N.Z. Agronomy Society
Special Publication No. 5

Paper 62

IMPROVEMENT OF FRUIT VARIETIES BY MUTAGENESIS

L. Decourtye, Y. Lespinasse and M. Duron
Angers Fruit Breeding Station,
France

ABSTRACT

During the last 25 years, resources at the Angers Fruit Breeding Station have been shared equally between apple cultivar improvement by hybridisation and by mutagenesis. On the basis of the amount of graftwood delivered to nurserymen today, it is clear that results of mutagenic work have come into practice much more quickly than the results of hybridisation.

An efficient method for isolating mutant cell strains into a stable mutant has been developed and characters which can be easily changed are experimentally known. Due to the financial advantage that the recent law of breeders' rights offers the owner of a new clone, the method will be used by nurserymen.

Compared with spontaneous mutation, induced mutagenesis has the advantage that it focuses the job in time and in space; in return, it needs more money input.

Progress can still be made in the methodology of mutagenic treatment, choice of organ to be treated, and screening methods. The combined used of *in vitro* techniques with mutagenic practices is particularly promising.

Despite results already obtained and the need for improvement of the method, very few research teams in the world are still working in this field.

KEYWORDS

Apple cultivars, screening methods, black currant, *Ribes nigrum*, pear, *Pyrus communis*.

INTRODUCTION

Most present day world fruit cultivars are sports of older original varieties, improved for phytotechnic behaviour (spur types) or attractiveness of fruit (skin colour, russett free, shape). Among new varieties coming on the market Angers Fruit Breeding Station examines some 150 new clones every year. The fact that about 40% of these clones come from mutation limits the genetic diversity of fruit varieties because the mutants have similar genetic parentage, but it simplifies handling of material for the different partners involved in the fruit industry, i.e. nurserymen, growers, retailers and consumers. On the other hand, the law on breeders rights, issued from the Paris Convention in 1961, is progressively coming into practice and spreading over more countries. Nurserymen now have the economical advantages of exclusive rights for propagation of a mutant. The situation can be compared with that in the car industry; although a new basic model exists which is easily recognised by a characteristic set of features, many different types are available fulfilling the requirements of a wider range of consumers.

Such is the present situation for fruit varieties that at least in the short or middle term, the fruit industry will remain based on a small number of varieties grown worldwide, each of them being offered with different characteristics. Development of a good new variety will be facilitated if different cultivars are available with different adaptations to soils, climate, or training methods, and due to the financial incentive of ownership.

The likelihood of finding a spontaneous mutation among commercial orchards is proportional to the variety under cultivation. However, production and selection of such varieties can be sped up by induced mutagenesis which reduces time and space requirements.

MATERIALS AND METHODS

During the last 25 years, a mutagenic programme has been run at the Angers Station, mainly on pip fruits, and, by extension, to black currant. Some experiments have also been carried out on cherries. French colleagues have also applied mutagenic methods to plums (Renaud, 1981), apricots (Legave, 1985), and citrus (Legave, pers. comm.).

After an international research effort between 1960 and 1980, the latest efficient method has been established by our staff (Decourtye and Lantin, 1971). In summary it consists of applying a mutagenic treatment, usually a gamma ray irradiation at a dose range from 30 to 60 grays, to dormant scions. Top-worked on vigorous and virus free seedlings, an irradiated bud develops into a shoot V_1, from which five successive buds are budded on a clonal rootstock, to give the generation V_2. Selections for mutations is made on these trees, first in the nursery and later in orchards.

RESULTS

With irradiation at dosages described above, the frequency of mutation is very high. If the number of

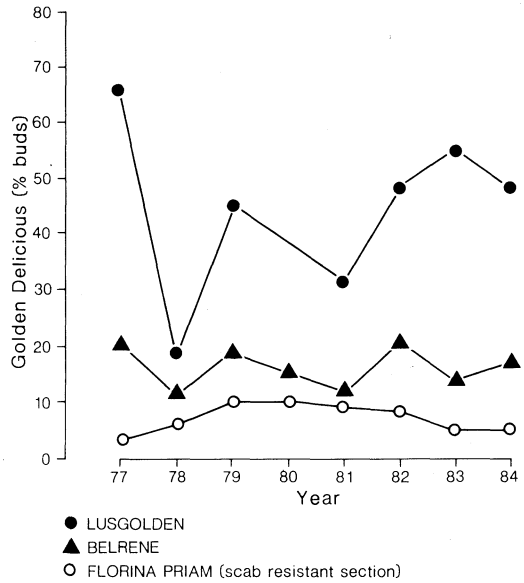

Figure 1. The number of buds of the new INRA varieties expressed as a percentage of Golden Delicious buds distributed to propagators by the National Technical Institute on Fruit and Vegetables (CTIFL).

characters tested was increased, all material would be found to have at least one observable mutation. The efficiency of the method is dependent on the ability to screen for the desirable characters and the possibility of screening at an early stage. Experience has shown that modification for vigour, growth habit, spur type, time of blooming or ripening fruit attractiveness (more colour, russett free), can be reasonably expected from a mutagenic programme.

Lysgolden

Some of the mutants that we have obtained have been taken up commercially to a significant level (Fig. 1). The most important mutant is the russett-free mutant of Golden Delicious, named Lysgolden® Goldensheen. The amount of budwood distributed by the National Technical Institute on Fruit and Vegetable (CTIFL), the French organisation responsible for registered varieties, has reached 30% of the total amount of Golden Delicious propagated. This mutant was initially selected for its high resistance to russetting (more than Smoothee), but further observations have shown that many other characters are also distinct from the original Golden Delicious (Le Lezec *et al.*, 1981). The fruit develops some pink colour on the sunshine face, mainly on young-bearing trees. Weight losses during storage are reduced by up to 20% due to a more waxy cuticle and resistance to bruising is better than in Golden Delicious. Picking time is delayed by about 10 days in the Loire Valley, which allows harvesting over a longer period. Fruit shape is slightly more elongate. The tree is less vigorous, with narrower leaves. Mildew susceptibility is increased. Blooming time is delayed a few days which may allow escape from late frost. The distribution of blossoms according to shoot age differs from Golden Delicious, so that pruning has to be modified to maintain yield. Many different characters are observed after one mutagenic event in both induced and spontaneous mutants (Lantin and Decourtye, 1973).

Belrêne

The second most successful mutant that we have released is a variant of Reine des Reinettes (syn. King of the Pippin), and has been named Belrêne. Fifty thousand scions are propagated each year. The main advantage is a more regular grade of crop which ripens over a shorter period. The entire harvest can be picked in two passages over the crop instead of three, without yield reduction; a distinct advantage for the grower. While most of our mutation work involves the use of gamma irradiation, Belrene was produced by treatment with ethyl methane sulfonate.

Other mutants released

Two other mutants of Reine des Reinettes have more recently been selected and released for commercial production.

Prérêne is earlier ripening (1 week), with the same fruit colour, and Tardirêne is later ripening (1 week). Harvesting can be made in one crop, and red fruit colour is increased.

Reine des Reinettes is an old European variety, grown for a long time on quite a significant area, which gives an opportunity to compare spontaneous and induced mutagenesis. Spontaneous mutations have not produced as much variation as induced mutagenesis, although the induced mutagenesis programme has been limited to a 10-year period and one hectare.

Some other mutants have been named which may have a commercial use: Courtagold and Courtavel. These are dwarf sports with very short internodes, derived respectively from Golden Delicious and Starking Delicious. They make small trees, convenient for city gardens, or even potted trees on balconies or terraces.

Other species

Besides our main work on apples, some results have been obtained in other species. In black currant (*Ribes nigrum*) we have a sport from the high quality cultivar Noir de Bourgogne. This sport ripens one week earlier which extends the harvest period. For pears (*Pyrus communis*), several mutants with potential interest have been induced. Because of the present reluctance to replant pear trees which are attacked by fire blight in France, we have not been encouraged to release them.

These results, obtained over a limited period of time, have encouraged some private nurseymen to contract with our laboratory to perform mutagenic programmes on some promising varieties.

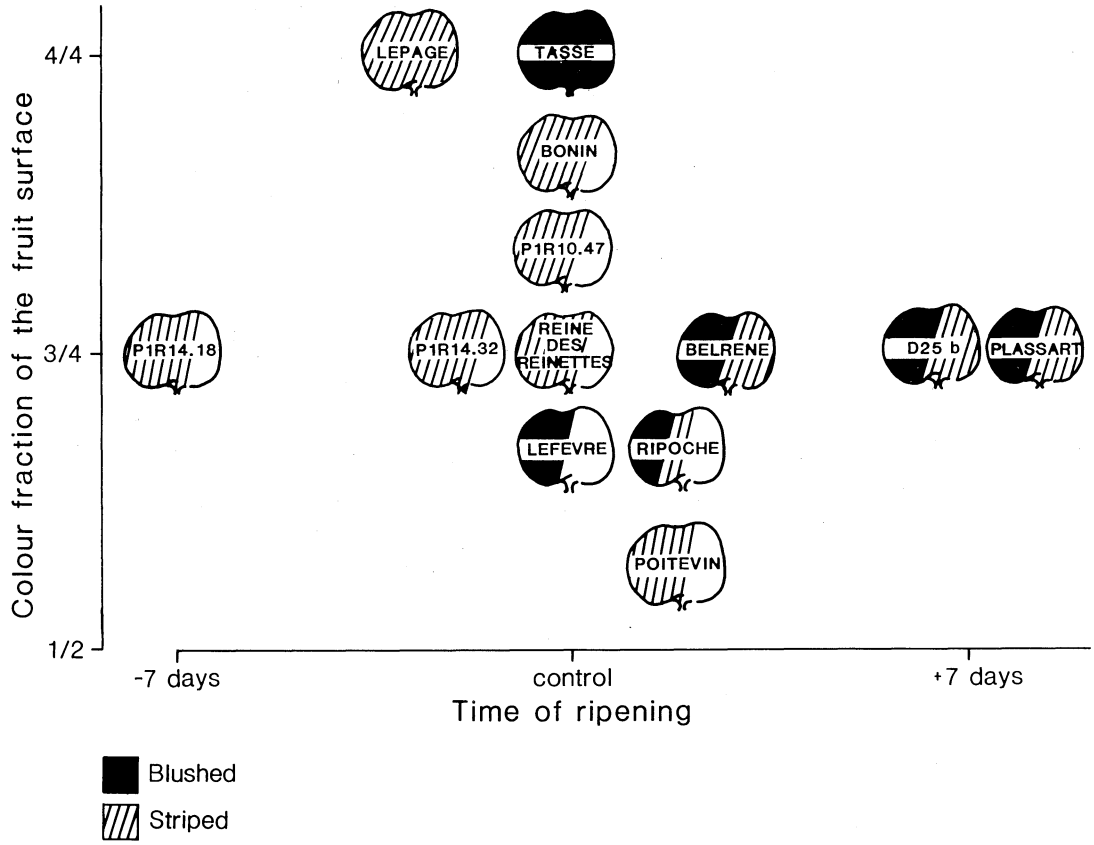

Figure 2. Classification of spontaneous and induced mutants of Reine des Reinettes, according to ripening period and fruit colour. Fruit colour is expressed in terms of fraction of the fruit surface and blushed or striped cover.

Besides the morphological modifications previously described, which are easily recognised, new results have been obtained which suggest more unexpected changes. For example, two mutants of Golden Delicious show some resistance to scab with only late contamination in the autumn. In addition, we have found a mutant with increased self-compatibility in Golden Delicious, a mutant with reduced evapotranspiration due to smaller number of stomata in Starking Delicious, two mutants of the pear Beure Bosc with an improved graft compatibility on quince, and a mutant for increased yield in Melrose apple.

As yield is supposed to be under polygenic control, we have controlled this characteristic in a statistical design experiment on a new generation V_3. Table 1 gives the results obtained on the V_2 generation from 1976 to 1979, for yield per tree, girth of the trunk, and productivity index. The mutant yields 16% more than the control and is smaller so that the productivity index is nearly 30% higher. In the next generation, for the first four crops, yield is increased by 20% and the productivity index by 24%. Table 2 gives the grading distribution of the V_3 crop over three years. It is clear that the mutant has smaller fruits than the control, with a much higher proportion in the best commercial grades. Fruit qualities are not changed, as confirmed by sugar and acid contents. This mutant will be released soon.

FURTHER WORK

More ambitious aims can be set for a mutagenesis programme, and the methodology can be improved.

An ambitious aim of our programme is to obtain new clones of the Passe-Crassane pear with less susceptibility to fire blight. Two different steps for screening are involved. The first step, which followed the method described above, was to look for a reduction in secondary blossoming, which allows bacteria to be present throughout the growing season. Results are already available which show induced differences for this character. The second step involves obtaining resistance to bacterial attack in pear tissue. This programme is going to be carried out with the help of *in*

Table 1. Yield, vigour, and index productivity of a Melrose mutant in V_2 and V_3 generations.

	Total yield (y) per tree (kg)	Girth (g) (mm)	Productivity index (i) i = y/g x 100
V_2 generation[1]			
Control	256	436	59
Mutant	298	391	76
V_3 generation[2]			
Control	67	227	29
Mutant	81	222	36

[1] Yield measured 1976-1979.
[2] Yield measured 1980-1984.

Table 2. Grading distribution (%) of fruits for control and mutant V_3 generation during 1982 and 1984.

	65mm	65-80mm	80mm
Control	1	56	43
Mutant	5	77	18

vitro techniques.

In vitro techniques appear to be a very useful tool used in conjuntion with mutagenesis. A working group is acting under the patronage of the International Atomic European Agency and met in Vienna in August 1985. Contributions have been made from our laboratory using ornamental woody plants (Duron and Decourtye, 1985).

Targets other than vegetative meristems may be worth treating with mutagenic agents. By irradiation of pollen mother cells, we are trying to obtain a self fertility gene, Sf, for apples, as this has been obtained by Lewis on cherry trees. This cherry self fertility gene has been used in crossing programmes and is now present in new cultivars such as Stella (Brown, 1983) or Starkrimson. The egg cell of apomictic species is also a desirable target which allows avoidance of chimera development (Decourtye *et al.*, 1983). It is clear that apple rootstock which could be propagated homogeneously through seeds would have some advantages over the present vegetative propagation method. Some Malus species have apomictic progeny, but results of Schmidt (1982) shows that many of them induce excessive vigour. It is another of our aims to obtain a more dwarfing rootstock in the *Malus hupehensis* species.

CONCLUSION

During these last decades, induced mutagenesis has proved its efficiency in apple variety improvement in the short or middle term. Characters that can be reasonably expected to be changed by this method are known, and it is not, as some people have said, a pure gamble. Success is probably more dependent on the availability of an efficient screen, rather than on the more or less complex genetic control of the character to be improved. Significant progress can still be achieved in the methodology of mutagenic treatment as well as in selection of the target organs and early or efficient screening of mutations.

Of course, the same results cannot be expected from mutation as from hybridisation programmes. Hybridisation is the best way to obtain a large genetic mixing and to originate a true novelty. But, whatever the qualities of the hybrid, commercial acceptance will remain a difficult and long term process. If many different sports were available commercially, different market needs would be met. Different mutants would also provide nurserymen with the opportunity of sole ownership of sports and the incentive of commercial profit.

ACKNOWLEDGEMENTS

I would like to thank Dr Harvey Hall and Tracy Williams for improving the English of the manuscript.

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SYMPOSIUM DISCUSSION

Mr G. Pringle, Division of Horticulture & Processing, DSIR

You showed a slide on the change of fruit maturity with natural and induced form. Has that change in maturity been in both directions and only up to seven days?

Decourtye

In both directions and maybe up two weeks. There is some Italian work where flowering two weeks later was achieved.

Pringle

Do different dosages achieve different results?

Decourtye

No. It is usually said that one should work at LD_{50} but this is really too much — I always work below that, but you just irradiate and pick up what is of interest to you.

Mr H.K. Hall, Crop Research Division, DSIR

Would there be any difference between the results in irradiation or mutagenic treatment of diploids or polyploids.

Decourtye

I cannot really answer your question. It is usually said that apples and pears are polyploid, so I should be a polyploid specialist, but they behave just like diploids.

Dr F. Alston, East Malling Research Station

I believe there is another russet-free sport of Golden Delicious available from natural mutation. Is this a character which you would pick up anyway without embarking on a mutation breeding programme?

Decourtye

There is another russet-free mutant. There is one coming from the United States, which is much less resistant than ours, intermediate for russeting susceptibility and like Golden Delicious for most of its characters. There are two other mutants which are adapted in France. An induced mutation will not produce anything other than a natural mutation could produce. However, I have developed my mutation on one hectare and spontaneous mutation has been obtained on 30 000 hectares and over 30 years.

Alston

In Britain, it has been found that there is a longer period to get establishment and stability of a mutation breeding line.

Decourtye

I have not found any big problem of stability in this mutant. Some mutants of course are unstable, but they are recognizeable and you will not propagate them in the field. Nobody has been able to show me that this mutant is unstable. Some people have claimed to have russetting but we have not been sure of the origin of the tree. So I have never been convinced of any reverse mutation or even chimera structure. Another point is that it is much easier to introduce this in the trade — we are able to stay with an established variety and introduce a desired character.