The development of (new) *in vivo* and *in vitro* techniques of significance for mutation breeding of vegetatively propagated crops*

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Abstract

Mutation breeding in vegetatively propagated plants is of great potential value 1) to improve the leading results of cross-breeding by altering one or a few important characters, without the rest of the genotype, 2) to induce variability where none is existing or difficult to be introduced in highly developed species and 3) to induce variability in sterile crops or in apomicts.

One of the main stumbling-blocks is the chimera formation following the irradiation of the multicellular apices in buds and the subsequent prolonged time and increased labour needed before a mutation can be detected, recovered and compared with the existing cultivars. This problem can be solved by producing plants, ultimately originating from one mutated cell, resulting in solid mutants.

The *in vivo* adventitious bud technique, using detached leaves, has proven its value for mutation breeding. It has been demonstrated in several species that commercial results can be obtained in a relatively short time. Experiments are underway to study the factors which control the process of adventitious bud formation and to make more crops accessible to this method. So far, however, with little success.

Many and increasingly more crops can be propagated clonally by *in vitro* methods, using plant parts (explants of leaves, flowers, flower stalk), callus or other plant material. In some cases it is expected that adventitious plantlets also will originate from one cell. In other cases it is to be investigated which method is of potential value for being used in a mutation breeding programme.

In a cooperative project (C. Broertjes, S. Roest and Miss G.S. Bokelmann) it is under investigation which plant part (young flowerheads, flowerstalks and leaves) is to be preferred in *Chrysanthemum morifolium*. Preliminary results will be presented at the meeting.

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Introduction

Mutation breeding offers large possibilities in vegetatively propagated plants, such as potatoes, sweet potatoes, cassave, sugar cane, numerous fruit crops, forest trees, ornamentals, peppermint, and various apomicts (Poa; grasses). The main advantage is the possibility to improve one or a few important characters of an otherwise excellent cultivar, without basically altering the remaining genotype. Thus, outstanding cultivars, often being the result of a time-consuming and painstaking cross breeding programme, can be further perfected within a reasonable time-period. And it is the only way to induce variability in sterile plants and in apomicts.

In ornamentals an additional advantage is the fact that selection of visible changes generally offers no serious problems and a favourable change soon may lead to the commercialization of the mutants. This holds true also for visible changes in other crops, like fruit colour and spurtype in apples and pears, skin colour of potato tubers as well as growth pattern, size, form, and many other directly perceptible characters in various crops.

Most attention is paid, in this paper, to mutation (breeding) experiments in ornamentals because it serves as an excellent model to demonstrate techniques and possibilities and because most experience has been obtained, using these plants.

Methods

The main stumbling-block of mutation breeding in vegetatively propagated species is the phenomenon that the irradiation of multicellular apices in buds of plants, rooted cuttings, tubers, rhizomes, or bulbs results in the formation of chimeras. Since, moreover, a mutated cell is subjected to intrasomatic selection and may also get lost as a consequence of chimera formation and of the structure of the apex, the final result is a low frequency of mericlinal chimeras which by repeated pruning (fruit trees; Chrysanthemum) or repeated asexual propagation (potatoes, 2 or 3 years) have to be transformed into periclinal chimeras (so-called bud sports) before selection can be carried out.

In spite of these complications and the laborous procedures involved, a fairly great number of succesful mutation breeding programmes, resulting in commercial varieties, have been recorded. Most of them are ornamentals, such as an unknown, but probably numerous number of Chrysanthemum mutant cultivars, various Dahlia,

several Alstroemeria and a few carnation mutants (Sigurbjörnsson and Micke, 1969, 1974: see also: Induced Mutations in Vegetatively Propagated Plants, IAEA, Vienna, 1973: 179-220; Broertjes and Ballego, 1967; Broertjes and Verboom, 1974). But also mutants in fruit crops are increasingly reported, such as spurtypes and fruit skin mutants in apple, pear, cherry and others (Campbell and Lacey, 1973; Decourtye, 1969; Lapins, 1965, 1970; Visser, Verhaegh and de Vries, 1971). (We recommend again to consult also the articles, literature list and recommendations in Induced Mutations in Vegetatively Propagated Plants, IAEA, Vienna, 1973).

The difficulties related to chimera formation can be overcome by growing plants from single cells, in vivo or in vitro, which automatically would lead to a high(er) percentage of solid, non-chimeral mutants.

For the plant breeders a promising in vivo method is the adventitious bud technique, using detached leaves. This makes use of the phenomenon that (the apex of) adventitious buds, formed at the base of the petiole, ultimately originate from a single epidermal cell. This has been demonstrated in Achimenes (Broertjes, 1972a), Begonia (Doorenbos and Karper, in press), Kalanchoë (Broertjes and Leffring, 1972), tobacco (de Nettancourt et al., 1971), Saintpaulia (Broertjes, 1968a and 1972b; Sparrow et al., 1960), Streptocarpus (Broertjes, 1969) as well as in Lilium and Peperomia (Broertjes, unpublished). In Achimenes and Streptocarpus hundreds and in Begonia even many more solid, non-chimeral mutants have been produced. Several of these, generally completely stable mutants, were introduced into commerce in no more than approx. three years after the very beginning of the project; in terms of plant breeding a real short cut.

Many plants can be propagated from adventitious plantlets on detached leaves. Broertjes, Haccius and Weidlich (1968) list over 350 species, covering a variety of families, reported in the literature to belong to that group. This does not mean that plants not listed cannot be propagated that way; many have been tried without success but many more have never been tried.

The breeder therefore should, with today's-knowledge, always make an attempt with the cultivar(s) he is interested in. Many variables, however, play a role, such as the rooting medium, the use of plant hormones to promote rooting (if necessary), environmental conditions, leaf-factors (age, position, influence petiole, length (monocots), etc. Broertjes and Leffring, 1972; Roest and Bokelmann, in press). In bulbcrops modified leaves (bulb-scales) are widely used. In Lilium exclusively solid, non-chimeral mutants are obtained when bulb-scales are irradiated,

immediately after scaling (Broertjes, unpublished). The use of wounded bulbs (Fracinsky) or of artificial bulb-scales may also result in solid mutants.

In vivo and in vitro experiments with Chrysanthemum morifolium.

(a cooperative project of C. Broertjes, S. Roest and Miss G.S. Bokelmann).

Chrysanthemum has been selected for studying in vivo and in vitro propagation methods and their value for mutation breeding since detached leaves produce (few) adventitious shoots after rooting and because it was expected that in vitro propagation, using various explants, could be developed within a reasonable time-period. Moreover, the plant is easily handled and propagated whereas flower induction under short day conditions can be carried out all year. We mainly used the pink flowered cultivar 'Bravo', of which numerous flower colour mutations can be produced (Jank, 1957), in order to be able to decide whether or not a mutant has a chimeral structure. Furthermore it was known that cv. Bravo occasionally develops adventitious plantlets on rooted leaves (most cultivars produce fewer plantlets or no plantlets at all).

Adventitious shoot formation in vivo could be improved by using leaves of stockplants grown in the greenhouse, instead of leaves from stockplants grown in growth chambers. Moreover, a mineral nutrition with 'pokon' (N-, P- and K-components) turned out to be favourable for the production of adventitious shoots. Under the most optimal conditions 100 % of the leaves developed adventitious shoots within a period of 2-4 months after leaf excision and with an average of 3-4 adventitious shoots per leaf.

In vitro, the cv. Bravo regenerated adventitious shoots on explants of the flower-head, the leaf and the flower-stalk. Flower-head and leaf-explants developed, usually via callus, the first adventitious shoots 3 weeks after incubation, whereas explants of the flower-stalk yielded a direct regeneration of the first adventitious shoots, which emerged over the whole length of the explant, 10 days after incubation. The development of the shoots was almost completed 2-3 months after incubation in vitro. Shoots with a length of at least 0.3 cm were then excised from the explants (up to 85 shoots at one explant!) and transferred to induce root formation. Adventitious roots were initiated within 2 weeks after transfer and thus complete plantlets were produced in approximately 3 months...

The next step was to grow adventitious plantlets, in vivo and in vitro, from irradiated material. Just mature, detached leaves were

irradiated with 500 rad X-rays and then rooted. The rooted leaves were potted 3 weeks later and started to produce the first adventitious shoots approx. 3 months after potting. During a few months all shoots were cut off, after having reached a certain size, rooted and potted. When the lateral shoots on the adventitious plant(s) on the mother-leaf bud reached a length of approx. 15 cm they were brought under short day conditions together with the rooted cuttings (by taking cuttings and thus forcing the original adventitious plantlet to produce side-shoots it was expected to obtain more information about the possible chimeral situation of the adventitious shoots). The results were very complex and confusing and attempts to order the data in such a way that a clear picture of the process of adventitious bud formation could be obtained, failed.

Of the approx. 400 adventitious plantlets, produced on 247 irradiated leaves (125 leaves produced 1 shoot, 93 leaves produced 2 shoots, 25 produced 3 shoots, and four produced 4 and 5 shoots), a great number showed mutations: from (part of) a single floret to completely (looking) mutants. All kind of situations in-between could be observed, such as part of the inflorescence mutated, only one flower mutated whereas also mutations were scored in the original shoot (the rooted cutting) and not in the lateral shoots or vice versa. Of the 177 plants carrying a mutation, 73 were solid looking. But in a number of cases such a 'solid' mutant was a cutting taken from an adventitious plantlet which was not or only partly mutated and the reverse. With other words, the number of adventitious plantlets, carrying the mutation in both the original plantlet and its rooted top-shoot, was restricted and amounted to approx. 20 % of the total number of mutated adventitious plantlets.

It is clear that the <u>in vivo</u> production of adventitious plantlets on callus, which is developing at the base of the petiole of leaves of <u>Chrysanthemum</u>, is <u>not</u> the method of propagation which we are looking for to be used in mutation breeding. Such an <u>indirect</u> regeneration of plantlets is too slow and, since obviously more than one cell is involved in the formation of the apex of an adventitious plantlet, does not produce enough 'complete' mutants (the 'complete' mutants were not checked upon possible periclinal chimerism).

The explants used <u>in vitro</u> were irradiated with a series of X-ray doses to determine the radiosensitivity, the mutation frequency and thus the optimum dose. The preliminary results can be seen in Table 1 and can be summarized as follows:

1. The highest production of adventitious plantlets is obtained when flower stalk segments are used as compared to tiny leaves and flower heads.

2. The optimum dose lies around 800 - 1000 rad X-rays (No. of plantlets per explant; no. of mutants; mutation frequency).
3. No chimeras have been found.

Consequently, the results, so far obtained, look very promising. The next steps will be to repeat the experiment, using flower stalk segments, irradiated with approx. 800 rad X-rays. Furthermore it is under investigation, whether or not the mutants found really are solid.

What also can be seen in the table is that in many cases more than one phenotypically identical mutant plant were found, which always regenerated from the same explant. In a few cases, even all plantlets, regenerated on a given piece of explant, were identical mutants, 22 mutants per 22 plantlets being the most extreme case. It seems that a given cell very rapidly may grow out into a multi-apical meristem (especially when by radiation-damage the number of cells which are able to regenerate plantlets, is reduced?).

Table 1. Preliminary results of *in vitro* propagation of various types of (irradiated) explants of *Chrysanthemum morifolium* cv. Bravo.

Explant type Dose	% of uncontaminated explants producing adventitious shoots	Total no. of adventitious shoots ≥ 0.3 cm	No. of adventitious shoots per shoot-forming explant	No. of plantlets potted **	No. of mutant plantlets	No. of different mutant genotypes	No. of solid non-chimeral mutant plantlets
Tiny leaves							
control	86	132	11	43	-		
400 rad X-rays	77	58	6	30	1 (3.3%)	1 (3.3%)	1 (100 %)
600 " "	92	85	7	46	2 (4 %)	2 (4 %)	2 "
800 " "	60	29	5	26	9 (35 %)	1 (4 %)	9 "
1000 " "	27	47	12	38	19 (50 %)	2 (5 %)	19 "
1200 " "	40	15	4	11	2 (19 %)	2 (19 %)	2 "
Flower stalk segments							
control	100	580	30	161	-		
400 rad X-rays	100	470	28	179	-		
600 " "	100	390	22	157	8 (5 %)	3 (2 %)	8 (100 %)
800 " "	100	264	15	144	45 (31 %)	8 (5.5%)	45 "
1000 " "	94	144	10	74	29 (39 %)	6 (8 %)	29 "
1200 " "	100	110	6	74	-	-	
Flower heads *							
control	80	16	4	13	-	-	
400 rad X-rays	100	15	3	11	-	-	
600 " "	55	15	3	12	-	-	
800 " "	25	-	-	-	-	-	
1000 " "	40	5	3	2	-	-	
1200 " "	33	3	2	3	-	-	

* Figures not very significant because of high contamination

** Generally only part of the rooted shoots were potted

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