MUTAGENESIS EN FITOMEJORAMIENTO DE PLANTAS DE PROPAGACION VEGETATIVA

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RESUMEN. Las plantas vegetativamente propagadas están divididas en dos grupos: cultivos obligados a la propagación vegetativa y cultivos opcionalmente propagados en forma vegetativa. Para los cultivares estériles, triploides y los apomícticos obligados, las mutaciones son las únicas fuentes de variación. La principal ventaja del mejoramiento mutacional en las plantas propagadas vegetativamente es tener la posibilidad de cambiar uno o más características, que podrían ser las únicas características, sin cambio en la parte restante del genotipo. Uno de los problemas importantes del mejoramiento mutacional de este grupo de plantas es el quimerismo. Este es un resultado de aplicaciones de agentes mutagénicos en piezas multitécnicas de yemas adventicias. El agente mutagénico más comun para inducir una mutación en plantas propagadas vegetativamente es la radiación con rayos X o rayos gamma. Generalmente, el intervalo de dosis es entre 5 y 100 Gy, dependiendo de la especie, cultivar y el tipo de material inicial. Hasta ahora 480 cultivares mutados han sido obtenidos.

PALABRAS CLAVE: mutaciones, quimeras, mutaciones homogéneos, yemas adventicias, logros prácticos.

MUTATIONAL BREEDING OF VEGETATIVELY PROPAGATED PLANTS

SUMMARY. Vegetatively propagated plants are divided into 2 groups: obligate vegetatively propagated crops and facultative vegetatively propagated crops. For sterile cultivars, triploides and obligate apomicts, mutations are the only source of variation. The main advantage of mutation breeding in vegetatively propagated plants in the possibility of changing one or two characteristicas of an otherwise outstanding cultivar without changing the remaining part of the genotype. One of the important problemas of mutation breeding of this group of plants is chimerism. This is a result of treating with mutagens buds with multicellular apices composed of a number autonomus cell layers. One of the methods to overcome this problem is the adventitious bud technique. The most common mutagenic agents used for induction of mutations in vegetatively propagated plants is radiation. X or gamma rays. Generally doses range between 5 and 100 Gy, depending on the plant species, cultivar and kind of starting material. Until now about 480 mutant cultivars have been realased.

KEY WORDS: Mutations, chimeras, homogeneous mutants, adventitious buds, practical achievements.

INTRODUCTION

Vegetatively propagated plants are a specific group of plants. To this group belong fruit trees, many ornamental plants, root and tuber plants, some essential oil plants (e.g. mentha species), fiber plants, sugar cane, tea, several grasses etc. In this group of plants useful spontaneous mutants have been utilized since the beginning of plant domestication. Such mutants have been described in literature in XIX century (Carriére, 1865; Darwin, 1868). According to publications from the IAEA (International Atomic Energy Agency) in Vienna, more than 1300 cultivars, derived from induced mutations, were registered up to 1989 (Anonymus, 1989). In this number about 480 mutant cultivars have been realised in vegetatively propagated plants (Micke *et al.*, 1990), from wich more than 400 belong to the ornamental plants.

General statements

Vegetatively propagated plants can be divided into two groups. One is obligate vegetatively propagated crops (relatively few) for which no alternative to use of mutants as a source for genetic variation exists. The second one is facultative vegetatively propagated crops. To the first we can include apomicts like *Poa pratensis* (Kentucky bluegrass) or man- made sterile triploid hybrids like Bermuda grass *(Cynodon sp.)* and several

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ornamentals, as many cultivars of *Alstroemeria*. For several plants it was assumed in the past that they were obligate apomicts, but detailed investigations or expeditions to remote areas revealed the existence of plants with fertile flowers. Garlic *(Allium sativum)* is an example of such a case (van Harten, 1991). The second group contains many economically important plants like cassava, fruit trees, grasses, ornamentals, pineapple, potato, sweet potato. Many of these are characterized by a high level of heterozygosity, often in combination with a high ploidy level, the presence of aneuploidy, sometimes a long juvenile period (fruit trees) and other factors which make conventional breeding difficult and time consuming. The aforementioned circumstances are less important from mutational breeding point of view or can be even advantageous in this respect. In vitro techniques have resulted in increasing numbers of species that can be propagated vegetatively, as a consequence of which interest in mutational breeding techniques for such plants has increased.

In some cases, e.g. in sterile triploid cultivars or obligate apomicts, mutations are the only source of variation. However, the main advantage of mutational breeding in vegetatively propagated plants is the possibility of changing one or few characteristics of otherwise outstanding cultivar without changing the remaining and often unique, part of genotype.

There are some problems of mutation breeding, specific for this group. The absence of "meiotic sieve" in vegetatively propagated plants may result in cells with damaged chromosomes; these cells despite this damage may remain in the tissue. As the consequence bad- functioning plants (physiological damage) may be produced. Useful mutations may be accompanied by unfavorable side-effects, either caused by close linkage of such traits or pleiotropic effects. This cannot be corrected in vegetatively propagated plants. The only solution is to try induce many independent mutants of the desired type and select for genotypes with the lowest level of undesired side-effects. A practical problem in discerning mutations in vegetatively propagated plants is that often virus diseases occur, which sometimes are difficult to distinguish from mutagenic effects and which always cause loss of plant material.

Chimeras and stable mutations

One of the most serious problems to solve is how to manipulate chimerism. Early examples of chimerism refer to so-called artificial graft chimeras of two species, such as in the case with so-called Bizzarria-orange, consisting of two species, which is probably a periclinal chimera (sour orange + citron) and known since 1644; and the chimeric combination of *Laburno-cytisus* and *Crataego-mespilus*. In the 3 cases a mantle of tissue of one partner surrounds a core with tissue of the other partner. This is the original meaning of the word "chimera" in plants. In later times the expression chimera was used for all stable combinations of two or more genetically-different somatic tissues.

The most desired situation is homogeneous mutation, when plants are completely mutated. A stable situation also arises when periclinal chimeras occur. In that case all cells within one layer (or 2 layers) are mutated. This periclinal condition is reached after one or a few cycles of vegetative propagation, starting from a so-called mericlinal chimeras, when plant carries a mutated area (originally even only one mutated cell) within a non-mutated cell layer.

Many commercial mutants of vegetatively propagated plants are periclinal chimeras, like in fruit-trees (e.g. large group of cultivars belonged to "Red Delicious" family) or ornamentals (e.g. many cultivars of *Chrysanthemum)*. Due to different types of stress, like variation in temperature etc. in stabilised cultivars reversions appear to the mother form. A chimeric situation can never be accepted in a cultivar for characters like resistances against diseases or for characters, for which screening is difficult. For flower colour mutants, periclinal chimeras are sufficiently stable from the point of view of practical cultivation and much easier to obtain than homogeneous mutants.

The disadvantage of periclinal chimeras is, that in many cases, especially in fruit trees, they cannot be used for cross- breeding, if mutation did not occur in L-2 layer, responsible for production of gametes. They cannot be also propagated true-to type by adventitious buds.

Adventitious buds

The use of newly formed buds, indicated as adventitious buds, which arise on places which were not predestinated for bud development, is much interest to the breeder of vegetatively propagated plants. In this way mutations, induced in plant tissues outside the apical area, can be utilized. The resulting adventitious plants may trace back to a few or even only one initial cell, hence, may show large mutated areas or even be homogeneous mutants.

It was reported in 1960 by Sparrow *et al.* that, when plants of the African violet *(Saintpaulia ionantha)* were propagated by detached leaves, new plantlets may derive from single epidermal cells at the petiole base. In this way solid, homogeneous mutants can be obtained after mutagenic treatment of leaves. Similar results were reported by Przybyla *et al.* (1974) when apple and cher-

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ry root cuttings were treated with gamma rays and chemical mutagens, as EMS, NMH, etc.

Plant material for mutagenesis

Various plant parts can be used for vegetative propagation. They are either already present in nature, like tubers, bulbs, rhizomes, apomictic seeds or stolons, or methods are developed by man, which can be either in vivo methods, like the use of stem- or leaf cuttings, or in vitro methods, with individual cells or various types of tissue culture. Genetically heterozygous starting material is preferable (Aa in diploids, or Aaaa in tetraploids), as most mutations are recessive, whereas dominant mutations seldom occur.

Starting from the tubers, bulbs, rhizomes, cuttings, leaves or leaflets, most mutation induction work has been performed with gamma or X rays. Radiation doses differ considerably, depending on plant species, cultivar, the kind of starting material, e.g.for rhizomes of Alstroemeria optimal doses of gamma rays are at range 3-7 Gy (Przybyla I992), for apples and apple rootstocks at the dormant stage 25-50 Gy (Przybyla *et al.,* 1989). Generally dosages range between 5 and 100 Gy (van Harten, I991).

On the basis of many experiments it can be suggested, that the best results give low dosages, lower than LD_{50}. It results of course in the induction of relatively lower mutation frequencies than after heavier treatments. But the advantage of low dosages is that they cause relatively less chromosomal damage and other negative side effects. Such effects in vegetatively propagated plants are difficult to get rid of because of the absence of a meiotic system, which could sort out such aberrations.

The use of chemical mutagens in vegetatively propagated material is complex, because of problems with dosimetry and uptake of chemicals. Treatments could be performed for instance by submersion of petioles of detached leaves in a solution of mutagen, by submersion of root cuttings in a solution of mutagen (Przybyla *et al.,* I974), by deposing a cotton plug with mutagen on axillary buds of whole plants, by using of vacuum pump method (Przybyla *et al.,* I988), by injection by means of syringe, etc. The chemical mutagens used were e.g.EMS,EI,NMH, sodium azide (Przybyla *et al.,*I974,I988).

Further steps and practical achievements

Alstroemeria is one from the good example of succesful breeding work. Mutational breeding stared in Poland in I980 (Przybyla, I982). In practice, actively growing rhizomes were irradiated with 3-7 Gy of gamma rays from ^{60}Co source in March. On the base of many experiments (Przybyla, I992) this range of doses appeared optimal for induction of useful mutations and this season of irradiation the most optimal for further plant development. Several doses were applied, on several dozen of young plants per dose as well mutants as hybrids. After irradiation plants were divided in cuttings containing few buds. In most cases irradiated plant material was observed for not less than two years. Selection was performed during flowering. Mutants were isolated by cutting them from rhizomes, followed by observation and further selection in the next flowering period. Mutants with very attractive flowers or other interesting features were cloned. Stable clones were planted on beds in the glasshouse for evaluation of their productivity and usefullness for production (Przybyla, I992). From the many mutants obtained in this way, seven have been registered and commercialized (Przybyla, I992,I994).

The other example of mutation breeding of vegetatively propagated plants, other than ornamentals, are fruit trees. Mutation breeding started in Poland in the sixties (Zagaja, Przybyla, I973). Dormant scions of the most important commercial cultivars apples, sweet- and sour cherries and vegetatively propagated rootstocks were irradiated in March with gamma rays from ^{60}Co source or with fast neutrons (Przybyla, Huczkowski, I978). Per treatment 100 or more dormant scions from each cv investigated were irradiated yearly.25 Gy, lower than LD_{50} ,appear to be very efficient dose. The length of irradiated shoots was 50 cm. After irradiation 3 bud scions from the center of each shoot were grafted on the rootstocks. Selection was performed on MV_1 shoots at the end of vegetation, for 3 years. All MV_1 shoots were measured and compact shoots, with shortened internodes were selected. Each compact shoot was cloned on the rootstocks. On MV_2 the next selection was performed. Stable clones were budded on the rootstocks in the summer and the last selection was performed on MV_3 plants. Trees of stable, compact mutants were planted in the orchard for estimation of their value in comparison with trees of standard cvs, and dwarf mutants of rootstocks were planted in the nursery for estimation of their practical value (Przybyla *et al.*,I989).

The highest percent of mutations was obtained in the second year after irradiation. Since third year a decrease of mutations was observed. Also root cuttings were used for irradiation and treatment with chemical mutagens (EMS,EI,NMH) to obtain homogeneous mutants (Przybyla *et al.*,I974).

Results of commercial mutational breeding in several vegetatively propagated plants are presented in table 1.

TABLE 1. Vegetatively propagated plants, in which commercial mutants were obtained till 1993

Group of crop	Name of plant	Number of the commercial mutants	Kind of mutagen used	Dose
1	2	3	4	5
I. Root and tuber crops	Potato-**Solanum tuberosum**	1	X- rays	30 Gy
II. Ornamental plants				
a. tuber and bulb crops	Dahlia-**Dahlia variabilis**	34	X- rays (18 cvs) gamma (16 cvs)	10-40 Gy 20-30 Gy
	Gladiolus	1	X-rays	40-70 Gy
	Lilium	2	X-rays	2.5 Gy
	Tuberose-**Polyanthes tuberosa** L.	2	gamma	20 Gy
	Tulipa	8	X-rays	2.5-3.5 Gy
b. flowering pot plants	Achimenes	8	X-rays (7 cvs) fast neutrons (1 cv)	20-40 Gy 10 Gy
	Begonia			
	-**Begonia elatior**	19	X-rays (5 cvs) gamma (11 cvs)	15-25 Gy 25 Gy
	-**Begonia rex**	2	gamma	100 Gy
	-**Begonia masoniana**	4	gamma	10-100 Gy
	Bougainvillea	5	gamma	2.5-10 Gy
	Bromeliaceae-**Guzmania peacockii**	1	gamma	33 Gy
	Calathea-**Calathea crocata**	1	X-rays	9 Gy
	Chrysanthemum	7	gamma	17.5
	Hoya-**Hoya carnosa**	4	X- or gamma rays	50 Gy
	Kalanchöe	3	X-rays	10-30 Gy
	Rhododendron			
	-**R. simsii (syn. Azalea indica)**	12	X-rays (3 cvs) X + gamma (8 cvs) gamma (1 cv),	20-25 Gy
	-**R. obtusum**	1	X-rays	15 Gy
	Saintpaulia-**S. ionantha**	1	gamma	20-30 Gy
	Streptocarpus	31	X-rays (26 cvs) gamma (4 cvs) colchicine (1)	30 Gy
c. foliage pot plants	Ficus-**F.benjamina exotica**	2	X + gamma rays	25 + 20 Gy
d. cut flowers	Alstroemeria	31	X-rays (24 cvs) gamma (6 cvs) fast neutrons (1 cv)	3.4-5 Gy 3-7 Gy
	Carnations-**Dianthus caryophyllus**	12	gamma (2 cvs) X- rays (7 cvs) EMS (3 cvs)	50 Gy 80 Gy 2.5 %
	Chrysanthemum	several hundreds	X-rays gamma	10-25 Gy 17.5 Gy
	Euphorbia-**E.fulgens**	1	X-rays	40 Gy

Group of crop	Name of plant	Number of the commercial mutants	Kind of mutagen used	Dose
1	2	3	4	5
	Roses	24	gamma (10 cvs) X-rays (13 cvs) EMS (1 cv)	30- 100 Gy 80 Gy 0.25%
e.vegetatively propagated garden plants	Portulaca	11	gamma	10-40 Gy
f. woody plantas	Abelia (ornamental shrub)-**A. grandiflora**	1	gamma	30 Gy
	Forsythia	2	gamma	70 Gy
	Malus (ornamental)	1	X-rays	30-35 Gy
	Weigela	3	gamma	40-50 Gy
III. Woody perennials and forest trees	Populus	1	gamma	0.5- 1.5 Gy
	Mulbery-Morus	1	gamma	
IV. Fruit crops				
a. fruit trees	Apple	6	gamma (5 cvs) EMS (1 cv)	50 Gy 1 %
	Apricot	1	thermal neutrons	
	Fig-**Ficus**	1	gamma rays (?)	50-70 Gy
	Olive-**Olea europea**	1	gamma	40 Gy
	Peach	2	gamma,	
	Pomegrante-**Punica grantum** L.	2	gamma (?)	50-70 Gy
	Sweet cherry **Prunus avium** L.	5	X-rays (3 cvs) gamma (2 cvs)	40 Gy
	Sour cherry **Prunus cerasus** L.	4	X-rays (1 cv) gamma (3 cvs),	
b. small fruits	Black currant	2	X-rays (1 cv) gamma (1 cv)	15 Gy
c. tropical fruits	Citrus	2	thermal neutrons (1 cv) gamma (1 cv) 100 Gy	
	Papaya	1	gamma (?)	
V. Other crops				
a. essential oil crops	Mentha	3	X + neutrons (1 cv) neutrons (1 cv) gamma (1 cv) 195 Gy	
b. fiber crops	Cyperus (Chinese mat grass) -**C. malaccensis**	1	gamma	1150 Gy
	Juncus (mat rush) -**J. decipiensis**	2	gamma	680 Gy
c. sugarcane	Sugarcane	3	gamma	
d. grasses	Bermuda grass -**Cyndon dactylon x C. transvaalensis**	1	gamma	90 Gy
	Centipede grass -**Eremochloa ophiuroides**	1	gamma	300-400 Gy
	St. Augustin grass -**Stenotaphrum secundatum**	1	gamma	58.3 Gy

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