SPONTANEOUS MUTATION AS A SOURCE OF CLONAL VARIATION ON DECIDUOUS FRUITS

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Abstract

Trees of a clone of the same age and growing under the same conditions should be identical. Variations within a clone are largely due to topophysis, virus infection and genetic instability. As an introduction to the symposium the latter aspect is briefly reviewed. Mutations may affect genomes, chromosomes or genes. Their expression depends on the genes involved and the genetic background. Instability of a gene may depend on other (mutagenic) genes or on the conditions in the tissue (paramutation). Because of the periclinal structure of the meristem, somatic mutations are usually limited to certain histogenetic layers. Separation from the normal tissue is possible by adventitious bud formation. Various methods are mentioned. When tissue culture in volving regeneration of buds from an undifferentiated callus is used, the incidence of mutations may be quite high as it seems to be affected by the presence of synthetic growth regulators and their quantitative ratios. The practical implications are briefly discussed.

Introduction

Most if not all of our woody fruit species are propagated asexually by taking pieces of stem and using these as cuttings or scions. In this way one overcomes the three disadvantages of sexual propagation: that this method is not always possible, as some varieties form little or no seed, that seedlings usually have a sterile juvenile period that lasts several years, and that seedlings often show a very high and undesirable genetic diversity.

Ideally, the clone formed by asexual propagation is highly uniform, modifications being due only to plant age and external conditions. On closer inspection, however, a certain amount of variation is found that must be due to other causes. One of these is genetical instability, but two other possible causes should be mentioned briefly.

Topophysis

The nature of the plant may depend on the original position of the cutting or scion on the stock plant. Molisch (1916) called this topophysis. The classical examples are those where a lateral shoot, growing more or less horizontally, and dorsiventrally symmetrical, has lost the capacity to generate an upright, radially symmetrical shoot. This occurs in certain conifers (e.g. Abies, Araucaria) but also in coffee and cacao. This particular case of topophysis has not been reported from temperate fruits, but one should not be too hasty in excluding the possibility. Some years ago a quite unexpected case of topophysis came to light in tulip, where it found to be responsible for considerable differences in bulb formation (Hekstra, 1968).

Seedlings of apple and other woody species pass through consecutive

ontogenetical stages, differing in way of branching, size and shape of the leaves and capacity to flower. The changes apparently take place in the meristem and do not affect the parts already formed, so that when the top of a seedling reaches maturity (i.e., starts to flower), its base is still juvenile. Consequently, plants propagated from different parts of a seedling tree show topophysis: those from the top are completely mature, but those from the basis show a juvenile zone just like the seedling tree from which the scions were cut.

A seedling tree not only passes from juvenility to maturity, but it ages further until it becomes senescent and dies. If as in our standard varieties scions are cut from the periphery of the tree for many generations, one may wonder if the whole clone would not eventually become senescent just like the individual tree. This idea was first put forward by Thomas Knight in 1795 and was still supported by Molisch in 1916. Since then, however, the prevalence of virus infection and mutations has been discovered, and almost all modern authors ascribe the detoriation of clones to these causes rather than to ontogenetical ageing.

Virus infection

In the forties study of the remarkable rubbery wood disease of 'Lord Lambourne' and certain other apple varieties led to the insight that the varieties of our deciduous fruits as well as of their clonal rootstocks have in the course of time become infected by a host of viruses and mycoplasms causing a wide variety of symptoms. Incidentally, it came to light that a number of disorders characterised by symptoms usually associated with virus were not transmissable by grafting. These disorders (e.g. in the apple: leaf curl, narrow leaf, leaf variegation, flat fruit and fruit cracking (Posnette, 1963)) are therefore thought to be of genetical origin, i.e. caused by spontaneous mutation. This brings us to our main subject.

Mutations

For obvious reasons, mutations reported in the literature are primarily those which are readily observed and involve characteristics of practical importance. Many concern fruit characters: size, skin colour and russeting. More recently there is also a lively interest in internode length, particularly in mutations with short internodes resulting in a compact growth habit (spur types).

It is plausible to presume that mutation may affect every possible characteristic of the plant. Therefore, one might look for mutations for a better disease resistance, later flowering, self fertility, earlier ripening and better storage qualities. One of the reasons that very few of such mutations have been reported is that suitable methods for detection have not yet been developed.

Mutations may affect the genome, the chromosome or the gene. Genome mutations affect the ploidy, raising it from diploid to tetraploid (or from triploid to hexaploid, as the case may be). In deciduous fruits this gives rise to the 'giant' sports with thicker shoots and shorter internodes, broader and thicker leaves and larger fruits. These have been described for apple, pear, prune and apricot, but apparently not for peach, cherry and plum although in these species polyploidy could be induced artificially, so it seems plausible that it may also occur spontaneously (Janick and Moore, 1975).

Other mutations affect the chromosomes. They consist of the loss of

a chromosome (or in rare cases the inclusion of an extra chromosome) or of chromosome breakage followed by deletion, duplication or inversion of a chromosome section. I have found no evidence in the literature of the occurrence of this type of chromosome or structural mutations in deciduous fruits, but this may well be due to the difficulties which these plants present to intensive genetic studies.

Mutations in deciduous fruits not involving visible changes of genome or chromosomes are thought to affect single genes although the proof of this, i.e. simple Mendelian segregation of the characters involved, is usually lacking. This is again a result of the fact that in trees the amount of time, space and labour required to produce outcross generations is usually prohibitive.

Frequency of mutation

The frequency of mutation may vary considerably between species, cultivars or even individual plants. Loss of parts of or whole chromosomes mutations may be expected to occur mainly in polyploid plants, where such rather drastic changes in the nuclear structure are less likely to lead to a loss of vitality. This may explain the high incidence of chromosome mutations in polyploid ornamental plants, e.g. hexaploid Chrysanthemum (Sampson et al., 1958).

Single gene mutations also are more frequent in some plants than in others. Mutations causing a red skin colour, considered a desirable characteristic in apple, frequently occur in varieties like Delicious, Rome Beauty, Cox's Orange Pippin and James Grieve, but are extremely rare or non-existent in varieties like Golden Delicious, Sturmer's Pippin and Granny Smith.

For such a difference, several possible causes can be envisaged. The gene in question may not be there: our apple varieties which are supposed to be have arisen from hybrids of Malus pumila Mill. and M. silvestris Mill., may be expected to have only part of the genes of the parent species. When the gene is present, the expression of a mutation is determined by heterozygosity and ploidy: for the effect of the mutation A $\rightarrow$ a the genetical constitution of the plant (AA, Aa, AAa etc.) will be decisive. It is of course also determined by the presence or absence of other genes which suppress or promote the expression of a.

Mutagenic genes

There is a more interesting possible effect of the other genes on the mutation of a particular gene. In 1950 Darrow concluded that June yellows in strawberries was due to a frequently mutating or unstable gene and that the tendency to mutate was inherited as a recessive. This concept was worked out by Wellensiek (1960) with Cyclamen persicum L. In this plant colouration of the flower depends on a gene W, when this is recessive (ww) the flowers are white. In certain white flowered families, but not in others, the mutation w $\rightarrow$ W occurs which causes coloured flowers. Wellensiek selected a 'ever mutating' and a 'never mutating' line, and after crossing, selfing or back crossing, and two outcross generations he found evidence of a simple Mendelian segregation. He explained this by postulating a 'mutagenic' gene Mut which when recessive would cause w to mutate to W (or stabilize w when dominant).

Paramutation

In maize, a colour mutation in the aleuron cells is not only predictable as to the gene involved (as in the case of gene W in Cyclamen), but also to the site in the plant where it will occur: most of the red spots in the kernels are very small, indicating that the change occurs at a late stage in the development of the inflorescence. This process has been called paramutation. It has been suggested that it would be caused by labile components in the chromosomes, regulating the action of the genes, and tuned specifically to the intraorganic environments that evolve during ontogeny. Paramutation has also been held responsible for phase changes characteristic for many woody plants (Brink, 1962). Recently, the observation that a colour mutation in Chrysanthemum flowers occurs mostly at the base of the petals (leading to narrow streaks rather than large sectors of colour) has been ascribed to paramutation (Bush et al., 1976).

Chimeras

It is a well known fact that the outer layers of the primary meristems of Angiosperms are very distinct, because in these mantle layers only anticlinal cell walls are formed. As a consequence, a mutation in a meristem remains limited to the layer in which it occurred, and a periclinal chimera results. In deciduous fruits there are usually 2 mantle layers covering a central region where cell divisions occur in all directions, so mutations can be represented as M-N-N, N-M-N or N-N-M (M= mutated, N= non-mutated), dependent on the histogenetic layer where the mutation occurred. In apple there is some evidence for a third, a fourth and even a fifth mantle layer, but mutations in these layers are rather unstable, in the sense that they are more apt to invade other layers, or be pushed aside by N-cells from other layers, than mutations in the first and second mantle layer or the core.

Through this hand in glove arrangement of the various tissues of the meristem and thus of the whole plant, the effect of a mutation is dependent on the layer in which it originated (M-N-N may differ from N-M-N and this again from N-N-M). Sometimes a mutation may not become visible at all, e.g. a mutation affecting a characteristic of the epidermis will pass unnoticed when it occurs in the deeper layers.

Dermen, who did much work on ploidy mutations, devised a method to separate the various zones by inducing the development of what he called 'endogenous shoots'. These shoots are initiated adventitiously on callus swellings (sphaeroblasts) which develop after removal of all terminal, axillary and adjacent buds. As this callus originates in the cortex and outer phloem, the buds which arise on it have the constitution of the internal histogenetic layers. A N-N-M plant will thus give a M-M-M shoot, although other arrangements (e.g. N-M-M) also occur. Dermen used this method to separate layers of different ploidy, Dayton (1969) used it to uncover growth habit and skin colour mutants.

The meristems of adventitious shoots which are regenerated on leaf cuttings originate from a very small number of cells, probably only from a single cell (Broertjes et al., 1968). This means that if the stock plant was a chimera, the constituent genotypes are drastically separated, and the ensuing plants are non-chimeral. This may partly account for the high number of spontaneous mutations found in some of the ornamental plants propagated in this way. In Begonia, percentages between 18 and 35 have been found (Doorenbos and Karper, 1975). Dayton (1969) ascribed the relatively high incidence of mutations in the Delicious apple to the fact that in comparison to other varieties Delicious

forms adventitious shoots rather easily. On the other hand, the raspberry which is the only fruit crop routinely propagated by adventitious buds, does not seem to be distinguished by a high frequency of mutations, although several have been found (Jennings, 1966).

It is therefore not surprising that when modern methods of in vitro propagation are used, particularly those involving regeneration of buds from an undifferentiated callus, the incidence of mutations may be quite high. A recent, rather extreme example is a hybrid Aechmea, a Bromeliad, where up to 50% of mutants were found among plantlets from a one year old subcultured callus tissue (Zimmer and Pieper, 1976).

Mutation in vitro

Evidence is accumulating that this mitotic unstability in vitro is not wholly due to 'unmasking' of mutations already present in the plant, but that the changes are induced by the conditions prevailing in the culture. Many authors have shown that the genetic disturbances are a consequence of the unorganised growth of the cells, especially when the cells or the callus are cultured for a long period. In old cultures, visible mitotic irregularities (genomic changes) are quite frequent. Mehra and Mehra (1974) found triploid, tetraploid and aneuploid cells in cultures of almond callus. There is evidence that the presence of certain synthetic growth regulators like 2,4-D and the synthetic cytokinins, and their quantitative ratios can greatly influence the genetic instability of the cell population (Bennici et al., 1971).

As soon as differentiation occurs, the incidence of mitotic irregularities is reduced (Bayliss, 1973: Bush et al., 1976). In methods of in vitro propagation where apical or axillary meristems are used, very few mutations are found. In a recent review, D'Amato (in Reinert & Bajaj, 1977) concluded that 'in higher plants, the only cell line endowed with genetic stability is the meristematic cell line'.

Although sofar the almond appears to be the only temperate fruit where adventitious plantlets have been obtained, it is to be expected that in the near future methods of micropropagation will be developed also for other deciduous fruit species. If these methods involve regeneration of buds from undifferentiated tissue, it will be of primary importance to find cultural conditions which ensure stability of the cells (Street, 1973).

On the other hand, several authors have already suggested that the use of tissue culture might render the application of irradiation or chemicals to stimulate mutation superfluous. It is too early to try to answer this question for deciduous fruit trees, but we may nevertheless presume that if better methods for the detection of mutations and the isolation of mutated cells or tissue could be developed, the mutation frequency might prove to be quite high.

Stability of mutants

Genetical instability is not limited to genotypes resulting from the generative process. Spontaneous or induced mutations also are often far from stable. Spur types are notorious in this respect, and certain skin colour mutants are also known to revert frequently to the original type. Several causes for this instability can be envisaged. The most plausible hypothesis is that the mutation in question is limited to cells of the outer mantle layer, and that the mutated cells are less vigourous than the normal ones. The latter could than rather easily invade the outer mantle layer and push aside the weaker mutated cells.

Further research is necessary to prove (or refute) that this is in fact what happens.

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