L-I by deeper layers.

Solid red tubers are derived from L-II or L-III. Solid yellow tubers must have originated from L-I only. It must be mentioned that only (assumedly) histogenic effects of irradiation were counted. Undoubtedly some 'real' mutations occurred as well. Table 11 clearly demonstrates that the proportion of aberrant tubers increased with increasing doses of irradiation.

We checked whether there were linear relationships between the percentages of normal, red (including red 'sectored') and yellow tubers, respectively and the size of radiation dose. Results, calculated by the Student *t*-test, are presented in Table 12. All regression coefficients were significant at at least the 5% level.

Table 12. (Exp. 1). Regression coefficients and *t* values for different categories of tuber-skin colour after X-irradiation of mutant Clone M 52 from cv. Désirée with 750, 1500 and 3000 rad.

Tuber-skin colour	Regression coefficient	*t* value
Yellow/red-splashed	–0.015	-4.58^{x}
Red (+ red 'sectored')	0.013	7.10^{xx}
Yellow	0.003	3.42^{x}

Degrees of freedom (n-2) : 2.
^{xx} = P < 0.01, ^{x} = P < 0.05

To determine whether the control was a random sample, the number of aberrant tuber-skin types in nature was counted in a large commercial stock of so-called Bonte Désirée (= mutant Clone M 52). From 185 283 tubers (counted in 1973), 445 tubers were completely red, 553 tubers were red 'sectored' with mainly red colour, 225 tubers showed smaller red 'sectors' and 6 tubers were solid yellow. In this stock about 0,3% of the tubers was solid red. It cannot be excluded completely that solid yellow tubers (which are traced back to L-I only) are due to contamination by tubers from other cultivars, although this is mostly unlikely. On the other hand yellow tubers of the stock may have been removed on purpose by the wholesaler.

If one compares the percentage of aberrant tuber-skin types in the control series of the irradiation experiment with the results of countings in the commercial stock, it is clear that the percentages of aberrant types in the stock are considerably lower. A χ^2 -test for homogeneity (Snedecor & Cochran, 1968, p. 240) showed that the composition of the stock differed very significantly ($P < 0.01$) from the control. It must be concluded that the composition of the control in the present irradiation experiment (353 tubers), is not representative for the proportion of different tuber-skin colours in the much larger stock. Very significant differences, of course, also exist between the composition of the stock and the irradiated material of the experiment. When comparing results, one has to keep in mind, however, that tubers of the control and commercial stock in fact are not the same vegetative generation. The work of selectors during multiplication may have affected the proportion of aberrant types.

Attention was also paid to the proportion of different tuber-skin colours in single plants. These results, however, are of minor importance only, as for one plant stolons may arise from several meristems. The proportion of plants with only yellow/red-splashed tubers, decreased from about 80% in the control to 40% in the 3000 rad treatment. The percentage of plants which only produced aberrant tubers, remained below 5% in all treatments except for the 3000 rad one, in which this percentage increased to almost 30%. Within the progeny of one plant, practically all skin colours were found. Also plants with only one tuber-skin colour were found, except for red 'sectored' tubers which always occurred together with other types.

6.4.2 Radiation damage and early recovery in unscraped tuber eyes (Exp. 2)[4]

6.4.2.1 Experimental details

The experiment was started on October 12, 1972 with 250 tubers. This time unscraped tuber eyes could be taken as all tubers were dormant and showed no signs of sprouting. The stolon end of 125 tubers was cut off to break the seasonal dormancy. Tuber eyes were scooped out one week later. On 31 October, 4 x 90 eyes were irradiated with 500, 1000, 2000 and 4000 rad of X-rays, respectively. A control of 90 eyes was added. Only eyes that looked uniformly with one central and two lateral buds and showing early sprouting activity were irradiated.

4. This work was carried out in co-operation with Mrs. M.P.P. de Senerpont Domis-Hoos, former student of plant breeding at the Agricultural University of Wageningen.

Fixations were made after 3, 6, 12, 24, 72, 96 and 120 h. For each exposure rate and fixation time two eyes were sectioned. From each eye the approximate median section and two adjoining sections were studied.

To determine microscopically degrees of irradiation damage, a scale was designed. Three different degrees of damage were distinguished, for example with respect to amount of vacuolation, damage to the protoplasma, to the cell walls, etc. The damage in the central bud and both main lateral buds was scored per apical layer in each section. Then the damage per apex and per radiation treatment was calculated by summing the values and by dividing the sum by the number of sections studied.

For observation of whole plants, in addition use was made of eyes of the other 125 tubers, which were pretreated differently, i.e. the eyes were scooped out and put in humid sand for two weeks before irradiation to break dormancy in this way. This different treatment did not affect the further course of the experiment.

6.4.2.2 Results and comments

Microscopic part Microscopiy of the potato apiced revealed that despite careful checking with a hand lens, the starting material still showed some heterogeneity. Sometimes more than three well developed buds were found because of the growth of axillary sprouts at the lower part of the central bud. Such observations were made in the control as well as in the irradiated eye-pieces and, therefore, can not be attributed to post-irradiation reac-

Fig. 12. (Exp. 2). Scanning electron photograph of central bud of potato eye of mutant Clone M 52 from cv. Désirée. Some leaf primordia have been severed. (Fotograph TFDL, Wageningen.)

tions. Countings made 3 h after irradiation showed that the central bud normally was surrounded by 6-7 leaf primordia (see also Fig. 12). For the two main lateral buds on an average 4-5 leaf primordia were counted. The other axillary buds carried 4-7 leaf primordia.

The results of determining the amount of apical damage according to the scale (Section 6.4.2.1) are presented in Table 13. Although for each fixation-time, sections of only two apices were studied in detail, a clear relation can be seen between the degree of damage and the radiation dose. Due to a technical mistake, the results on the fixations at 120 h in the control and in the 500 rad treatment were lost.

Apical damage was mostly observed after 24 h. In the 500 rad treatment maximum damage was reached already after 48 h. Table 13 further shows that for larger doses, there is a rather regular increase in damage after 24-48 h. The results obtained suggest that for the largest doses of X-rays, maximum damage was not reached even after 120 h so that in future experiments later fixation times have to been chosen. A comparison between the damage in the central bud and the main lateral buds showed that at a given moment (say after 96 h) the central apex was more damaged than the lateral ones. Explanations for this result must be looked for in differences in their respective stages of development and in the apical dominance of the central apex.

In all fixations damage seemed to be of the so-called 'scattered' type, following the classification of Pratt (1968). According to the scale, various degrees of damage were found for the different histogenic layers. Especially for low exposures, L-I showed a slightly higher proportion of damaged cells than L-II and L-III. In general, L-II showed more damage than L-III. These findings seem to support the preliminary conclusions drawn in the literature survey and further seem to be in line with the 'outer to inner pattern of decreasing radiosensitivity', as reported by Miksche et al. (1962). As the maximum damage was not reached for the largest doses, the results of these treatments could not be adequately compared. The results, however, suggest a rapid increase in the amount of damage of especially L-III, after large doses of X-rays. It cannot yet be said whether different mechanisms govern sensitivity of layers to irradiation, at low and high doses.

Some scattered active cell divisions were found in all apices of the control and in the 500 rad treatment after 12-24 h. The 1000 rad treatment showed a start of mitotic activity after 48 h. In the 2000 rad treatment a very low mitotic activity was found after

Table 13. (Exp. 2). Estimation of total radiation damage (in %) in X-irradiated unscraped tuber eyepieces of mutant Clone M 52 from cv. Désirée. Results from central and lateral buds have been pooled.

Treatment	Hours after irradiation							
	3	6	12	24	48	72	96	120
500 rad X-rays	1	5	7	7	14	9	11	•
1000 rad X-rays	7	5	3	13	8	21	26	28
2000 rad X-rays	10	0	2	13	13	21	27	32
4000 rad X-rays	4	2	2	8	7	17	25	120
Control	2	0	2	1	1	1	3	•

72 h with a considerable increase in the number of divisions after 96 h. No divisions at all were observed in the apices of the 4000 rad treatment. It seems that increased mitotic activity indicates the beginning of post-irradiation recovery, but it must be kept in mind that mitoses are also found in the control. Moreover, a dose of 500 rad may stimulate mitotic activity (low-dose stimulation).

Recovery may take place in different ways, i.e. either from different parts and layers of the apex proper, from existing lower situated axillary buds or from deeper situated plant tissue. Some early signs of recovery (divisions, more intense staining) were found in the latest fixations of the 1000 rad treatment, which suggests that for higher exposures the process of recovery may have just started. Because of the low level of recovery in the sections studied, it was not possible to determine degrees of recovery in the same way as was done for radiation damage.

Periclinal divisions in L-I occurred very seldom and were observed only in the latest fixations of exposures of 1000 rad. It would be premature to conclude that L-I does not, or only to a very small extent, participate in regeneration of the apex via replacement of damaged cells of L-II. However the low number of periclinal divisions and results from further morphological work point in this direction. Periclinal divisions in L-II in low frequency were found in all fixations, except the 4000 rad treatment. In the apices studied it seemed that early (i.e. during the first five days) periclinal divisions occurred mainly in the central apical part of the control and along the flanks of the irradiated apices.

Histogenic effects (like layer replacement, etc.) after irradiation occurred seldom. The results suggest that in further experiments fixation has to be done later for the data to be reliable.

Finally it must be remembered that few scientists have experience with distinguishing and classifying different degrees of damage. It is therefore necessary to make more detailed investigations and to compare various systems of classification. As it has been found repeatedly that one can obtain contradictory conclusions by using different methods of calculation, it seems necessary to be somewhat reticent in making conclusions and in judging results of others.

Morphological part The proportion of emerged plants was determined at different times after irradiation. After 4 weeks, the control showed 44% emergence compared with 48% in the 500 rad treatment, 50% in the 1000 rad treatment, 18% in the 2000 rad treatment and 0 in the 4000 rad treatment. These results suggest some effect of radio-stimulation on early growth after low exposures. As only 35-40 plants were studied per treatment, one cannot consider these results as definite proof. After 3 months about 100% of the potato tuber eyes had emerged in all treatments.

Larger doses of X-rays seem to completely inactivate the central apex of the potato eye. Probably some of the lower situated axillary buds are still active. Especially after exposure to 1000 and 2000 rad, a rosette-like growth of the plants was observed (Type B-10), with outgrowth of several axillary sprouts. The proportion of such plants reached a peak (28%) at 2000 rad and declined at larger doses.

In addition to the (presumed) histogenic effects of irradiation on tuber-skin colour, which will be discussed below, some 'real' mutations were found in the different radiation treatments, e.g. for skin colour, flesh colour and tuber shape. White flesh was found in one

Table 14. (Exp. 2) Number and proportion (in %) of different types of tuber-skin colour after X-irradiation of tuber eye-pieces of the yellow/red-splashed mutant Clone M 52 from cv. Désirée.

	Control	500 rad	1000 rad	2000 rad	4000 rad
Total number of tubers	107 (100%)	121 (100%)	95 (100%)	125 (100%)	92 (100%)
Number of yellow/red-splashed tubers	107 (100%)	121 (100%)	94 (99%)	99 (80%)	35 (38%)
Number of solid red tubers	– (–)	– (–)	– (–)	17 (13%)	46 (50%)
Number of tubers with red 'sectors'	– (–)	– (–)	1 (1%)	4 (3%)	– (–)
Number of solid yellow tubers	– (–)	– (–)	– (–)	6 (4%)	11 (12%)

yellow tuber of three plants of the 2000 rad treatment. Tubers with light red tuber skin were found in the 2000 and 4000 rad treatments in two plants (with one light red tuber each) and four plants (with in total 14 red tubers), respectively. All four tubers of one plant of the 4000 rad treatment were abnormally long. From previous experiments it can be concluded that this effect is genetically determined. Several mutations were also found in the aerial parts of the plants but these mutations are not discussed here.

Patterns of tuber-skin colour The number of different tuber-skin colours was counted in the same way as in Exp. 1. Results are presented in Table 14. Regression coefficients and t values (see also Section 6.4.1.2) are given in Table 15. This time all regression coefficients were significant at the 1% level.

In this experiment very few aberrant tuber-skin types were found at doses of X-rays lower than 2000 rad. The results further showed a sharp decrease in the percentage of tubers with a yellow/red-splashed tuber-skin with increasing doses of irradiation. The percentage of tubers with a red skin, with red 'sectors' and with a yellow skin increased accordingly. The percentage of tubers with red 'sectors' remained low for all applied doses.

Table 15. (Exp. 2) Regression coefficients and t values for different categories of tuber-skin colour after X-irradiation of mutant Clone M 52 from cv. Désirée with 500, 1000, 2000 and 4000 rad.

Tuber-skin colour	Regression coefficient	t value
Yellow/red-splashed	−0.016	-7.10^{xx}
Red (+ red 'sectored')	0.013	7.13^{xx}
Yellow	0.003	6.93^{xx}

Degrees of freedom (n-2): 3.
^{xx} = $P < 0.01$

6.4.3 A microscopic investigation of radiation damage and recovery in unscraped tuber eyes (Exp. 3)

6.4.3.1 Experimental details

On 16 July 1975, tubers of mutant Clone M 52 from cv. Désirée were obtained from the experimental farm of the Agricultural University in the new Flevopolder, and stored in Wageningen in a dark, cool room as usual. The tubers initially were kept at 5°C and from 10 October onwards at 3°C to prevent precocious germination. On 1 December, 225 healthy tubers with uniformly looking eyes were selected and for acclimatization transferred to a greenhouse (average air temperature 18°C), where they were kept for one day under a black plastic cover.

The next day 240 very uniform eyes, all situated in the centre of the tubers, were scooped out from these tubers. They were irradiated on December 4 with 1200 and 2400 rad of X-rays. The size of the doses given was based on the outcome of previous experiments. These indicated that irradiation with 2400 rad of X-rays is very effective to study direct regeneration from irradiated apices. At 1200 rad preliminary observations showed that the overall apical organization was still maintained.

Per treatment 80 eyes were irradiated. In addition 80 eyes were used as a control. Immediately after irradiation all eyes were put in humid riversand in a greenhouse at an air temperature of about 18°C. Per fixation time (2, 4, 6, 8, 11, 14, 17 and 20 days after irradiation) 10 eyes were available for each treatment (control, 1200 rad, 2400 rad), the four most uniform eyes being selected for further microscopy. Details on methods of fixation and staining were given in Section 6.3.

In Exp. 3, detailed observations were made. Results for central buds and (main) lateral buds were recorded separately. From each bud 8 near median sections (10 μm thick) were studied. Results were pooled per bud, per fixation time or per dose rate. A distinction was made between the flanks and the central part of each apex in such a way that the number of cells in L-I of both flanks together equals about the number of cells in L-I of the central part. In addition results for each histogenic layer were collected independently.

Observations included the occurrence of irregularities in different zones and layers, cell layer duplications, loose layers, numbers of cells, mitotic activity, periclinal divisions, plasmolysis, cell collapse, the occurrence of empty and necrotic cells, idioblasts, aberrant cell walls and degree of stainability of cytoplasma and nucleus. Time permitted a detailed microscopic study of only two tuber eye-pieces per fixation time. Radiation damage in leaf primordia surrounding the apical area, a common feature, was not studied in detail.

6.4.3.2 Results and comments

Sometimes observations were considerably complicated by certain reactions of the apices to irradiation which disturbed the normal pattern of apical organization. For example in the 1200 rad treatment, new (pseudo-) adventitious apices developed from cells within the area of the original, damaged central apices. They started as a small cluster of meristematic cells, apparently mainly of L-III origin (Fig. 13). In such cases, often more sections were studied to obtain a good impression of the apical structure. Calculations, nevertheless, always were made on the base of 8 sections per eye-piece per fixation time.

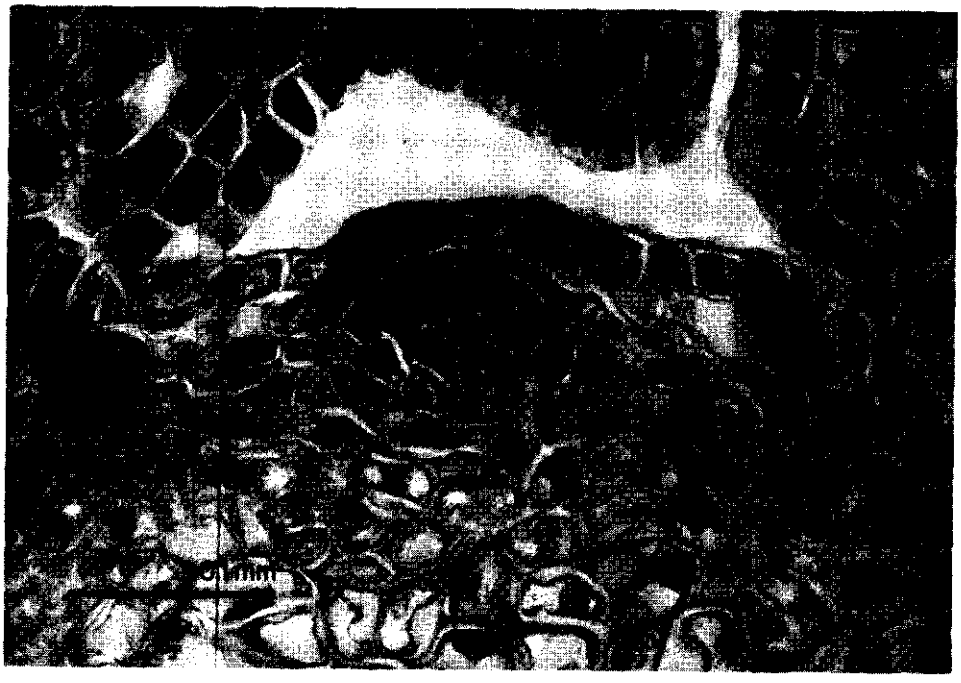

Fig. 13. (Exp. 3). Formation of a cluster of meristematic cells in a damaged lateral apex after irradiation of tuber eye-pieces of mutant Clone M 52 from cv. Désirée with 1200 rad of X-rays. Fixation after 8 days.

Number of cells The number of cells, present in L-I of each central and lateral bud studied, was determined by calculating the average of the countings for the most median sections of each apex. Because of the disorganization of the apices in late fixations from the 1200 rad treatment, no proper countings could be made in such apices.

Practically all central buds of the selected eyes contained 14-18 cells in L-I of each section. In the control, there were 4 central apices containing more L-I cells, i.e. up to 23 and one very small apex with only 12 L-I cells. Lateral buds mostly had 12-16 cells in L-I. The highest number observed here was 19. No significant effect of the fixation time was observed. The averages for central as well as for lateral buds were slightly lower in the irradiated series, but differences again were not significant. As the number of divisions in L-I remained low (see below under 'normal cell divisions'), enlargement of apices during the experiment must be due mainly to cell enlargement.

Shape of the apical dome The results of the present experiment confirmed the general observations that the shape of the apical dome often seems to be affected by irradiation (Yamakawa & Sekiguchi, 1968; Katagiri, 1973). Plastochron changes also affect changes in apex configuration after irradiation (Iqbal, 1969). In most of our experiments flattening was observed in irradiated apices. Sofar no proper explanations are known for this phenomenon. No specific differences in this respect were observed between the 1200 and the 2400 rad treatment.

Axillary meristems The number of meristems present in axils of central and lateral buds was determined. As in both irradiation treatments axillary meristems were virtually absent in lateral buds, only results on central buds are presented in Fig. 14. The increasing number of axillary meristems in later fixations of the control was according to expectations. Some axillary meristems have been present at the moment of harvesting in the summer preceding the experiment. Afterwards the tuber eyes went into dormancy which was terminated at the beginning of the experiment, after which new axillary meristems are formed again.

The 1200 rad treatment showed a significantly slower increase in number of axillary meristems than the control. The low number of meristems after 20 days most probably is incidental. In the 2400 rad treatment, the formation of new axillary meristems was almost completely suppressed during the first weeks. After 17 days, a small increase in frequency was observed, which could be considered as a sign of recovery. It is most likely that axillary bud formation is delayed temporarily and not permanently, because in irradiated plants often more aerial branches and more subterranean stolons are produced than in unirradiated material.

Data on axillary meristems present in lateral buds showed that in the control the average number increased from 0.75 after 2 days to 5.75 after 14 days. In both irradiation treatments axillary meristems were absent in lateral buds.

Root initiation Lateral roots develop from the lower parts of the (suppressed) tuber sprouts before the latter have reached the earth surface. Formation of root primordia was observed clearly in the central sprouts of the control and in the 1200 rad treatment after 6 days. On the average 4.3 root primordia were counted in the control and 6.2 in the

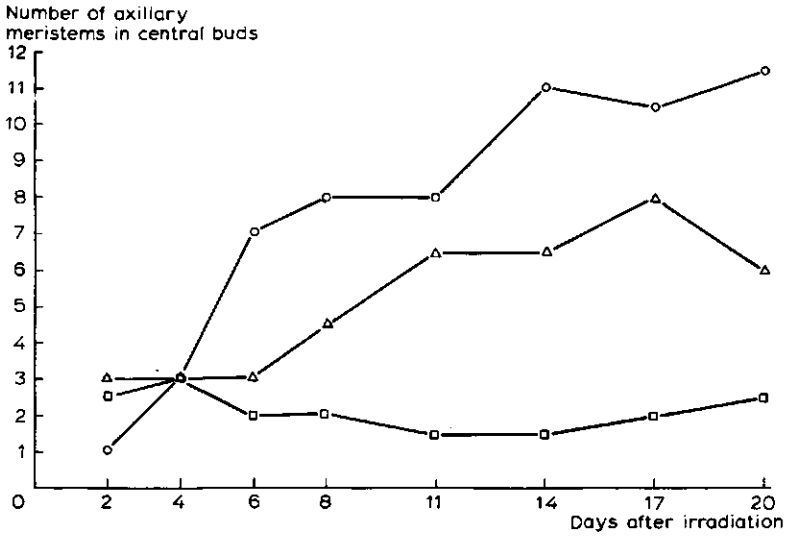

Fig. 14. (Exp. 3). Average number of axillary meristems in central buds of X-irradiated tuber eyepieces of mutant Clone M 52 from cv. Désirée. ○ = control, △ = 1200 rad, □ = 2400 rad.

1200 rad treatment. The results on the 1200 rad treatment suggest that this dose stimulates root initiation.

No root primordia at all were observed in the central sprouts of fixations from the 2400 rad treatment. It is known from other experiments that most tuber eye-pieces survive a dose of about 2-3 krad. Therefore the absence of root primordia in the 2400 rad treatment of Exp. 3 must be due to a considerable deceleration of their development, caused by irradiation. In lateral sprouts, root initiation at the base was not observed in the control nor in the irradiated apices.

Discreteness of cell layers Irregularities in the course of cell layers are indicative for the degree of stability of the apex as a whole and for the relative stability and independence of the distinct layers. In the calculations it has been taken into account whether a whole layer or part of it was irregular. As practically no differences were found between the flanks and the central part, these findings have been taken together. The results presented in Fig. 15 (a en b) have been expressed in percentages. 100% indicates that the layer concerned is completely irregular in all (i.e. 2 x 8) sections studied for a certain fixation time.

When considering the 6 graphs together, one can see that L-I is nearly always the most stable layer, followed by L-II. In central buds of the control, L-I showed no disturbance at all and in lateral buds only incidentally irregularities were observed. In L-III always a considerable amount of irregularity occurred. In the control a maximum was reached after 8 days, i.e. 12 days after transfer of the buds from a low level of metabolic activity (due to storage conditions) towards a considerable higher level. In a still later stage the metabolic activity seems to be stabilized at a somewhat lower level. The graphs of the controls after 6 days showed a depression for central as well as lateral buds. This may be explained by metabolic changes in the buds after termination of dormancy.

The results for the 1200 rad treatment differed considerable from those of the control. After 6 days an average level of layer disturbance has been reached which is about three times that of the control apices. After 13-17 days the apices gradually seemed to recover. During the first two weeks, the 2400 rad treatment showed significantly less layer disturbances than the 1200 rad treatment. The very low values observed during the first 8 days can be explained by inactivation of the apices due to high dose of X-rays. The results further suggest that even 20 days after irradiation treatment, the maximum level of layer disturbance had not been reached.

A comparison of the behaviour of central and lateral buds for the control showed no big differences. For the 1200 rad treatment the lateral buds showed less irregularities. An explanation, most probably, can be found in the protective effect by the apical dominance from the central bud (Grodzinskii & Gudkov, 1969). This phenomenon, however, did not occur in the 2400 rad treatment. Perhaps the effect of apical dominance was completely suppressed at this dose.

Whether the increased number of irregular cell layers after irradiation was a direct consequence of the radiation treatment or a reaction of the apices in order to repair radiation damage, is difficult to say.

Cell layer duplications The frequency of cell layer duplication has been determined as a percentage in the same way as for irregular cell layers. Duplications were absent in L-I of

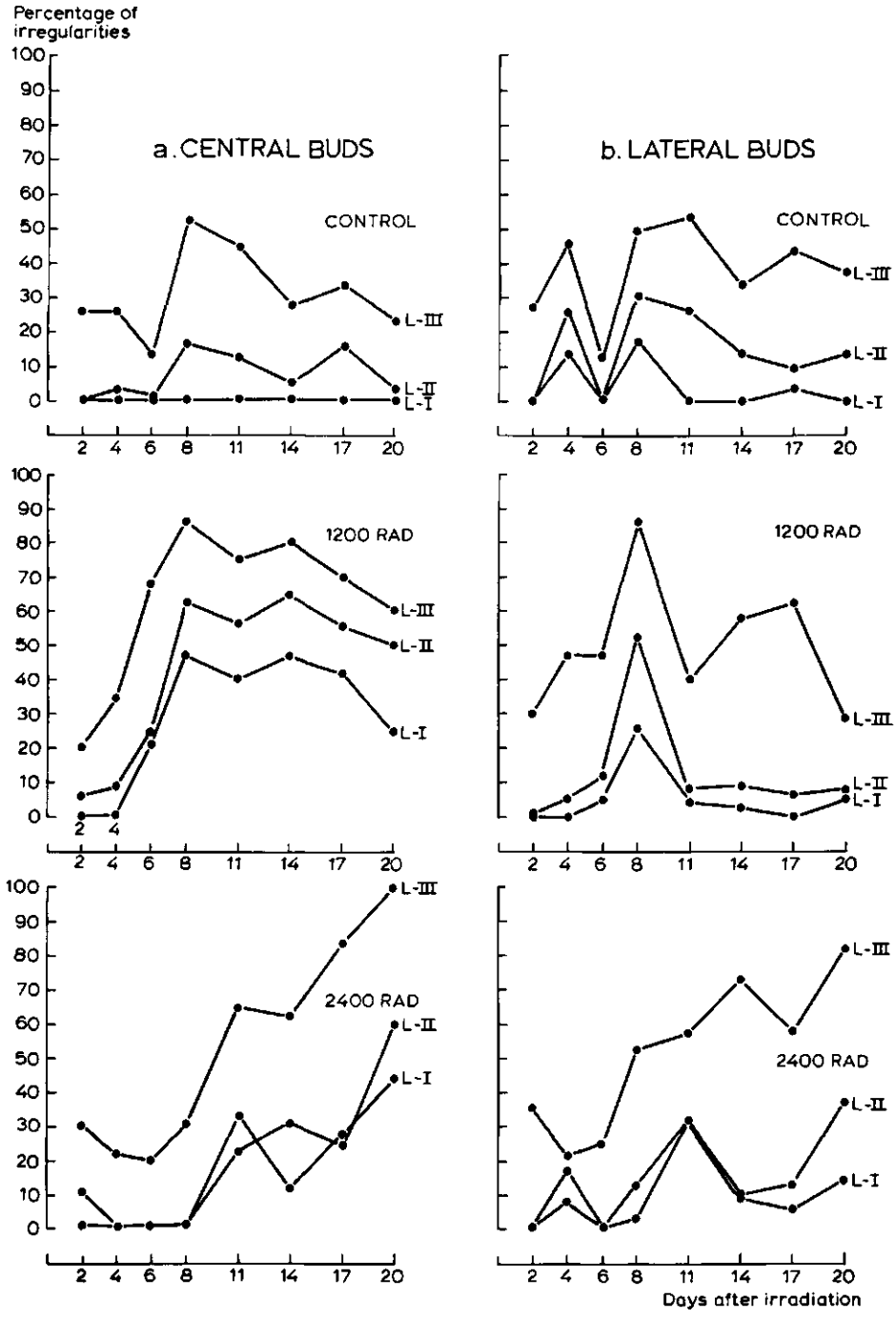

Fig. 15. (Exp. 3). Irregularities (in %) in different cell-layers of central and lateral buds from X-irradiated and unirradiated tuber eye-pieces of mutant Clone M 52 from cv. Désirée.

central as well as lateral buds of the control and occurred at very low rates only in subepidermal layers.

In the 1200 and 2400 rad treatments only two clear-cut examples of duplication were found in L-I and, although slightly more than in the control, again very few in deeper layers. Duplication occurred twice as often in the central part as along the flanks. Late fixations after the 1200 rad treatment again were difficult to interpret because of the formation of new meristematic centres in the apices.

Taken together the results confirm the generally accepted opinion that after treatment with X-rays some cell layer duplications may occur in L-I. For the other layers only a small increase in frequency is observed after irradiation. Data are too few to draw any conclusion about a possible relationship between dose of X-rays and frequency of duplication.

Loosened cell layers In the control, almost all cell layers remained in close contact. In only one case, after 8 days, did one central bud show spacing between all three layers in several sections. Some more examples were observed in lateral buds. Irradiated buds did not differ from the controls in this respect.

Normal cell divisions Cells from early metaphase to telophase were counted to determine the number of cells in mitosis. Results were expressed as a percentage of the number of cells in L-I of the section concerned. In the control as well as in the irradiated treatments, the number of mitotic divisions remained low. Despite this result, the commonly observed effect of reduction or complete suppression of mitosis after irradiation (Sparrow, 1961) was also observed in our experiments during the first two weeks in the 1200 rad treatment and during the whole period studied in the 2400 rad treatment (Fig. 16). A remarkable increase in mitotic activity was found after 14 days in the 1200

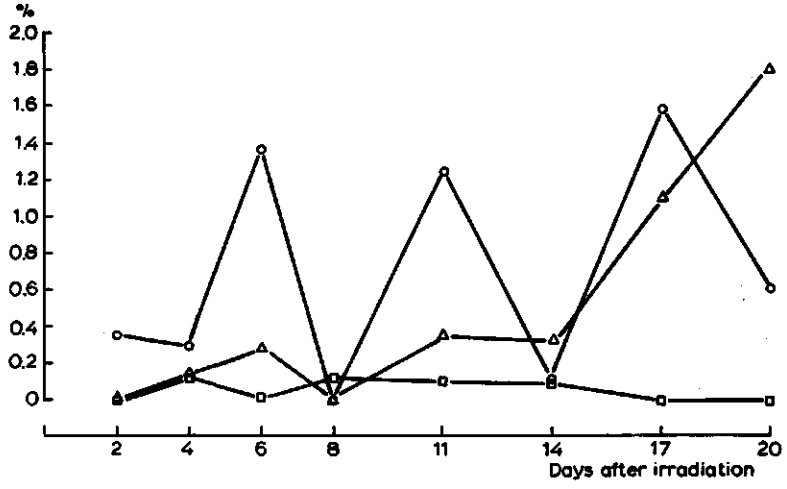

Fig. 16. (Exp. 3). Mitotic divisions (in %) in central buds of X-irradiated tuber eye-pieces of mutant Clone M 52 from cv. Désirée. ○ = control, △ = 1200 rad, □ = 2400 rad.

rad treatment. In the 2400 rad treatment the frequency of mitoses remained below 0.15% for all times of fixation.

On the average scores were slightly lower in lateral buds than in the corresponding central buds. The control showed no differences in mitotic rate between the central areas and the flanks. In the 1200 rad treatment considerably more divisions were found in late fixations in the central apical parts. Perhaps this higher number of divisions is a reaction of the central part after damage of the flanks which are supposed to be more radiosensitive. The 2400 rad treatment showed no differences in rate of mitosis. L-I and L-II had about the same rate of mitosis whereas L-III always scored slightly higher.

Finally the peaks in Fig. 16 after 6, 11 and 17 days in the control suggest that potato could have a mitotic cycle length of between 2 and 6 days. As far as known the length of the mitotic cycle has never been carefully determined for potato up to now (Ramanna, pers. commun.). The data on axillary bud formation in the control, mentioned before (See Fig. 14) do not disagree with the present findings.

Plasmolysis The amount of plasmolysis in a layer was determined by calculating the percentages of plasmolytic cells, expressed in percents of the number of L-I cells in the section concerned. To take into account various degrees of plasmolysis, results were multiplied by a factor $\frac{1}{4}$, $\frac{1}{2}$, $\frac{3}{4}$ or 1. The highest figure corresponds with the most severe degree of plasmolysis.

In Fig. 17 results for central buds are presented. Those from the lateral buds are not

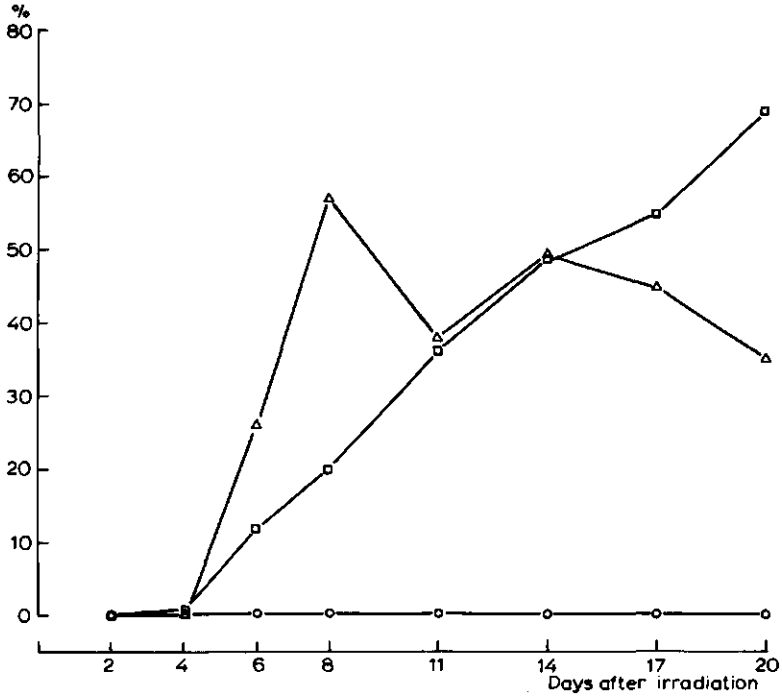

Fig. 17. (Exp. 3). Plasmolysis (in %) in central buds of X-irradiated tuber eye-pieces of mutant Clone M 52 from cv. Désirée. ○ = control, Δ = 1200 rad, □ = 2400 rad.

given as hardly any differences were found between central and lateral buds. The same can be said for the different layers, and the central part and flanks. In this experiment plasmolysis seemed an 'all or none' reaction (Stein & Steffensen, 1959a).

No signs of plasmolysis were found in the eyes of the control. In the 1200 rad treatment plasmolysis reached a maximum after 8 and after 14 days. The high value observed after 8 days must be considered incidental as this effect has been caused by only one of both apices. Moreover lateral buds showed a value intermediate between the observations for 4 and 8 days. After 14 days the number of plasmolysed cells again declined, probably a sign that post-irradiation recovery of the apical region had started. In the 2400 rad treatment the maximum amount of damage was found in the latest fixation. It is quite probable that here the maximum amount of plasmolysis had not been reached, but no later fixations were made.

Cell collapse Collapse of cells is a very drastic reaction to radiation (Lapins & Hough, 1970) and normally occurs less often than does plasmolysis, given a certain dose of irradiation. In Fig. 18, the average percentages for central buds are given. Frequency of collapse found for lateral buds was considerably lower.

There were no collapsed cells in all layers of the control. In the 1200 rad treatment a maximum was reached already after 8 days. There is a second peak in the graph after 17 days. However, one of both apices studied for this fixation time was heavily damaged and showed very deep vertical cracks in the apical region. Moreover this kind of abnormality has never been observed since. Therefore one might expect that normally the number of collapsed cells at 1200 rad at 17 days would be somewhat lower than that observed. When comparing cell collapse in different layers of the 1200 rad treatment, we observed that L-II had more collapsed cells than L-I and L-III. These two layers did not differ in this respect.

In the 2400 rad treatment, maximum cell collapse was observed after 14 days. In this series not L-II but L-I contained slightly more collapsed cells, but differences were not significant.

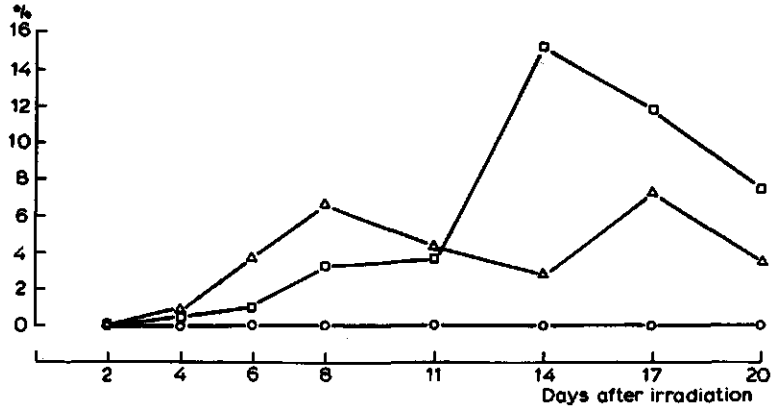

Fig. 18. (Exp. 3). Collapsed cells (in %) in central buds of X-irradiated tuber eye-pieces of mutant Clone M 52 from cv. Désirée. o = control, Δ = 1200 rad, □ = 2400 rad.

Cell collapse within apices can be expressed in relative figures, in which case the control scores 0, the 1200 rad treatment 7 and the 2400 rad treatment 10. For both doses of X-rays significantly more collapsed cells were observed along the flanks than in the centre. The ratios are about 3.7 : 1 for the 1200 rad treatment and 2.5 : 1 for the 2400 rad treatment. The differences observed were probably caused by more mitotic divisions along the flanks and, therefore, a higher degree of radiosensitivity. This difference in rate of mitosis indeed was found for the 1200 rad treatment but not for the 2400 rad treatment.

In lateral buds of the control collapsed cells, according to expectations, were absent. In all irradiated eye-pieces, lateral buds showed considerably lower numbers of collapsed cells than the central ones. Their frequency nearly always remained below 3%. Often no collapsed cells at all could be found in such buds. There was a slight indication that the percentage of collapsed cells in lateral buds increased in the latest fixations. From the results obtained one could conclude that lateral buds are protected in a certain way against this type of radiation damage. It seems logical to relate this phenomenon to the apical dominancy of the central bud.

When comparing results on plasmolysis and collapsed cells, one expects the maximum degree of plasmolysis to be reached earlier than the peak of collapsing. This expectation, however, was not to fulfilled in our material. In literature, no references were found to relationships between both types of damage.

Idioblasts The occurrence of excessive numbers of idioblasts after irradiation has been reported by several authors like e.g. Lapins & Hough (1970). In the present experiment counts were also made, but in the irradiated series only low frequencies were found, e.g. one or two only per 40-50 cells. In lateral buds this frequency was still lower.

Empty and necrotic cells The frequency of empty cells has been used sometimes as a parameter to measure radiation damage, e.g. by Lapins & Hough (1970). In the present experiment empty cells were virtually absent in the control and occurred at very low frequencies in all fixations at 1200 rad, with slightly higher figures for the fixations made at 11, 14 and 17 days after irradiation. The 2400 rad treatment sharply increased the number of empty cells at fixation 17 and 20 days after irradiation.

Necrotic cells were observed very occasionally, i.e. in the 1200 rad treatment in two apices, fixed after 8 and 14 days and in the 2400 rad treatment after 11, 14 and 20 days. No necrotic cells were found in any of the lateral buds.

Thickness of cell walls Thickening of the primary cell wall has been used for example by Sekiguchi et al. (1971) as a parameter to determine radiation damage in cells. We observed aberrant cell walls in up to 20% of the sections made after 11 days in the 1200 rad treatment. In the 2400 rad treatment even 20-75% of the sections made for the latest two fixation times contained aberrant cell walls. Earlier fixations did not show any sign of this phenomenon.

Stainability of cytoplasm and nucleus Reduced stainability of the cytoplasm, according to most authors, is due to vacuolation. Stainability of the cytoplasm after different doses of X-rays and at various fixation times is shown in Fig. 19. The results clearly show a

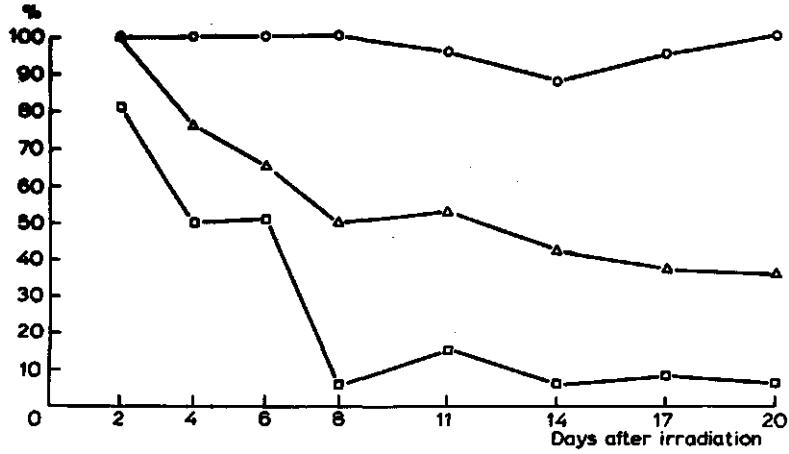

Fig. 19. (Exp. 3). Stainability of cytoplasma (in %) in central buds of X-irradiated tuber eye-pieces of mutant Clone M 52 from cv. Désirée. ○ = control, △ = 1200 rad, □ = 2400 rad.

relationship between dose and injury, as well as between time and injury. In several apices of the 2400 rad treatment, staining was even completely absent. A comparison of the staining results in central and in lateral buds revealed that practically no difference existed in this respect. Also no notable differences were observed between the various histogenic layers and between central parts and flanks.

More difficult to interpret are the results on stainability of the nuclei (Fig. 20). A survey of the literature on this subject gave rather contradictory results. Lapins & Hough (1970), for example, mentioned contracted, dense nuclei after heavy irradiation of leaf buds of peach. Such nuclei, undoubtedly, will have shown intense staining. On the other hand, Langenauer et al. (1973) observed either unstained or very dark nuclei after irradia-

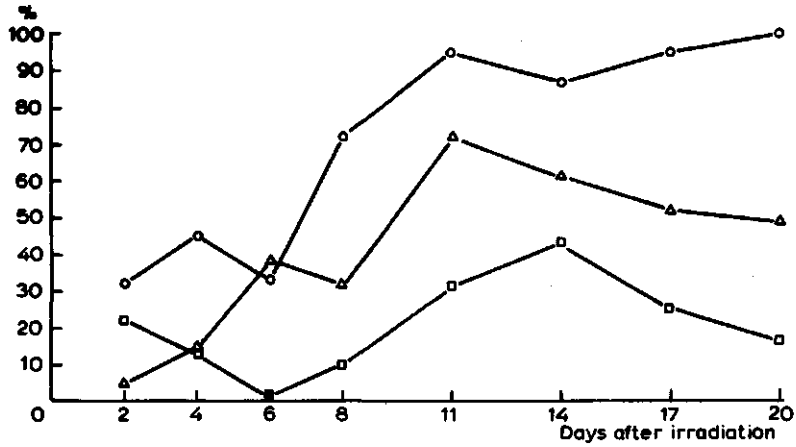

Fig. 20. (Exp. 3). Stainability of nuclei (in %) in central buds of X-irradiated tuber eye-pieces of mutant Clone M 52 from cv. Désirée. ○ = control, △ = 1200 rad, □ = 2400 rad.

tion of axillary buds of *Parthenocissus tricuspidata*, whereas Iqbal (1972) referred to reduced or no staining of nuclei of irradiated seedlings of *Capsicum annuum*.

In the present experiment considerably variation in stainability of nuclei was observed within the control as well as within irradiated material. Differences between both apices studied for a certain dose and fixation time were considerable. As the data on lateral buds were nearly always in accordance with those on central buds, only results on central buds have been included in Fig. 20.

The remarkably low stainability observed in the earliest fixations of the 1200 rad treatment most probably was due to statistics, because the corresponding lateral buds did not give such low values, these being the only exceptions to the similarity between central and lateral buds. The most likely explanation for the low degree of staining in early fixations of the control seems to be that such cells still are in stage G-I of the mitotic cycle and gradually are transferred to stage S or G-II. The decline in stainability after 11 and 14 days, in the 1200 and 2400 rad treatments respectively, was probably due to direct chromosomal damage or was caused by the effect of irradiation treatment on the rate of mitosis.

Changes in cell size after irradiation Irradiated potato apices followed the pattern that cells surrounding damaged cells may become hypertrophic and afterwards may crush the damaged cells (e.g. Pratt, 1968; Iqbal, 1969). The distribution of such large cells throughout the apices in our experiment was very irregular and their frequency remained very low.

It was mentioned before that the normal increase in size of the apices during the experiment is caused by cell enlargement and not by increase of the number of apical cells.

Periclinal divisions Periclinal divisions, expressed as a percentage of the number of cells in L-I of the section concerned, were absent in L-I, very rare in L-II and occurred frequently in L-III of the control. In the 1200 rad as well as in the 2400 rad treatment,

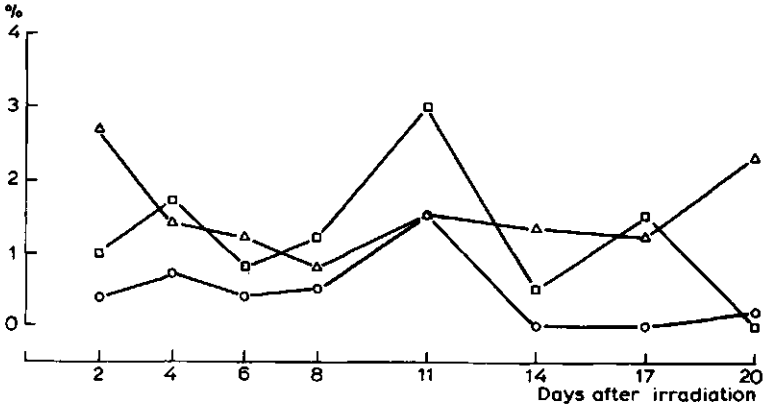

Fig. 21. (Exp. 3). Periclinal divisions (in %) in L-I + L-II of central buds of X-irradiated tuber eye-pieces of mutant Clone M 52 from cv. Désirée. ○ = control, △ = 1200 rad, □ = 2400 rad.

the L-I showed some periclinal divisions, although at a low frequency, which never exceeded 1.4%. Thus in all 8 sections of an apex at a given dose of X-rays and a given time of fixation only 1 or 2 periclinal divisions were observed. The 1200 and 2400 rad treatments did not differ significantly. The latter gave even slightly lower results. No differences were observed between flanks and central area.

To compare the frequencies of periclinal divisions at different times of fixation and for different irradiation treatments, average percentages for L-I and L-II together in central buds were determined. Results are presented in Fig. 21. Data on L-III have been left out on purpose, because irradiation only leads to some increase in the frequency of such divisions in this layer, whereas for L-I and L-II the occurrence of periclinal divisions may change fundamentally the regular pattern of clearly distinct and independent outer histogenic layers, which normally show anticlinal divisions only.

Percentages of periclinal divisions in L-I and L-II, after using an arcsin transformation (Snedecor & Cochran, 1968, p. 327), were further studied via an analysis of variance. The Student *t*-test (Snedecor & Cochran, 1968, p. 59) showed that differences between irradiated and non-irradiated series were significant ($P < 0.05$). No significant differences were observed with respect to the time of fixation. One relatively high value in the control (1.5% after 11 days) must be due to periclinal divisions in an apex with relatively few L-I cells. In this way the frequency of periclinal divisions was overestimated. In conclusion one can say that after irradiation the rate of periclinal divisions in L-I increased from zero to a very low value, in L-II from a rather low rate to about three times as much and in L-III from a rather high value to a still slightly higher one.

Why are the values for periclinal divisions higher than those for mitotic divisions? The answer most probably lies in periclinal divisions being scored by counting periclinal cell walls, whereas the rate of mitosis is determined by counting cells showing a clear metaphase, anaphase or telophase. Such cells can only be observed during a limited time whereas periclinal cell walls are permanent. A comparison of the figures, therefore, is not a fair one.

Our observations on periclinal divisions basically are in accordance with those of Iqbal (1969) for *Capsicum annuum*. Iqbal found about 2% periclinal divisions in L-I and L-II of irradiated apices, whereas such divisions were lacking in L-I and L-II of unirradiated apices. Iqbal thought such divisions to be a symptom of recovery. He supported this opinion by his findings that periclinal divisions only occur in the outer layer when adjoining cells in the second layer are dead. Furthermore he found that such divisions only occur at low exposures (1-3 kR) and that they are not observed before 15 days. Our own observations do not fully support the opinion of Iqbal. Periclinal cells in L-I are not necessarily associated with dead cells in L-II. They occurred in the 1200 rad as well as in the 2400 rad treatments after 11 days. Whether application of larger doses of X-rays would completely suppress periclinal divisions in L-I and L-II was not studied in the present experiment.

In lateral buds periclinal division was observed only once in L-I of an apex of the control. The frequency of such divisions in irradiated series also remained very low. No effect of the time of fixation was observed. Results for L-II were very inconsistent. In the control this layer was less stable in the lateral buds than in the central buds. After irradiation high as well as lower values for the number of periclinal divisions were observed. The time of fixation had no effect.

New meristematic centres Such centres – which could be called pseudo-adventitious – always arose in the apical part of the bud and mainly consisted of L-III tissue. They normally started as a cluster of, mostly small, dividing cells. In a later stage these clusters may form new apices, arising in the old apical region (Fig. 13). After irradiation formation of new meristematic centres was observed. Substitute meristems have been found in irradiated apices by several other investigators like Iqbal (1970) and Lapins & Hough (1970). They may be considered as islands of active cells within apices which are otherwise temporarily or permanently inactivated.

In our experiment this way of recovery, as Fig. 22 shows, especially is found in central apices after 11 days in the 1200 rad treatment. As expected substitute meristems were not formed in the control. Irradiation of potato eye-pieces with 2400 rads seems to damage the apices too much so that regeneration by new meristematic centres is not possible, at least in all fixations except the last ones. In lateral buds no meristematic centres were found in the control, whereas the lateral apices of the 1200 rad treatment on the average contained two 'meristem balls' in later fixations. In the 2400 rad treatment only the last three fixations showed some substitute meristems in lateral buds.

The results suggest that regeneration after exposure of potato eyes to moderate doses of irradiation occurs via the formation of substitute meristems in the original apical zone, as well as from axillary meristems. After treatment with larger doses the apical areas may have been damaged too much to regenerate from within such areas, at least for the period studied. Regeneration of plants irradiated with 2400 rad of X-rays, mainly occurs from axillary buds (mostly from lower axils) and sometimes from adventitious shoots originating from callus. Probably more meristem balls are formed later.

Additional morphological observations Investigation of plant types revealed that the 1200 rad treatment consisted for 100% of the so-called B-10 type (Fig. 12). After treatment with 2400 rad of X-rays, 95% of the plants could be classified as belonging to this type. In the 2400 rad treatment, the lowest two or three internodes were also suppressed, so that regeneration from subterranean axils occurred. This suppression was also observed

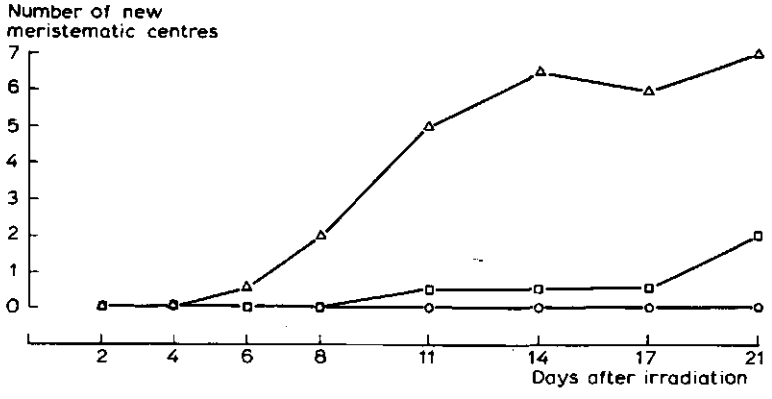

Fig. 22. (Exp.3). Number of new meristematic centres in the apical region of central buds of X-irradiated tuber eye-pieces of mutant Clone M 52 from cv. Désirée. ○ = control, Δ = 1200 rad, □ = 2400 rad.

Table 16. (Exp. 3) Number and proportion (%) of different types of tuber-skin colour after X-irradiation of tuber eye-pieces of the yellow/red-splashed mutant Clone M 52 from cv. Désirée.

	Control	1200 rad	2400 rad
Total number of tubers	218 (100%)	219 (100%)	344 (100%)
Number of yellow/red-splashed tubers	212 (97%)	209 (95%)	294 (85%)
Number of red and red 'sectored' tubers	6 (3%)	10 (5%)	43 (13%)
Number of solid yellow tubers	– (–)	– (–)	6 (2%)

in Exp. 2. The type of regeneration described here occurs more often than has been realized in the past,

All plants regenerated in the 1200 rad treatment not earlier than 32 days after irradiation and in the 2400 rad treatment 47 days after irradiation. This is later than normal, as a retardation of one week for 1 krad of X-rays is common, plus one week for each additional krad.

The average number of tubers per plant (75 plants per treatment) were 3.4 for the control, 3.3 for the 1200 rad treatment and 4.2 for the 2400 rad treatment. These numbers seem rather low but it must be kept in mind that plants were raised as a winter generation in a greenhouse. Increased tuberization, often in combination with the production of smaller tubers after irradiation has been reported on several occasions for potato (Section 2.3.2).

Patterns of tuber-skin colour As in both previous experiments (Exps. 1 and 2), different colours of tuber skin were distinguished: yellow/red-splashed (the starting material), red (including red 'sectored') and yellow. Numbers and proportions (in %) are given in Table 16. It was verified whether a linear relationship existed between the different colours and the size of the radiation dose. Results were calculated by the Student *t*-test, but regression coefficients (-0.00458 for yellow/red-splashed and 0.00375 for red skinned tubers) were not significant because of the low number of degrees of freedom (1 only). Linear regression analysis could not be applied to yellow tubers. Correlation coefficients, determined for yellow/red-splashed and red skinned tubers, showed very high values ($r = -0.94$ and $r = 0.95$, respectively).

6.5 Discussion

In Experiments 1, 2 and 3, information was collected about the behaviour of potato shoot apices after irradiation. Most useful microscopic information was obtained in Exp. 3 from the detailed investigations of sections made during 20 days. The results of Exp. 3 did not always confirm the preliminary conclusions and ideas of Exps. 1 and 2.

One of the problems was to examine whether radiation damage of apices could be indicated by one value. Iqbal (1970), for example, distinguished four different degrees of

damage, based on observations of phenomena like plasmolysis, stainability of nuclei and cytoplasma, and cell death. From Exp. 3 it became clear that it is very difficult to categorize irradiated apices successfully in this way. The problem is that the different parameters may show considerable variation in their reaction to various doses of irradiation, especially in relation to the time it takes before different effects manifest themselves and reach their maximum value. In my opinion, a more useful approach would be to assess the behaviour of the various parameters after irradiation more or less separately.

In normally developing tuber shoots, the number of axillary buds increases in time, especially after termination of dormancy. Axillary bud formation is affected by radiation. In Exp. 3, it was considerably retarded after irradiation with the (moderate) dose of 1200 rad during the whole period of observation (20 days). Formation of new axillary buds was almost completely suppressed by the 2400 rad treatment, except for the last fixations.

After irradiation with 2400 rad of X-rays, root formation from the lower part of the sprout was also retarded in Exp. 3. In the 1200 rad treatment, on the contrary, slightly more roots were produced than in the control. This dose is only slightly above what is normally considered a stimulation dose for potato and therefore this increased number of roots probably could be seen as an effect of radiostimulation. However radiostimulation is still a rather controversial subject and will not be discussed here.

The stability of histogenic layers in the apical region was assessed from observations on cell layer duplications, periclinal divisions and layer discreteness. As expected L-I was by far the most stable layer in all experiments. Sometimes the course of L-II was difficult to follow, especially after irradiation. Layer disturbances after treatment with 1200 rad (Exp. 3) were mainly observed between 8 and 14 days. Apparently the apices recovered afterwards. In the 2400 rad treatment of the same experiment, the level of disturbances increased throughout the whole period of observation. The percentage of periclinal divisions increased in all layers after irradiation. The frequency in L-II always considerably surpassed that in L-I. In L-I of the control such divisions were virtually absent. In the three experiments, the effects of dose on these parameters were rather inconsistent as were the differences observed between flanks and central apical area. Very probably still more material has to be studied to obtain reliable conclusions.

In Exp. 2, after irradiation with 4000 rad no periclinal divisions were observed in L-II. Probably L-II has a limited role in regeneration processes after irradiation with large doses. If this supposition indeed were true, it would indicate a lower degree of independence of L-II. However there are two restrictions: first in Exp. 2 fixations were made for five days only and second, with the present starting material, it was not possible to check the role of L-II e.g. via analysis of suitable periclinal chimeras. It is not very likely that periclinal divisions in L-II would still appear in late fixations of the 4000 rad treatment because in the treatments with large doses in Exp. 1 (3000 rad) and Exp. 3 (2400 rad) such divisions were found already after two days. Finally, in Exp. 3 no effect of the time of fixation on the frequency of periclinal divisions was observed.

Irradiation clearly leads to a reduction in the number of mitotic divisions. In Exp. 3, a 2400 rad treatment almost completely suppressed mitosis for the whole period of fixation. In the 1200 rad treatment, after an initial period of suppression, a considerable increase in mitosis was observed again after 14 days. This indicates that these apices recovered after two weeks. Rate of mitosis in lateral buds was lower than in central buds.

Differences between the central parts and the flanks were observed, but results were too inconsistent to allow reliable conclusions.

Results on the degree of plasmolysis as determined in Exp. 3 support the opinion that, after exposure to 1200 rad, recovery starts after about 14 days. In the 2400 rad treatment maximum values for plasmolysis were found after three weeks. Probably the real peak is reached somewhat later. Plamolysis seems a typical 'all or none' reaction. Somewhat unexpectedly it was found in Exp. 3 that the highest values for cell collapse were reached earlier than those for plasmolysis. For the 1200 rad treatment the maximum was observed at 8 days and for the 2400 rad treatment at 14 days. To explain these observations one could assume, for example, that cells that are damaged heavily after irradiation quickly show a strong reaction, i.e. cell collapse, whereas plasmolysis builds up in cells which are relatively less damaged. Cell collapse and plasmolysis are useful parameters to indicate radiodamage. Both phenomena were completely absent in the control.

Another useful parameter was the degree of stainability of the cytoplasma. Staining in the control was about 100% in all fixations. After exposure to 1200 rad of X-rays a quick decline was observed during the first two weeks. Afterwards this level of staining remained practically constant. In the 2400 rad treatment, the lowest rate of staining was found already after 8 days, after which it remained constant at this level. Stainability of the nuclei also showed a clear dose effect but in general these results are more difficult to interpret.

After two weeks, on the average about 7 new meristematic centres were observed within the original apices of the 1200 rad treatment. They probably develop into new sprouts and then take over (part of) the activity of the original apex and its axillary buds. Exposure to 2400 rad of X-rays seems too heavy a treatment for the formation of new meristematic centres, at least during the three weeks that fixations were made.

The above results show that in central buds after exposure to 1200 rad of X-rays, the maximum level of damage was reached about two weeks after irradiation. Afterwards there were progressively more signs of recovery and regeneration such as increased mitotic activity, periclinal divisions and formation of new meristematic centres. The maximum level of damage after exposure to 2400 rad was found about three weeks after irradiation. There were some signs of recovery in the last fixations of this treatment. This again indicates that it would have been worthwhile to study some later fixations.

A comparison of central and lateral apices showed that lateral buds reacted to irradiation less for most parameters like layer irregularities, number of collapsed and necrotic cells and number of idioblasts. For some other phenomena like plasmolysis and stainability of the cytoplasma, no differences were found between central and lateral buds. Although in the lateral buds of the control, slightly more loose cell layers and layer irregularities were found than in central buds, there is not enough evidence that the contribution of the different histogenic layers to sprout development in lateral (i.e. in fact axillary) buds within potato eyes diverges from the situation in central buds as Howard et al. (1963) believed.

Although the preliminary results from Exp. 1 suggested that radiation damaged the central apical area more than the flanks, this was not confirmed in later experiments (N.B. in Exp. 1 desprouted tuber eyes were used). Between Exps. 2 and 3 no significant differences were found for most parameters. Sometimes opposite effects were observed. Cell layer duplications, for example, in Exp. 3 occurred significantly more often in the

central area than along the flanks. As this was observed in all sections, irrespective of the time of fixation, this result cannot be considered as proof for the opinion that the central part becomes more active after inactivation of the flanks. On the other hand the observations on the mitotic rate in the 1200 rad treatment do point to decreased activity of the flanks because the rate of mitosis increased in the central part of especially late fixations. In the 2400 rad treatment no differences were found in this respect. Additional evidence for a higher radiosensitivity of the flanks could be obtained from the significantly higher number of collapsed cells along the flanks in the 1200 rad treatment as well as in the 2400 rad treatment. However results clearly show that the problem is complex and that one should be very careful when drawing conclusions about a shift in the activity within apices after irradiation.

Care should also be taken when interpreting results from observations on differences in radiosensitivity between the histogenic layers. In Exps. 1 and 2, some indications were found for the existence of a localized, outer to inner pattern of decreasing radiosensitivity. However, after studying the results from Exp. 3, one is more inclined to classify radiation damage in potato as 'scattered' (Pratt, 1968). Up to now there has been insufficient proof for the existence of differences in radiosensitivity between the various layers. Most publications, describing this subject have been based on few observations. Most probably the differences observed seem due to mitotic activity differing between cells, layers or regions.

How do results of the microscopic work fit in with the literature on potato? Our observations confirm that in most apices both outer layers (at present commonly indicated as L-I and L-II) normally show a clear pattern of stratification, whereas the third one (L-III) often is more difficult to recognize as such. Our findings further are in agreement with the general opinion that L-I is more stable than L-II.

The unpublished results of Montezuma and Ghatnekar, referred to in Section 6.2.4, agree with our results. Ghatnekar found, as expected, less mitotic activity with relatively more mitoses in the central part after irradiation. He also could not establish layer differences. Temporary inactivation of X-irradiated apices was also observed by Korableva (1961).

Although microscopy of shoot apices certainly provides interesting information, this approach is not ideal for explaining histogenic effects in detail. In my opinion, it is more useful to study wellknown irradiated periclinally chimeric plants and tubers derived from them. An additional comment is that in literature most investigators have not provided sufficient evidence for their opinions about the processes of regeneration after irradiation. Thus their conclusions, for example, about the relative participation of different histogenic layers are sometimes unjustified or at least incomplete.

Although the tubers of the starting material (mutant Clone M 52 from cv. Désirée) showed physiological variations at the moment of irradiation in our three experiments and even though different doses of irradiation were used, the results are in good agreement as is shown in Fig. 23. With increasing doses of X-rays, a decrease in the percentage of original periclinally chimeric (yellow/red-splashed) tubers and an increase in the percentage of solid (red or yellow) tubers was observed. (N.B. In the calculations tubers which are mericlinally red for L-I were recorded as 'red', because the occurrence of tubers of both these groups to a large extent refers to the same type of histogenic effects.) Tubers with yellow/red-splashed tuber skin occur when stolons arise from axillary buds

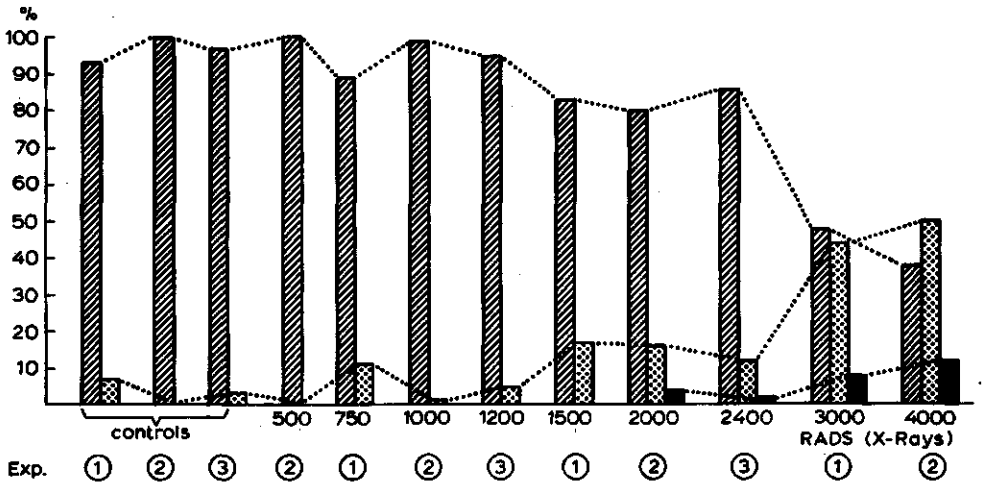

Fig. 23. Types of tuber-skin colour (in %) after X-irradiation of tuber eye-pieces of mutant Clone m 52 from cv. Désirée in three experiments. Hatched columns = yellow/red-splashed tuber skin (starting type), dotted shading = solid + mericlinally red tuber skin, solid black = solid yellow tuber skin.

which reproduce the (periclinal) structure of the original apex. One expects that with increasing doses more axillary buds are inactivated or destroyed, whereas increasingly more adventitious buds or substitute meristems may develop and participate in regeneration.

The proportion of tubers with a yellow skin increases slowly with increasing dose. Such tubers originate only from cells which can be traced back to (genetically yellow) L-I. Two processes may be involved: either direct regeneration of a complete sprout from an L-I cell, or reduplication of L-I, i.e. when L-I cells penetrate deeper situated layers and take the function of cells in those layers, followed by a more or less regular kind of regeneration. Solid yellow tubers are virtually absent after exposure to doses of X-rays below 2000 rad, probably because L-I only becomes independent when the dose of irradiation passes a minimum.

The frequency of yellow tubers does not give at the same time a value for the rate of replacement because also tubers with the genetic constitution L-I yellow, L-II yellow, L-III red, represent effects of replacement. Such tubers, however, cannot be easily recognized and were mostly recorded as yellow/red-splashed. Thus the effect of replacement is underestimated. This problem does not exist with respect to displacement (L-II and/or L-II penetrating in outer layers) as tubers with red stripes in L-I can be recognized as such. In fact the percentage of mericlinally red tubers (L-I partly red and partly yellow, L-II + L-III red) was found to be fairly constant and never exceeded 3-4%.

In calculations about the rate of displacement, however, another source of error is introduced, which is ignored by practically all investigators. Red tubers not only arise after displacement but they can also originate from a large reservoir of subepidermal cells, from within the real apical zones as well as from outside this area. When new meristematic centres occur in inactivated apices or when a substitute meristem is formed, (e.g.

from a callus originating in extra-apical tissue) the cells of such meristems will, for reasons of statistics, consist mainly or exclusively of cells that are genetically red.

In the control of all three experiments, 95% or more of the tubers was found to have the original periclinally chimeric structure. The proportion of tubers representing the genetic constitution (for tuber-skin colour) of deeper layers ranged from a few per thousand to a few percent, whereas tubers representing the constitution of L-I were virtually absent.

In Exp. 1 a large commercial stock was counted. In this stock consisting of about 185,000 tubers, less than 1% of the tubers was red + red 'sectored' and only 6 tubers were solid yellow. It was concluded at that time that the percentage of aberrant (i.e. not yellow/red-splashed) tubers significantly differed from the control (353 tubers) of this experiment. If, however, the results for the control in the three experiments are pooled. the results agree reasonably well with the totals recorded for the 'stock'. In addition, as was mentioned before, 'stock' and starting material do not refer to the same vegetative regeneration, and the work of the clonal selector may have had some impact on the proportion of different tuber-skin colours. Some contamination of the commercial stock cannot be excluded.

Aberrant types in nature may arise in different ways. Most aberrations are caused by mechanical damage, e.g. due to harvest or storage, but the occurrence of solid yellow tubers cannot be explained in this way. The microscopic work showed that also in non-irradiated tubers some irregularities occur. It is peculiar, however, that after irradiation with 4000 rad of X-rays, the highest proportion of solid yellow tubers was found, but no periclinal divisions were scored in L-I. The only likely explanation is that such divisions remained unobserved due to the relatively small number of sections studied.

Other investigators of potato also studied the kind of effects described above. Mostly they applied doses of about 3000-3500 rad of X-rays. Chimeras for tuber-skin colour were examined by Howard (1964b) and Klopfer (1965a) who also studied leaf-shape chimeras. In these experiments, between 45 and 65% of the tubers or plants after irradiation maintained the chimeral structure of the starting material. Mutants representing the structure of L-I only, (corresponding with solid yellow tubers in our experiment) were found with a frequency of 5-10%. These values are in good agreement with results after irradiation with relatively large doses of X-rays in our experiments.

Despite differences in starting material, dose and method of irradiation, characters studied, etc., the proportion of the different types of tuber-skin colour, leaf shape, etc. can be predicted to a certain extent. However research up to now has shown that microscopically observed effects of radiation cannot be related directly to effects like changes in tuber-skin colour, because of the limitations of present methods and material.

Reference has already been made to plantlets of Type B-10. Initially we thought that such plants especially occurred after irradiation with moderate doses. Later they also were recognized, be it in a somewhat modified form, after exposure to higher doses. The idea prevailed that sprouts of rosette-like plants had developed from new meristematic centres in the inactivated apices. However, if this were so, the majority of tubers obtained from cuttings of these sprouts would be solid red, as these sprouts arise mainly from subepidermal plant tissue which is genetically red for tuber-skin colour. Very surprisingly 95% or more of the tubers were identical to the starting material, i.e. yellow/red-splashed, also after treatment with larger doses. This result can only occur when the sprouts, or more

exactly the cuttings from which the tubers arose, have the same periclinally chimeric constitution as the main apex and are therefore of normal axillary origin. Apparently the role of new meristematic centres within inactivated apices in potato is rather limited as long as axillary buds are able to regenerate.

Another explanation could be that axils or areas giving rise to aerial sprouts (which usually manifest themselves as B-10 types) and to subterranean sprouts (from which the tubers arise) behave differently after irradiation. However Klopfer's results (1965a) on proportions of leaf-shape and tuber-skin colour make this explanation most unlikely. This interesting topic, as well as many other subjects related to the behaviour of potato shoot apices after irradiation, deserves further research.

Summary

Chapter 1 Unlike the induced mutations in various other vegetatively propagated crops, those in potato (*Solanum tuberosum* L.) have not contributed much so far to the breeding of new varieties. At the Institute of Plant Breeding (IvP), Wageningen, mutation breeding in potato has been studied since 1961 to determine whether it offers sufficient prospects for commercial breeding. This publication describes part of the investigations.

Chapter 2 A review of the literature on mutation work in potato is given. From these data it is concluded that structures with small apices like young single-eye tuber pieces or cuttings, when irradiated with 2-3 krad of X-rays or γ-rays, give sufficiently high mutation rates and show a low level of chimerism. If the total number of induced mutations is high enough, sufficient positive mutations without adverse side-effects can be obtained. In earlier publications the amount of material under treatment was often too limited and experiments were not continued long enough to allow reliable conclusions.

Chapter 3 Efforts to produce a di(ha)ploid tester clone (2n=2x=24) for the collection of reliable data on mutation frequency are described. Different sources with genes for synthesis and distribution of pigment, resistance to late blight, leaf shape and leaf margin colour, were crossed in many combinations. The progenies, which carried 4-5 marker genes in heterozygous condition, were examined for general growth, expression of marker genes, radiosensitivity and mutability. Because of a high susceptibility to diseases, poor growth, high radiosensitivity or low general or gene-specific mutability, none of these clones seemed promising. Thus the original goal of using such a tester clone for all further studies on mutation breeding in potato, had to be abandoned.

Chapter 4 The formation of adventitious roots and shoots from potato leaves, leaflets and stem parts in vivo was studied. In mutation breeding the adventitious shoot technique has been successful, especially with shoots of single-cell origin. Shoots from epidermal cells at the petiole base often originate from single cells. For potato such a method is not yet available.

In our experiments we mostly used potato material that was periclinally chimeric for tuber-skin colour, so that it was possible to determine immediately whether shoots of (assumedly) adventitious origin could be traced back to the outer histogenic layer (L-I). At first we thought that rooting of the starting material before adventitious shoot formation, offered the best prospects. Conditions affecting root formation were studied. A system was devised in which leaves were kept in trays of liquid media. We observed that the choice of cultivars had a strong effect on rooting and longevity. Single leaflets rooted considerably better than compound ones. Application of auxins stimulated rooting. Kinetin, gibberellic acid (GA_3) and 6-benzylaminopurine (BA), added because of their

positive effect on adventitious shoot formation in many experiments, suppressed root formation. Rooting was also suppressed by prolonged dark periods.

From more than 100 000 leaves and leaflets, only about 120 shoots of possible adventitious origin were obtained, but hardly any of them were of L-I origin. Shoots were also produced from eye-less stem parts. However, these were definitely not of single-cell origin, so that the method was useless for mutation breeding. Hence, for potato, stimulation of adventitious shoot formation in vivo does not produce results of sufficient practical value. It was then decided to start investigations on techniques in vitro, in cooperation with the Institute of Atomic Sciences in Agriculture (ITAL) in Wageningen. The first results (not discussed in this publication) are very encouraging and meet the requirements of mutation breeding.

Chapter 5 The literature on shoot apices is reviewed, with emphasis on post-irradiation behaviour. Different hypotheses on the behaviour of normal apices are mentioned like the Histogen theory, the Corpus/Tunica theory and the anneau initial/méristème d'attente theory. Formation of chimeras, systems of classifying different types of periclinal chimeras and the increased incidence of various histogenic effects after irradiation are discussed. Various patterns of radiodamage and recovery in shoot apices are reported in literature and some examples are reviewed in this context.

Chapter 6 Damage and recovery of irradiated potato tuber eyes was investigated in three experiments. Potato tuber eyes were irradiated with doses, ranging from 500-4000 rad of X-rays. In Exps 1 and 2 the conclusions were mainly based on studies of whole plants and on the frequency of changes in tuber-skin colour, supplemented with results from some microscopic observations of fixations made during the first days after irradiation. In Exp. 3 emphasis was laid on studies of microscopic sections made during a 20-day period after irradiation. Frequencies of plasmolytic, collapsed, empty and necrotic cells were compared between and within histogenic layers. Stainability of cytoplasm and nuclei was estimated in the same way. The frequency of periclinal divisions was determined. Formation of axillary buds and of lateral roots at the base of central apices was suppressed after irradiation with 2400 rad. L-I was the most stable layer in the control as well as in all treatments. After irradiation with 1200 rad, mitotic activity increased after 14 days, whereas in the 2400 rad treatment mitotic activity remained very low throughout the experiment. Within irradiated apices, new 'meristem balls' formed in late fixations of the 1200 rad treatment. The role of such meristematic centres in regeneration processes was discussed. In the three experiments changes in tuber-skin colour, caused by histogenic effects were very similar. With increasing doses, the percentage of tubers of the (periclinally chimeric) starting type with splashed tuber skin decreased, whereas the frequencies of solid red or yellow tubers, derived from different histogenic layers, increased. The results are discussed in relation to present knowledge.

Samenvatting

Het gebruik van mutaties bij het kweken van plantenrassen heeft tot op heden geleid tot een aantal duidelijke successen. In de aardappelveredeling kent men al sinds meer dan een eeuw rassen die spontaan zijn ontstaan als zogenaamde knopmutanten of 'bud sports'. Enkele van dergelijke rassen, zoals Russet Burbank, Red Pontiac en Red laSoda in de V.S. en Red Craigs Royal in Engeland, nemen een niet onaanzienlijk deel van het totaal aardappelareaal in. Kunstmatige opwekking van mutaties heeft daarentegen bij de aardappel, voor zover bekend, tot op heden slechts één nieuw aardappelras opgeleverd: het ras Konkei in Japan. In de literatuur treft men diverse verklaringen aan voor het uitblijven van succes, zoals het optreden van chimerie, diplontische selectie en ongewenste erfelijke koppelingen, en van te lage mutatiefrequenties en ongunstige mutatiespectra. Deze publikatie is het resultaat van onderzoek naar enige aspecten van de mutatieveredeling bij aardappel welke van belang worden geacht voor het verkrijgen van meer inzicht en voor het vergroten van de kans op praktisch waardevolle resultaten.

Uit het literatuuroverzicht over aardappelmutaties (hoofdstuk 2) blijkt dat chimerie een belangrijk obstakel vormt bij de bruikbaarheid van (kunstmatige) mutaties voor vegetatief vermeerderde gewassen, waarvan de aardappel er één is. Een chimere plant ontstaat na mutatie – als regel een gebeurtenis die plaats vindt in één cel – en vegetatieve vermeerdering als gevolg van de veelcellige en laagsgewijze bouw van de plant. Het is tegenwoordig mogelijk een methode voor behandeling van het materiaal te kiezen waarbij vrijwel geen chimerie optreedt. Deze methode is echter tijdrovend en arbeidsintensief, zodat voor praktische toepassing naar een meer efficiënte werkwijze moet worden omgezien. Met name wordt hier gedoeld op het stimuleren van adventief-spruitvorming aan bladstelen, welke methode bij veel gewassen met succes wordt toegepast, maar bij aardappel nog niet systematisch is onderzocht.

Sinds 1923 zijn pogingen ondernomen om aardappelmutaties kunstmatig op te wekken. Oorspronkelijk werden hierbij hele of halve knollen als uitgangsmateriaal genomen. Geleidelijk aan groeide het inzicht dat het gebruik van structuren met kleinere groeipunten zoals jonge knolletjes, knolstukjes met slapende ogen en stekken o.a. gunstig is voor vermindering van de chimerie en vergroting van de uniformiteit van de mutagene behandeling. Op het Instituut voor Plantenveredeling te Wageningen wordt sinds 1965 meestal gewerkt met aardappelknolstukjes met slechts één oog.

De mutagene behandeling bestaat in de praktijk veelal uit een bestraling; daarnaast worden ook chemische mutagentia aangewend. Bestraling van vegetatieve plantedelen wordt vooral uitgevoerd met X- en γ-stralen, meestal met een dosis van 2 – 3 krad. Soms wordt ook gebruik gemaakt van neutronen en heel incidenteel van protonen, radio-isotopen en U.V.-straling. Chemische mutagentia, waarvan ethylmethaansulfonaat (EMS) de belangrijkste is, worden vrij veel toegepast in Oosteuropesche landen. Duidelijke voordelen ten opzichte van bestraling zijn echter niet aangetoond.

Voor de veredeling is het merendeel van de verkregen mutaties onbruikbaar. Waar-

genomen veranderingen zijn vooral morfologisch of fysiologisch van aard en betreffen verder eigenschappen als vroegheid, diverse resistenties en zetmeelgehalte. Uit de literatuur blijkt dat het mogelijk is bij aardappel hoge mutatiefrequenties te verkrijgen, terwijl ook het spectrum voldoende geschakeerd is. Hoofdstuk 2 wordt afgesloten met de constatering dat bij de aardappel mutatieveredeling moet worden beschouwd als een aanvullende veredelingsmethode, welke, mits juist toegepast, in bepaalde gevallen succes kan opleveren, met name in latere stadia van veredelingsprogramma's.

De overerving van eigenschappen wordt bij de aardappel bepaald door het tetraploïde karakter van het gewas (2n=4x=48) hetgeen de genetische analyse van kruisingsresultaten bemoeilijkt. Er bestaat een sterk groeiende belangstelling voor onderzoek over en met gereduceerde genoomaantallen (2n=2x=24 of zelfs 2n=x=12). Een dihaploïde aardappelkloon (2n=2x=24) zou ideaal basismateriaal vormen voor verder aardappelmutatieonderzoek. Aangezien de meeste mutaties van dominant naar recessief verlopen, kan men, uitgaande van een heterozygote di(ha)ploïde plant, reeds in de mutageen behandelde generatie het effect van de behandeling bepalen.

Bij het onderzoek, beschreven in hoofdstuk 3, werd uitgegaan van 5 verschillende bronnen van diploïd of dihaploïd materiaal, die onder meer genen bevatten welke de synthese van pigment, de distributie van pigment over de plant, resistentie tegen bepaalde fysio's van *Phytopthora infestans* en het optreden van gele bladranden regelen. Onderlinge kruisingen werden uitgevoerd teneinde zoveel mogelijk testkenmerken in een kloon te combineren. De uit deze kruisingen verkregen planten werden vervolgens getoetst op aanwezigheid en expressie van de testkenmerken, algehele groei, stralingsgevoeligheid en mutabiliteit.

De ontwikkelde klonen leverden niet de gewenste resultaten op vanwege een veelheid van oorzaken. Besloten moest worden dit onderdeel van het programma te beëindigen en voor uitvoering van de verdere onderzoeksplannen gebruik te maken van ander (tetraploïd) aardappelmateriaal. Vanzelfsprekend leidde dit ook tot bijstelling van het verdere onderzoek.

Bij het onderzoek naar de vorming van adventiefspruiten aan bladeren en stengels van aardappel (hoofdstuk 4) stond centraal het ontstaan van dergelijke spruiten aan de basis van bladstelen in vivo. De adventiefspruitmethode heeft bij een aantal andere gewassen dan aardappel geleid tot vorming van spruiten waarvan de apex uiteindelijk te herleiden is tot slechts één oorsprongscel. Indien men deze methode beschikbaar heeft in de mutatieveredeling, is het chimerievraagstuk definitief opgelost. Een beschrijving wordt gegeven van de factoren welke de vorming en differentiatie van adventieve organen beïnvloeden. Reeds bekende gegevens over adventieve orgaanvorming bij aardappel in vivo en in vitro worden samengevat.

Voor het experimentele gedeelte werd naast het bekende ras Bintje voornamelijk gebruik gemaakt van periclinaal chimere aardappelklonen, aangeduid als B 165 en M 52, welke respectievelijk afkomstig zijn van de rassen Burmania en Désirée. Knollen van deze klonen hebben genetisch verschillende groeipuntlagen voor knolschilkleur (constitutie L-I geel, L-II + L-III rood) en hebben een geel/rood gevlekt uiterlijk. Omdat de meeste adventiefspruiten tot één cel uit de L-I te herleiden zijn, kan men via dergelijke periclinaal chimeren eenvoudig vaststellen uit welke laag (of lagen) nieuwe spruiten afkomstig zijn. In het onderhavige geval verraadt de schilkleur van de knollen welke onstaan aan gestek-

te adventiefspruiten, uit welke laag van de oorspronkelijke plant de adventiefspruiten zijn geweest.

Omdat aanvankelijk werd aangenomen dat beworteling voorwaarde is voor adventiefspruitvorming, werd aandacht besteed aan factoren welke bladsteelbeworteling en vitaliteit van het blad beïnvloeden. Een arbeidsbesparend systeem werd ontwikkeld om series blaadjes onder identieke omstandigheden te laten opgroeien in kweekbakken met vloeibare media.

Ras Bintje toonde zowel de beste beworteling als de hoogste vitaliteit. De fysiologische leeftijd van bladeren en blaadjes had geen duidelijk effect op beworteling. Jongere blaadjes bleven in het algemeen wel langer vitaal. De omstandigheden waaronder het oudermateriaal werd opgekweekt speelden doorgaans geen grote rol. Enkelvoudige blaadjes gaven een aanzienlijk betere beworteling dan samengestelde bladeren.

De samenstelling van het medium had grote invloed op de beworteling. Auxinen bevorderden de vorming van wortels aan blaadjes, kinetine en benzyladenine hadden hierop een remmend effect, terwijl gibberelline (GA_3) de wortelvorming vrijwel onderdrukte. De werking van GA_3 kon goeddeels worden opgeheven door auxinen. 2,4-D, abscissinezuur en citroenzuur leverden geen significante effecten op. Daglengte en temperatuur hadden in het algemeen slechts een geringe invloed op de bewortelingsgraad. Het blootstellen van aardappelblad aan een donkere periode van bijvoorbeeld een week werkte duidelijk remmend op beworteling.

Uit experimenten met meer dan 100.000 bladeren en blaadjes werden in totaal slechts 120 adventiefspruitjes verkregen. In een aantal gevallen was bovendien de ontstaanswijze van deze spruiten aan twijfel onderhevig. De ontstane knollen hadden in vrijwel alle gevallen een geel/rood gevlekte knolschil en waren soms zuiver rood, hetgeen aantoont dat deze knollen niet tot L-I te herleiden waren. Doordat geen reproduceerbare resultaten konden worden verkregen en niet duidelijk was in welke richting het onderzoek zou kunnen worden voortgezet, werden de proeven inzake adventiefspruitvorming aan bladstelen in vivo in 1974 beëindigd.

In beperkte proeven met stengels en stengeldelen bleek dat op oogloze stukjes van stengelleden geregeld callus-vormig en adventiefspruitvorming optrad. Aangezien de ontstane spruiten niet tot slechts één cel te herleiden zijn, heeft deze methode geen praktische betekenis voor de mutatieveredeling. Om dezelfde reden moest negatief geoordeeld worden over spruiten, verkregen via de zogenaamde Jørgensen-methode. Bij het onderzoek werd weer gebruik gemaakt van het eerder beschreven periclinaal chimere uitgangsmateriaal.

In samenwerking met onderzoekers van het Instituut voor Toepassing van Atoomenergie in de Landbouw te Wageningen werd omstreeks 1975 een begin gemaakt met een studie van de mogelijkheden van het toepassen van in vitro technieken in de aardappelmutatieveredeling. De eerste resultaten van deze aanpak zijn veelbelovend.

In hoofdstuk 5 worden allereerst verschillende systemen ter verklaring van de bouw en het functioneren van groeipunten zoals de Histogeentheorie, het Tunica/Corpus concept en het 'Anneau initial / Méristème d'attente'-concept beschreven. Meningsverschillen bestaan over de rol en de eventuele situering van zogenaamde initiaalcellen. Momenteel heerst overwegend de opvatting dat een apicaal meristeem bestaat uit een dynamische celpopulatie, waarin een zekere gelaagdheid te herkennen valt. De wijze van ontstaan van

periclinale chimeren bij vegetatief vermeerderde gewassen na het optreden van een mutatie wordt besproken. Aandacht wordt besteed aan de klassificatie van periclinale chimeren en aan het optreden van histogene effecten. Bestraling leidt tot het optreden van meer histogene effecten. De bestralingsdosis en de doseringssnelheid hebben mogelijk invloed op de aard van de histogene effecten.

Morfologische stralingsschade in groeipunten wordt vaak beschreven als 'gelokaliseerd', 'verspreid' of 'uniform'. Soms meent men het bestaan van een 'outer to inner pattern' van afnemende stralingsgevoeligheid te kunnen aantonen. Een aantal stralingsschadebeelden uit de literatuur wordt geanalyseerd. Verschillen in fysiologische of mitotische activiteit tussen cellen of zones bieden waarschijnlijk de beste verklaring voor verschillen in stralingsgevoeligheid.

Hoofdstuk 6 begint met een beschrijving van de ogen op aardappelknollen. In een aardappeloog zijn meestal een centrale knop en twee laterale knoppen te onderscheiden. Tussen de bladprimordia welke iedere knop omringen bevinden zich okselknoppen. De apicale zone van elke knop bestaat uit drie redelijk stabiele histogene lagen. Het aantal initiaalcellen is tot nu toe niet met zekerheid vastgesteld.

Bij bestraling van knoloogstukjes wordt meer dan één groeipunt tegelijk bestraald. De reactie van groeipunten op bestraling wordt mede bepaald door de apicale dominantie van het aardappelgroeipunt. Er is slechts zeer weinig informatie over stralingsschade in aardappelgroeipunten en over het optreden van herstel.

Het onderzoek werd uitgevoerd met de periclinaal chimere kloon M 52 van het ras Désirée. In twee inleidende proeven en een daaropvolgende grote proef – hierna aan te duiden als exp. 1, 2 en 3 – werden in totaal 1020 knoloogstukjes bestraald met X-stralen variërend van 500 – 4000 rad. Onderzoek aan planten en knollen betrof vooral de groeiwijze van uit bestraalde knolstukjes ontstane planten en de knolschilkleur. Microscopische waarnemingen (aan coupes van 8 – 10 μm dik) hadden o.m. betrekking op kleuring van celkernen, cytoplasma en celwanden; celgrootte; delingen en deelwanden; plasmolyse; lege, samengeklapte en necrotische cellen; zelfstandigheid van cellagen en het optreden van nieuwe meristeem'nesten'. Getracht werd om uit deze gegevens een beeld te krijgen van de primaire (fysiologische) stralingsschade; waaronder we globaal verstaan het totaal aan niet-genetisch bepaalde effecten van de bestralingsbehandeling. Verder werd nagegaan in hoeverre bestraalde okselknoppen eenzelfde reactiepatroon vertonen als centrale knoppen. Gepoogd werd om verband te leggen tussen de resultaten van het microscopisch en het morfologisch onderzoek.

In exp. 1 lag het accent op het morfologisch onderzoek. Spruiten en knoloogstukjes werden 6 dagen voor bestraling afgesneden teneinde uniform uitgangsmateriaal te krijgen. Ondanks deze voorzorg bleken de ogen later toch aanzienlijk te verschillen in ontwikkeling. Fixaties werden gemaakt gedurende 2 dagen. Microscopische stralingsschade werd eerst na 12 uur waargenomen in de behandelingen met 750 en 1500 rad en al na 3 uur in de behandeling met 3000 rad. Bestraling had minder mitotische activiteit tot gevolg. Enkele periclinale delingen werden waargenomen in de L-I. De L-II was meermalen moeilijk als doorlopende laag te onderscheiden.

Na bestraling ontstond vooral in de behandeling met 750 rad een gedrongen, rozetachtig planttype, aangeduid als B-10. Bij een stijgende dosis X-stralen nam het percentage knollen van het uitgangstype (geel/rood gevlekt) af tot 48% in de behandeling met 3000 rad, terwijl het aantal volledig rode knollen steeg tot 42%. Het percentage geheel gele

knollen steeg tot maximaal 8%. Rode knollen ontstaan indien uitsluitend L-II- en L-III-weefsel bijdragen tot de knolvorming, terwijl gele knollen te herleiden zijn tot uitsluitend L-I-weefsel.

In exp. 2 werd de periode van microscopische waarnemingen verlengd tot 5 dagen. Niet-geschraapte ogen werden bestraald met doses X-stralen van 500, 1000, 2000 en 4000 rad. In behandeling met 500 rad werd de maximale stralingsschade bereikt na 48 uur. Na 5 dagen was bij de twee hoogste doses (2000 en 4000 rad) de maximale schade nog niet bereikt. Het centrale groeipunt toonde steeds grotere stralingsschade dan de laterale groeipunten van eenzelfde oog. Stralingsschade trad meestal verspreid op in de apex. De L-I leek meer stralingsgevoelig dan de meer naar binnen gelegen lagen. Bij hogere stralingsdoses steeg – na een aanvankelijke onderdrukking – het aantal delingen weer aan het einde van de waarnemingsperiode. Enige periclinale delingen werden weer waargenomen in de L-I na bestraling.

Het ook in exp. 2 aangetroffen planttype B-10 lijkt te kunnen worden verklaard uit inactivatie van het centrale groeipunt t.g.v. de bestralingsbehandeling en het daaropaansluitende uitlopen van meerdere okselscheuten. De resultaten van tellingen betreffende veranderde knolschilkleurtypes waren in lijn met de waarnemingen in exp. 1.

In exp. 3 werden bestralingen met doses X-stralen van 1200 en 2400 rad uitgevoerd. De doses werden vastgesteld aan de hand van eerdere proeven waaruit was gebleken dat bij 1200 rad lichte schadebeelden optreden, echter zonder verstoring van de apicale organisatie, terwijl de empirisch vastgestelde, optimale dosis voor mutatie-inductie bij aardappel ligt tussen 2000 en 3000 rad. Fixaties werden gemaakt na 2, 4, 6, 8, 11, 14, 17 en 20 dagen; gedetailleerde microscopische waarnemingen werden verricht aan 2 ogen per fixatietijdstip voor elke bestralingsdosis (0, 1200 en 2400 rad).

Centrale knoppen bevatten gemiddeld 14 – 18 L-I cellen, laterale knoppen 12 – 16. Tijdens de observatie periode namen de cellen in de betreffende groeipuntlagen niet significant toe in aantal maar wel in omvang. Na bestraling vertoonden de meeste groeipunten een wat afgeplatte bovenzijde.

Bestraling leidde tot vertraging in, of tijdelijke onderdrukking van de vorming van okselknoppen in centrale groeipunten. Het effect was duidelijk sterker na de behandeling met 2400 dan die met 1200 rad. In laterale knoppen werden na bestraling geen okselknoppen aangetroffen. Vorming van laterale wortels aan de basis van de spruiten afkomstig van de centrale knoppen werd voor alle fixatietijdstippen niet waargenomen in de behandeling met 2400 rad. Een dosis van 1200 rad leek daarentegen stimulerend te werken in dit opzicht.

L-I bleek na bestraling duidelijk de meest stabiele laag te zijn en L-III de minst stabiele. Bestraling met 1200 rad leidde aanvankelijk tot verhoging van het aantal laagonregelmatigheden, maar na twee weken trad herstel op. In de behandeling met 2400 rad werden laagverstoringen eerst na een week aangetroffen. Nadien werd gedurende de gehele waarnemingsperiode een constante stijging waargenomen. Duidelijke laagduplicaties kwamen slechts zelden voor.

Het aantal mitoses steeg in de behandeling met 1200 rad na 14 dagen sterk, terwijl dit aantal in de behandeling met 2400 rad laag bleef in de gehele periode van fixatie. Verschillen in aantallen delingen tussen flanken en centraal apicaal deel werden vrijwel niet waargenomen. In laterale knoppen werd minder mitotische activiteit aangetroffen dan in centrale knoppen. De resultaten wijzen op een mitotische

cyclus in onbestraalde aardappelgroeipunten met een duur van niet langer dan 6 dagen.

Plasmolyseverschillen werden alleen gevonden na bestraling. In de behandeling met 1200 rad werd een maximum bereikt tussen 8 en 14 dagen na bestraling, terwijl bij 2400 rad het niveau voortdurend steeg gedurende de gehele waarnemingsperiode. Het aantal samengeklapte cellen bereikte bij 1200 rad een maximum na 8 dagen, bij 2400 rad na 14 dagen. Meestal werden meer samengeklapte cellen waargenomen langs de flanken dan in het centrum. Laterale knoppen bevatten aanzienlijk minder samengeklapte cellen dan centrale knoppen.

De frequentie van idioblasten en van lege en necrotische cellen bleef na bestraling opvallend laag. Het aantal lege cellen steeg echter sterk in de laatste fixaties van de behandeling met 2400 rad. Bestraling leidde tot aanzienlijk minder kleurbaarheid van het cytoplasma, waarbij tevens een duidelijk dosiseffect optrad. De resultaten inzake kernkleuring vertoonden vrij veel variatie. In bestraalde groeipunten werden slechts zelden periclinale delingen aangetroffen in L-I. Het gemiddelde aantal periclinale delingen voor L-I + L-II was echter significant hoger dan in de controle.

De microscopische resultaten doen vermoeden dat na bestraling met 1200 rad regeneratie zowel via nog functionerende okselknoppen als via nieuwgevormde meristeemnesten binnen bestaande groeipunten verloopt. Bij hogere doses zou regeneratie aanvankelijk vooral voorkomen van okselknoppen gelegen op enige afstand van het eindgroeipunt en mogelijk later ook van adventiefknoppen.

In tegenstelling tot de in exp. 1 verkregen indruk, werden ook planten van het type B-10 aangetroffen na hogere stralingsdoses, zij het dat dan ook de laagste internodiën samengedrukt waren. Tellingen van de knollen behorende tot de verschillende categorieën knolschilkeur bevestigden de uitkomsten van exp. 1 en exp. 2.

Hoofdstuk 6 wordt afgesloten met een algemene discussie over de resultaten van de drie bestralingsexperimenten. Het blijkt dat niet alle aanvankelijke waarnemingen door de resultaten van de derde proef worden bevestigd. De belangrijkste conclusies lijken dat L-I ook na bestraling de meest stabiele laag blijft en dat bij regeneratie nieuwe pseudo-adventieve meristeemnesten een rol kunnen spelen. Verschillen in stralingsgevoeligheid tussen lagen en zones binnen een groeipunt blijken moeilijk aan te tonen.

De opvallend uniforme resultaten inzake verschillen in knolschilkleurfrequenties mogen niet rechtstreeks worden gerelateerd aan de resultaten van het microscopisch groeipuntonderzoek. De afname van het aandeel van de bonte knollen bij stijgende doses X-stralen kan worden verklaard door toenemende inactivatie van okselknoppen en een stijgende bijdrage van de adventiefknoppen welke als reactie op genoemde inactivatie van de bestaande knoppen worden gevormd. Deze adventiefknoppen zijn op statistische gronden grotendeels afkomstig uit het binnenste weefsel.

Het aandeel van gele knollen geeft geen directe informatie omtrent de hoeveelheid 'layer replacement' (cellen van de buitenste laag of lagen verdringen meer naar binnen gelegen cellen) na bestraling. Knollen met de schilkleur L-I + L-II-geel, L-III-rood kunnen bijvoorbeeld niet worden onderscheiden van bonte knollen, zodat het aantal gevallen van 'replacement' te laag wordt geschat. Bij het tegenovergestelde proces: 'displacement' (verdringing naar buiten toe) zijn de in sommige publikaties gegeven frequenties naar alle waarschijnlijkheid te hoog. Immers, verschijnselen met een geheel andere achtergrond, zoals adventiefspruitvorming uit endogeen ontstaan callusweefsel, leiden tot uitwendig waarneembare veranderingen welke niet van 'displacement' te onderscheiden zijn.

De aanvankelijke verklaring voor de ontstaanswijze van zgn. B-10-planten, namelijk via vorming van nieuwe, pseudo-adventieve meristematische centra binnen bestaande groeipunten, blijkt verrassend genoeg niet te worden bevestigd door de uitkomsten van knolschilkleurbepalingen. In tegenstelling tot het verwachte overheersend voorkomen van knollen met rode schil (nl. uit endogeen weefsel) bleken de meeste knollen van gestekte scheuten van B-10-planten overeen te komen met die van het uitgangstype waarvan de schil gevlekt is. Een afdoende verklaring hiervoor kon tot op heden niet worden gegeven.

References

Anonymous, 1857. Gardeners Chronicle, p. 613, 629.

Anonymous, 1963. (Report of the Faculty). Annls Fac. agr. Pisa (Italy) 23: 124.

Anonymous, 1970. Manual on mutation breeding, IAEA, Vienna, 237 p.

Anonymous, 1973. Isotopes and radiation in agricultural research in the Soviet Union. Rep. Study Tours, IAEA, Vienna (p. 22), 108 p.

Appelgren, M. & O.M. Heide, 1972. Regeneration in *Streptocarpus* leaf discs and its regulation by temperature and growth substances. Physiologia Pl. 27: 417–424.

Artschwager, E.F., 1918. Anatomy of the potato plant, with special reference to the ontogeny of the vascular system. J. agric. Res. 14: 221–252.

Artschwager, E.F., 1924. Studies on the potato tuber. J. agric. Res. 27: 809–836.

Asseyeva, T., 1927. Bud mutations in the potato and their chimerical nature. J. Genet. 19: 1–26.

Asseyeva, T , 1930. (Vegetative mutations in the potato). Proc. USSR Congr. Gen., Pl. and Animal Breed. (1929), p. 141–154.

Asseyeva, T., 1931. (Bud mutations in the potato). Bull. Appl. Bot., Genetics, and Plant Breed. (Leningrad) 27: 135–218.

Asseyeva T. & M. Blagovidova, 1935. (Artificial mutations in the potato). Bull. Appl. Bot., Genetics, and Plant Breed. (Leningrad) Ser. A (15): 81–85.

Asseyeva, T.V. & I.M. Yashina, 1968. (Somatic mutations in potato). Genetics (USSR) 3: 145–164.

Bajaj, Y.P.S., 1971. Direct and indirect effects of gamma irradiation on plant tissue cultures. Int. symp. on the use of isotopes and rad. in agric. and animal husb. res. New Delhi, 285–301.

Bajaj, Y.P.S. & L.A. Dionne, 1968. The continuous culture of excised potato roots. N. Z. J. Bot. 6: 386–394.

Baker, R.E., 1943. Induced polyploid, periclinal chimeras in *Solanum tuberosum*. Am. J. Bot. 30: 187–195.

Balkema, G.H., 1971. Chimerism and diplontic selection. Thesis, Landbouwhogeschool, Wageningen. A.A. Balkema. Rotterdam, Kaapstad, 173 p.

Ball, E., 1972. Differential tagging of the shoot apex. (Abst.) Meeting Bot. Soc. of Amer., Am. J. Bot, 59: 647.

Bateson, W., 1916. Root-cuttings, chimaeras and 'sports'. J. Genet. 6: 75–80.

Bateson, W., 1921. Root-cuttings and chimaeras. II. J. Genet. 11: 91–97.

Baur, E., 1909. Das Wesen und die Erblichkeitsverhältnisse der 'Varietates Albomarginatae hort' von *Pelargonium zonale*. Z. indukt. Abstamm. u. Vererblehre 1:330–351.

Baur, E., 1910. Propfbastarde. Biol. Zbl. 30: 497–514.

Behnke, M., 1975. Regeneration in Gewebekulturen einiger dihaploider *Solanum tuberosum*-Klone. Z. PflZücht. 75: 262–265.

Behnke, M., 1976. Kulturen isolierter Zellen von einigen dihaploiden *Solanum tuberosum*-Klonen und ihre Regeneration. Z. Pflanzenphysiol. 78: 177–181.

Bennici, A., 1974. Cytological analysis of roots, shoots and plants regenerated from suspension and solid *in-vitro* cultures of haploid *Pelargonium*. Z. PflZücht. 72: 199–205.

Benvenuti, A., M. Buiatii, F. D'Amato & R. Ragazzini, 1963. Mutazioni somatiche nella patata indotte dalla radiazione gamma. Agricoltura Italiana 4: 449–456.

Bergann, F., 1954. Praktische Konsequenzen der Chimärenforschung für die Pflanzenzüchtung. Wiss. Z. Univ. Leipzig, Math.–nat. Reihe 4: 281–291.

Bergann, F., 1955. Einige Konsequenzen der Chimärenforschung für die Pflanzenzüchtung. Z. Pfl-Zücht. 34: 113–124.

Bergann, F., 1957a. Gelungene experimentelle Entmischungen und Umlagerungen bei bekannten oder vermuteten Periklinalchimären. Ber. dt. bot. Ges. 70: 335–360.
Bergann, F., 1957b. Die züchterische Auswertung der intraindividuellen (somatischen) Variabilität von Kulturpflanzen durch bewußte Auslösung von Regenerationsvorgängen. Wiss. Z. Päd. Hochsch. Potsdam. Math. – Naturw. Reihe 3: 105–109.
Bergann, F., 1965. Wächst *Epilobium* mit Scheitelzellen? Ber. dt. bot. Ges. 78: 405–410.
Bergann, F., 1967. The relative instability of chimerical clones – the basis of further breeding. In: H. Stubbe (ed.), Induced mutations and their utilization. Erwin-Baur-Gedächtnisvorlesungen IV (1966), 287–300.
Bergann, F., 1968. Mutations-chimären: Rohmaterial züchterischer Weiterbehandlung. Umschau 24: 791–797.
Bergann, F. & L. Bergann, 1959. Über experimentell ausgelöste vegetative Spaltungen und Umlagerungen an chimärischen Klonen, zugleich als Beispiele erfolgreicher Staudenauslese I. *Pelargonium zonale* Ait. 'Madame Salleron'. Züchter 29: 361–374.
Bergann, F. & L. Bergann, 1962. Über Umschichtungen (Translokationen) an den Sproßscheiteln periklinaler Chimären. Züchter 32: 110–119.
Bergonie, J. & L. Tribondeau, 1906. Interprétation de quelques résultats de la radiothérapie et essai de fixation d'une technique rationelle. C. r. Acad. Sci., Paris 143: 983–985.
Bersillon, G., 1951. Sur le point végétatif de *Papaver somniferum* L.: structure et fonctionnement. C. r. Acad. Sci., Paris 232: 2470–2472.
Bishop, C.J., 1950. Differential X-ray sensitivity of *Tradescantia* chromosomes during mitotic cycle. Genetics 35: 175–187.
Blixt,S., 1970. Studies of induced mutations in peas. XXV. Genetically conditioned differences in radiation sensitivity. 4. Agri. Hort. Gen. 28: 55–116.
Blixt, S., 1972. Mutation genetics in *Pisum*. Agri. Hort. Gen. 30: 1–293.
Brabec, F., 1960. Über eine Mesochimäre aus *Solanum nigrum* L. und *Lycopersicon pimpinellifolium* Mill. Planta 55: 687–707.
Brabec, F., 1965. Propfung und Chimären, unter besondere Berücksichtigung der entwicklungsphysiologischen Problematik. Hdb. PflPhysiol. 15, 2: 388–498. Springer Verlag, Berlin.
Brand, R. & C.J. Venverloo, 1973. The formation of adventitious organs. II. The origin of buds formed on young adventitious roots of *Populus nigra* L. 'Italica'. Acta bot. neerl. 22: 399–406.
Breukelen, E.W.M. van, M.S. Ramanna & J.G.Th. Hermsen, 1975. Monohaploids (n= x = 12) from autotetraploid *Solanum tuberosum* (2n = 4x = 48) through two successive cycles of female parthenogenesis. Euphytica 24: 567–574.
Broertjes, C., 1969. Mutation breeding of *Streptocarpus*. Euphytica 18: 333–339.
Broertjes, C., 1972a. Improvement of vegetatively propagated plants by ionizing radiation. In: Induced mutations and plant improvement. IAEA, Vienna. p. 293–295.
Broertjes, C., 1972b. Mutation breeding of *Achimenes*. Euphytica 21: 48–63.
Broertjes, C., B. Haccius & S. Weidlich, 1968. Adventitious bud formation on isolated leaves and its significance for mutation breeding. Euphytica 17: 321–344.
Broertjes, C. & L. Leffring, 1972. Mutation breeding of *Kalanchoë*. Euphytica 21: 415–423.
Broertjes, C., S. Roest & C.S. Bokelmann, 1976. Mutation breeding of *Chrysanthemum morifolium* Ram. using in vivo and in vitro adventitious bud techniques. Euphytica 25: 11–19.
Brown, J.A.M., J.P. Miksche & H.H. Smith, 1964. An analysis of H^3-thymidine distribution throughout the vegetative meristem of *Arabidopsis thaliana* (L.) Heynh. Radiation Botany 4: 107–113.
Buder, J., 1928. Der Bau des phanerogamen Sproßvegetationspunktes und seine Bedeutung für die Chimärentheorie. Ber. dt. bot. Ges. 46: 20–21.
Buvat, R., 1952. Structure, évolution et fonctionnement du méristème apical de quelques dicotylédones. Annls Sci. nat. (Bot) 11^e sér. 13: 202–303.
Buvat. R., 1955. Le méristème apical de la tige. Année biol. 3, 31: 596–656.
Carrière, E.A., 1865. Production et fixation des variétés dans les végétaux. Paris, 72 p.
Catesson, A.M., 1953. Structure, évolution et fonctionnement du point végétatif d'une monocotylédone: *Luzula pedemontana*. Boiss. et Rent. (Joncacées). Annls Sci. nat. (Bot) 11^e sér. 14: 253–291.

Champagnat, M., 1961. Recherches de morphologie descriptive et experimentale sur le genre *Linaria*. Annls Sci. nat. (Bot) 12e sér. 2: 1–170.
Chauhan, Y.S. & R.P. Singh, 1975. Morphological studies in safflower (*Carthamus tinctorius* Linn.) with special reference to the effect of 2,4-D and gamma rays. I. Vegetative shoot apex. Radiation Botany 15: 68–77.
Clark, G.L., 1955. Applied X-rays. McGraw-Hill, New York, 4th ed. 843 p.
Clowes, F.A.L., 1957. Chimeras and meristems. Heredity, Lond. 11: 141–148.
Clowes, F.A.L., 1961. *Apical meristems*. Blackwell, Oxford, 217 p.
Clowes, F.A.L. & E.J. Hall, 1966. Meristems under continuous irradiation. Ann. Bot., N.S. 30: 243–251.
Cooper, L.S., D.C. Cooper, A.C. Hildebrandt & A.J. Riker, 1964. Chromosome numbers in single cell clones of tobacco tissue. Am. J. Bot. 51: 284–290.
Cramer, P.J.S., 1907. Kritische Übersicht der bekannten Fälle von Knospenvariation. Natuurk. verh. v.d. Holl. My d. Wetensch., Haarlem 3. VI, 474 p.
Crockett, L.J., 1957. A study of the tunica, corpus and anneau initial of irradiated and normal stem apices of *Nicotiana tabacum* L. Bull. Torrey bot. Club 84: 229–236.
Crockett, L.J., 1968. The effects of chronic gamma radiation on the internal apical configurations of the vegetative shoot apex of *Coleus blumei*. Am. J. Bot. 55: 265–268.
Cross, G.L. & T.J. Johnson, 1941. Structural features of the shoot apices of diploid and colchicine-induced, tetraploid strains of *Vinca rosea*. Bull. Torrey bot. Club 68: 618–635.
Cuany, R.L., A.H. Sparrow & A.H. Jahn, 1958a. Spontaneous and radiation induced somatic mutation rates in *Antirrhinum, Petunia, Tradescantia* and *Lilium*. Proc. 10th Int. Congr. Gen. (Montreal) 2: 62–63.
Cuany, R.L., A.H. Sparrow & V. Pond, 1958b. Genetic response of *Antirrhinum majus* to acute and chronic plant irradiation. Z. indukt. Abstamm.-u. Vererblehre 89: 7–13.
Cutter, E.G., 1965. Recent experimental studies of the shoot apex and shoot morphogenesis. Bot. Rev. 31: 7–113.
D'Amato, F., 1975. The problem of genetic stability in plant tissue and cell cultures. In: O.H. Frankel & J.G. Hawkes (eds), Crop genetic resources for today and tomorrow. Cambridge University Press: 333–348.
Darwin, Ch., 1868. The variation of animals and plants under domestication. 10th impr. of the 2nd ed. Vol I (1921). Murray, London; 473 p.
De Jong, H., 1971. Inbreeding in cultivated diploid potatoes. Ph. D. thesis, University of Wisconsin, Madison, 114 p.
De Jong, H. & P.R. Rowe, 1972. Genetic markers in inbred clones of cultivated diploid potatoes. Potato Res. 15: 200–208.
De Loose, R., 1970. Het bekomen van 'sporten' voor de bloemkleur bij de Belgische hybrieden van *Rhododendron simsii* Planch. (= *Azalea indica*) door middel van ^{60}Co-gammastralen. Meded. v.d. Fac. Landbouww. Gent 35: 1047–1074.
Demidovic, A., 1934. (A new possibility in breeding). Semenovodstvo (Seed Growing) 4: 8–10.
Dermen, H., 1945. The mechanism of colchicine-induced cytohistological changes in cranberry. Am. J. Bot. 32: 387–394.
Dermen, H., 1947a. Periclinal cytochimeras and histogenesis in cranberry. Am. J. Bot. 34: 32–43.
Dermen, H., 1947b. Histogenesis of some bud sports and variegations. Proc. Amer. Soc. Hort. Sci. 50: 51–73.
Dermen, H., 1951. Ontogeny of tissues in stem and leaf of cytochimeral apples. Am. J. Bot. 38: 753–760.
Dermen, H., 1960. Nature of plant sports. Amer. Hort. Mag. 39: 123–173.
Dermen, H., 1967. Colchiploidy and cytochimeras in the study of ontogenic problems. Proc. XVIIth Int. Hort. Congr., Vol. II: 3–14.
Dertinger, H. & H. Jung, 1970. Molecular radiation biology. Springer Verlag, New York, 236 p.
Devreux, M., 1973. In-vitro culture and mutation breeding. In: Induced mutations in vegetatively propagated plants. IAEA, Vienna, 41–52.
Dobzhansky, T., 1970. Genetics of the evolutionary process. Columbia University Press, New York, 505 p.

Dodds, K.S., 1962. Classification of cultivated potatoes. In: Correll, D.S.: The potato and its wild relatives. Texas Research Found., p. 517–539.
Dodds, K.S. & D.H. Long, 1955. The inheritance of colour in diploid potatoes-I. Types of anthocyanidins and their genetic loci. J. Genet. 53: 136–149.
Dodds, K.S. & D.H. Long, 1956. The inheritance of colour in diploid potatoes-II. A three-factor linkage group. J. Genet. 54: 27–41.
Dodds, K.S. & G.J. Paxman, 1962. The genetic system of cultivated diploid potatoes. Evolution 16: 154–167.
Dommergues, P., 1961. Action des rayons gamma sur les bourgeons de la varieté de poirier Max Red Bartlett. Annls Amélior. Plantes 11: 349–356.
Dommergues, P., 1962. Mutagénèse experimentale. Annls Amélior. Plantes 12: 67–78.
Dommergues, P., 1964. La destinée de la cellule mutée: Consequences dans le cas des plantes à multiplication végétative et dans le cas des plantes à reproduction sexuée. Proc. 3rd Congr. Eucarpia, Paris (1962), p. 115–139.
Dommergues, P. & J. Gillot, 1965. Variation de la réaction des boutures d'oeillet à l'irradiation gamma. In: The use of induced mutations in plant breeding. FAO/IAEA meeting, Rome (1964), p. 713–719.
Dore, J., 1955. Studies in the regeneration of horseradish I. A re-examination of the morphology and anatomy of regeneration. Ann. Bot. N.S. 19: 127–137.
Dormer, K.J., 1972. Shoot organization in vascular plants. Chapman and Hall, Londen, 240 p.
Dorst, J.C., 1924. Knopmutatie bij den aardappel en hare betekenis voor den landbouw. Genetica 6: 1–123.
Dulieu, H., 1970. Les mutations somatiques induites et l'ontogénie de la pousse feuillée. Annls Amélior. Plantes 20: 27–44.
Dunwell, J.M. & N. Sunderland, 1973. Anther culture of *Solanum tuberosum* L. Euphytica 22: 317–323.
East, E., 1908. A study of the factors influencing the improvement of the potato. Illinois Agr. Exp. Sta. Bull. 127: 375–456.
East, E., 1910. The transmission of variations in the potato in asexual reproduction. Connectic. Agric. Exp. Sta., Ann. Rpt 33: 119–160.
East, E.M., 1917. The bearing of some general biological facts on bud-variation. Am. Nat. 51: 129–143.
Esau, K., 1965. Plant anatomy. Wiley and Sons, New York (2nd ed.), 767 p.
Fellenberg, G., 1963. Über die Organbildung an in vitro kultivierten Knollengewebe von *Solanum tuberosum*. Z. Bot. 51: 113–141.
Ferwerda, F.P., 1965. Mutagenic effects of X-rays and ethyl methane sulphonate on potato sprouts. In: The use of induced mutations in plant breeding. FAO/IAEA meeting, Rome (1964), p. 687–690.
Foard, D.E. & A.H. Haber, 1961. Anatomic studies of gamma irradiated wheat growing without cell division. Am. J. Bot. 48: 438–446.
Folsom, D., 1923. Mutations of the potato: two somewhat unstable leaf-form sports of the Irish potato. J. Hered. 14: 45–48.
Fosket, D.E. & J.P. Miksche, 1966. A histochemical study of the seedling shoot apical meristem of *Pinus lambertiana*. Am. J. Bot. 53: 694–702.
Foster, A.S., 1938. Structure and growth of the shoot apex in *Ginkgo biloba*. Bull. Torrey bot. Club 65: 531–556.
Foster, A.S., 1939. Problems of structure, growth and evolution in the shoot apex of seed plants. Bot. Rev. 5: 454–470.
Foster, A.S., 1941. Comparative studies on the structure of the shoot apex in seed plants. Bull. Torrey bot. Club. 68: 339–350.
Frandsen, N.O., 1967a. Chimären verschiedener Ploidiestufen aus haploiden Kartoffelklonen. Z. Pfl-Zücht. 57: 123–145.
Frandsen, N.O., 1967b. Chromosomenverdoppelung und Chimärenbildung nach Colchicinbehandlung haploider Kartoffelsamen. Eur. Potato J. 10: 1–15.

Fruwirth, C., 1929. Über eine durch spontane Variabilität entstandene Kartoffelform und über spontane Variabilität der Kartoffel überhaupt. Z. PflZücht. 14: 35–79.
Galston, A.W. & P.J. Davies, 1969. Hormonal regulation in higher plants. Science, N.Y. 163: 1288–1297.
Garrison, R., 1955. Studies in the development of axillary buds. Am. J. Bot. 42: 257–266.
Gaul, H., 1959. Über die Chimärenbildung in Gerstenpflanzen nach Röntgenbestrahlung von Samen. Flora, Jena. Bd 147: 209–241.
Gerlach, D., 1969. Botanische Microtechnik. Thieme Verlag, Stuttgart, 298 p.
Gifford Jr, E.M., 1954. The shoot apex in angiosperms. Bot. Rev. 20: 477–529.
Gifford Jr, E.M., S. Kupila & S. Yamaguchi, 1963. Experiments in the application of the H^3-thymidine and adenine-8-C^{14} to shoot tips. Phytomorphology 13: 14–22.
Gifford Jr, E.M. & G.E. Corson Jr., 1971. The shoot apex in seed plants. Bot. Rev. 37: 143–229.
Gomez Cuervo, P.L. & R. Nelson Estrada, 1972. Artificial induction of mutants in the Criolla potato (*Solanum phureja* Juz. et Buk.). In: Proc. Study Group on Induced Mutations. Buenos Aires (1970). IAEA, Vienna, p. 457–468.
Gorter, C.J., 1968. Hormone translocation and rooting. In: Y. Vardar (ed.), The transport of plant hormones. North-Holland Publ. Company, Amsterdam, p. 293–308.
Grégoire, V., 1938. La morphogénèse et l'autonomie morphologique de l'appareil floral. Cellule 47: 287–452.
Gröber, K., 1962. Chimärenbildung bei der Tomatenmutante gilva von *Lycopersicon esculentum* Mill. nach Behandlung von heterozygoten Samenmaterial mit Colchicin und Röntgenstrahlen. Die Kulturpflanze Bd 10: 293–311.
Grodzinskii, D.M. & I.N. Gudkov. 1969. (Apical dominance and regeneration in vegetating plants after γ-irradiation). Radiobiologiya. Moscow, Vol IX (2): 249–256.
Guern, J. & M. Usciati, 1972. The present status of the problem of apical dominance. In: H. Kaldewey & Y. Vardar (eds.), Hormonal regulation in plant growth and development. Proc. Adv. Study Inst. Izmir (1971). Verlag Chemie, Weinheim, p. 383–400.
Gunckel, J.E., 1957. The effects of ionizing radiation on plants: Morphological effects. Symp. on the Effects of Ionizing Radiation on Plants. Quart. Rev. Biol. 32 (1): 46–56.
Gunckel, J.E., 1965. Factors affecting the morphogenesis of plant organs. In: P.R. White & A.R. Grove (eds.), Proc. Int. Conf. Pl. Tiss. Cult. Penn. State. Un. (1963), p. 251–268.
Gunckel, J.E. & A.H. Sparrow, 1954. Aberrant growth in plants induced by ionizing radiation. Brookhaven Symp. in Biol. 6 (1953), p. 252–279.
Gunckel, J.E. & A.H. Sparrow, 1961. Ionizing radiations: Biochemical, physiological and morphological aspects of their effects on plants. In W. Ruland (ed.), Encyclopedia of Plant physiology. Springer Verlag, Berlin, p. 551–611.
Günther, E., 1957. Die Nachkommenschaft von Solanaceeen – Chimären (1. Mitteilung). Flora, Jena 144: 497–517.
Günther, E., 1962. Die Nachkommenschaft von Solanaceeen – Chimären (2. Mitteilung). Flora Jena 152: 196–226.
Haberlandt, G., 1881. Über Scheitelzellwachstum bei den Phanerogamen. Mitt. naturw. Ver. Steiermark, p. 129–156.
Haccius, B. & H. Reichert, 1963. Restitutionsscheinungen an pflanzlichen Meristemen nach Röntgenbestrahlung I. Die Genese strahleninduzierter Sprossgabelungen. Planta 60: 289–306.
Hagberg, A. & N. Nybom, 1954. Reaction of potatoes to X-irradiation and radiophosphorus. Acta Agric. scand. 4: 578–584.
Hagemann, A., 1932. Untersuchungen an Blattstecklingen. Gartenbauwissenschaft 6: 69–195.
Hanstein, J., 1868. Die Scheitelzellgruppe im Vegetationspunkt der Phanerogamen. Festschr. Niederrhein Ges. Nat. – u. Heilkunde, Bonn, p. 1–26 (original not consulted).
Hara, N., 1973. Structure of the shoot apex with special reference to chimera formation. In: Induced mutation and chimera in woody plants. Gamma Field Symposia 12: 97–110.
Harris, G.P. & M.H. Hart, 1964. Regeneration from leaf squares of *Peperomia sandersii*: A relationship between rooting and budding. Ann. Bot. 28: 509–526.
Harten, A.M. van, 1970. Probleme und Perspektiven der Mutationszüchtung bei der Kartoffel. In: Bericht Arbeitstagung 1969, Gumpenstein (Österreich): 116–133.

Harten, A.M. van, 1972. A suggested method for investigating L-I constitution in periclinal potato chimeras. Potato Res. 15: 73–75.
Harten, A.M. van, H. Bouter & A. van Ommeren, 1972. Preventing chimerism in potato. Euphytica 21: 11–21.
Harten, A.M. van & H. Bouter, 1973. Dihaploid potatoes in mutation breeding: some preliminary results. Euphytica 22: 1–7.
Harten, A.M. van, H. Bouter & B. Schut, 1973. Ivy leaf of potato (*Solanum tuberosum*), a radiation-induced dominant mutation for leaf shape. Radiation Botany 13: 287–292.
Harten, A.M., van & H. Bouter, 1974. The 'Jørgensen' method, does it produce adventitious sprouts from potato? Potato Res. 17: 340–343.
Hawkes, J.G., 1956. A revision of the tuber-bearing Solanums. Annual Report Scott. Plant Br. St., p. 37–109.
Hawkes, J.G., 1963. A revision of the tuber-bearing Solanums (second edition). Scott. Pl. Br. St. Record 1963: 76–181.
Heide, O.M., 1964. Effect of light and temperature on the regeneration ability of *Begonia* leaf cuttings. Physiologia Pl. 17: 789–804.
Heide, O.M., 1965a. Photoperiodic effects on the regeneration ability of *Begonia* leaf cuttings. Physiologia Pl. 18: 185–190.
Heide, O.M., 1965b. Interaction of temperature, auxins and kinetins in the regeneration ability of *Begonia* leaf cuttings. Physiologia Pl. 18: 891–920.
Heide, O.M., 1968. Auxin level and regeneration of *Begonia* leaves. Planta 81: 153–159.
Heide, O.M., 1972. The role of cytokinin in generation processes. In: H. Kaldewey & Y. Vardar (eds.), Hormonal regulation in plant growth and development. Proc. Adv. Study Inst. Izmir (1971), Verlag Chemie, Weinheim, p. 207–219.
Heiken, A., 1960. Spontaneous and X-ray induced somatic aberrations in *Solanum tuberosum* L. Almquist and Wiksell, Stockholm, 125 p.
Heiken, A., 1961. Induction of somatic changes in *Solanum tuberosum* by acute gamma irradiation. Hereditas 47: 606–614.
Heiken, A. & G. Ewertson, 1963. The chimaerical structure of a somatic *Solanum* mutant revealed by ionizing radiation. Genetica 33 (1962): 88–94.
Heiken, A., G. Ewertson & L. Carlström, 1963. Studies on a somatic subdivided-leaf mutant in *Solanum tuberosum*. Radiation Botany 3: 145–153.
Helgeson, J.P., 1968. The cytokinins. Science N.Y., 161: 974-981.
Henshaw, P.S. & D.S. Francis, 1935. A consideration of the biological factors influencing the radiosensitivity of cells. J. cell. comp. Physiol. 7: 173–195.
Hermsen, J.G.Th. & A.J.E. de Boer, 1971. The effect of colchicine treatment on *Solanum acaule* and *S. bulbocastanum*; A complete analysis of ploidy chimeras in *S. bulbocastanum*. Euphytica 20: 171–180.
Hermsen, J.G.Th., M.S. Ramanna & J. Vogel, 1973. The location of a recessive gene for chlorophyll deficiency in diploid *Solanum tuberosum* by means of trisomic analysis. Can. J. Genet. Cytol. 15: 807–813.
Heyn, R.F., A. Rörsch & R.A. Schilperoort, 1974. Prospects in genetic engineering of plants. Quart. Rev. of Biophysics 7 (1): 35–73.
Hildering, G.J. & J.H. van der Veen, 1966. The mutual independence of M_1-fertility and mutant yield in EMS treated tomatoes. Euphytica 15: 412–424.
Hilding, A., 1974. (Effects of day-length and temperature at propagation of *Begonia* x *hiemalis* by leaf cuttings). Rep. Roy. Agr. Coll. Sweden Ser. A, 209: 1–15.
Hofmeister, W., 1852. Zur Entwickelungsgeschichte der *Zostera*. Bot. Ztg. 10: 121–131, 137–149, 157–158.
Hougas, R.W. & S.J. Peloquin, 1958. The potential of potato haploids in breeding and genetic research. Am. Potato J. 35: 701–707.
Hougas, R.W., S.J. Peloquin & R.W. Ross, 1958. Haploids of the common potato. J. Hered. 49: 103–106.
Hougas, R.W., S.J. Peloquin & A.C. Gabert, 1964. Effect of seed parent and pollinator on the frequency of haploids in *Solanum tuberosum*. Crop. Sci. 4: 593–595.

Howard, H.W., 1958. Transformation of a monochlamydius into a dichlamydius chimaera by X-ray treatment. Nature, Lond., 82: 1620.
Howard, H.W., 1959. Experiments with a potato periclinal chimera. Genetica 30: 278–291.
Howard, H.W., 1961a. Mericlinal chimeras in the potato variety Gladstone. New Phytol. 60: 388–392.
Howard, H.W., 1961b. The production of hexaploid *Solanum* x *juzepczukii*. Euphytica 10: 95–100.
Howard, H.W., 1962. Experiments with potatoes on the effect of the pigment-restricting gene M. Heredity, Lond. 17: 145–156.
Howard, H.W., 1964a. Experimental production of buds on the roots of potatoes. Nature, Lond. 203: 1303–1304.
Howard, H.W., 1964b. The use of X-rays in investigating potato chimeras. Radiation Botany 4: 361–371.
Howard, H.W., 1966. A sectorial entire-pinnate leaf chimera in the potato variety Majestic. New Phytol. 65: 284–287.
Howard, H.W., 1967. Further experiments on the use of X-rays and other methods in investigating potato chimeras. Radiation Botany 7: 389–399.
Howard, H.W., 1970a. Genetics of the potato *Solanum tuberosum*. Logos Press, London, 126 p.
Howard, H.W., 1970b. The eye-excision method of investigating potato chimeras. Potato Res. 13: 220–222.
Howard, H.W., 1972. The stability of an L3 mutant potato chimera. Potato Res. 15: 374–377.
Howard, H.W., J. Wainwright & J.M. Fuller, 1963. The number of independent layers at the stem apex in potatoes. Genetica 34: 113–120.
Hülsmann, B., 1936. Der Einfluß der Stecklingsform auf die Nachkommenschaft einiger gärtnerischen Zierpflanzen. Landw. Jahrb. LXXXII, p. 925–1000.
Ichikawa, S. & A.H. Sparrow, 1967. Radiation induced loss of reproductive integrity in the stamen hairs of a polyploid series of *Tradescantia* species. Radiation Botany 7: 429–441.
Ichikawa, S. & A.H. Sparrow, 1968. The use of induced somatic mutations to study cell division rates in irradiated stamen hairs of *Tradescantia virginiana* L. Jap. J. Genet. 43: 57–63.
Ichikawa, S., A.H. Sparrow & K.H. Thompson, 1969. Morphologically abnormal cells, somatic mutations and loss of reproductive integrity in irradiated *Tradescantia* stamen hairs. Radiation Botany 9: 195–211.
Iqbal, J., 1969. Radiation induced growth abnormalities in vegetative shoot apices of *Capsicum annuum* L. in relation to cellular damage. Radiation Botany 9: 491–499.
Iqbal, J., 1970. Recovery from cellular damage in vegetative shoot apices of *Capsicum annuum* L. after acute gamma irradiation. Radiation Botany 10: 337–343.
Iqbal, J., 1972. Effects of acute gamma radiation on the survival, growth and radiosensitivity of the apical meristems of *Capsicum annuum* L. at different stages of seedling development. Radiation Botany 12: 197–204.
Irikura, Y., 1975. Induction of haploid plants by anther culture in tuber-bearing species and interspecific hybrids of *Solanum*. Potato Res. 18: 133–140.
Irikura, Y. & S. Sakaguchi, 1972. Induction of 12-chromosome plants from anther culture in a tuberous *Solanum*. Potato Res. 15: 170–173.
Isbell, C.L., 1931. Regenerative capacities of leaf and leaflet cuttings of tomato and of leaf and shoot cuttings of potato. Bot. Gaz. 92: 192–201.
Jacobson, M., 1923. Die Wirkung der Röntgenstrahlen auf das Wachstum der Pflanzen. Beilage zum Rigaschen Rundschau 54: 5.
Jank, H., 1957a. Zur Anwendung der experimentellen Mutationsauslösung in Zierpflanzenbau. Dt. Gartenbau 4: 210–212.
Jank, H., 1957b. Experimentelle Mutationsauslösung durch Röntgenstrahlen bei *Chrysanthemum indicum*. Züchter 27: 223–231.
Jauhar, P.P., 1969a. Induction of some rare somatic mutations in *Solanum tuberosum* L. by ionizing radiations and radiophosphorus. Eur. Potato J. 12: 8–12.
Jauhar, P.P., 1969b. Morphological and physiological effect of radiations and radioisotopes on potato, *Solanum tuberosum* L. Indian J. agric. Sci. 39: 88–100.
Jauhar, P.P. & M.S. Swaminathan, 1967. Mutational rectification of specific defects in some potato varieties. Curr. Sci. 36: 340–342.

Jentsch, R., 1957. Untersuchungen an den Sprossvegetationspunkten einiger Saxifragaceen. Flora, Jena 144: 251–289.
Johnson, E.L. 1928. Tuberization of potatoes increased by X-rays. Science, N.Y. 68: 231.
Johnson, E.L., 1933. The influence of X-radiation on *Atriplex hortensis* L. New Phytol. 32: 297–307.
Johnson, E.L., 1937. Tuberization of the Colorado wild potato as affected by X-radiation. Plant Physiol., Lancaster 12: 547–551.
Jolivet, E., 1969. Physiologie de la tubérisation. Annls physiol. vég., 11: 265–301.
Jørgensen, C.A. & M.B., Crane, 1927. Formation and morphology of *Solanum* chimaeras. J. Genet. 18: 247-273.
Kaplan, R.W., 1953. Über Möglichkeiten der Mutationsauslösung in der Pflanzenzüchtung. Z. Pfl-Zücht. 32: 121–131.
Kaneko, I., 1975. (Mutations in potatoes induced by radiation I. Mutations by gamma irradiation). Bull. Hokkaido Agr. Exp. Sta. 31: 28–33.
Katagiri, K., 1973. Radiation damage in winter buds in relation of shoot cuttingback to mutationfrequencies and spectra in acutely gamma-irradiated mulberry. Induced mutations and chimera in woody plants. Gamma Field Symposia 12: 63–79.
Katagiri, K. & K.O. Lapins, 1974. Development of gamma-irradiated accessory buds of sweet cherry, *Prunus avium* L. Radiation Botany 14: 173–178.
Kessel, R., 1972. Production and use of inter- and intraspecific aneuploids of the genus *Solanum*. Ph. D. thesis, Univ. of Wisconsin, Madison, 312 pp.
Kessel, R. & P.R. Rowe, 1974. Inheritance of two qualitative traits and a proposed genetic map for their linkagegroup in diploid potatoes. Potato Res. 17: 283–295.
Kimber, G & R. Riley, 1963. Haploid angiosperms. Bot. Rev. 29: 480–535.
Kishore, H., Pushkarnath & G. Singh, 1963. The effect of radiation on potato tubers. Ind. Potato J. 5: 86–92.
Kishore, H., B. Das, K.N. Subramanyan, R. Chandra & M.D. Upadhya, 1975. Use of induced mutations for potato improvement. In: Improvement of vegetatively propagated plants through induced mutations (Tokai, Japan, 1974). IAEA, Vienna, p. 77–82.
Klopfer, K., 1965a. Erfolgreiche experimentelle Entmischungen und Umlagerungen periklinalchimärischer Kartoffelklone. Züchter 35: 201–214.
Klopfer, K., 1965b. Über den Nachweis von drei selbständigen Schichten im Sproßscheitel der Kartoffel. Z. PflZücht. 53: 67–87.
Klopfer, 1965c. Histogenetische Untersuchungen am Sproßscheitel der Kartoffel. Flora, Jena Abt. B, 156: 50–77.
Klopfer, K., 1967. Methods of demonstrating and breeding chimerical potato clones. In: H. Stubbe (ed.), Induced mutations and their utilization. Erwin-Baur-Gedächtnisvorlesungen IV (1966), 305–309.
Korableva, N.P., 1961. (Bestrahlungswirkungen auf anatomisch-physiologische Besonderheiten der Vegetationspunkte von Kartoffelklone). Dokl. Akad. Nauk. SSSR 137: 454–457.
Krantz, F.A., 1951. Potato breeding in the United States. Z. PflZücht. 29: 388–393.
Krenke, N.P., 1933a. Wundkompensation, Transplantation und Chimären bei Pflanzen. (translated from Russian into German). Springer Verlag, Berlin. 934 p.
Krenke, N.P., 1933b. Die Methode der Auslösung von Adventivsprossen bei der Kartoffel zwecks Bildung polyploider Sorten und Chimären (Russian, German summary). In: N.P. Krenke (ed.), Phänogenetische Variabilität, Moscow. Vol 2, p. 173–222.
Krythe, N., 1946. De invloed van de bewaring der aardappelknollen op de bouw van de knoppen en op de ontwikkeling tot volwassen plant I. Meded. LandbHogesch. Wageningen 47: 6, 36 pp.
Krythe, N., 1962. Ontwikkeling en groei van aardappelknol en aardappelplant. Sticht. Aard. Stud. Centr. 1: 1–12; 2: 21–30.
Kuehnert, C.C., 1962. Cytological and morphological changes induced in tomato as a result of thermal neutron irradiation. Radiation Botany 2: 81–88.
Kukimura, H., 1967. (Intra-varietal differenee of radiosensitivity of potatoplants). Am. Rep. I.R.B. (Japanese, original not consulted).
Kukimura, H., 1972. Effects of gamma rays on segregation ratios in potato families. Potato Res. 15: 106–116.

Kukimura, H., & T. Takemata, 1975. Induced quantitative variation by gamma-rays and ethyleneimine in tuber bearing plants. Gamma Field Symposia 14: 25–38.
Kumar, D. & P.F. Wareing, 1972. Factors controlling stolon development in the potato plant. New Phytol. 71: 639–648.
Kupfer, E., 1907. Studies in plant regeneration. Mem. Torr. bot. Club 12: 195–241.
Lam, S., 1975. Shoot formation in potato discs in tissue cultures. Am. Potato J. 52: 103–106.
Lam, S.L. & H.T. Erickson, 1971. Location of a mutant gene causing albinism in a diploid potato. J. Hered. 62: 207–208.
Langenauer, H.D., T.S. Osborne & D.A. Haskell, 1972. Effects of accute X-irradiation upon growth of *Parthenocissus tricuspidata* axillary buds I. Morphological damage and recovery. Radiation Botany 12: 297–309.
Langenauer, H.D., D.A. Haskell & T.S. Osborne, 1973. Effects of accute X-irradiation upon growth of *Parthenocissus tricuspidata* axillary buds II. Anatomical damage and recovery. Radiation Botany 13: 197–205.
Langton, F.A., 1974. A re-evaluation of the Dionne method of vegetatively doubling the chromosome number in potato. Potato Res. 17: 296–306.
Lapins, K.O., C.H. Bailey & L.F. Hough, 1969. Effects of gamma-rays on apple and peach buds at different stages of development I. Survival, growth and mutation frequencies. Radiation Botany 9: 379–389.
Lapins, K.O. & L.F. Hough, 1970. Effects of gamma-rays on apple and peach leaf buds at different stages of development. II. Injury to apical and axillary meristems and regeneration of shoot apices. Radiation Botany 10: 59–68.
Lauer, F.I., 1963. Influence of high and low levels of N and K on adventitious bud formation in the potato. Am. Potato J. 40: 302–307.
Lauer, F.I., 1967. Factors affecting adventitious bud formation in the potato. Am. Potato J. 44: 87–94.
Lauer, F.I. & F.A. Krantz, 1957. Formation of buds from callus tissue in the potato. Am. Potato J. 34: 158–164.
Lea, D.E., 1962. Actions of radiations on living cells. 2nd ed. Cambridge University Press, 416 p.
Levitt, J., 1972. Responses of plants to environmental stresses. Academic Press N.Y., 697 p.
Lindemuth, H., 1903a. Vorläufige Mitteilungen über regenerative Wurzel- und Sprossbildung auf Blättern (Blattstecklingen) und ihre Bedeutung für die Pflanzenvermehrung. Gartenflora 52: 479–485.
Lindemuth, H., 1903b. Weitere Mitteilungen über regenerative Wurzel- und Sprossbildung auf Laubblättern. Gartenflora, 52: 619–625.
Link, G.K.K. & V. Eggers, 1946. Mode, site and time of initiation of hypocotyledonary bud primordia in *Linum usitatissimum* L. Bot. Gaz. 107: 441–454.
Lunden, A.P., 1937. (Inheritance studies in the potato (*Solanum tuberosum* L.)). Meldinger Norges Landbrukshøgskole, 156 p.
Lunden, A.P., 1960. Some more evidence of autotetraploid inheritance in the potato (*Solanum tuberosum*). Euphytica 9: 225–234.
Lunden, A.P., 1974. Inheritance of tuber and flower colour in the potato (*Solanum tuberosum* L.). Meldinger fra Landbrukshøgskole 53 (18): 1–19.
MacDaniels, L.H., 1953. Anatomical basis of so-called adventitious buds in the apple. New York Agric. Expt. Sta. Mem. 325, 22 p.
Majumdar, G.P., 1942. The organization of the shoot in *Heracleum*. Ann. Bot. 6: 49–81.
McCrory, G. & P. Grun, 1969. Relationship between radiation, dose rate and lethality of diploid clones of *Solanum*.. Radiation Botany 9: 27–32.
McKelvie, D., 1921. Bud variation. Rep. Int. Potato Conf. Roy. Hort. Soc., London: 35–40.
Melchers, G., 1965. Einige genetische Gesichtspunkte zu sogenannten Gewebekulturen. Ber. dt. bot. Ges. 78: 21–29.
Melchers, G. & L. Bergmann, 1959. Untersuchungen an Kulturen von haploiden Geweben von *Antirrhinum majus*. Ber. dt. bot. Ges. 71: 459–473.
Melikyan, N.M. & Zh. V. Tsovyan, 1969. (Bud formation on potato tuber). (Russian). Biol. Zhur. Arm. 2: 32–38.

Mergen, F. & B.A. Thielges, 1966. Effects of chronic exposures to Co^{60} radiation on *Pinus rigida* seedlings. Radiation Botany 6: 203–210.

Mericle, L.W. & R.P. Mericle, 1967. Genetic nature of somatic mutations for flower color in *Tradescantia*, clone 02. Radiation Botany 7: 449–464.

Mezentzev, A.V., 1970. (The effect of gamma-irradation on the frequency of chromosome aberrations in potato seeds *(Solanum tuberosum* L.)). Genetics (USSR) 6, 11: 52–56.

Mezentzev, A.V. & I.M. Yashina, 1971. (The effect of dose rate of gamma-irradiation on the frequency of somatic mutations affecting the tuber colour in potato). Genetics (USSR) 7, 4: 5–12.

Mezentzev, A.V. & I.M. Yashina, 1973. (The effect of the dose rate of gamma-irradiation on the variability of quantitative characters in the potato in progeny vM_2 and M_1). Genetics (USSR) 9, 1: 39–45.

Micke, A., 1976. Introduction. In: Induced mutations in cross-breeding. Proc. Meeting Advisory Group FAO/IAEA 1975. IAEA, Vienna, p. 1–4.

Miedema, P., 1967. Adventitious buds on potato roots. Euphytica 16: 163–166.

Miedema, P., 1973a. The use of adventitious buds to prevent chimerism in mutation breeding of potato. Euphytica 22: 209–218.

Miedema, P., 1973b. A physiological study of adventitious bud formation in potato. Agric. Res. Rep. 787 (Pudoc, Wageningen), 67 p.

Miksche, J.P., A.H. Sparrow & Anne F. Rogers, 1962. The effects of chronic gamma irradiation on the apical meristem and budformation of *Taxus media*. Radiation Botany 2: 125–129.

Miller, C.O., F. Skoog, M.H. von Saltza & F.M. Strong, 1955. Kinetin, a cell-division factor from desoxyribonucleic acid. J. Am. chem. Soc. 78: 1375–1380.

Miller, J.C., 1954. Selection of desirable somatic mutations, a means of potato improvement. Am. Potato J. 31: 358–352.

Moreno, B.G. & R. Nelson Estrada, 1973. (Induction of mutants in species of potatoes (*Solanum tuberosum* L.) by means of ionizing radiations). Rev. Inst. Col. Agrop. 8: 117–129.

Murashige, T., 1974. Plant propagation through tissue cultures. Ann. Rev. Plant Physiol. 25: 135–166.

Nayar, N.M., 1969. Considerations of overcoming intrasomatic selection during mutation breeding of vegetatively propagated plants. Theor. Appl. Gen. 39: 99–103.

Nayar, N.M., Puskarnath & K.L. Hakim, 1965. Frequencies of certain types of gamma ray-induced mutations in potato. Ind. Potato J. 7: 76–80.

Nayar, N.M. & H.S. Chauhan, 1968. A chemically induced systematic mutation in the potato (*Solanum tuberosum* L.) produced as a sectorial chimera. Japan. J. Genet. 43: 389–392.

Nayar, N.M. & T.R. Dayal, 1970. A method of securing high frequencies of induced mutations in the potato. Z. PflZücht. 63: 155–160.

Naylor, E.E. & B. Johnson, 1937. A histological study of vegetative reproduction in *Saintpaulia ionantha*. Am. J. Bot. 24: 673–678.

Newman, I.V., 1965. Patterns in the meristems of vascular plants. III. Pursuing the patterns where no cell is a permanent cell. J. Linn. Soc. (Bot.) 59: 185–214.

Nitsch, C., 1968. Induction *in vitro* de la floraison chez une plante de jour courts *Plumbago indica* L. Ann. Sci. Nat. Bot. Paris 9: 1–92.

Nougarède, A., 1965. Organisation et fonctionnement du méristème apical des végétaux vasculaires. In: Travaux dédiés au Lucien Plantefol. Masson et Cie, Paris. 520 p.

Nougarède, A., 1967. Experimental cytology of the shoot apical cells during vegetative growth and flowering. Int. Rev. Cytol. 21: 203–351.

Overbeek, J. van, 1966. Plant hormones and regulators. Science, N.Y. 152: 721–731.

Partanen, C.R. & E.M. Gifford Jr., 1958. Application of autoradiographic techniques to studies of shoot apices. Nature, Lond. 182: 1747–1748.

Partanen, C.R., 1963. Plant tissue culture in relation to developmental cytology. Int. Rev. Cytol. 15: 215–243.

Pavek, J.J., 1972. A preliminary trial with chemical mutagens applied to potato eyes. (Abst.). Am. Potato J. 49: 362.

Péreau-Leroy, P., 1969. Effect de l'irradiation gamma sur une chimère complexe d'oeillet sim. In: Induced mutations in plants. Proc. Symp. FAO/IAEA. Pullman, USA, IAEA, Vienna, p. 337–344.

Péreau-Leroy, P., 1975. Recherches radiobiologiques sur des chimères d'oeillet *Dianthus caryophyllus* L. (Thèse) Univ. de Clermont-Ferrand, 169 p.
Phillips, I.D.J., 1969. Apical dominance. In: M.B. Wilkins (ed.), Physiology of plant growth and development. McGraw-Hill, London, p. 163–202.
Plantefol, L., 1947. Hélice foliaire, point végétatif et stéle chez les dicotylédones. La notion d'anneau initial. Revue gen. Bot. 54: 49–80.
Popham, R.A. & A.P. Chan, 1950. Zonation in the vegetative stem tip of *Chrysanthemum morifolium* Bailey. Am. J. Bot. 39: 329–339.
Pötsch, J., 1966a. Über die Auslösung extramutativer Strahlungseffekte an Klonsorten von *Euphorbia pulcherrima* Willd. Züchter 36: 12–25.
Pötsch, J., 1966b. Das Verhalten von *Abutilon hybridum* hort. 'Andenken an Bonn' nach einmaliger und fraktionierter Röntgenbestrahlung. Z. PflZücht. 55: 183–200.
Pötsch, J., 1967. On the dissociation of chimerical shoot-variants by the use of X-rays. In: H. Stubbe (ed.), Induced mutations and their utilization. Erwin-Baur-Gedächtnisvorlesungen IV (1966), p. 301–304.
Pötsch, J., 1969. Die Abhängigkeit röntgeninduzierter Histogenese-anomalien von der Höhe der Bestrahlungsdosis bei *Pelargonium zonale* Ait. 'Madame Salleron'. Wiss. Z. Päd. Hochsch. Potsdam 13: 129–137.
Pratt, C., 1959. Radiation damage in shoot apices of Concord grape. Am. J. Bot. 46: 103–109.
Pratt, C., 1960. Changes in structure of a periclinal chromosomal chimera of apple following X-irradiation. Nature, Lond. 186: 255–256.
Pratt, C., 1963. Radiation damage and recovery in diploid and cytochimerical varieties of apples. Radiation Botany 3: 193–206.
Pratt, C., 1967. Axillary buds in normal and irradiated apple and pear. Radiation Botany 7: 113–122.
Pratt, C., 1968. Radiation damage in shoots of sweet cherry (*Prunus avium* L.). Radiation Botany 8: 297–306.
Pratt, C., J. Einset & Mohammad Zahur, 1959. Radiation damage in apple shoot apices. Am. J. Bot. 46: 537–544.
Priestley, J.H. & C.F. Swingle, 1929. Vegetative propagation from the standpoint of plant anatomy. U.S. Dept. of Agric., Techn. Bull. 151, 98 p.
Rappaport, L., S. Blumenthal-Goldschmidt, M.D. Clegg & O.E. Smith, 1965. Regulation of bud rest in tubers of potato *Solanum tuberosum* L.I. Effect of growth substances on excised potato buds. Pl. Cell. Physiol. 6: 587–599.
Rechinger, C., 1894. Untersuchungen über die Grenzen der Theilbarkeit im Pflanzenreich. Verh. Zool.-bot. Ges. Wien 43: 310–334.
Reinert, J., 1972. Control of morphogenesis in plant tissue cultures by hormones and nitrogen compounds. In: D.J. Carr (ed.), Plant growth substances. Proc. 7th Int. Conf. Canberra, Australia (1970). Springer Verlag, Berlin, p. 686–694.
Renner, O., 1936. Zur Entwicklungsgeschichte randpanaschierter und reingrüner Blätter von *Sambucus, Veronica, Pelargonium, Spiraea, Chlorophytum*. Flora, Jena 30: 454–466.
Richter, A. & W.R. Singleton, 1955. The effect of chronic gamma radiation on the production of somatic mutations in carnations. Proc. natn. acad. Sci. (U.S.A.) 41: 295–300.
Riley, R., 1974. The status of haploid research. In: K.J. Kasha (ed.), Haploids in higher plants. Proc. 1st Int. Symp., Univ. of Guelph, p. 3–9.
Roer, L., 1967. Mutations in potatoes induced by gamma irradiation. Euphytica 16: 283–292.
Roest, S. & G.S. Bokelmann, 1976. Vegetative propagation of *Solanum tuberosum* L. in vitro. Potato Res. 19: 173–178.
Romberger, J.A., 1963. Meristems, growth and development in woody plants. U.S. Dept. Agric. Tech. Bull. 1293, 214 p.
Ross, R.W., L.A. Dionne & R.W. Hougas, 1967. Doubling the chromosome number of selected *Solanum* genotypes. Eur. Potato J. 10: 37–52.
Rudno, G. von, 1925. Beobachtungen über vegetative und geschlechtliche Aufspaltung bei Kartoffeln. Die Kartoffel 1, 17: 215–216.
Rudorf, W. & K. Wöhrmann, 1963. Versuche zur Auslösung von Mutationen durch Co^{60}-Bestrahlung auf vorgetriebene Augenkeime bei der Kartoffel. Z. PflZücht. 49: 397–414.

Rünger, W., 1959. Über den Einfluß der Temperatur und der Tageslänge auf die Bildung und Entwicklung der Adventivwurzeln und -triebe and Blattstecklingen von Begonia 'Konkurrent' und 'Marina'. Gartenbauwissenschaft 24: 472–487.

Sachs, T. & K. Thimann, 1967. The role of auxins and cytokinins in the release of buds from dominance. Am. J. Bot. 54: 136–144.

Sacristán, M.D., 1971. Karyotypic changes in callus cultures from haploid and diploid plants of *Crepis capillaris* (L.) Wallr. Chromosoma 33: 273–283.

Sagawa, Y & G.A.L. Mehlquist, 1957. The mechanism responsible for some X-ray induced changes in flower color of the carnation *Dianthus caryophyllus*. Am. J. Bot. 44: 397–403.

Salaman, R.N., 1925. Genetic studies in potatoes: McKelvie's Arran Victory mutations. J. Genet. 15: 267–300.

Salaman, R., 1926. Potato varieties. Cambridge University Press, 377 p.

Salaman, R.N., 1931. Somatic mutations in the potato. Report Proc. IXth Int. Hort. Congr. Roy. Hort. Soc. London, p. 117–140.

Satina, S., A.F. Blakeslee & A.G. Avery, 1940. Demonstration of the three germ layers in the shoot apex of *Datura* by means of induced polyploidy in periclinal chimeras. Am. J. Bot. 27: 895–905.

Satina, S. & A.F. Blakeslee, 1941. Periclinal chimeras in *Datura stramonium* in relation to development of leaf and flower. Am. J. Bot. 28: 862–871.

Sax, K., 1963. The stimulation of plant growth by ionizing radiation. Radiation Botany 3: 179–186.

Sax, K. & C.P. Swanson, 1941. Differential sensitivity of cells to X-rays. Am. J. Bot. 28: 52–59.

Schmidt, A., 1924. Histologische Studien an phanerogamen Vegetationspunkten. Bot. Arch. 7/8: 345–404.

Sekiguchi, F., K. Yamakawa & H. Yamaguchi, 1971. Radiation damage in shoot apical meristems of *Anthirrhinum majus* and somatic mutations in regenerated buds. Radiation Botany 11: 157–169.

Semerdzhyan, S.P. & N.G. Nor-Arevyan, 1971. (On the role of sulphydryls in the determination of natural radiosensitivity of *Vicia faba* and *Triticum* sprouts). Radiobiologiya (USSR) 11: 278–281.

Simmonds, N.W., 1964. Observations on potato callus and adventitious shoot formation. Am. Potato J. 41: 129–136.

Simmonds, N.W., 1965. Mutant expression in diploid potatoes. Heredity, Lond. 20: 65–72.

Skoog, F. & C.O. Miller, 1957. Chemical regulation of growth and organ formation in plant tissues cultured in vitro. Symp. Soc. exp. Biol. 11: 118–131.

Smith, L., 1942. Hereditary susceptibility to X-ray injury *Triticum monococcum*. Am. J. Bot. 29: 189–191.

Snedecor, G.W. & W.G. Cochran, 1968. Statistical methods. Iowa State Univ. Press, 6th ed., 2nd impr., 593 p.

Solomko, E.A., 1962. (Ways of bringing induced somatic mutations in potato to light). Radiobiologiya (USSR) 2: 634–638.

Solomko, E.A., 1965a. (Potato mutations induced by irradiation of vegetative parts of the plant). Radiobiologiya (USSR) 5: 547–551.

Solomko, E.A., 1965b. (Useful artificial mutations in potatoes). Genetics (USSR) 1: 233–239.

Soma, K., 1973. Experimental studies on the morphogenesis in the vegetative shoot apex. Induced mutation and chimera in woody plants. Gamma Field Symposia 12: 83–94.

Soma, K. & E. Ball, 1964. Studies on the surface growth of the shoot apex of *Lupinus albus*. Brookhaven Symp. Biol. 16: 13–45.

Sparrow, A.H., 1951. Radiationsensitivity of cells during mitotic and meiotic cycles with emphasis on possible cytochemical changes. Ann. N.Y. Acad. Sci. 51: 1508–1540.

Sparrow, A.H., 1961. Types of ionizing radiation and their cytogenetic effects. Mutation and plant breeding, NAS-NCR 891: 55–119.

Sparrow, A.H. & E. Christensen, 1950. Effects of X-ray, neutron and chronic gamma irradiation on growth and yield of potato (Abstr.). Am. J. Bot. 37: 667.

Sparrow, A.H. & E. Christensen, 1953. Tolerance of certain higher plants to chronic exposures to gamma-radiation from Cobalt-60. Science, N.Y. 118: 697–698.

Sparrow, A.H. & V. Pond, 1956. Some cytogenetic and morphogenetic effects of ionizing radiation on plants. Proc. Conf. Radioact. Isot. in Agric. USAEC Rep. TID 7512: 125–139.

Sparrow, A.H., R.C. Sparrow & L.A. Schairer, 1960. The use of X-rays to induce somatic mutations in *Saintpaulia*. Afr. Violet Mag. 13: 32–37.

Sparrow, A.H., R.L. Cuany, J.P. Miksche & L.A. Schairer, 1961. Responses of plants to acute and chronic radiation exposures. Radiation Botany 1: 10–34.
Sparrow, A.H., R.C. Sparrow, K.H. Thompson & L.A. Schairer, 1965. The use of nuclear and chromosomal variables in determining and predicting radiosensitivities. In: The use of induced mutations in plant breeding. FAO/IAEA meeting, Rome (1964), p. 101–132.
Sprague, H.B. & M. Lenz, 1929. The effect of X-rays on potato tubers for 'seed'. Science, N.Y. 69: 606.
Stadler, L.J., 1930. Some genetic effects of X-rays in plants. J. Hered. 21: 3–19.
Stanton, W.R. & W.K. Sinclair, 1951. Effect of high concentrations of phosphorus-32 on growth of potato. Nature, Lond. 167: 234–235.
Stebbins, G.L., 1950. Variation and evolution in plants. Columbia University Press, New York, 643 p.
Steffensen, D.M., 1968. A reconstruction of cell development in the shoot apex of maize. Am. J. Bot. 55: 354–369.
Stein, O.L. & D.M. Steffensen, 1959a. The activity of X-rayed apical meristems: a genetic and morphogenetic analysis in *Zea mays*. Z. VererbLehre 90: 483–502.
Stein, O.L. & D.M. Steffensen, 1959b. Radiation induced genetic markers in the study of leaf growth in *Zea*. Am. J. Bot. 46: 485–489.
Steinberg, C., 1950. Ricerche sull'istogenesi dell'apice vegetativo di alcune specie del genere *Solanum*. Nuovo G. bot. ital. 57: 319–334.
Stewart, R.N. & L.G. Burk, 1970. Independence of tissues derived from apical layers in ontogeny of the tobacco leaf and ovary. Am. J. Bot. 57: 1010–1016.
Stewart, R.N. & Haig Dermen, 1970a. The origin of adventitious buds in *Chrysanthemum*. (Abst.). Am. J. Bot. 57: 734–735.
Stewart, R.N. & Haig Dermen, 1970b. Determination of number and mitotic activity of shoot apical initial cells by analysis of mericlinal chimeras. Am. J. Bot. 57: 816–826.
Stewart, R.N., Pete Semeniuk & Haig Dermen, 1974. Competition and accomodation between apical layers and their derivatives on the ontogeny of chimeral shoots of *Pelargonium* x *hortorum*. Am. J. Bot. 61: 54–67.
Sussex, I.M., 1955. Morphogenesis in *Solanum tuberosum* L.: Apical structure and developmental pattern of the juvenile shoot. Phytomorphology 5: 253–273.
Sutton, A., 1918. Do potatoes give rise to new and distinct varieties by bud variation? Bull. No. 9, Messrs Sutton and Sons (U.K.).
Swaminathan, M.S. & H. Howard, 1954. The cytology and genetics of the potato (*Solanum tuberosum*) and related species. Biblphia genet. 16 (1953), p. 1–192.
Tarasenko, N.D., 1965. (Experimental somatic mutations in some potato cultivars). Genetics (USSR) 5: 145–149.
Tarasenko, N.D., 1977. Mutagenic efficiency of high-energy protons in potato. Z.PflZücht. 79: 79–81.
Thielke, C., 1951. Über die Möglichkeiten der Periklinalchimärenbildung bei Gräsern. Planta 39: 402–430.
Thoday, D., 1939. The interpretation of plant structure. Nature, Lond. 144: 571–575.
Tilney-Bassett, R., 1963. The structure of periclinal chimeras. Heredity, Lond. 18: 265–285.
Torrey, J.G., 1966. The initiation of organized development in plants. Advan. Morphog. 5: 39–91.
Toxopeus, H.J., 1954. Leaf testing as a method of genetical analysis of immunity from *Phytophtora infestans* in potatoes. Euphytica 3: 233–240.
Turnquist, O.C., 1960. Production of certified seed potatoes by varieties 1959. In Potato handbook 1960, Potato Assoc. Am., New Brunswick, New Yersey, p. 55–59.
Udai Singh, 1970. Radiation induced hooded eye mutants in potato. Sci. Cult. 36: 609–610.
Umaerus, M., 1966. Somatic aberrations in *Solanum tuberosum* induced by ethyl methane sulfonate and X-irradiation. Z. f. PflZücht. 55: 238–245.
Upadhya, M.D. & A.N. Purohit, 1973. Mutation induction and screening procedure for physiological efficiency in potato. In: Induced mutations in vegetatively propagated plants. Proc. Meeting FAO/IAEA, Vienna (1972), p. 61–66.
Upadhya, M.D., T.R. Dayal, B. Dev., V.P. Chaudhri, R.T. Sharda & R. Chandra, 1974a. Chemical

mutagenesis for day-neutral mutations in potato. In: Polyploidy and induced mutations in plant breeding. Proc. Meeting FAO/IAEA, Bari (1972), p. 379–383.

Upadhya, M.D., S.K. Anand & S. Pandey, 1974b. Mutation induction for resistance to bacterial wilt in potato. In: Induced mutations for disease resistance in crop plants. Proc. Meeting FAO/IAEA/SIDA, Novi Sad (1973), p. 172–173.

Venverloo, C.J., 1973. The formation of adventitious organs. I. Cytokinin-induced formation of leaves and shoots in callus cultures of *Populus nigra* L. 'Italica'. Acta bot. neerl. 22: 390–398.

Vig, B.K. & E.F. Paddock, 1970. Studies on the expression of somatic crossing over in *Glycine max*. Theor. Appl. Genet. 40: 316–321.

Von Guttenberg, H., 1960. Grundzüge der Histogenese höherer Pflanzen. I. Die Angiospermen. In: Handbuch der Pflanzenanatomie VIII, 3. Borntraeger, Berlin- Nikolassee.

Vries, H. de, 1878a. Keimungsgeschichte der Kartoffelknollen. Landwirthsch. Jahrb. 7: 217–249.

Vries, H. de, 1878b. Wachsthumgeschichte der Kartoffelpflantze. Landwirtsch. Jahrb. 7: 591–682.

Walther, F., 1969. Strahlenbiologische Untersuchungen an Getreidearten IV. Chromosomen-aberrationen und Sterilität in den X_1-generation unterschiedlich strahlenempfindlichen Gerstenarten. Radiation Botany 9: 231–240.

Weaver, G.M., 1963. The effect of Cesium-137-gamma irradiation of plant growth and flower colour of greenhouse *Chrysanthemum* varieties. Can. J. Genet. Cytol. 5: 73–82.

Weaver, R.J., 1972. Plantgrowth substances in agriculture. Freeman and Cy, San Fransisco, 594 p.

Went, F.W., 1938. Specific factors other than auxin affecting growth and root formation. Plant Physiol. 13: 55–80.

Wickson, M. & K.V. Thimann, 1958. The antagonism of auxin and kinetin in apical dominance. Physiol., Lancaster II: 62–74.

Winkler, H., 1903. Über regenerative Sprossbildung auf den Blättern von *Torenia asiatica* L. Ber. dt. bot. Ges. 21: 96.

Winkler, H., 1907. Über Propfbastarde und pflanzliche Chimären. Ber. dt. bot. Ges. 25: 568.

Wurm., 1960. Vergleichende Untersuchungen über Wachstum und Organbildung an Segmenten pflanzlicher Speicherorgane bei Kultur in vitro. Flora 149: 43–76.

Yamagata, H., Y. Kowyama & K. Syakudo, 1969. Radiosensitivity and polyploidy in some non-tuber bearing *Solanum* species. Radiation Botany 9: 509–521.

Yamakawa, K. & F. Sekiguchi, 1968. Radiation-induced internal disbudding as a tool for enlarging mutation sectors. Gamma Field Symposia 7: 19–39.