PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Induced Gene and Chromosome Mutants

R. A. Nilan

Phil. Trans. R. Soc. Lond. B 1981 **292**, 457-466
doi: 10.1098/rstb.1981.0040

Phil. Trans. R. Soc. Lond. B **292**, 457–466 (1981)
Printed in Great Britain

Induced gene and chromosome mutants

By R. A. Nilan

Department of Agronomy and Soils and Program in Genetics, Washington State University, Pullman, Washington 99164, *U.S.A.*

Plant scientists, including breeders, can use an arsenal of physical and chemical mutagens and appropriate selection techniques to 'manufacture' in their experimental plots gene and chromosome mutants to compensate for the erosion of natural sources of genetic variability. They also have the capability of generating in this type of genetic manipulation the entire array of genetic variation inherent in all loci controlling each plant trait, and thus in a relatively short time producing most, if not all, of the genetic variants that have ever occurred in the evolution of a given agricultural plant.

This capability is required not only for the breeder concerned with developing new cultivars to meet the numerous and varied demands of the modern farmer, processer and consumer, but also for the geneticist, physiologist, anatomist and biochemist concerned with unravelling important plant processes and their genetic control. In short, these scientists need inexhaustible supplies of genetic variability, often never before selected in Nature or by earlier plant breeders.

Numerous experiments demonstrate that induced mutants have considerably extended the genetic variability of a phenotype. An outstanding example is *eceriferum* ('waxless' plant surfaces) in barley. Spontaneous mutations produced several well known variants controlled by about six loci. Genetic analyses of over 1300 induced and the few spontaneous mutants have determined that this trait is controlled by at least 77 loci (Lundqvist 1976, and personal communication). There are numerous alleles at some of these loci. Other examples are described in this paper.

The quantity and quality of artificially induced genetic variability in plants is in no small part due to the contributions of improved mutagens, mutagen treatments and selection techniques. A new potent and unique mutagen, sodium azide, is particularly successful in inducing putative point mutations. Recent experiments with barley and *Salmonella* have revealed that it is not azide *per se* but an activated metabolite that is the mutagenic agent. The metabolite has been isolated and crystallized and can now be synthesized *in vitro*. These findings usher in a new category of mutagens and suggest new avenues for understanding the interaction of mutagens with chromosomes and genes and for greater control of the induction of genetic variability in plants.

The considerable success of varietal development through induced mutants is well documented: 465 culvitars of sexually and vegetatively reproducing crops have been released that owe some of their production advantage to an induced gene or chromosome mutant. These cultivars have led to considerable economic impact in a number of countries.

In breeding research, induced mutants are indispensable for probing and elucidating the pathway and genetic control of important plant processes such as wax synthesis and deposition (von Wettstein-Knowles 1979), nitrogen assimilation (Kleinhofs *et al.* 1980), photorespiration and different facets of photosynthesis (Somerville & Ogren 1980; Miles *et al.* 1979; Simpson & von Wettstein 1980).

In the manipulation of plant genes (genetic engineering) in breeding research, it becomes increasingly necessary to pinpoint these genes on chromosomes. For this endeavour, an abundant array of induced chromosome mutants such as trisomics, telotrisomics, acrocentrics, inversions, translocations and deletions is required. This important activity can now be complemented by ever-improving chromosome banding techniques.

Introduction

Genetic variability, as derived to a greater extent from mutations comprising extragenic and possibly intragenic events (Nilan & Vig 1976; Brock 1980), and to a lesser extent from chromosome mutations (changes in chromosome number and structure), is the raw material of plant improvement. Until recently, genetic variability was secured by the plant breeder entirely from 'Nature's improvement programme' (rarely arising spontaneous mutations, subsequent recombination and natural selection) through collections of crop germ plasm, and wild species and even genera. In Nature, the genetic variants are end-products of thousands of years of evolution and were selected primarily for survival and reproductive capability.

Presumably during evolution, myriads of variants resulted from the variability potential of every locus and every chromosome break and many of the variants now needed by the plant breeder probably occurred. However, most were discarded since they were of little value for the plant's survival and fecundity. Evolutionary processes were not concerned with preserving the numerous traits that are now required from plants by the modern farmer for higher yield and adaptation to advanced farming practices and to new environmental niches; by the modern processor of food and fibre; and by the animal and human consumers who require an increased variety of foods with improved nutrition, palatability and attractiveness. These requirements are increasing while at the same time natural genetic variability in some crops is eroding.

The plant breeder needs now, and will do more so in the future, a broad array of genetic variation, possibly for every locus, whether it be a structural or a regulatory gene, for every plant process and phenotype. This is required most assuredly for the relatively few plants upon which we now base our production of food and fibre and for those plants that have the potential of broadening the food and fibre base and for fulfilling special needs. This genetic variation, plus all the conceivable changes in plant karyotypes that can be achieved through chromosome mutants, will eventually be required to reconstruct, so to speak, plants for man's needs. Obviously, the breeder cannot wait for new and usable spontaneous mutations.

Fortunately, plant geneticists and breeders, using an arsenal of physical and chemical mutagens and appropriate selection techniques, can now 'manufacture' gene and chromosome mutants to compensate somewhat for the depletion of natural sources of genetic variability. They also have the capability in this type of genetic manipulation of generating experimentally the entire array of genetic variation inherent in all loci controlling each plant trait, and thus in a relatively short time producing most, if not all, of the genetic variants that have ever occurred or may yet occur in the evolution of agricultural plants. The bases for the above concepts have been previously recounted (Brock 1980; Nilan *et al.* 1977).

This paper considers the current and future impact of induced gene and chromosome mutants on plant improvement. It also examines the impact of induced mutants on breeding research, especially towards providing new knowledge about the genetics, physiology, anatomy and biochemistry of cellular processes that produce all the traits so necessary for successful cultivars now and in the future.

Induced and spontaneous mutations

A breeder contemplating induced mutations might first ask, 'Do induced and spontaneous mutations and mutants differ?' and 'What is the value of induced mutants when they are so "raw" and have not been moulded by evolution and the various recombination and selection

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

processes that have developed spontaneous mutants into useful adapted complexes?' It has been amply demonstrated in a wide variety of organisms, including plants, that there are actually no major detectable differences between induced and spontaneous gene and chromosome mutations (Brock 1980; Nilan *et al.* 1977; Nilan & Vig 1976; Konzak *et al.* 1977). Of interest to the breeder is that the older spontaneous mutants have been moulded by recombination and natural selective forces into useful co-adaptive complexes. Newly arisen spontaneous mutants have not had time to be moulded into such complexes. However, the artificial acceleration of recombination plus refined and discrete selection techniques in the hands of the plant breeder can soon lead to useful trait complexes whether the origin of the trait is by induced or spontaneous mutation. Indeed, Brock (1980), on several pieces of evidence, questions the value of co-adapted complexes in plant improvement.

Induced 'new' variability

Another question often asked by plant scientists contemplating the use of induced mutations is, 'Can induced mutations produce "new" forms of traits that have not been observed among the spontaneous genetic variability?' The answer is that they can. Artificial mutagens can produce mutants that have not arisen in recent evolutionary history and thus have never been encountered by the breeder. However, they are probably not 'new' to a given plant because such variants may have occurred during its evolution.

In plants such as barley and peas, and to a lesser extent maize and wheat, that have been used extensively in basic and applied mutagenesis research, the extension of genetic variability by induced mutations and mutants is well documented. In these examples, induced mutants have uncovered hitherto unknown or 'new' loci controlling a phenotype and have revealed much about the potential variability (alleles) of many loci.

One of the most striking examples of the induction of 'new' variability and loci for a phenotype is represented by variants for the *eceriferum* ('wax-less' plant surface) phenotype of barley. Natural variability for this trait was confined to a few spontaneous mutants at six controlling loci. By using a wide variety of mutagens, 1302 mutants for this trait have been induced (Lundqvist 1976; Lundqvist *et al.* 1968; Lundqvist, personal communication). These numbers of independent gene changes differentially affect the wax composition of the leaf blade and sheath, spike and stem. They also lead to remarkable and distinct differences in fine structure and chemical composition of the surface wax molecules (von Wettstein-Knowles 1976, 1979). Appropriate genetic tests of the induced and spontaneous mutants have revealed at least 77 loci mapped to each arm of the seven barley chromosomes and numerous alleles, over 100, occurring at each of several loci. Similarly, in barley, induced chlorophyll-deficient mutants have revealed 600–700 loci controlling chlorophyll development (von Wettstein *et al.* 1974; Nilan & Velemínský 1981; Simpson & von Wettstein 1980) and 26 loci, some with numerous alleles, for the *erectoides* trait (Persson & Hagberg 1969). Moreover, genetic variability has been greatly broadened through induced mutation techniques for such phenotypes in barley as anthocyanin development (Jende-Strid 1978), nitrate reductase (Kleinhofs *et al.* 1980), mildew resistance (Jørgensen 1976), spike development (Gustafsson & Lundqvist 1980), and for lysine content (Doll *et al.* 1974). That the examples above are in barley testifies to the fact that among all of the crop plants, and indeed higher plants, there has been no other plant that has received so much investigation in the area of mutagenesis. In short, it has been the plant model of choice for experimental mutagenesis and mutation breeding. Examples of how induced

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

mutants have revealed 'new' loci in other plants have been given previously (Nilan *et al.* 1977; Konzak *et al.* 1977; Brock 1980).

Improved techniques

The increasing success of induced gene and chromosome mutants in breeding and breeding research can be attributed to improvements in mutation induction and selection techniques. These, along with relevant literature and descriptions of the most useful physical and chemical mutagens, recipes for their use on appropriate plant parts, e.g. seeds, buds, pollen, tissue and cells, and techniques for inducing and selecting mutants in sexually and vegetatively reproducing crops, are presented in the International Atomic Energy Agency's *Manual on mutation breeding* (1977).

There are numerous data and well documented technology that can lead to greater mutagen effectiveness (frequencies of mutations per dose of mutagen), efficiency (frequencies of desired events such as gene mutations in relation to such undesirable or unwanted events as sterility and, in some cases, chromosome aberrations), and specificity (group mutability (spectrum alteration), interlocus and non-random chromosome breakage) (Konzak *et al.* 1965; Nilan 1972; Brock 1980). With judicial selection of mutagens and manipulation of mutagen treatments, the breeder can influence the kind of genetic events that he may wish to induce as sources of genetic variability for his improvement programme. For instance, all of the physical mutagens such as X-rays, γ-rays and neutrons, as well as certain chemicals such as myleran, can induce high ratios of chromosome aberrations to mutations. There are other mutagens, e.g. ethyleneimine, that can induce about equal frequencies or proportions of both. Finally, there are mutagens such as ethyl methanesulphonate, diethylsulphate, sodium azide and certain base substitution and nitroso compounds that appear to induce higher proportions of mutations to chromosome aberrations (Konzak *et al.* 1977). As more knowledge is obtained about the mechanism, action and specificity of mutagens, and the nature of the mutations that they induce, the breeder will acquire even more precision for advantageously inducing and manipulating mutants in plant improvement.

At Pullman, 10 years of extensive basic research has developed a relatively new mutagen, sodium azide (Sideris *et al.* 1973; Nilan *et al.* 1973), which is one of the most potent available for higher plants. The research with this mutagen has been recently summarized by Kleinhofs *et al.* (1978*a*).

Azide is unique in that it induces in plants very high frequencies of gene mutations but is ineffective in producing major chromosomal changes. Experiments with bacteria indicate that azide is a base substitution mutagen, and in eukaryotes it appears to induce changes on the order of point mutations. Whether these mutations are small deletions or true base changes has not yet been resolved. We are attempting to answer this question by mapping numerous alleles induced by azide at the waxy pollen locus of barley (Rosichan *et al.* 1981; Nilan *et al.* 1981). Here the nature of the mutant alleles can be genetically resolved to near the base-pair level, since rare interallelic recombination events can be detected on a per million pollen basis. Preliminary results suggest that distances between alleles of about 50 base pairs can be detected, indicating that at least some mutational events do not involve large DNA deletions.

Recently, we have determined that it is not the inorganic azide *per se* but an organic metabolite synthesized in azide-treated barley and bacteria cells that is the mutagenic agent (Owais *et al.* 1978, 1979). This metabolite has been isolated, purified, crystallized and partly

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

characterized chemically (Owais *et al.* 1981 *b*) and its *in vitro* synthesis from cell-free extracts of *Salmonella typhimurium* has been accomplished (Owais *et al.* 1981 *a*). Furthermore, the pathway by which this metabolite is synthesized is being revealed (Owais *et al.* 1981 *c*; Cieśla *et al.* 1980).

Although activation of numerous chemicals to mutagenic metabolites is well known in mammalian mutagenesis, very little research in this area has been conducted in plants. Indeed, the azide metabolite is one of only three (atrazine (Plewa & Gentile 1976) and 1,2-dibromoethane (Scott *et al.* 1978) being the others) that have been detected, and the only one in plants that has been isolated and purified to crystal form and about which knowledge of its synthesis is becoming available. It is now suspected that additional chemicals may act the same way in plants.

This type of research is providing a greater insight about the interactions of mutagens with genes and chromosomes and the nature of induced genetic change. It is also providing the breeder with new knowledge and technology with which he can 'manufacture', with considerable deliberation, greater genetic variability.

Induced mutants for cultivar improvement

There is now overwhelming evidence that induced mutants have contributed most significantly to breeding new cultivars of crops (Sigurbjörnsson 1976; Gustafsson 1975; Sigurbjörnsson & Micke 1974; Broertjes & Van Harten 1978; A. Micke, personal communication). By September 1980, at least 224 cultivars of self and cross-pollinating crop species had been released for commercial production around the world (table 1). These cultivars, possessing at

Table 1. Released induced mutant cultivars
(September 1980.)

	number	
type of crop	direct	cross
cereals	74 (total)	57 (total)
bread wheat	12	5
durum wheat	5	7
rice	28	9
barley	25	33
oats	4	3
legumes	18	10
fruit trees	8	1
other crops	46	10
total crops	**146**	**78**
ornamentals	237	4
total	**383**	**82**

After Sigurbjörnsson & Micke (1974), Sigurbjörnsson (1976), and A. Micke (personal communication).

least one improved trait due to an induced mutation, include 131 cereals, 28 legumes, and 9 fruit trees. In addition, 241 new strains of vegetatively reproducing species, mostly ornamental have been released. Cultivars that owe their advantage to induced mutants have been developed in 37 countries and grown successfully on millions of hectares, and thus have had considerable economic impact in numerous countries. In some countries induced mutant cultivars have enjoyed most of the acreage devoted to a given crop species.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

The techniques for utilizing induced mutant genes for both qualitatively and quantitatively inherited traits and chromosome mutants in breeding have been adequately described in numerous reviews (for instance Brock 1980; Gaul 1964; Nilan *et al.* 1965) and publications from the International Atomic Energy Agency, especially the *Manual on mutation breeding* (1977).

In sexually propagating species, induced mutants can be used in two principal ways: directly, or in crosses or hybridization. In the former, a mutant that exhibits at least one improved trait with no new undesirable traits as a result of the induced genetic changes is multiplied directly. Once the mutant has been sufficiently tested with positive results, then it can be released to growers as a new cultivar. Among the 224 crop cultivars developed through induced mutants, 78 have been developed by direct multiplication of the mutant line (table 1). One advantage of the method is the short time required for developing a new cultivar. An example is the breeding of the six-row winter barley 'Luther' at Washington State University. Only 6 years elapsed from the time of mutagen treatment of seeds of the parent cultivar 'Alpine' to release of the mutant cultivar to growers in the Pacific Northwest of the U.S.A.

The plant breeder also can effectively use induced mutants in crosses – a necessity when the desired improved trait is closely linked or associated with undesirable spontaneous or induced traits. Furthermore, even directly useful mutant cultivars have proved to be outstanding parents for cross-breeding. The latter is well illustrated by barley. Of the 58 barley cultivars released with an induced mutant in their backgrounds, 33 have resulted from crossing mutants with other varieties or lines (table 1). Six successful cultivars were developed from the Swedish mutant cultivar Mari (Gustafsson 1975). In our barley breeding programme, the mutant cultivar Luther has been the parent of one released cultivar in Washington and of several advanced selections pending release in the states of Oregon, Washington, and Idaho.

In vegetatively propagated species (ornamentals, including cut flowers, bulbs, trees and shrubs; fruits; potato; sweet potato; sugar cane; cassava), much plant improvement has been based on the selection of 'sports' or spontaneous mutants. Thus, induced mutants are an obvious supplementary source of genetic variability. According to Broertjes & Van Harten (1978), the main advantage of mutation induction in vegetatively propagated plants is the ability to change one or a few characteristics of an outstanding genotype or cultivar without altering the remaining phenotype. In such plants, selection and propagation of useful mutants is relatively easy and development of mutant cultivars quite rapid.

The decision to use induced mutants in breeding will depend on the available supply of natural variability (and for some crops this is rapidly being depleted), the potential for success, and the effort and cost, especially where the utility of induced mutants are compared with securing the needed variants from related species or genera. These aspects of mutation breeding have been thoroughly analysed and discussed by Brock (1971, 1980).

Induced mutants for breeding research

The molecular basis of the genetic, biochemical, physiological and anatomical processes leading to those traits that comprise a successful crop cultivar are little understood. Yet the plant breeder today, and especially in the future, must learn to control and manipulate these processes if new cultivars to meet the requirements of the farmer, processer and consumer are to be met. Some of the progress and problems in understanding these processes, which are in the realm of plant molecular biology, have been recently reviewed (Walbot 1980) and are described elsewhere in this symposium.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

In microorganisms, and even certain well studied animal species, one of the requirements for advancing knowledge about the molecular bases of cell processes is an array of mutant lines, often induced, that modify or block steps in the process under study. Until recently, this approach has been neglected in plants and probably accounts for the lack of knowledge and slow development of technology in plant molecular biology.

Examples of the role of induced mutants in probing developmental and cellular processes and their genetic control use a broad spectrum of induced mutants with specific defects. Some facets of this approach have been reviewed by Rice & Carlson (1975) and Scholz & Böhme (1980). The former also present some valuable ideas about the use of induced mutants in analysing seed development and relevant biochemical and physiological processes.

The use of numerous *eceriferum* ('waxless' plant surface) mutants (von Wettstein-Knowles 1979) in barley is elucidating the pathway of wax synthesis and deposition; mutants lacking in serine–glyoxylate aminotransferase activity in *Arabidopsis* are permitting an understanding of photorespiration and its genetic control and regulation (Somerville & Ogren 1980); detailed genetic, biochemical and ultrastructural analyses of innumerable chlorophyll-deficient mutants in barley (von Wettstein *et al.* 1974; Simpson & von Wettstein 1980), and of high chlorophyll fluorescence mutants in maize (Miles *et al.* 1979), are providing an understanding of the regulation, genetic control and metabolic pathways involved in various facets of photosynthesis; ten induced nitrate-reductase deficient mutants in barley are permitting biochemical, genetic and physiological investigations toward an understanding and control of the nitrate assimilation pathway (Kleinhofs *et al.* 1978*b*; Warner & Kleinhofs 1974; Kleinhofs *et al.* 1980); waxy pollen mutants in barley are being used to probe with classical and molecular genetic techniques the nature of induced mutations, the composition of a eukaryotic locus and the synthesis and deposition of starch, and to develop a mutagen monitoring system (Rosichan *et al.* 1981; Nilan *et al.* 1981; Hodgdon *et al.* 1981); and numerous induced anthocyanin-free mutants in barley are helping us to understand the pathway and genetic control of anthocyanin synthesis and to develop strains free of proanthocyanidins, which are responsible for permanent chill haze and instability in beer (von Wettstein *et al.* 1980; von Wettstein 1979; Jende-Strid 1978; R. A. Nilan & A. L. Hodgdon, unpublished).

Success in using induced mutants in cell cultures, protoplasts and pollen for analysing basic processes has been very limited. Recent developments, and the problems inherent in mutant induction and selection, and especially plant regeneration, have been reviewed (Rice & Carlson 1975; Brock 1980) and are described in more detail by Davies (this symposium).

Another important area of breeding research involves chromosome mutants and the location of genes on chromosomes. The efficient assembly of necessary genotypes for analyses of biochemical and physiological processes and progress in manipulating genes in breeding and breeding research (genetic engineering) requires that each gene or set of genes contributing to a trait be pinpointed on the chromosome. Success in this endeavour will require a vast array of induced chromosome mutants and improved chromosome banding techniques. In short, this area of cytogenetics should become as important for plant improvement as it has been for advancing knowledge and technology in human genetics and medicine.

Locating genes on a specific chromosome is facilitated by trisomics (Khush 1973; Lewis *et al.* 1980; Tsuchiya 1969), monosomics (Kimber & Sears 1980; Law *et al.*, this symposium) and translocation break points. The latter may be recognized and used in mapping through partial sterility (Ramage *et al.* 1961) or cytologically (Tuleen 1971). In barley, over 300 translocations,

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

mostly induced, are available for cytologically locating genes (Nilan 1974). Induced translocation break-points can often provide cytological markers in chromosome regions lacking suitable genes.

To locate genes on a specific arm, telocentrics (Sears & Sears 1978; Kimber & Sears 1980; Singh & Tsuchiya 1977) and translocation break points are indispensable. In maize, numerous induced B–A translocations have been useful (Beckett 1978). To pinpoint genes cytologically within chromosome arms, translocation break-points as well as deletions, as so elegantly demonstrated in tomato by Khush & Rick (1968), and acrocentrics (Tsuchiya & Hang 1980), are necessary. Chromosome banding, now being used for locating genes (Kimber & Sears 1980; Linde-Laursen 1979) will be a powerful complementary tool in this endeavour.

Conclusion

Improved mutagen treatments, along with increased precision in selection of resulting mutants, are rapidly increasing the use and success of induced mutations in plant improvement. Such mutations are substituting for and even extending the variability obtained from natural germ plasm sources. As natural variability becomes further depleted and a much greater supply of variants is needed to create new cultivars of the future, artificially induced variability will assume greater importance. Indeed, the relative ease of producing and the suitability of induced variability for some crops may reduce or even negate the need for collection and preservation of natural germ plasm.

Induced gene and chromosome mutants are already proving indispensable for elucidating new basic knowledge about physiological, biochemical and genetic processes composing phenotypes and their control and for pinpointing genes on chromosomes.

Results attributed to me and my colleagues were obtained from investigations supported by the College of Agriculture Research Center, Washington State University, Pullman, Washington, Project nos 1006 and 1068, Department of Energy Contract no. DE-AM06-76RL02221, National Institute of Environmental Health Sciences Grant ES02224, and the Carlsberg Research Foundation, Copenhagen, Denmark, DOE/RL/02221-53.

References (Nilan)

Beckett, J. B. 1978 B–A translocations in maize. I. Use in locating genes by chromosome arms. *J. Hered.* **69**, 27–36.

Brock, R. D. 1971 The role of induced mutations in plant improvement. *Radiat. Bot.* **11**, 181–196.

Brock, R. D. 1980 Mutagenesis and crop improvement. In *The biology of crop productivity* (ed. P. S. Carlson), pp. 383–409. New York: Academic Press.

Broertjes, C. & Van Harten, A. M. 1978 *Application of mutation breeding methods in the improvement of vegetatively propagated crops*. Amsterdam: Elsevier Scientific Publishing Company.

Cieśla, Z., Filutowicz, M. & Klopotowski, T. 1980 Involvement of the L-cysteine biosynthetic pathway in azide-induced mutagenesis in *Salmonella typhimurium*. *Mutat. Res.* **70**, 261–268.

Doll, H., Køie, B. & Eggum, B. O. 1974 Induced high lysine mutants in barley. *Radiat. Bot.* **14**, 73–80.

Gaul, H. 1964 Mutations in plant breeding. *Radiat. Bot.* **4**, 155–232.

Gustafsson, Å. 1975 Mutations in plant breeding – a glance back and a look forward. In *Radiation research* (*Proc. Fifth Int. Cong. Radiat. Res.*) (ed. O. F. Nygaard, H. I. Adler & W. K. Sinclair), pp. 81–95. New York: Academic Press.

Gustafsson, Å. & Lundqvist, U. 1980 Hexastichon and intermedium mutants in barley. *Hereditas* **92**, 229–236.

Hodgdon, A. L., Marcus, A. H., Arenaz, P., Rosichan, J. L., Bogyo, T. P. & Nilan, R. A. 1981 The ontogeny of the barley plant as related to mutation expression and detection of pollen mutations. *Environ. Hlth Perspect.* **37**. (In the press.)

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Jende-Strid, B. 1978 Mutations affecting flavonoid synthesis in barley. *Carlsberg Res. Commun.* **43**, 265–273.
Jørgensen, J. H. 1976 Identification of powdery mildew resistant barley mutants and allelic relationship. In *Barley genetics III* (ed. H. Gaul), pp. 446–455. Munich: Karl Thiemig.
Khush, G. S. 1973 *Cytogenetics of aneuploids.* New York: Academic Press.
Khush, G. S. & Rick, C. M. 1968 Cytogenetic analysis of the tomato genome by means of induced deficiencies. *Chromosoma* **23**, 452–484.
Kimber, G. & Sears, E. R. 1980 Uses of wheat aneuploids. In *Polyploidy: biological relevance* (ed. W. H. Lewis), pp. 427–443. New York: Plenum Publ. Corp.
Kleinhofs, A., Kuo, T. & Warner, R. L. 1980 Characterization of nitrate reductase-deficient barley mutants. *Molec. gen. Genet.* **177**, 421–425.
Kleinhofs, A., Owais, W. M. & Nilan, R. A. 1978*a* Azide. *Mutat. Res.* **55**, 165–195.
Kleinhofs, A., Warner, R. L., Muehlbauer, F. J. & Nilan, R. A. 1978*b* Induction and selection of specific gene mutations in *Hordeum* and *Pisum*. *Mutat. Res.* **51**, 29–35.
Konzak, C. F., Nilan, R. A. & Kleinhofs, A. 1977 Artificial mutagenesis as an aid in overcoming genetic vulnerability of crop plants. In *Genetic diversity in plants* (ed. A. Muhammed, R. Aksel & R. C. von Borstel), pp. 163–177. New York: Plenum Publ. Corp.
Konzak, C. F., Nilan, R. A., Wagner, J. & Foster, R. J. 1965 Efficient chemical mutagenesis. In *The use of induced mutations in plant breeding* (*Radiat. Bot., Suppl.* **5**), pp. 49–70. Oxford: Pergamon.
Lewis, E. J., Humphreys, M. W. & Caton, M. P. 1980 Chromosome location of two isozyme loci in *Lolium perenne* using primary trisomics. *Theor. appl. Genet.* **57**, 237–239.
Linde-Laursen, I. 1979 Giemsa C-banding of barley chromosomes. III. Segregation and linkage of C-bands on chromosomes 3, 6 and 7. *Hereditas* **91**, 73–77.
Lundqvist, U. 1976 Locus distribution of induced *eceriferum* mutants in barley. In *Barley genetics III* (ed. H. Gaul), pp. 162–163. Munich: Karl Thiemig.
Lundqvist, U., Wettstein-Knowles, P. von & Wettstein, D. von 1968 Induction of *eceriferum* mutants in barley by ionizing radiations and chemical mutagens. II. *Hereditas* **59**, 473–504.
Manual on mutation breeding 1977 Tech. Reps Ser. no. 119, 2nd edn. Vienna: I.A.E.A.
Miles, C. D., Markwell, J. P. & Thornber, J. P. 1979 Effect of nuclear mutation in maize on photosynthetic activity and content of chlorophyll-protein complexes. *Pl. Physiol.* **64**, 690–694.
Nilan, R. A. 1972 Mutagenic specificity in flowering plants: Facts and prospects. In *Induced mutations and plant improvement*, pp. 141–151. Vienna: I.A.E.A.
Nilan, R. A. 1974 Barley (*Hordeum vulgare*). In *Handbook of genetics* (ed. R. C. King), vol. 2, pp. 93–100. New York: Plenum Press.
Nilan, R. A., Kleinhofs, A. & Konzak, C. F. 1977 The role of induced mutation in supplementing natural genetic variability. *Ann. N.Y. Acad. Sci.* **287**, 367–384.
Nilan, R. A., Konzak, C. F., Wagner, J. & Legault, R. R. 1965 Effectiveness and efficiency of radiations for inducing genetic and cytogenetic changes. In *The use of induced mutations in plant breeding* (*Radiat. Bot., Suppl.* **5**), pp. 71–89. Oxford: Pergamon.
Nilan, R. A., Rosichan, J. L., Arenaz, P., Hodgdon, A. L. & Kleinhofs, A. 1981 Pollen genetic markers for detection of mutagens in the environment. *Environ. Hlth Perspect.* **37**. (In the press.)
Nilan, R. A., Sideris, E. G., Kleinhofs, A., Sander, C. & Konzak, C. F. 1973 Azide – a potent mutagen. *Mutat. Res.* **17**, 142–144.
Nilan, R. A. & Velemínský, J. 1981 Mutagenicity of selected chemicals in barley test systems. In *Proc. Comparative Chemical Mutagenesis Workshop* (ed. F. J. de Serres), ch. 11. (In the press.)
Nilan, R. A. & Vig, B. K. 1976 Plant test systems for detection of chemical mutagens. In *Chemical mutagens. Principles and methods for their detection* (ed. A. Hollaender), vol. 4, pp. 143–170. New York: Plenum Press.
Owais, W. M., Kleinhofs, A. & Nilan, R. A. 1979 In vivo conversion of sodium azide to a stable mutagenic metabolite in *Salmonella typhimurium*. *Mutat. Res.* **68**, 15–22.
Owais, W. M., Kleinhofs, A. & Nilan, R. A. 1981*a* Effects of L-cysteine and *O*-acetyl-L-serine in the synthesis and mutagenicity of azide metabolite. *Mutat. Res.* **80**, 99–104.
Owais, W. M., Kleinhofs, A. & Nilan, R. A. 1981*b* In vitro synthesis of the mutagenic metabolite by cell-free bacterial extracts. (Submitted.)
Owais, W. M., Kleinhofs, A., Ronald, R. C. & Nilan, R. A. 1981*c* Isolation of an azide mutagenic metabolite in *Salmonella typhimurium*. *Mutat. Res.* (In the press.)
Owais, W. M., Zarowitz, M. A., Gunovich, R. A., Hodgdon, A. L., Kleinhofs, A. & Nilan, R. A. 1978 A mutagenic in vivo metabolite of sodium azide. *Mutat. Res.* **50**, 67–75.
Persson, G. & Hagberg, A. 1969 Induced variation in a quantitative character in barley. Morphology and cytogenetics of *erectoides* mutants. *Hereditas* **61**, 115–178.
Plewa, M. J. & Gentile, J. M. 1976 Mutagenicity of atrazine: a maize–microbe bioassay. *Mutat. Res.* **38**, 287–292.
Ramage, R. T., Burnham, C. R. & Hagberg, A. 1961 A summary of translocation studies in barley. *Crop Sci.* **1**, 277–279.
Rice, T. B. & Carlson, P. S. 1975 Genetic analysis and plant improvement. *A. Rev. Pl. Physiol.* **26**, 279–308.
Rosichan, J., Arenaz, P., Blake, N., Hodgdon, A., Kleinhofs, A. & Nilan, R. A. 1981 An improved method for the detection of mutants at the waxy locus in Hordeum vulgare. *Environ. Mutagenesis.* **3**. (In the press.)

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Scholz, G. & Böhme, H. 1980 Biochemical mutants in higher plants as tools for chemical and physiological investigations – a survey. *Kulturpflanze* **28**, 11–32.

Scott, B. R., Sparrow, A. H., Schwemmer, S. S. & Schairer, L. A. 1978 Plant metabolic activation of 1,2-dibromoethane (EDB) to a mutagen of greater potency. *Mutat. Res.* **49**, 203–212.

Sears, E. R. & Sears, L. M. S. 1978 The telocentric chromosomes of common wheat. In *Proc. 5th Int. Wheat Genet. Symp.*, New Delhi, pp. 389–407.

Sideris, E. G., Nilan, R. A. & Bogyo, T. P. 1973 Differential effect of sodium azide on the frequency of radiation-induced chromosome aberrations vs. the frequency of radiation-induced chlorophyll mutations in *Hordeum vulgare*. *Radiat. Bot.* **13**, 315–322.

Sigurbjörnsson, B. 1976 The improvement of barley through induced mutation. In *Barley genetics III* (ed. H. Gaul), pp. 84–95. Munich: Karl Thiemig.

Sigurbjörnsson, B. & Micke, A. 1974 Philosophy and accomplishments of mutation breeding. In *Polyploidy and induced mutations in plant breeding*, pp. 303–343. Vienna: I.A.E.A.

Simpson, D. J. & von Wettstein, D. 1980 Macromolecular physiology of plastids. XIV. *Viridis* mutants in barley: genetic fluoroscopic and ultrastructural characterisation. *Carlsberg Res. Commun.* **45**, 283–314.

Singh, R. J. & Tsuchiya, T. 1977 Morphology, fertility, and transmission in seven monotelotrisomics of barley. *Z. PflZücht.* **78**, 327–340.

Somerville, C. R. & Ogren, W. L. 1980 Photorespiration mutants of *Arabidopsis thaliana* deficient in serine-glyoxylate aminotransferase activity. *Proc. natn. Acad. Sci. U.S.A.* **77**, 2684–2687.

Tsuchiya, T. 1969 Status of studies of primary trisomics and other aneuploids in barley. *Genetica* **40**, 216–232.

Tsuchiya, T. & Hang, A. 1980 Acrocentric chromosome $4S^{4L}$ in barley. *Barley Genet. Newsl.* **10**, 72–73.

Tuleen, N. A. 1971 Translocation-gene linkages from F_2 seedlings in barley. In *Barley genetics II* (ed. R. A. Nilan), pp. 208–212. Pullman: Washington State University Press.

Walbot, V. 1980 Molecular biology of higher plants. In *The biology of crop productivity* (ed. P. S. Carlson), pp. 343–409. New York: Academic Press.

Warner, R. L. & Kleinhofs, A. 1974 Relationships between nitrate reductase, nitrite reductase, and ribulose diphosphate carboxylase activities in chlorophyll-deficient mutants of barley. *Crop Sci.* **14**, 654–658.

Wettstein, D. von 1979 Biochemical and molecular genetics in the improvement of malting barley and brewers yeast. In *Proc. 17th Congr. European Brewery Convention*, West Berlin, pp. 587–629.

Wettstein, D. von, Jende-Strid, B., Ahrenst-Larsen, B. & Erdal, K. 1980 Proanthocyanidin-free barley prevents the formation of beer haze. *MBAA tech. Q.* **17**, 16–23.

Wettstein, D. von, Kahn, A., Nielsen, O. F. & Gough, S. 1974 Genetic regulation of chlorophyll synthesis analyzed with mutants in barley. *Science, N.Y.* **184**, 800–802.

Wettstein-Knowles, P. von 1976 Tracking down β-diketone synthesis with the aid of the *eceriferum* mutants. In *Barley genetics III* (ed. H. Gaul), pp. 20–22. Munich: Karl Thiemig.

Wettstein-Knowles, P. von 1979 Genetics and biosynthesis of plant epicuticular waxes. In *Advances in the biochemistry and physiology of plant lipids* (ed. L.-Å. Appelqvist & C. Liljenberg), pp. 1–26. Elsevier/North-Holland Biomedical Press.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES