Considerations on Overcoming Intrasomatic Selection During Mutation Breeding of Vegetatively Propagated Plants*

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Summary. When seeds and vegetative tissues are irradiated, a competition occurs in meristematic tissues between lethally and sublethally affected cells on one hand and unaffected cells on the other hand to the advantage of the latter. This phenomenon was first recognized by FREISLEBEN and LEIN (1943) and termed intrasomatic selection by KAPLAN (1951) and diplontic selection by GAUL (1957). The work done on the nature of this phenomenon in seed-propagated plants is summarized along with suggested methods to reduce its intensity.

The generally unencouraging results obtained so far in vegetatively propagated plants have been attributed to the more intensive operation of this phenomenon in this group of plants, consequent on the more complex nature of apical meristems and propagating materials. The work of ZWINTZSCHER (1959) in apples and BAUER (1957) in black currants have shown that it is possible to overcome its effects with suitable handling of irradiated materials.

The radiogenetic studies done by the author in the potato have been reviewed in this context. Low intensity irradiation, dose fractionation and irradiation of physiologically dormant tubers gave higher survival value. The method of isolating sprouts from irradiated tubers and growing them, and scoring for mutations in sprouting progenies was found to increase mutation frequency considerably. Other methods proposed for minimizing the effects of intrasomatic selection in vegetatively propagated plants are chronic irradiation, neutron irradiation, chemical mutagenesis and lastly, mutagenesis of isolated single cells and growing them into whole plants.

In one of the first papers reporting on the genetic effects of radiations on plants, STADLER (1930) mentioned about the possibilities of using induced mutagenesis as a breeding method in vegetatively propagated plants. Yet, we find nearly four decades later, that this method has hardly found any significant use in breeding programmes, nor even being used for any genetic or morphogenetic studies to any appreciable degree. Perhaps one of the most important reasons for this may be the more intensive operation of intrasomatic selection following mutagenic treatment in this group of plants. In this paper, we shall examine the mechanics of operation of this phenomenon and consider ways and means of overcoming, or at least minimizing, its unfavourable effects.

In a radiation breeding programme, what is more important is not the induction of mutations — these are generally produced in adequate numbers — but, their survival through successive cycles of cell division and selection for further propagation. Induced changes to be useful must be firstly heritable, secondly, must be viable through somatic and/or meiotic division, and finally, must come to exist in a high proportion of cells, so that the mutated descendents are not overgrown by the unmutated normal cells (CONGER, 1960). CONGER has pointed out that while the first step of getting a viable and useful mutation in a single cell is purely chance, the second one of getting this cell to develop into a new plant is a biolog-

ical problem. It is about this latter aspect that we shall discuss presently.

There is considerable variability in the organization and structure of apical meristems among angiosperms which include most of the agricultural plants. In their simplest form, meristems consist histologically of a one to few layered tunica enclosing a corpus. The outer layer of the tunica gives rise to the epidermis. Not much is known about the number of cells which are true leaders or initials in either layer of the shoot apex. Estimates run from two to fifteen. It may be varying with the stage in life history. A mutant cell may or may not have the power to establish itself or its progeny as leaders or partners in the shoot apex. This would depend very much on the extent of inviability associated with the genetic nature of mutations (CUANY, 1960).

Irradiation of a multicellular tissue or organ results in a chimera of mutated and unmutated cells. There would then ensue a competition between mutated and unmutated cells and also among mutated cells within a tissue. This competition between cells in a developing tissue or organ following irradiation is termed intrasomatic selection. The existence of this phenomenon was first recognized by FREISLEBEN and LEIN (1943). It was termed intra-individual selection or intrasomatic selection by KAPLAN (1951). GAUL (1957), who did most work on this phenomenon, suggested the term diplontic selection for this.

GAUL (1959, 1964), working with barley, found that the first five culms possess a greater mutation frequency than those arising later, and that with increasing dosage, the size of the mutated M_1 spike

sectors also increases. With excessive tillering, the mutation frequency was lower, but mutated chimeras, if and when formed, were found to include entire and sometimes even more than one spike.

Histogenetical studies have shown that the embryo in a barley seed has in it the initials of the main culm and first four tillers. GAUL (1959, 1964) proposed a constancy in the number and function of L_{II} initial cells which are the ones that give rise to the generative tissues. Their number is about or exactly four, and they are already present for the first five culms in dormant barley seeds. These initial cells are genetically independent. Consequently, there is a greater chance for mutations to appear in the first five tillers, since intrasomatic selection is restricted to these four initial cells only in each of them. The dose-dependence of sector size was suggested to be due to the greater killing of initial cells with increasing dosages. With higher dosages, more and more spikes originate from three, two and finally even from a single cell, which then result in a corresponding increase in size of mutated spike sectors. At the same time, the initial cells of spikes arising after the first five spikes are not yet differentiated at the time of seed irradiation. They belong to a larger cell complex, and consequently, there will be greater cell competition, and this would lead to fewer mutated cells in ontogenetically later-formed ones. Though, GAUL's explanation would appear to be rather simple a model to explain this phenomenon, these general principles have been found to hold good in all cases where such studies were made. Some of these are SWAMINATHAN (1961) in wheat and cotton, EHRENBERG, GUSTAFSSON and LUNDQVIST (1961) in barley, BEKENDAM (1961) and YAMAGUCHI (1962) in rice, and, D'AMATO et al. (1962) and D'AMATO (1964) in durum wheat. To overcome the intensity of this selection process, GAUL suggested close planting of M_1 seedlings to reduce tiller number, and egg cell or zygote irradiation.

Several other workers, notably in Sweden, have also examined this problem in seed-propagated plants and suggested measures for overcoming this elimination process. For example, EHRENBERG et al. (1953) found that seed irradiation in a nitrogen atmosphere did not result in any detectable cell elimination, whereas an oxygen atmosphere accentuated it drastically, and further that elimination processes were easily influenced by various other processes such as presoaking, oxygen pressure, temperature and dose. MACKEY (1951), and NYBOM, GUSTAFSSON and EHRENBERG (1952) observed that its intensity was reduced after neutron irradiation. Contrary to the common assumptions, chronic irradiation did not help in overcoming intrasomatic selection in barley (NYBOM et al. 1956) and Antirrhinum (CUANY et al., 1958).

Several workers (CUANY, 1960; NYBOM, 1960; GAUL, 1964) have proposed that irradiation at game-

tic, zygotic or embryonic stages could be expected to eliminate intrasomatic selective forces. The earlier work has been reviewed by MERICLE and MERICLE (1961) and BREWBAKER and EMERY (1961). The former workers observed considerable differences in radiosensitivity of developing embryos. Their studies indicated that even as late as at mid-proembryo stages, a single cell may be giving rise ultimately to all the above-ground portions of the plant. GAUL (1964) irradiated barley spikes at meiotic prophase, three-nuclei pollen stage and soon after fertilization. Though these did not lead to very high mutation rates, he did observe that for comparable M_1 sterility, irradiation of egg cells and zygotes led to higher chlorophyll mutation rates. However, more studies are required on this aspect.

To understand the operation of intrasomatic selection in vegetatively propagated plants, we have to examine their modes of origin and the structure of vegetative organs. Here, we will consider only the higher plants. The vegetative organs include a whole range of structures as underground stems — like rhizomes (eg: ginger), tubers (potato), bulbs (onion) and corms (amorphophallus) —, subaerial stems — like runners (oxalis), stolons (colocasia), offsets (pistia) and suckers (bananas) —, adventitious buds (rose, bryophyllum) and bulbils (yams). The organs of economic use may be the same as the vegetative organs (eg: potato, onion) or different (eg: bananas, most ornamentals and fruit plants). This is also important, as we would see later.

The structure of vegetative organs in asexually propagated plants is also very variable and complex. Let us take the case of the potato as an example. It is propagated by tuber. This is an underground stem. Each tuber has many eyes — usually about five to ten — with each eye capable of producing normally three sprouts. The tubers are formed at the tip of stolons which are themselves produced by the growth of buds present in the axils of leaves. The apical meristems of axillary buds trace back to the L_I and L_{II} layers of the mainstem (SUSSEX, 1955). These two layers contain six and two cells, respectively and they are genetically independent (HOWARD, WAINWRIGHT and FULLER, 1963). To bring about an improvement, changes will have to be induced in both the layers. With the multiplicity of eyes and sprouts in a tuber, and of axillary meristems in each sprout, with the double layered nature of apical meristems and also the multicellular structure of these two layers, this would indeed be a very formidable task. In contrast with these is the situation in a cereal crop like rice where the entire panicle (which carries both seed and grain) is produced by the L_{II} layer only. It is true that a dormant seed contains initials of five tillers with each initial containing two to four cells. But then, even if only one of these cells is mutated, it could produce a 25% mutated sector in a tiller, and with about 150 spikelets per

panicle, it should not be very difficult to pick out a mutant plant in the R_2 generation.

Other difficulties also add to the problem. Between harvest in one season and planting in the next, a tuber, unlike a dormant embryo in a seed, undergoes morphogenetic development, and considerable cell division and differentiation go on during this period. Depending on storage conditions, the number and development of sprouts will vary tremendously. It is known that differentiated tissues show much variations in radiosensitivity and that consequently the problems of selection also become correspondingly more complicated (NYBOM, 1960; MERICLE and MERICLE, 1961).

These would show how much more complex is the problem of intrasomatic selection following mutagenic treatment in vegetatively propagated plants.

Several German workers have paid attention to the problem of recovery and isolation of induced mutations in this group of plants. The best known work is that of BAUER (1957) in black currants. His method consisted essentially of pruning back the first primary shoot coming from the irradiated buds to the originally irradiated stock and thereby forcing new shoots to develop, when a certain proportion of these were seen to show mutational changes. These second year shoots were then removed, transplanted and new shoots forced to develop again. This process was repeated for several years, and after four years of selection, 324 mutated plants were obtained from 343 originally irradiated shoots. This work has shown that with suitable procedures, it should be possible to obtain encouraging results in vegetatively propagated plants also. BAUER (1957) himself concluded that "this method may be useful for all those crop plants in which dormant buds build up a root stock from which shoots are regenerating for several years and in which X-irradiation tests so far seemed to be ineffectual or gave only small rates of sporting". This method has been used successfully in other fruit trees and ornamentals also (ZWINTZSCHER, 1959; GRÖBER, 1959; JANK, 1957).

It was in this background that radiation genetical work was initiated in the potato at the Central Potato Research Institute three years back. Only little work has been done in this crop plant so far and our first step was to study the response of the potato to gamma radiation under different conditions. We have found that lower dose rates, fractionated dosages and irradiation of physiologically dormant tubers result in higher germination (NAYAR and PUSHKARNATH, 1966).

In another experiment, half tubers were irradiated and the other half kept as control — and set to produce successive crops of sprouts. The number of tubers producing sprouts was about the same as when tubers were planted as such, but the recovery of sprouts — that is, the number of sprouts per tuber — was considerably improved with low inten-

sity and fractionated treatments (NAYAR and DAYAL, 1967).

Observations were also taken on the branching pattern of sprouts (NAYAR, 1966). They were either unbranched or branched. When branched, these came either from the base, or from above as in normal ones. Most of those that branched from the base were of the twinned or forked type resulting from a bifurcated nature of branching. About 60% of first-emerging branches were unbranched and 20% each base-branched or above-branched. In the second-emerging sprouts 34% were unbranched and about 48% base-branched. There was a progressive increase in the proportion of unbranched sprouts and a corresponding decrease in that of base-branched sprouts from the third-emerging sprouts until the seventh, when their proportions were again about 60% and 20%, respectively. The proportion of above-branched sprouts remained around 20% throughout. We couldn't take observations beyond the seventh sprouts, as the number of tubers giving more than seven sprouts was very low. A more interesting observation was that when we considered the last-emerging sprouts, 247 of them were unbranched and only one base-branched and two above-branched. In control tubers, 94 sprouts were unbranched and one above-branched. The branching behaviour of sprouts produced by control tubers followed approximately a similar pattern irrespective of their sequence of origin. In them, about 65% sprouts were unbranched, 24% above-branched and 6% base-branched. Sprouts with twinned or forked branching were only rarely seen in them.

A twinned or formed branching indicates that there was a major chromosomal aberration in the apical cell and this forced the two adjacent cells to develop into shoots (BISHOP and AALDERS, 1955; MERICLE and MERICLE, 1961). One would also expect the first one or two sprouts originating from a tuber to be non-mutated and consequently unbranched. This is because non-mutated apical meristems can be expected to multiply and develop into shoots faster than mutated ones. This situation was also realized in a majority of cases. But there was a good number of branched sprouts also among them. The potato tuber shows strong apical dominance and hence a good many of the damaged sprouts would also have managed to emerge first in the competition with the unaffected sprouts, if these were the ones to emerge first consequent on the operation of apical dominance. It would also appear that the unbranched sprouts emerging towards the end would be the ones to be looked for potentially useful mutant types. This can, however, be confirmed only by genetic studies in the vM_2 generation. This has since been found to be true (unpublished data).

The method described above of taking successive crops of sprouts from irradiated tubers and growing them appears to be a satisfactory technique for

obtaining mutant types from irradiated vegetative organs containing several primordia. Some of the other methods which could be profitably tried for overcoming intrasomatic selection, as would be shown by a higher recovery of viable mutant types, are use of chronic or subacute irradiation, sprout irradiation, recurrent irradiation, neutron irradiation and chemical mutagenesis (NAYAR, PUSHKARNATH and HAKIM, 1965). Work has been taken up on these lines.

And, before I conclude, I may make a reference about one of the most exciting developments in biology in recent years. I am referring to the success obtained in the growth and development of isolated somatic cells *in vitro* into entire plants as has been done by STEWARD in carrots and others (cf. CUTTER, 1965). They have proved the totipotency of somatic cells. This means that every cell in an organism possesses the inherent capacity to develop into a whole plant of that genotype. We might as well consider the possibilities of isolating single cells from irradiated embryos or meristematic tissues and using them for developing whole plants. If we can adopt this technique, say, with as much facility as embryo culturing, we may then have gone a long way in at least minimizing — even if not overcoming — the effects of intrasomatic selection following seed or vegetative tissue irradiation.

Zusammenfassung

Bei Bestrahlung von Samen und vegetativen Geweben konkurrieren in den meristematischen Geweben letal und subletal geschädigte Zellen einerseits und unbetroffene Zellen andererseits, wobei die letzteren überwiegen. Diese Feststellung wurde zuerst von FREISLEBEN und LEIN (1943) gemacht und durch KAPLAN (1951) als intrasomatische Selektion und durch GAUL (1957) als diplontische Selektion bezeichnet. In der vorliegenden Arbeit sind die an samenvermehrten Pflanzen vorgenommenen Untersuchungen dieses Phänomens zusammengestellt mit den vorgeschlagenen Methoden zur Verminderung dieser Erscheinung.

Die im allgemeinen wenig ermutigenden Ergebnisse, die bisher bei vegetativ vermehrten Pflanzen erzielt wurden, sind einem verstärkten Auftreten der intrasomatischen Selektion als Folge der komplexeren Art der apikalen Meristeme und der Fortpflanzungsorgane bei dieser Pflanzengruppe zugeschrieben worden. ZWINTZSCHER (1959) und BAUER (1957) haben durch ihre Arbeiten an Äpfeln bzw. Schwarzen Johannisbeeren gezeigt, daß durch eine entsprechende Behandlung des bestrahlten Materials eine Ausschaltung dieses Effektes möglich ist.

In diesem Zusammenhang wird über die Bestrahlungsuntersuchungen des Verfassers an Kartoffeln berichtet. Höhere Überlebenswerte wurden durch geringere Bestrahlungsintensität, durch Dosisfraktionierung und Bestrahlung physiologisch ruhender

Knollen erreicht. Eine Isolierung und Aufzucht von Keimen bestrahlter Knollen ergab eine erhebliche Steigerung der Mutationshäufigkeit in den Nachkommenschaften. Andere zur Verminderung der Wirkungen der intrasomatischen Selektion vegetativ vermehrter Pflanzen vorgeschlagene Methoden sind Dauerbestrahlung, Bestrahlung mit Neutronen, chemische Mutagenese und schließlich Mutagenese isolierter einzelner Zellen und ihre Aufzucht als ganze Pflanze.

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