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Mutation Breeding Review

ECONOMIC AND AGRICULTURAL IMPACT OF MUTATION BREEDING IN FRUIT TREES

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Abstract

Constraints of conventional cross breeding in fruit trees, wide market acceptance of definite cultivars, especially in apple, pear, citrus and wine grape, and the increased impact of natural mutants provide incentives for mutation breeding. Only few induced mutants in fruit trees have been commercialized and are being planted on a large scale, contrary to the situation in ornamentals. Thermal neutrons, x or gamma rays have yielded commerical mutants, with only isolated cases of success with chemical mutagens or with chronic irradiation. The latter method often results in severe cumulative injury to plants. The main method followed in mutation breeding of tree fruit has been acute irradiation of meristematic multicellular buds. Chimera formation and reversion present a serious problem. Selection is usually performed in the second vegetatively propagated progeny, mV_2, in an attempt to overcome chimerism. Numerous mutants for the same trait need to be selected and compared in

further clonal trials, in order to assess reversion and comparative freedom from modification of other traits beside the selected one. Main characters selected were shorter stems and internodes, fruit colour, self - compatibility, seedlessness and in some cases, extension of time of fruit maturity. So far, little work has been performed with chemical or physical mutagens in vitro. Induced mutants of commercial significance include highly coloured seedless grapefruit (Star Ruby, Riored) seedless Minneola tangelo, self compatible and in some cases, compact sweet cherries, late blooming almond, reduced tree size in apple, self compatible Cox and Queen Cox apple, russet free Golden Delicious type apple (Lysgolden) succession of evenly ripening apple (Belrene and two other mutants). Isolated cases of better colour, resistance to fungi and lower fruit acidity have also been reported. Somaclonal variation and the creating of transgenic plants may provide important alternative methods for inducing new valuable mutants. Some induced mutants have already been used as parents in conventional cross breeding programs. Future targets for mutation breeding will probably not deviate essentially from those formulated in the past, with more emphasis on easily selectable simple traits and the problem of reversion.

1. INTRODUCTION

Conventional cross breeding in fruit trees has been successful with many crops. Limitations have become increasingly evident namely a long juvenile period, heterozygosity, often polyploidy, high cost and space requirement [1,2]. Lately another factor has gained in importance. With increasing international trade from both hemispheres certain cultivars attained a generally wide acceptance by trade and consumers, thus making it often increasingly difficult to launch new, different cultivars. This trend has paved the way for many natural mutants and is best exemplified in apple and citrus cultivars. Natural mutants of major impact have been those with compact or spur type growth, better fruit colour, spread of time of fruit maturity, seedlessness in citrus. The quest for natural mutants has reached in some instances the stage of an organized and publicized search. Success seems to depend to a large extent on number of trees, easily identifiable traits, frequent presence in the orchard, climatic effects, cultural practices (pruning) and frequency of mutations per clone.

The success and acceptance of natural mutants has served as an incentive to try to obtain mutants by planned mutation breeding, eliciting mutations in a much shorter time and often by making use of a sizable vegetative progeny.

Up to now only few commercial induced mutants have been produced in fruit trees, in comparison with ornamentals

TABLE I. COMMERCIAL MUTANTS IN ORNAMENTALS AND FRUIT CROPS
(AFTER VAN HARTEN AND BROERTJES, 1987)

Ornamentals	Number of commercial mutants			
	1960-1969	1970-1979	1980-1986	Total
Tuber and bulb crops	13	36	5	54
Pot plants	7	54	12*	73
Cut flowers	18	121	40	179
Other ornamentals	-	20	4	24
Fruit crops	4	14	4	22

* In chrysanthemum, induction of mutations is everyday
  practice and may exceed several hundreds (Broertjes and Van
  Harten 1987).

(table 1). However, making use of conventional cross
breeding in fruit trees, only a relatively small fraction of
varieties bred does reach wide commercial adaptation, for
reasons discussed before, as well as because of inadequate
testing, changes in market preference since the inception of
such programs. Moreover, information on new varieties bred by
mutation breeding is far from complete - as is the case with
those obtained by conventional cross breeding. Some of the
more promising and successful varieties obtained by mutation
breeding will de dealt with in a later chapter. Similarly to
natural mutants the problem of reversion is an important
consideration. Nurserymen are reluctant to propagate unstable
mutants. Growers of spur type trees with a tendency to
reversion may face crowding in the orchard, while in the case
of reverting self-compatible mutants the need to introduce
pollenizers will be inevitable.

    Comparison of success and use of mutation breeding in
fruit trees and ornamentals, undoubtedly point to better
methods (e.g. adventitious bud techniqe) and shorter time
required in ornamentals.(see table I) One of the main
difficulties in fruit trees is chimera formation, since
mutation is a one-cell event and generally multicellular
apices are treated. For a detailed discussion consult
Broertjes and Van Harten [3] Lapins [4] Pratt [5] and Tilney
-Bassett [6]. In many ornamentals heterozygosity is very high
and often polyploidy is also involved. Vegetative period is
short, allowing several crops per year in many cases.
Selection for colour, form and size is much easier than
selection for fruit yield and quality. The greenhouse
environment can be kept constant, and there is no need as in
fruit trees to transfer results to often widely differing
climatic and production patterns. The adventitious bud
technique, in vitro and in vivo has been widely applied,
yielding solid mutants [3,7,8]. With fruit trees - the large
size of plants requires ample space,though it seems that

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tree spacing with mutation breeding can be somewhat reduced compared to that required for hybrids in recombination breeding. The length of the vegetative period may also be shorter, in many cases with material used in mutation breeding relative to hybrids derived from conventional cross breeding. However, the problem of chimerism may impose more than one period of further selection (beyond mV_2 and often mV_3). In citrus in particular, the breeding cycle compared to that of sexual hybrids is shortened because of the prolonged juvenility - beyond bearing age - in the latter case.

Vegetative propogation in fruit is slower than with most ornamentals. Selection for quantitatively inherited characters is relatively difficult. Even with more easily selectable traits, e.g. fruit colour, spur type, seedlessness several fruiting periods are required to observe further aspects of fruit quality as well as yield performance.

2. Choice of mutagen and treatments

2.1. Mutagenic agents

Best results so far have been obtained by using heavy particles like neutrons and mainly ionizing radiation, X or gamma rays. Successful applications of chemical mutagens has been very rare [3,4,9,10]. The new apple, Belrene did result however from treatment with a chemical mutagen. Wider application of in vitro methods may improve chances for more successful applications of chemical mutagens, often lacking in reproducibility and ease of penetration.

Radiation has been found to induce many non lethal chromosome aberrations affecting often more than one gene. Mutant genotypes may be obtained, improved in one trait and poorer as to other characteristics. This imposes the need for large vegetative progenies, the selection and follow-up observation of a larger number of mutants [11]. As side effects are very often negative, mutants having the least negative effects on characters other than the character selected for, are desired mutants. Periclinal chimeras may be still prone to reversion; Solid mutants tend to be stable, and can be used as parents in conventional breeding programs. A disadvantage is the possibility of expression of several new characters due to pleiotropic effects and close linkage of genes, often detracting from conformity with the original cultivar and calling for larger populations to select from.

While valuable information has been gathered on radiosensitivity of different fruit tree species (mainly with deciduous fruits - table 2) less information exists on organ sensitivity (see table 3 and references [12,13,14] on citrus). In contrast, use of dose rates shows wide divergence [4] and may have significantly influenced results and bud or seed survival. Differences, in radiosensitivity of different

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TABLE II. DOSES AND EXPOSURES SUGGESTED OR USED IN MUTAGENIC
TREATMENTS. (Acute treatments with physical mutagens)
after Lapins 1983

Fruit	Plant material	Exposure[z]	Dose (Gy/min)	Mutagen
Almond	dorm. seed	2×10^{13} N/cm² sec.		th.neut.
Apple	dorm. buds	LD_{50}=61 Gy	15 Gy	X rays
	dorm. buds	LD_{50}=50 Gy	0.05-0.17	y rays
	dorm. buds	$3.9-15.6 \times 10^{12}$ N/cm²		th.neut.
	active buds	LD_{50}=43 Gy	7.5 Gy	y rays
	strat. seed	50-60 Gy	0.2-0.4 Gy	y or X
	PMC	20-30 Gy		X rays
Apricot	dorm. buds	LD_{50}=40 Gy	1.85 Gy	X rays
Banana	rhizomes	20-40 Gy		y rays
Blackberry	plants	70-150 Gy		
	dorm. seed	10-200 Gy		
Black currant	dorm. seed	LD_{50} 45 Gy	0.1 Gy	y rays
	dorm. seed	30 Gy		X rays
	Pollen	150-200 Gy		y rays
Cherry, sweet	dorm. buds	LD_{50} 40 Gy	1.85 Gy	X rays
	dorm. seed	1.5×10^{13} N/cm² sec		th.neut.
	PMC	8 Gy	1.1 Gy	X rays
Fig	dorm. buds	LD_{50}=25 Gy	0.1 Gy	y rays
Grape	dorm. buds	LD_{50}=50 Gy	0.35 Gy	X rays
	dorm. buds	LD_{50}=30 Gy	0.04 Gy	y rays
	dorm buds	4.8×10^{11} N/cm²	0.09 Gy	neutrons
	act. plants	24-44 Gy		y rays
	strat. seed	100 Gy		y rays
Hazel	drom. buds	70 Gy	0.1 ˍy	y rays
Mandarin	act. plants	20 Gy		y rays
	PMC	5 Gy		
Olive	act. plants	LD_{50}=35Gy	0.0.3-0.05	X rays
Orange	active buds	LD_{50}=55 Gy	0.17 Gy	y rays
	seeds	LD_{50}=100 Gy	1.66 Gy	y rays
	callus/vitro	LD_{50}=120-160 Gy	1.66 Gy	y rays
	protoplasts	LD_{50}=34 Gy		X rays
Peach	dorm. buds	LD_{50}=35 Gy	0.1-0.33	X rays
	summer buds	LD_{50}=61Gy	7.5 Gy	y rays
	act. plants	LD_{50}=37 Gy	8 Gy	y rays
	act. plants	LD_{50}=150 Gy	0.12 Gy	y rays
	dorm. seed	5 hr at $7-8 \times 10^{8}$ N/cm² sec		th.neut.
Pear	dorm. buds	LD_{50}=55 Gy	0.1 Gy	y rays
Plum	dorm. buds	LD_{50}=40 Gy	0.1 Gy	y rays
	dorm. seed	1.5×10^{13} N/cm²-sec.		th.neut.
Raspberry	dorm. buds	LD_{50}=50 Gy	0.1 Gy	y rays
	suckers	50-70 Gy		y rays
Walnut	dorm. buds	LD_{50}=42 Gy	0.1 Gy	y rays

[z]Or average flux of neutrons.

TABLE III. RADIOSENSITIVITY OF DIFFERENT CITRUS SPECIES
(AFTER SPIEGEL-ROY AND VARDI 1989)

species	Seed or buds	LD 50 (Gy)	
Citrus sinensis	buds	50	Spiegel-Roy and
Citrus paradisi	"	40-50	Kochba 1975
Citrus limon	"	50-60	
Citrus reticulata, sensitive cultivars	"	25-35	
Citrus reticulata, medium radiosensitivity	"	35-40	
Citrus reticulata, tolerant cultivars	"	45-50	
Citrus grandis, Chandler cultivar	"	25	
Citrus sinensis	seed	100	Spiegel-Roy and
Citrus reticulata,			Padova 1973
C. sinensis	seed	100-120	
Citrus reticulata	seed	80-100	

cultivars have also been noted. Comparing experience of
chronic irradiation with that obtained by acute irradiation,
chronic irradiation of potted plants does enable treatment to
be performed during specific, critical periods of flower
differentiation, flower and fruit development. Interesting
work on embryo irradiation has been performed by Decourtye
[15]. Chronic irradiation is much more difficult to perform
than acute irradiation and is accompanied also by an
accumulation of radiation injury. Kawai [16] stresses the
need for basic studies on the action of radiation on living
plant cells, including repair systems, in view of the
severity of cumulative effects found.

      As to type of mutations elicited, this has been dealt
exhaustively in several publications [9,17]. Most mutational
events are of a recessive nature. Aa - aa or a.Frequency of
aa - Aa is very low [18]. To distinguish heterozygotes from
homozygous dominants (AA - Aa) will be possible only in few
cases. Most fruit crops have a very high degree of
heterozygosity, thus recessive mutations can be frequently
induced. Polyploidy, frequent in fruit tree crops is also
considered generally favourable in eliciting mutations.

2.2. Methodological considerations

      Chimera formation following mutagenic treatment of plant
parts like buds having a multicellular apex constitutes a
severe constraint of mutation breeding in fruit crops.

This is in contrast to the facility by which interesting
homohistont mutations can be produced in a crop like

TABLE IV. SCHEDULES FOR PORPAGATION AND SELECTION OF MUTAGENS
TREATED FRUIT PLANT MATERIAL (AFTER LAPINS 1983)

Year	Season	Procedure
	Schedule	A. Original selection in V_2
1	Winter	Irradiation: grafting in a greenhouse. V_1 generation.
1	Summer	Budding, three to five buds from each primary shoot.
2	Summer	First selection in V_2. Budding, 10 buds from each selection.
3	Autumn	Observations and measurements in V_3. Second selection.
4	Spring	Planting potential mutants in an orchard for the first test.
5-9-11		Evaluation of trees and fruit, analyses of data. Selection.
10-12		Propagation of promising mutants for the second orchard test.
	Schedule	B. Original selection in V_1
1	Spring	Irradiation: grafting in the nursery. V_1 generation.
1	Summer	First selection in V_1. Budding, 10 buds from each selection.
2a-9a 11a		Continue as in Schedule A (years 3 to 10 or 12).
2b	Spring	Pruning back all V_1 shoots.
2b	Summer	Reducing V_2 shoots to one per stock. First selection in V_2 budding, 10 buds from each selection.
3b-10b- 12b		Continue as in Schedule A (years 3 to 10 or 12)

chrysanthemum. The foundation of the methodological work,
aiming to circumvent to a large extent formation of chimeras
was laid by Bauer [19] and Zwintscher [20] and has been
described in detail later. [4,11,21,22]. The isolation of
individual buds from mV_1 is instrumental in creating better
prospects for avoidance of chimera formation in mV_2. That
this is not always the case was shown by Lacey and Campbell
in the apple [23] by re-irradiation performed on mutants.
Backpruning in mV_1 has also been recommended and found as
an effective means in experiments with bush fruits [19].
Lapins [4] illustrates two possible schemes, one involving
initial selection in mV_1 followed by selection in mV_2,
the other based entirely on selection in mV_2 (see table 4).
On the whole, selection in mV_2 has been followed in more
cases than inital selection in mV_1. The method has been
studied most exhaustively in apple, applied to other crops as
well (cherry, grape, citrus). Two important improvements have
been described. The one involves shielding the bases of
irradiated scions or cuttings by inserting into holes of a

lead block or covering by lead pellets. Shielding
substantially increased chances of success of subsequent
propagation and enables use of higher irradiation doses.
Campbell and Lacey [22 also report in the apple covering of
scions by a shallow layer of water (6 mm) during irradiation,
and use of higher doses in this case. The use of seed in
irradiation even in apomictic citrus seems less justified
than using buds, in view of the much prolonged juvenile
period. The classical work of Lewis and Crowe [24] for
obtaining self compatibility in the sweet cherry was
performed with irradiation of pollen at the PMC stage.
Millions of gametes can be thus screened through the self
incompatibility sieve by using mutagen treated pollen on an
incompatible style, as only mutant pollen grains will set
fruit. The temporary drawback of the method is that because
of heterozygosity no SI (self incompatible) variety turned
into self compatible and further conventional breeding is
required. Successful irradiation of buds of an established
variety would not necessitate similar further breeding
efforts.

2.3. Use of in vitro methods.

Extensive work with in vitro methods in plants for
regeneration, resulted also in considerable efforts
consecrated to fruit tree species. Successful application of
such methods may facilitate in the future certain aspects of
mutation breeding, such as early preselection for certain
characters, lesser chimera formation, better use and testing
of chemical mutagens. Lane and Looney [25] demonstrated that
shoots from a compact type of sweet chery were much less
sensitive to excessive cytokinin in the medium. Initial
selection for a similar vegetative character may be thus
aided by similar techniques. Of even greater interest and
potential are more recent findings on regeneration from leaf
and petiole in vitro in several fruit species such as grape
[26,27] apple [28] blueberry [29]. These may ultimately lead
to better isolation of monohistonts and perhaps also enable
preselection for certain characters.

3. Economical characters affected and studied

Economic characters studied in fruit trees in connection
with mutation induction include shorter stems on internodes,
fruit colour, self fertility, seedlessness, time of fruit
maturity. Work in France [30] has shown that numerous fruit
characters may be affected. Possibilities of decreasing acid
levels in fruit have also been raised [31] as well as few
cases involving disease tolerance [32,33,34] and cases of
induction of polyploids [35,36].

3.1 Compact tree growth

This characteristics is much sought after in fruit
trees. Many spontaneous mutants have been found and

propagated. It seems that tree vigour and compact growth type
are affected independently by mutagenic treatment. Compacity
is often induced in different degrees. In cases studied in
detail, 1-2 recessive genes seem to be involved. Of
spontaneous mutants studied, McIntosh Wijcik apple behaves as
a single dominant [37] while the dwarf peach behaves as
single recessive (dwdw) [38] Cases of failure to transmit the
characterstics gave rise to the assumption that mutation
arose in L III and that, only induction of adventitious buds
developing on roots would result in tissue - homogeneous
trees and transmission. The most exhaustive work on the
subject has been summarized with the triploid apple cultivar,
Bramley's Seedling [23] In sweet cherry several compact types
have been produced by irradiation in the U.S., Canada and
Italy. While the subject has been of considerable economic
interest and mutants have been produced of the universal
cultivars such as Bing, Lambert, Van), in some cases at least
reversion to tree types with "normal" growth has proven a
setback. Mutation breeding would be still of considerable
promise, as probably dwarf, compact or spur types can be
successfully induced even in cultivars that have not produced
such mutants spontaneously. Successful use of a natural dwarf
mutant in peach, in breeding high quality peaches and
nectarines has been demonstrated [39] with similar work on
the columnar apple being carried out in England [37].

3.2. Self compatibility

        The importance of self compatibility for achieving
better crops at least in certain seasons and environments,
and for better orchard management (dispensing with lower
value pollenizers) is well recognized. Self incompatibility
in fruit trees has been most widely studied in Prunus,
especially sweet cherry [24] but also in apple and pear.
Incompatibility is controlled by a series of alleles at the S
locus. Work in Australia [40] led to isolation of specific
glycoproteins for Salleles in Prunus avium.

        Mutations affect loss of activity at the S locus, Sl -
Sf and result in self compatibility (SC). Some mutations are
reversibble Sl - Sf [24]. Stable mutants have been induced
in the sweet cherry by irradiation of pollen mother cells in
the resting stage, before meiotic prophase. Permanent
mutations have been induced in the activity part of the S
locus, but no changes in specifity has been reported after
irradiation [24]. Most mutations are deletions; irradiation
does not induce constructive mutations, affecting the
specifity part of the S locus. Mutations of the pollen part
of the locus may be genetic losses as a result of deletions
or unequal crossing over [41]. Millions of gametes are
screened through the self-incompatibility sieve by using
mutagen treated pollen on an incompatible sieve. Only mutant
pollen grains cause fruit set. The disadvantage of this very
efficient technique - production of seedlings with problem of
juvenility, quality selection and need to proceed in creating

cultivars suitable for commerce. For a mutant to become a commercial cultivar it has to be induced in somatic tissue and mature trees have to be screened for self-compatibility [42]. In addition, large numbers have to be screened [43]. Moreover, Weinbaum [44] stressed the difference between self-compatibility and self fertility (self pollination). While in the almond new cultivars were bred using the self-compatibility allele in cultivars grown in Puglia in Italy, self compatibility in sweet cherry was introduced by Lewis and Crowe [24] after X ray treatment of PMC; selfcompatible seedlings produced set the basis for new cultivars with self compatibility. Later, also by irradiation, a two fold goal was achieved - self compatibility and compactness in Compact Stella [45,46]. Increased self fertility in the apple was obtained by chronic irradiation [47] as well as by selection in Cox apple after irradiation from several thousand of mV_2 plants. Self compatibility allows monocultivar planting, and will be particularly valuable if induced in a commercial cultivar. The opposite effect of mutating a self compatible apricot cultivar into a self-incompatible one has been reported [48,10] with the Blenheim apricot. The self incompatible mutant (early Blenheim) had also larger fruit, required less thinning and ripened somewhat earlier.

3.3. Sterility

One of the most frequent effects of mutagenic treatment observed were various degrees of pollen and ovule sterility. Deficient flowers, aborted pollen, egg cells, embryos and seeds are very often the result of mutagenic treatments. While pollen sterility is usually more severe than ovule abortion, the two phenomena are usually associated [49,45]. Various degrees of lowered pollen fertility and lower seed number have been found in mV_2 of Golden Delicious apple. Percentage of stainable pollen will give a rough estimate of viability. A simple approximate measure of ovule fertility can be attained by seed counts. In principle and sometimes in practice, lowered fertility can also be conducive to fruit quality improvement and reduced biennial bearing.

3.4 Seedlessness

In addition to lower pollen viability in seedless citrus mutants [14] increased ovule sterility as evidenced by lower seed number in citrus has been also noted [14,50,51,52]. Irradiation with 25 gray of Perlette grape cuttings caused sterile ovules and pollen, as well as poorer fruit set. [53]. The aim was to cause looser clusters in order to minimize fruit thinning. Successful treatment may ultimately contribute not only to commercial exploitation but to alleviation of overbearing, decreasing expenses for thinning. On the other hand, decreased seed number per fruit has been definitely detrimental to fruit quality in Golden Delicious in France; however quality, economic value and marketability in Citrus will increase with a decrease in number of

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seeds/fruit. Problems of parthenocarpic fruit set and size of
yield often linked in citrus to seediness, at least with
certain genotypes. These facts point to the need for making a
larger number of selections from the same genotype, when
selection of mutants is made for seedlessness. In view of
this the time (years) required for final selection and
assessment for economic use will have to be increased.

3.5 Other fruit characteristics

Improvements in fruit colour and extension of time of
maturity in established cultivars are of great potential
economic interest. Extending time of maturity will allow
exploiting a well accepted cultivar for a longer period in
the market. This is well exemplified by the span obtained
through three recent mutants of Reine de Reinettes in France
[30].

3.6 Fruit colour

Improved fruit colour in a highly competitive market is
of prime importance. Natural red mutants of Delicious, Cox
and many other apples have superseded less coloured strains.
The first red skinned and fleshed grapefruits had a
considerable impact in the latter case, colour is due
entirely to lycopenes and no anthocyanins are involved. The
genotype of L II determines transmission of colour of fruit
rind in citrus [54]. Most studies on high colour mutants have
been conducted in the apple. No useful mutants with
redskinned fruit were found in completely yellow or green
apples. Peel colour depends on first and second histogenic
layers. Visible colour was found to depend mainly on
anthocyanins in the epidermis and the 3 outer layers of
hypodermis [55,56]. Production of anthocyanins is dominant.
As to degree of red colour a pronounced cultivar specifity is
evident. The natural mutant Queen Cox has more red colour in
the skin compared to Cox, while stable self compatibility has
been attained hitherto mainly in regular Cox. Emphasis on
breeding for self-incompatibility has now moved to Queen Cox,
in view of the pronounced market preference for red fruit.

In citrus natural mutants with red peel and juice
vesicles have been found mainly in Texas grapefruit. Mutation
breeding by use of thermal neutrons has brought about a most
significant change from a deeply coloured, highly seeded
grapefruit to a seedless one. In another, more recent case
thermal neutrons and natural mutation have been instrumental
in producing a mutant with increased red colour, from an
established natural mutant having good peel and flesh colour.

Of other fruit characters mutants, for low acidity are
of considerable interest in citrus and in grapefruit
especially. In the apple low acid types depend on a single
homozygous recessive gene [57]. Modification of fruit acidity
in grapefruit is being investigated in Florida [31]. Data

from a set of radiation induced mutants [50] also point to the possibility of a single gene control.

Estimation of further fruit characteristics in radiation induced mutants have evidenced unexpected benefits, such as in the russet free apple Lysgolden (derived from Golden Delicious) [30]. Changes in some fruit characteristics, such as shape can be expected. While some of the changes affecting fruit parameters will turn out objectionable compared to control, others may prove of a certain commercial value without influencing other market characteristics.

Mutants with significant deviations as to time of fruit ripening have been successfully induced in the case of the Reine de Reinettes, a goal likely to be achieved in other cases as well. Mutation breeding by irradiation has also resulted in a most significant delay in time of bloom [30].

Such an effort in almond would be of great importance for ensuring more dependable fruit set and yields, in many environments. We have to take into account that time of maturity may be influenced by tree vigour and crop load. For this reason observations concerning time of maturity have to be pursued for several years.

Colchicine is the usual means for artificial induction of polyploidy. However irradiation with gamma rays induced polyploidy in muscadine grape [35] and mulberry [36].

3.7 Disease and pest resistance

While resistance to many diseases has been found to be polygenic, numerous cases of mono or oligogenic resistance are known. The situation may be complicated by several biotypes of the fungus and their interaction with the host.

In a few cases an effect of mutation inducers on disease resistance have been noted. Difference in susceptibility to apple powdery mildew (Podosphaera leucotricha) were noted in some induced mutants of McIntosh [58] and Cox [32]. Mutants resistant to Plasmopara viticola in grape after seed irradiation were claimed by Coutinho [33]. Thermal neutron treatment of Prunus seeds yielded seedlings of several species resistant to crown gall, Agrobacterium fumefaciens [34]. Commercial significance was not attained by any of these mutants.

3.8 Mutation breeding in rootstocks

There have been very few cases of mutation breeding in fruit tree rootstocks. An attempt to breed mutants of lesser vigour of the vigorous 12/1 cherry rootstock was made by Donini [59]. We have to take into account the relatively smaller volume of breeding work on rootstock compared to variety breeding. Of even greater significance is the fact,

that in the case of rootstock breeding, cross breeding using
wild relatives poses no problem and back crosses are also
more widely employed. The limitation on new cross-bred
varieties because of marketing problems and heterozygosity
does not apply, of course, for rootstocks. For these reasons
mutation breeding is not likely to assume great importance
in rootstock breeding.

4.   Case histories, cases of pronounced economic impact

       An attempt to analyze why certain projects of mutation
breeding met with success, while others turned out
unsuccessful, will be made by analyzing some case histories.
Obviously, success is more likely when cultivars are more
universally adapted, have been successfully established over
large surfaces, and the only changes affected involve 1-2
characters. For mutation breeding at present an easily
recognizable character, like fruit colour or seedlessness
seem essential. The scale of work has to be adequate, though
some cases of obtaining seedless citrus with relatively small
populations of mV_2 have also occurred [14,50,51].Careful
and sometimes quite lengthy trials to test the new mutant
for other characteristics such as yield, fruit size and
shape, freedom from reversion, are required. The time element
is of prime importance, as even well established cultivars
may decline in market demand and popularity. Successful cases
with economic impact save one case have resulted so far from
use of irradiation as a mutagenic agent. Concerning methods
employed, selection in mV_2 has proved best so far, in most
cases. Competition from breeders employing cross breeding
have proven strong and most effective in many cases, such as
peach, grape, cherry, blueberry. The need for using virus
free material as source for treatment has been justly
stressed. Exemplary management practices are essential, in
order to successfully select useful mutants and for valid
comparisons with control. Environmental hazards have also
played a role: heavy frost damage has adversely affected
valuable mutation breeding projects of citrus in Texas and
Florida.

       The list given below has to be rather incomplete. In
many cases no adequate information could be gathered, while
in others no information has been divulged. Still, the few
cases listed below will illustrate successful economic
results with mutation breeding in fruit trees. One has to
bear in mind that the cultivar picture is apt to change; the
latter holds, however, also for established cultivars and to
a considerable degree, even for newly bred cultivars by
methods other than mutation breeding.

4.1 Spur types of Bramley's seedling apple

       In a most exhaustive program and detailed publication
Lacey and Campbell [19] dealt also with selection stability,
propagation and the problem of reversion. In England in the

1970 and 1980's Cox and Bramley's seedling were highly
popular. A compact form of the overvigorous triploid Bramley
was certainly of interest. No natural compact forms were
found.

The project started in 1970. Scions were irradiated by 50 -
100 Gray. After initial selection (mV_2) and clonal
propagation (10 plants per variant), commercial trials
followed. Of the 12 mutants selected 7 clones were uniform,
with no linked detrimental changes, and a coefficient of
variation similar to the control. Crop weight per tree volume
was higher than in the control, though crop per tree was
below that of control. In recent trials, only one clone, No.
36 has shown consistent satisfactory cropping and reduced
plant vigour. This selection may turn out to be some type of
periclinal chimera. (A.D. Webster, personal communication).
As recently few Bramley trees are being replanted, even the
best mutant selections may not be planted widely in the
future.

     Further compact apples. Two selections have been made in
France, both dwarf with short internodes. Courtagold has been
obtained from Golden Delicious, Courtave from Starking. Both
are being used only for potted plants [30]. Chances of
induced apple mutants for restricted growth do not seem very
high, in view of similar natural mutants in numerous apple
cultivars and because of the possibilities and accumulated
experience for successful manipulation of tree size by use
of rootstocks in the apple.

4.2 Compact cherries

     Limitation of tree size of sweet cherries is obviously
of great interest in view of the high picking costs involved.
Over 5000 trees, mostly for home gardeners have been sold of
the compact form of the first self compatible cultivar,
Stella. Of more widely renowned cultivars, compact forms have
been developed through mutation breeding, and named Compact
Lambert, Compact Van (developed in Canada) Compact Bing
(developed in the U.S.). All three will manifest 1/3 less
growth than the control. High and early fruiting is also
claimed. Compact Lambert is still sold by nurseries, but is
not widely planted and is rather of low vigour. All three
cultivars, as well as Compact Stella are prone to reversion
and this has limited their expansion. New cultivars with a
tendency to compactness are also being recently developed
from cross breeding (Lane, personal communication).

     Compact cherries by irradiation, have been also produced
in Italy [60,61]. Burlat C 1 has been produced by
irradiation in 1969. Earlier fruiting is claimed (compared to
control) as well a 20% height decrease and 30% decrease in
trunk diameter, compared to control. Of wider acceptance are
compact forms of Durone Nero I and II of Vignola Durone Nero
II C 1 is claimed to be more productive, about half in

height compared to control and about 60% in diameter. Selections have been made already 2 years from treatment and later tested in clonal trials.

In contrast to compacts developed in the U.S. and Canada, it is claimed that no reversion occurred in the Italian mutants.

A certain effect towards compactness has been found also in the Fascionello K late flowering and self compatible almond, obtained by irradiation [62]. A compact olive mutant has also been induced.

4.3 Compact Shamouti

A semi compact form of Shamouti orange has been selected in mV_2 after irradiation with 20 Gys [14]. Two mV_3 trees showed a 20.6-23.7% reduction in tree size. With decreasing Shamouti plantings, there is little interest in these selections.

4.4 Self compatible mutants

Apple - Achieving a self compatible Cox has been of considerable interest in Britain and elsewhere. The work has proceeded uninterruptedly in spite of difficulties and results have been widely tested. The material was established after irradiation with   Gy. Two clones have performed well, no 7 and 8 [63]. These are going to be released in 1991. In a latest trial at East Malling, selection 8 stood out in cropping performance with somewhat less vigour than EMLA Cox. Two other clones also yielded better than EMLA Cox. A lower pollen viability of self compatible Cox compared to Golden Delicious (usually employed as pollenizer) has been found [64]. This may necesitate a higher proportion of pollenizers. Since the final selection of self compatible Cox, fruit growers started to favour Queen Cox, with fruit having more red colour in the skin than "regular" Cox. This has caused a decrease in interest for the self incompatible Cox. Work on attaining self compatibility in Queen Cox, though started later, has also progressed [64]. Fruit set records were taken on 5000 trees planted in 1975/6 and 20 selections planted in the fall of 1983. Two self compatible clones (no. 7 and 18) have been chosen. Further tests are rquired before the merits of the self incompatible Queen Cox selections can be fully appraised. There will be probably considerable grower interest in such clones.

Cherry - Self compatiblity in the sweet cherry was introduced through PMC irradiation [24]. The first self compatible cultivar, Stella was derived using the SC gene evolved. It is also a good pollinnator of cultivars with intermediate period of blooming. Since, new cultivars with self compatibility and higher fruit quality have been bred. The impact of self compatible sweet cherry cultivars at present is however less than expected.

Almond - A late flowering, self compatible almond (Sf), derived from Fascionello and lately named Supernova has been bred utilizing gamma rays (30 Gray) and selection performed in mV_2 [62] . The most striking characteristics is the bloom period, nearly 20 days later than Fascionello. Alternative methods of introducing self compatibility in almond by cross breeding (using the South Italian cultivar Tuono) have been successfully employed in France and Spain [65,66].

4.5   Fruit characters and colour, time of ripening

In the apple, one of the most successful mutants from irradiation, is the russet free Golden Delicious, named Lysgolden, developed in France [30]. Fruit russeting is a serious problem with Golden Delicious, at least in certain environments, detracting substantially from the market value of the fruit. 5 buds from V_1 shoots have been propagated, to form V_2 families. The selected mutant is more russet free than the Smoothee cultivar. Additional merits are a pink color on the sunshine face of the yellow fruit, mainly in young trees, and a lower weight loss in storage (up to 20%) because of a waxy cuticle, with subsequent less fruit bruising. Other characters also modified are a later picking period, lower tree vigour, more elongated fruit and increased mildew sensibility   Lysgolden trees sold in France during the last five years amount to about 30% compared to (100%) of Golden Delicious, the main apple cultivar in France [67], Decourtye (personal communication).

Further apple mutants developed and commercialized in France have been developed from the popular high quality summer apple in France. Reine de Reinettes. A drawback of the variety is uneven ripening of the fruit, necessitating numerous pickings. Belrene developed by mutation breeding necessitates 2 pickings only and has been planted on a modest scale (50000 plants). It is supplemented by two other mutants derived from Reine de Reinettes, propagated since 1988 and extending the ripening season. Prerene ripens 1 week earlier, Tardirene 1 week later. Both show also increased red skin colour [30].

4.6 Colour mutants in Citrus

Natural mutants with improved colour are of great significance in orange and grapefruit. Colour in grapefruit is derived from lycopene and associated pigments. Nucellar selection accompanied by thermal neutrons enabled to obtain two mutants of great commercial significance and prospects. Star Ruby exceeds all natural and induced mutants in color; it has been
derived from a highly seeded cultivar (Hudson Red) with intense internal and external colour. Rio Red, also seedless has been derived from Ruby Red a coloured, seedless mutant and is being ranked now by most experts as second in colour

only to Star Ruby with better adaptation possibilities to
environment and cultural practices.

4.7 Seedlessness

One case of induction of seedlessness in grape by
irradiating cuttings of a seeded grape has been described.
Seedlessness in grape is probably conditioned by two
complementary recessive genes [68]. Nearly all work on
induction of seedlessness by mutation breeding has been
performed in citrus. Most important natural seedless mutants
have been found in various citrus species, gaining wide
economic importance in most markets. Seedlessness is
usuallly, but not invariably associated with high pollen and
ovule sterility. A pronounced parthenocarpic tendency is
essential for satisfactory fruit set and yield. Effects of
seedlessness on decrease in fruit size are in numerous cases
not severe enough to detract from economic importance.
Mechanisms leading to seedlessness have been discussed by
Iwamasa [69] and Frost and Soost [70]. An asynaptic gene in
inbred Wilking has been located [71]. Induction of
seedlessness has been achieved by treatment of apomictic seed
with gamma rays [50] by treating seed with thermal neutrons
[72] and by treating scions with gamma rays [14,51,73,74].
Data on radiosensitivity by various citrus organs and species
have been published [13,14].Successful mutants have been
obtained in some cases with relatively small mV$_2$ progenies;
thus from 160 plant is Pineapple orange, 65 plants in Duncan
grapefruit (50) 60 plants in Minneola tangelo,   600
plants in Eureka lemon, 120 plants in Villafranca lemon [14].
This points to a low number of recessive genes involved, a
conclusion not supported by the very low proportion of
seedless progeny from most diploid crosses in Citrus, except
those performed with Satsuma as a parent [75].

As already mentioned, natural mutants for colour have
been found in grapefruit. These have in many cases
substituted white fleshed grapefruit as table fruit. Mutation
breeding, perhaps aided by nucellar selection has given risen
to two highly coloured economically important new grapefruit
cultivars, Star Ruby and Riored, Star Ruby has been developed
at A & I, Weslaco, Texas [72]. Seeds of Hudson Red were
treated with thermal neutrons and the seedlings budded on
rootstocks. Fruiting started 7 years from irradiation.
Seeds in the selected mutant, later named Star Ruby, averaged
4 compared to 50 in Hudson Red. Flesh colour was equal to
Hudson Red and 3 times redder than the most red natural
mutant then available. Actual seed number in fruit of trees
propagated since is is usually lower (0-3). Cultivar has been
released in 1971. Some cases of reversion to seediness have
been noted, especially in Texas in fruit borne in clusters.
Reversion sometimes attains 2% of the fruiting branches. No
severe reversion has been noted in Israel. The cultivar shows
a typical red coloration in the trunk and branches. The
leaves are sensitive to chlorophyll injury from herbicide

application and often show higher sensitivity to phytophtora, freeze damage and winter chlorosis. While the rind shows uniform blush, internal colour sometimes fades. The fruit is claimed to be somewhat sweeter and less bitter than Marsh Seedless. In an extremely hot climate (Coachella Valley, Calif.) the tree does well mostly in shade of date trees. It performs very well in Israel and the Bahamas and fairly well in the traditional grapefruit areas of Florida with plantings since 1982, and close to 100.000 trees sold anually. In Israel so far 1400 ha have been planted. The yield in 1988/9 amounted to about 33000 tons of which 18000 tons have been exported. Yields are usually 40-60 tons/ha and sometimes higher. The main problem with this still expanding cultivar is some tendency to small fruit and in certain areas, excessive acidity. Star Ruby has not been planted in recent plantings in Texas. Riored has been developed in a similar manner as Star Ruby, but from a different type of cultivar [76]. Seedlings of Ruby Red have been propagated on rootstock. Budwood has been irradiated with thermal neutrons or X ray. One tree grown from budwood treated with thermal neutrons produced fruit 3 x redder than Ruby Red. The selection was propagated clonally on rootstock and gave rise on a limb of one tree to a mutation with fruit 5 x redder than Ruby Red, and only somewhat less than Star Ruby. The mutant was named Riored in 1984.

The fruit and composition are similar in other respects to the natural mutants Ruby Red, Ray Ruby. It has been planted on 4000 acres in Texas between 1985 and 1989. Many trees of this cultivar as well as other grapefruit cultivars have been severely attained by the latest freeze in Texas (1989). Wutscher, personal communication. The tree does not seem to be sensible to herbicide, phytophtora, chlorosis in a manner similar to Star Ruby [77]. Some cases of colour not superior to the natural mutant Henderson have been claimed. The cultivar has been introduced to Israel, Florida, California (1000 acres in Coachella [74] and the Bahamas. Market preference for red grapefruit is definitely on the rise so that interest in this cultivar claimed to be much less sensitive to cultural disorders than Star Ruby will be pronounced.

At this time it is not clear whether the relatively low acid grapefruit obtained by mutation breeding in Florida, will be widely planted in the future.

There is little information on the seedless orange produced by irradiation in China [9,74]. In principle successful expansion of such new cultivars in lieu of older, seeded cultivars seems likely. No significant fruit size decrease to the seeded fruit is likely to occur, in many cases.

Completely seedless lemons have been selected from mV_2 plants developed from irradiated budwood in Israel [14]. Two

cultivars were developed 1. Eureka (originally 6-15 seeds). So far no reversion was found. 2. Villafranca (originally 22 seeds per fruit). Trees are very prolific; and precocious. Pollen germination practically nil. No reversion found so far. Fruit tends to be somewhat angular. Both cultivars will be planted, at least to a moderate extent. Seedlessness in lemon is not such an express demand by market as is the case with grapefruit, orange and more recently, easy peeling fruit.

Seedless Minneola has been attained in mV_2 from budwood irradiated by 50 Gy. Only 60 mV_2 trees were tested of which 44 have been surveyed in detail [14]. Of these 8 were seedless with 4.7-23.9 seed in other mV_2 trees. Seedless Minneola still retains a slight pollen viability. Average seed number per fruit is 0.6. Fruit size does not significantly differ from seeded Minneola and the typical shape is retained. Sugar, acidity and time of ripening are also similar. Fertility seems adequate. Seedlessness is retained also under conditions of ample cross pollination. Seedless Minneola can also be planted adjacent to other selfincompatible cultivars. Planting of commercial plots has been started. Provided further experience is satisfactory, fairly large plantings are envisaged, ultimately replacing regular Minneola in new groves.

5. Alternative technologies to mutation breeding

Somaclonal variation, as a term was introduced by Larkin and Scowcroft [78] to cover all types of variation occurring in plants regenerated from cultured cells or tissue. Several mechanisms may be responsible such as gross karyotypic changes, cryptic chromosomal rearrangements, somatic crossing over with sister chromatid exchange, transposable elements, gene amplification or diminution. Tissue culture environment may enhance the frequency of somatic crossing over.

Variant plants have been regenerated mainly in sugar cane [79],potato [80] and celery [81]. The possibility of uncovering variants retaining qualities of existing cultivars while adding an additional trait, exists.

In principle this is a much simpler technique than recombinant DNA and transfer to field involves usually fewer special problems. However, correlation between cellular and whole plant response is required [82], not always present, and in some cases controversial. Moreover this line of research is more promising in annuals than in plants with a long vegetative phase like fruit trees. Lately, advances have been made also in establishing callus and shoots from leaves or leaf parts in apple [28], blueberry [29] grape [26,27] and also in producing diploid plants from anthers of wine grapes of Vitis vinifera [83].

These methods could be also adapted for induction of mutations; thus apple leaves in vitro showed regeneration after treatment with gamma rays between 10-20 Gy [28]. Accumulation of further experience in tissue culture and plant regeneration will also be instrumental towards a more extended use of in vitro methods in conjunction with mutation breeding.

More attention is being focused on novel methods of genetic engineering, aiming to produce transgenic plants. Several transgenic plants have been already produced in fruit trees. [84,85,86].The ultimate aim is to incorporate genes for economically valuable characters, including resistance to viruses, pests and diseases, as well as herbicides in an otherwise highly desirable clone. This is a much more direct approach than present mutation breeding or somaclonal variation. Moreover, it serves as a much higher incentive for young researchers and funding agencies compared to mutation breeding. The latter suffers also from the image of lengthy clonal comparisons lack of publicized results, and though unjustly, perhaps also from the use of irradiation as a means of initial treatment. With genetic engineering field testing still poses problems. Genetic engineering still requires elucidation of genetic functions encoding useful agronomic traits. In spite of progress and versatility in introducing foreign DNA, progress to ensure more efficient transformation techniques as well as targetting into subcellular compartments is needed. The lack of genetic background, the long vegetative period in fruit trees, as well as preference given to the main food crops in genetic engineering studies will cause a delay in the application of this fast growing field to fruit breeding.

On the other hand, standard procedures in genetic engineering may prove particularly useful in fruit trees in which the very high degree of heterozygosity reduces our capacity to obtain genetic information by classical methods.

6. Use of induced mutants in conventional cross breeding

Use of induced mutants with improved characteristics as parents in crosses is definitely of interest. However, compact mutants may not have L II mutated; thus only monohistonts, peridinal chimeras (L II - L III different from L I) and ascertained cases of mutation in L II will be of use. In colour mutants, some may be peridinal chimeras with increased anthocyanin in L II and L III, while in other increased anthocyanin occurred in L I only.

As to citrus, nucellar cultivars can be depended upon to transmit new traits via pollen. Cultivars with new traits of seedlessness or low seed number per fruit may be used as alternative improved parents compared to the original cultivar; this will, of course, also depend on degree of pollen and ovule sterility.

Use of mutants with increased disease resistance might be of potentially high value. Unfortunately, very few mutants possessing such proven characteristics were produced so far by mutation breeding.

While natural self compatibility encountered in some almond cultivars did prove very valuable in cross breeding, the induced self-incompatiblity in sweet cherry made a likewise contribution to the breeding of self fertile sweet cherry cultivars.

7. Future targets for mutation breeding

Though interest in somaclonal variation is on the increase, it is not likely to attain very great importance in fruit trees as a source of new variation and new cultivars. Testing of grapes from anther culture (derived actually from diploid filaments) and other somatic tissues in vitro may perhaps provide interesting variants, especially for wine grapes in the traditional growing areas. Attempts to locate natural mutants will continue and in some cases more targetted and planned schemes for carrying out surveys will materialize. Transgenic plants with economically important characters will be implemented, in conjunction ith conventional cultivar testing, but the realization of this will take time, especially in plants with a long vegetative period like fruit trees.

The main aims for mutation breeding might not deviate essentially from the targets indicated in the past, though more emphasis will have to be placed on easily identifiable characteristics and on problems of chimerism and reversion in fruit trees [87]. Induction of dwarf and compact growth habit will still be of interest in cherry and numerous other fruit trees for fresh fruit. However, in the apple in which most work has been performed in the past for induced mutations, natural mutants for compact growth have been found. In addition very satisfactory tree size control has been attained in this crop through manipulation of scion/rootstock combinations. Attaining self fertility in view of preference for large plots of single cultivars in the modern orchard is still important in certain species. Obvious gains in seedlessness by mutation breeding in citrus can be envisaged, also as a second phase after selection from progeny obtained by cross breeding. Enhanced fruit colour and other characteristics, like changes in time of fruit maturity will also be of obvious interest.

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