Monographs on
Theoretical and Applied Genetics 7

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With 42 Figures and 45 Tables

Springer-Verlag
Berlin Heidelberg New York Tokyo 1983

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ISBN 3-540-12184-6 Springer-Verlag Berlin Heidelberg New York Tokyo
ISBN 0-387-12184-6 Springer-Verlag New York Heidelberg Berlin Tokyo

Library of Congress Cataloging in Publication Data. Gottschalk, Werner. Induced mutations in plant breeding. (Monographs on theoretical and applied genetics; 7) Bibliography: p. Includes index. 1. Plant mutation breeding. I. Wolff, G. (Gisela), 1943–. II. Title. III. Series. SB123.G67 1983 631.5'3 83-357

Offsetprinting and bookbinding: Brühlsche Universitätsdruckerei, Giessen
2131/3130-543210

Preface

Mutation breeding has been introduced into modern plant breeding in the early 1940's. In spite of pessimistic predictions, the application of experimental mutagenesis has led to encouraging results demonstrating that mutation breeding is a well-functioning method in many crops. So far, more than 500 varieties, developed by means of induced mutations, have been officially released; others have been approved for registration. Many mutants with characters of agronomic interest cannot be utilized directly because of their unsatisfying yielding capacities, or of other negative traits which are partly due to the pleiotropic action of the mutant genes. Sometimes their negative selection value can be overcome by transferring them into the genomes of other varieties. According to experience available, the efficiency of mutant genes can considerably vary depending on the genotypic background in which they become effective. The interactions between mutant genes and genotypic background cannot be predicted. Therefore, mutants with valuable traits should be crossed with many varieties and strains in order to discern positive and negative interactions. In this way, genotypes can be selected in which the mutant gene is able to express its action without showing negative by-effects. This procedure has been used for about 10 years by combining the methods of mutation and crossbreeding.

Mutation breeding is predominantly used in annual diploid and allopolyploid self-fertilizing crops, while it causes much more difficulties in cross-pollinating species. Especially rapid results have been obtained in ornamentals, because not only generative but also somatic mutations can be utilized in this material. Of special interest is applied mutagenesis in that small group of crops which are unsuitable for crossbreeding because of full sterility. In these cases, induction of mutations is the only way to increase the genetic variability within a short period.

A particularly intensively studied field of applied mutagenesis refers to the alteration of seed proteins, to some extent also of other storage substances. In spite of immense expenditure of time and work, the practical success with regard to the selection of "protein mutants" and related genotypes is small. The initiation of international research programs, however, has led to an immense broadening of our knowledge on the relations between gene action and metabolic processes. This holds true not only for this branch of mutation breeding. On the contrary, the application of experimental mutagenesis to crops in general

has provided a huge amount of basic information about the behavior of mutant genes, which is of interest for plant genetics in general.

Since 1940, thousands of papers have been published dealing with the various problems of applied mutagenesis. In the frame of the present book, it was not possible to discuss all the findings available in detail. We have considered the literature since 1965, but references on review papers are given containing older results. Moreover, references to plenty of additional information are given in the tables of the book.

Bonn, April 1983

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1 Introduction

The permanently increasing number of released or approved varieties, developed by means of mutant genes, demonstrates the effectiveness of the methods of applied mutagenesis in a convincing way (Fig. 1, Table 1). According to the mode of development, the agronomically utilized mutant material can be subdivided into three groups as follows:

1. Selected mutants with favorable properties are directly developed into varieties. This has preferably been done during the early period of mutation breeding; but even nowadays many mutant varieties derive directly from prospective mutants. This holds particularly true for those crops the breeding of which has not yet reached a high level. In such cases, the broadening of the genetically conditioned variability can be achieved relatively easily within a short period. Some of the new genotypes obtained may be of direct economic value. In general, however, this method proved to be not as successful as originally expected.

2. Prospective mutants are incorporated into crossbreeding programs in which the mutant genes are utilized indirectly. For the majority of the sexually propagated crops suited for mutation breeding findings are available, demonstrating thereby that the indirect use of mutant genes may be more propitious for reaching distinct aims of plant breeding than their direct use. This method is widely used, preferably in cereals. Impressive examples for the potentialities of this method are the development of a relatively large number of commercial *barley* varieties from a very small number of mutants in Sweden and Czechoslovakia. Many mutant genes of different crops are just being transferred into the genomes of varieties or strains, and the genotypes selected are tested with regard to their agronomic usefulness. There is no doubt that many of them will lead to new cultivars in near future. Findings are available in *bread* and *durum wheat, rice, peanuts, peas,* and *tomatoes* among others.

3. Different favorable mutants are crossed with each other in order to combine specific mutant genes in recombinant lines. This method is only used to a small extent at present. It will preferably be used in those crops in which collections with hundreds or even thousands of mutants are already available, i.e., in some cereals and pulses.

Abbreviations: EMS, ethyl methane sulfonate; MMS, methyl methane sulfonate; DES, diethyl sulfate; DMS, dimethyl sulfate; EI, ethylene imine; EO, ethylene oxide; HA, hydroxylamine; MH, maleic hydrazide; ENH, NEU, ethyl nitroso urea; MNU, NMU, N-methyl-N-nitroso urethane or urea, respectively

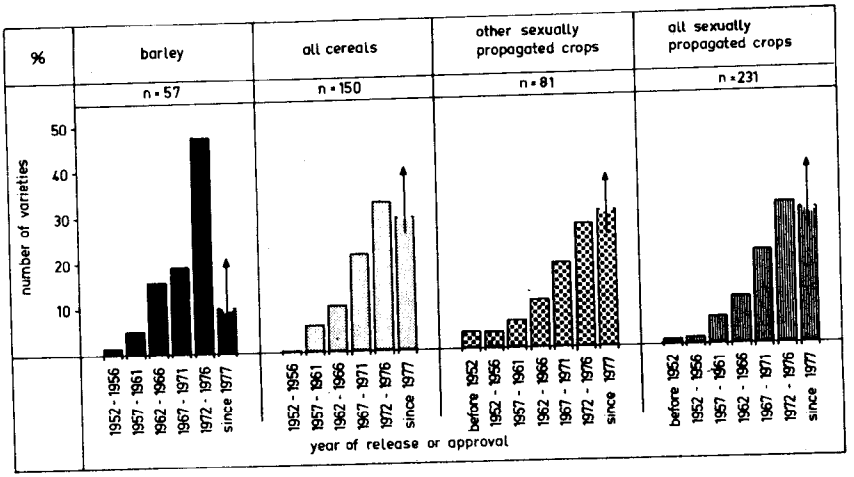

Fig. 1. Increase of the number of mutant varieties during the past decades.
Twenty-one released mutant varieties of sexually propagated crops are not considered in this graph, details of which have just been published in Mutation Breeding Newsletter 19:14–19, 1982. They belong to the following species:

– *Triticum aestivum:*	6 varieties	– *Glycine max:*	1 variety
– *Hordeum vulgare:*	4 varieties	– *Lupinus luteus:*	1 variety
– *Oryza sativa:*	2 varieties	– *Lycopersicon esculentum:*	1 variety
– *Zea mays:*	1 variety	– *Gossypium* sp.:	1 variety
– *Pisum sativum:*	2 varieties	– *Nicotiana tabacum:*	1 variety
– *Cicer arietinum:*	1 variety		

In spite of the positive results available, many geneticists and plant breeders are still very sceptical with regard to the effectiveness of the application of mutagenesis in plant breeding. It is true that only little success was had during a relatively long initial period. This is due to the fact that plenty of positive and negative experiences were necessary for learning to handle these methods in a promising way. Moreover, geneticists, plant breeders, phytopathologists, and biochemists had to be convinced of the usefulness of these methods; this needs time and patience. At present, about 500 officially released varieties, derived from experimentally induced mutants, are available. In many cases, the expenditure in time was considerably less than the time necessary for developing a variety by using conventional methods. The effectiveness of these methods becomes especially clear in some ornamentals from which whole groups of mutant varieties have been developed within a few years. All these findings and results demonstrate that the methods of applied mutagenesis are well suited for supplementing the conventional breeding procedures. This especially is valid for annual diploid and allopolyploid autogamous crops and for some vegetatively propagated species inclusive many ornamentals, whereas mutation breeding in allogamous species is only in its very beginnings.

Our knowledge on some fundamental aspects of the mutational process has been widened during the past years, but it is not yet clear, whether the findings obtained in

Table 1. Mutagens used for developing 485 released or approved varieties of different crops and ornamentals

Mutagens	Sexually propagated crops		Vegetatively propagated crops		All crops	
	Number of varieties	%	Number of varieties	%	Number of varieties	%
X rays	87	36.3	125	51.0	212	43.7
Gamma rays	94	39.2	103	42.1	197	40.6
^{32}P beta rays	2	0.8			2	0.4
Neutrons	29	12.1	9	3.7	38	7.9
Combination of different rays	2	0.8	5	2.0	7	1.4
Total physical mutagens	214	89.2	242	98.8	456	94.0
Chemical mutagens	23	9.6	2	0.8	25	5.2
Combination of physical and chemical mutagens	3	1.2	1	0.4	4	0.8
Grand total	240	100.0	245	100.0	485	100.0

distinct species treated with distinct mutagens can be generalized. An important theoretical and practical problem refers to the possibility that different mutagens may induce different kinds of effects at the chromosome level. It is the opinion of many geneticists that spontaneous and induced mutations do not differ qualitatively from each other. This hypothesis is supported by the fact that nearly the whole natural genetic variability of *barley* has been reproduced by means of induced mutations and by recombination following crossing (Stubbe 1967). Similar experiences have been made in many other crops. There are numerous examples demonstrating that experimentally obtained mutants proved to be identical with corresponding spontaneously arisen genotypes available in the collections of institutes or breeders. There are, however, certain discrepancies in this respect.

The question whether mutants, showing monohybrid segregations, are homozygous for "true" gene mutations or for minute deficiencies, is still an open problem. According to Ichikawa (1965), the majority of his gamma-ray-induced mutations in hexaploid *wheat* are deficiencies or other chromosomal aberrations rather than gene mutations. This seems to be also valid for X rays, neutrons, and alkylating mutagenetic chemicals such as EMS and EI (Bender 1970: Auerbach and Kilbey 1971). On the other hand, findings obtained by Dumanović et al. (1968) in *wheat* indicate, that low and medium gamma ray doses cause a relatively high proportion of useful mutants with normal yielding properties, whereas high doses lead to mutants with negative effects. Similar experiences with X rays, gamma rays, and EMS were made by Yonezawa and Yamagata (1977) in *rice* and *barley*. It is difficult to understand that plant organisms, homozygous for the loss of a certain amount of genetic material, are fully vital.

Our results in *Pisum* indicate likewise that many of our X-ray- and neutron-induced mutants are obviously not characterized by minute deficiencies. In the frame of our radiation genetic experiments, about 800 "gene mutations" and 120 translocations were isolated. A large number of them was maintained by propagating the translocation-heterozygous plants. In their offspring, plants homozygous for the translocated chromosomes were selected and developed into strains. Twenty-five different strains of this group were studied with regard to vitality and fertility. Only one of them was distinctly inferior to the mother variety. All the other ones were fully fertile and vital (Gottschalk 1978a). This is in contrast to the situation in *Drosophila* where translocation homozygosity leads often to lethality. The high degree of physiological effectiveness observed in our *Pisum* material could hardly be expected if the radiation-induced translocations would have led to the loss of genes.

Another important problem refers to the specificity of the mutation spectra produced by different mutagens. According to Smith (1972) there is little, if any, evidence in higher plants for mutagenetic specificity among physical mutagens. It may exist between specific physical and chemical mutagens. Some clear examples are available in *barley* (Gustafsson 1972; Lundqvist 1976, 1978). It is, however, very difficult to evidence this specificity because a large number of mutants, obtained in parallel treatments with different mutagens, is needed for obtaining the empirical data. The value of some experiences published in this field should therefore not be overestimated. Also the biochemical situation of the treated nuclei seems to be of importance with regard to the effects of distinct mutagens. DES, given at middle G_1 stage of the interphase nuclei of *barley* seeds, was found to give the highest rates of induced mutations. The most promising mutants for breeding purposes, however, were obtained when the mutagen was applied in late S stage (Yamaguchi et al. 1974).

For practical use of applied mutagenesis, it is of interest to consider that different cultivars of the same crop can differ markedly from each other with regard to their susceptibility against distinct mutagens. A specific variety of *Lens culinaris*, for instance, was found to be relatively resistant to gamma ray and NMU treatment, whereas two other cultivars were sensitive giving a wide range of different types of mutants (Sharma and Sharma 1979a). Similar experiences were made in *Cicer arietinum* (Ahmad and Godward 1981) and *Phaseolus vulgaris* (Al-Rubeai and Godward 1981) among others. There is no doubt that genetically conditioned differences with regard to both the radiosensitivity in general and the mutation rates in particular exist between different strains of many crops.

Many efforts are still necessary in order to clarify the problems just mentioned. This holds also true for the fact that often mutants are obtained which resemble the existing varieties, arisen by conventional cross breeding, without being identical with them. These cases are possibly not exclusively due to mutational processes but also to alterations in the pattern of gene arrangement and intragenic recombination (Kulshrestha and Mathur 1978; *Triticum aestivum*).

A considerable expenditure in space, time, and money is necessary if one intends to obtain a specific group of mutations or even a single desired mutation. This may be demonstrated by the following examples:

- Three blast-resistant *rice* mutants were selected in Japan out of an M_2 generation of 51,530 plants (Yamasaki and Kawai 1968).
- Screening of 951,000 M_2 *barley* plants in Denmark resulted in selecting 5 M_1 plants giving rise to powdery mildew-resistant M_2 seedlings (Jörgensen 1975).
- Testing more than 2.5 million M_2 plants of *barley* in the German Democratic Republic resulted in selection of 95 mildew-resistant mutants (Hentrich 1977).
- Seven mildew resistant *barley* mutants were selected from 1,200,000 M_2 plants in Japan (Yamaguchi and Yamashita 1979).
- From more than 6 million M_2 plants of *Mentha piperita*, grown in the United States, 7 wilt-resistant strains were developed (Murray 1969, 1971).
- 500,000 stems with dormant buds of *coastcross 1 bermuda grass* hybrids were irradiated, giving rise to one mutant with improved winterhardiness (Burton et al. 1980).

Similar estimates have been given by Yonezawa (1975): For the reliable selection of a mutation for a distinct quantitative character, at least 200,000–250,000 M_2 plants should be grown. Comprehensive M_2 generations of *barley*, obtained after gamma ray and sodium azide treatment, were evaluated in U.S.A. with the aim to select distinct single locus mutations. In these model experiments, the respective mutants, induced by NaN_3, occurred with the following frequencies:

- 2.7 per 10,000 M_2 seedlings for "waxy endosperm;"
- 1.0 per 10,000 M_2 seedlings for "vine (gigas)."

Similar results were obtained with regard to mutations causing distinct physiological anomalies in *barley* and *peas* (Kleinhofs et al. 1978). These experiments are insofar encouraging as they demonstrate that it is in principle possible to get a desired mutation in the frame of mutation treatments. The prospects are even better if we consider that many plant characters are controlled by polymeric systems. The desired character occurs already if one single gene mutates out of the polygenic group of 30, 50, or more genes. Impressive examples for the existence of such polygenic groups are:

- The *eceriferum mutants* of *barley* showing certain alterations of the wax coating on different organs such as stem, leaf blade or sheath, and spike. In Svalöf (Sweden), 1310 mutants of this type have been isolated so far. They were referred to 65 different loci randomly distributed over the seven chromosomes of the genome by diallel analysis. Thousand and sixty-one induced *eceriferum* mutants are recessive, 18 are dominant. Details concerning this giant amount of work have been published by Lundqvist et al. (1968), Lundqvist (1976, 1978), Fester and Søgård (1969).
- The *erectoides mutants* of *barley* showing shortened internodes combined with lodging resistance, dense spikes, certain alterations of the root system and some other characters. Until the end of the 1960's, more than 700 *erectoides* mutants have been genetically analyzed in Svalöf. They were found to refer to 26 different loci, some of them having more than 30 multiple alleles (Gustafsson 1969; Persson and Hagberg 1969).

Some of the *erectoides* mutants are of direct importance for barley breeding. It can be assumed that a similar polygenic situation is realized with regard to other traits of agronomic interest which should be obtained relatively easily in mutation experiments. This holds for instance true for male sterility. Another aid, not yet utilized so far in applied mutagenesis, is the pronounced mutagen specifity of distinct loci of polygenic systems. Results in this field are available in the *eccriferum* and *erectoides* mutants of *barley* (Gustafsson 1972; Lundqvist 1976, 1978).

Theoretically, each gene which is of any agronomic interest can mutate. Therefore, a wide spectrum of mutants can be expected in mutation experiments which have to be tested with regard to their usefulness for specific aims of breeding. This holds true for all yielding characters, furthermore for all kinds of resistance and tolerance, for the flowering and ripening behavior, and for many other traits influencing the breeding value of a crop directly or indirectly. It was already mentioned that desired mutants can be in principle obtained if the total number of the mutants selected is very high. It is, however, not yet possible in higher plants to induce specific mutations in distinct loci; the mutational event is still a matter of chance.

A comparably new aspect in applied mutagenesis is the quantitative and qualitative alteration of seed storage substances, such as proteins and carbohydrates, to some extent also of specific other substances deposited in various plant organs. Special emphasis is directed to seed proteins because a part of diseases due to malnutrition and undernourishment is related to insufficient protein supply. An increase and improvement of these substances could bridge the "protein gap." These considerations especially become valid under the aspect that 70% of consumed proteins derive from plants (IAEA 1979).

The importance of this problem becomes evident from the fact that the International Atomic Energy Agency (IAEA) in cooperation with the Food and Agriculture Organization of the United Nations (FAO) started a voluminous research program on this topic in 1968. The aim was to find ways for increasing the world's protein resources by means of selection and mutation. The findings published on *opaque* and *floury maize mutants* in the early 1960's (Mertz et al. 1964; Nelson et al. 1965) were the first promising results in this field, demonstrating that by gene mutation seed protein improvement is possible in principle. Later on, protein research programs were started in many countries and mutants were selected differing from their initial lines with regard to their seed proteins.

"Protein mutants" can be subdivided into two groups: Those genotypes which differ quantitatively from their control lines in seed protein content, and those in which deviations in the composition of seed protein subfractions are found. The distribution of amino acids in the latter group shows distinct deviations from the normal pattern, which in the first group cannot be detected.

Quantitative traits, such as protein content, are polygenically controlled and highly influenced by environmental factors. The genotype determines a certain potential of ontogenetic development; environment in the broadest sense determines the dimension of this development, resulting in the visible and measurable phenotype. In *wheat*, for example, Dhaliwal (1977) assumes that hundreds of structural genes may be involved in the control of seed protein content. Also the findings in other crops have shown that several genes are directly involved in protein production. Furthermore, various

plant characters, such as seed size and number of seeds per plant, which are likewise genetically controlled, can influence the amount of deposited seed proteins. In addition, certain environmental factors, such as temperature and manuring, have a proven effect not only on the amount but even on the composition of the seed proteins. These interactions are the reasons for the difficulties in analyzing the "quantitative protein mutants." The action of genes controlling the amount of seed proteins is influenced by environmental factors in a way that the reliable selection of mutants of this category becomes difficult. It is necessary to evaluate several replications of mutants and initial lines in a comparable way. These replications should be grown in the same year as well as in different years. In this way, variation ranges can be calculated which are a better criterion than single values for characterizing the respective genotypes. This presupposition, however, is often not fulfilled. Therefore, one should be careful in judging the protein situation of those mutants from which data of only one generation are available.

With regard to the "qualitative protein mutants," the results are more clear. In this field, extensive research work has been done during the last years and much basic knowledge about the biochemical interrelations in these mutants has been published. It is remarkable that qualitative deviations in mutants till now are preferably found in *cereals*, while there are hardly any in *pulses*. Furthermore, the comparison of the deviations found in these cereal mutants reveal that the mechanism which causes the qualitative deviations is rather similar in all the genotypes analyzed.

As proteins are direct gene products, mutations are immediately reflected in the respective polypeptides. With regard to other plant substances, the situation is different. They are synthesized under the influence of special enzymes which are likewise genetically controlled. The relations between deviations in these substances and the respective mutated genes are indirect and thus are more complicated.

Some examples may demonstrate that an immense expenditure in material, work, and time is necessary for selecting protein mutants. The following number of genotypes with favorably altered seed proteins was isolated in different cereals:

- 20 *barley* mutants with increased lysine content from 14,776 M_2 plants (Doll et al. 1974).
- 2 *barley* mutants from 10,000 M_2 or M_3 plants (Scholz 1972).
- 8 *barley* mutants from 2,455 M_2 plants (Krausse et al. 1974).
- Some *bread wheat* mutants from 25,000 M_2 plants (Parodi and Nebreda 1979), whereas no mutants of this category were found within 15,000 samples studied by Johnson et al. (1973).
- 2 *rice* mutants from 6,600 M_2 plants (Tanaka and Hiraiwa 1978).
- 1 *sorghum* mutant with increased lysine content from 23,000 M_2 heads (Axtell et al. 1974).
- From the *pearl millet*, 16,770 samples were analyzed, but no protein mutants could be isolated (Rabson et al. 1978).

Mutated genes that influence carbohydrates have been known for a long time. Of practical importance in this connection are genotypes, in which the sugar content is increased; these forms are utilized as vegetables such as *sugary maize*. In these genotypes in general, a block in starch synthesis is supposed being responsible for the higher

sugar content. Differences in the proportion of amylose and amylopectin in starch, as compared to normal conditions, are found as being caused by single mutant genes, too. In some of these cases deviations in the enzyme makeup, controlling starch synthesis, could be made responsible for the alterations.

Mutated genes with effect on seed oil content are hardly known and only little information is available in this field. Besides these three important groups of substances, several single mutants in various groups are known, where the respective genes alter the amount of special components. Most of these substances interfere with the nutritional value of the crop. Such substances are for example tannins in *sorghum*, that reduce the availability of the seed proteins. A particularly interesting example is BOAA (β-N-oxalyl-α,β-diamino-propionic acid) in *Lathyrus* seeds which acts as a neurotoxin if not completely destroyed by heat before consumption. Mutants in *Lathyrus sativus* are known in which this unfavorable component is reduced.

In the present survey, only gene mutations are considered. The utilization of chromosome mutations in plant breeding is still in its very beginnings. Some experiences are available in *barley* in Sweden and U.S.A. demonstrating that certain lines, homozygous for translocated chromosomes, are superior to their chromosomally normal mother varieties. The problem has been discussed by Gustafsson et al. (1966, 1971); Hagberg and Hagberg (1971); Hagberg et al. (1972); Künzel and Scholz (1972).

The number of papers, published in the field of the application of mutagenesis in plant breeding, is so large that not all of them can be considered in the present publication. The reasons for these restrictions are not only due to the huge number of data available but also to the difficulties in obtaining certain foreign periodicals. In order to restrict the number of references in a way appropriate to the present book, preferably papers published since 1965 are referred to. Some publications are available, in which the problems, methods, and results of mutation breeding are discussed and reviewed in general. Plenty of information on this field is compiled in publications presented by Gustafsson (1965, 1969), Gaul (1965a, c); Matsuo and Yamaguchi (1967); Khvostova (1967, 1978); Sigurbjörnsson (1968); Scarascia Mugnozza (1969a, b); Swaminathan (1969a, b, 1971); Černý (1970); Brock (1971); Favret (1972); Gaul et al. (1972); Gottschalk (1978f, 1979a, 1980b). This history of mutation breeding along with early results in *barley, rice, wheat,* and *soybean* was described by Sigurbjörnsson and Micke (1969, 1974); Brock (1965); Scossiroli (1965). They discussed the prospects of mutagenesis with regard to the alteration of quantitative characters. Surveys on the application of mutagenesis for reaching specific aims of plant breeding (resistance, straw stiffness and others) are mentioned in the various chapters of the present paper. Furthermore, review papers have been published considering the utilization of induced mutations in distinct crops. Examples are as follows:

- barley (Gaul 1965c; Sigurbjörnsson 1976);
- rice (Gustafsson and Gadd 1966);
- bread wheat (Khvostova et al. 1969; Konzak 1972, 1976; Konzak et al. 1973);
- durum wheat (Bagnara 1971);
- oats (Gustafsson and Gadd 1965e);
- sorghum (Sree Ramulu 1975);
- *Poa pratensis* (Gustafsson and Gadd 1965c);
- sugarcane (Heinz 1973);