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Induced Mutations and Barley Improvement

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Summary. Seven barley varieties, originating from three *X*-ray induced mutations, have been officially approved in Sweden since 1958. Some have gained a wide area of cultivation. The list is as follows: *Pallas*, isolated 1947, approved 1958, mutant *ert-k*^{32} of Bonus barley. — *Mari*, isolated 1950, approved 1960, mutant *mat-a*^{8} of Bonus. — *Hellas*, approved 1967, mutant cross of Pallas × Herta. — *Kristina*, approved 1969, mutant cross of Domen × Mari. — *Visir*, approved 1970, Pallas × Long Glumes back-crossed to Pallas. — *Mona*, approved 1970, mutant cross of Mari × Monte Cristo back-crossed to Mari. — *Gunilla*, approved 1970, hybrid cross of the mutant 44/3 arisen from Gull barley in 1939; evolved in a series of steps, using one six-row and four two-row varieties, with mutant characters prevailing and Gull genes reiterated. — After the first approval of Pallas in 1958, 12 more years have led to the approval of a second mutant case and five mutant crosses. In addition, chromosome translocations, induced by irradiation in Bonus, have been instrumental in the production of *hybrid barley* in USA and are used in the barley improvement program of Sweden, as well as for theoretical analysis in numerous countries.

"Similarly, for the practical breeder, it is hoped that the method will ultimately prove useful." H. J. Muller (1927)

"The practical value of induced mutations in the improvement of crop plants has been much overrated, at least as regards immediate application." L. J. Stadler (1930)

Introduction

In his paper of 1930, Stadler summarized his outstanding data on induced plant mutations. He also formulated a series of arguments on their practical utilization, coming to the conclusion quoted above. Although not entirely negative, especially with regard to fruit tree improvement, his criticism was so sharp and so logical that it no doubt retarded the development of the mutation method in practice. His arguments were quoted and reiterated in many ways by later authors. A partial counter-criticism of these can be found in a paper by Gustafsson (1963).

In the meantime, scientific work along this line was continuously carried out in the Soviet Union (up to the Second World War), in Germany, and in Sweden. Some of the Swedish results have reached a state permitting definite conclusions, so it seems appropriate to present them to a wide audience. It is now evident that mutation programs should be regularly included in breeding programs of crop plants. This implies that the mutation method, in accordance with Muller's view but in contrast to Stadler's arguments, is a method which under definite conditions, depending on the crop plants, the characters to be improved, and the available mass screening techniques, may turn out not only "useful" (Muller) but in special cases will be the most appropriate or the only method possible (*cf.* Sigurbjörnsson 1971; with special emphasis on *Mentha*).

Background

It was the original intention of Nilsson-Ehle, a founder of modern plant breeding and genetics, and the senior author of this article (Gustafsson), to find a way to induce and select "positive" variations of crop plants (barley, wheat, oats, flax) by means of seed irradiation and UV-treatment. Unfortunately, little was published by Nilsson-Ehle on his results (only two small papers in 1939 and 1948), but some oral comments were given in discussions with Gustafsson, relating to articles by him (1940 and onwards), concerning, for instance, the occurrence of so-called minor variations ("line mutations", "physiological mutations") and the profitable use of mutations in recombination work.

One sharp argument of Stadler's was presented in the following way (*l.c.*, p. 18): "The variations resulting from induced mutations are in most cases unfavorable . . . it is reasonable to expect that among artificially induced mutations a small proportion of favorable variations will be found. But the rare favorable mutation is likely to be accompanied by unfavorable mutations induced by the same treatment. The result is a plant heterozygous for several genes, mostly undesirable . . . in most cases a heterozygote of greater promise could be produced by well-directed hybridization."

A favorable mutation is an exception, it is true. But so is also a favorable recombinant in cross-breeding, leading to a new variety in high-bred crop plants. It is our aim in this paper to show that the mutation method can really be "well-directed" already from the beginning of a breeding project, and that it may lead both to the exceptional favorable mutation and to the exceptional, favorable mutant recombinant.

Choice of material in mutation breeding. Examples from barley

The induction of productive mutations of potential value in agriculture (and horticulture) should be well-planned in the sense that the characteristics of the parent materials chosen for experimentation have to be carefully evaluated beforehand. But the same is valid also with regard to the partners in cross-breeding programs, as elucidated by Nilsson-Ehle in an early paper of 1906 (reprinted and partially translated into English by O. Tedin 1950).

The first model variety in our irradiation work was Gull barley (Gull = Gold), selected for its well-defined characteristics. Also, it was a pure line, released by the Seed Association in 1913, and known to be highly uniform. Outcrossings and admixtures were readily detected. (In fact, this was a central precaution in our irradiation work also in oats and wheat. In the latter species, for instance, the "Pudel wheat" with its recessive characters, long awns and white kernels, formed the original material, permitting the differentiation of mutations from crossings and admixtures; *cf.* Gustafsson 1947.)

Another variety chosen for experimentation was the Danish Maja barley. Evident mutations, confirming the Gull results, were also obtained in this high-yielding variety. However, the chief plant material for many years consisted of the extremely high-yielding and high-tillering Bonus barley, approved in 1952. It became wide-spread in Denmark but did not reach the same area of cultivation in Sweden, owing to its inferior lodging resistance, as compared to other varieties (Herta, Rika, and Ingrid barleys from the plant breeding institute of Weibullsholm). Moreover, in some parts of Sweden the Bonus variety was rather late in maturity, especially in bad years. In consequence of this, it was planned, even before the official release of Bonus, to raise its lodging resistance and to increase its earliness by means of mutation. Results in Gull and Maja indicated that such a procedure would be justified (*cf.* the article by Borg 1959).

In fact, Bonus barley has become an international model variety with regard to induced rearrangements of chromosome structure, gene localization and linkage, studies of mutation specificity and quantitative inheritance. It has shown itself useful also for studies on irradiation and chemical mutagen effects.

In summary, in precise experimentation, aiming at the induction of high-productive mutations, the parent varieties should be selected with considerable care. Their advantageous characters should be carefully evaluated against inferior ones, always considering agrotechnical demands and exterior conditions. The successive improvement and change of Bonus barley and its mutants and the work on Gull barley may be used to illustrate this point.

First favorable mutants induced

The early mutation studies in Gull barley concerned chlorophyll mutations and a scheme for their classification. Soon, however, some other conspicuous mutations were isolated, among them the so-called *erectoides* mutants (Gustafsson 1941), easily detected by their dense-eared appearance. Nilsson-Ehle crossed mutants of this kind with the Danish Kenia variety, a superior malting barley, and obtained lodging-resistant and high-yielding recombinants (oral communication). These were tested in yield trials. In 1937, Gustafsson isolated some further cases, two of them denoted as *ert-c*^{1} and *ert-d*^{7}, both of a certain historical interest. They could not possibly be intermixtures of any kind, owing to their changed karyotypes (they contain reciprocal translocations; Hagberg and Tjio 1950). In fact, *ert-d*^{7} permitted the beginning of standardization of the barley chromosomes, since the points of breakage could be localized. Both mutants, and especially *ert-c*^{1}, were high-yielding. At the same time lodging resistance increased and nitrogen response was improved. (*Erectoides* mutants have been denoted as "nitrogen ecotypes".) In other properties the mutants remained unchanged in relation to the parent.

The finding of *ert-c*^{1}, with one distinct gene change (the *ert*-character) and two chromosome breakage and rearrangement points, one at or close to the *ert*-mutation, definitely showed Stadler's quoted argument not to be correct: *Favorable mutations, in no way associated with unfavorable side effects, do occur.*

Another factor mutation of Gull: "late, tall" had no detectable concomitant chromosomal rearrangement or disturbance. Under dry conditions it was especially high-yielding both in grain and straw and was denoted a drought-resistant mutant (making a new "ecotype").

However, we have to agree that favorable mutations are rare. Most conspicuous mutations result in a decrease in viability or are deleterious. The same is true with spontaneous mutations. An example of the combination of favorable and unfavorable traits, corroborating Stadler's quoted argument on this point, was discussed by Gustafsson (1947, p. 33). It involved a strikingly blueish-waxy, bushy, lodging-resistant mutant from Gull, having a fairly good yielding ability but drastically inferior in 1000-grain weight. After repeated and directed recombination work it has now given rise to the Gunilla variety of barley possessing a high 1000-grain weight.

This emphasizes again a view, advocated by Nilsson-Ehle, Åkerman and Gustafsson (*v.* the last author 1941), concerning the great potentialities of induced mutations in regular recombination breeding. Nilsson-Ehle apparently succeeded in his mutation-hybridization work, although for some reason no released variety resulted. Also with regard to this point, Stadler over-emphasized the range of his argument, *viz.* that similar varieties could, with "greater promise", have been "produced by well-directed hybridization". In the case of the Gunilla variety, for instance (*v.* below), the high lodging resistance and the bushy blue habit would not easily have been accomplished without using the inferior mutant. In addition, the unchanged Gull genes of the mutant may have added to the high yielding ability and adaptability of the new variety.

The isolation and release of Pallas barley, an *ert*-mutation

After a series of *erectoides* mutations had been induced from Gull and Maja barleys, it was clear that using a modern variety like Bonus, high in tillering and yield but lodging under severe conditions, *ert*-mutations would possibly become of direct practical importance, since some of them immediately and in one stroke increase the resistance to lodging. It was soon found that the erectoid character is caused by mutations not only in one or two gene loci but in a rather high number, 25—30 or more (*v.* Persson and Hagberg 1969). In some of these loci inferior changes always arise, but in some, as in loci *a, b, c, d, k, m, l, o,* mutants of high productivity appear. By mere phenotypical inspection it is often possible to decide which gene locus is mutated. For instance, most mutants of *ert*-loci *a, c, d* and *m* are rather easily diagnosed. However, in each locus a series of mutant gradations exist, from extreme to less noticeable allelic changes. The mutated character of ear density is of a more quantitative than qualitative type. Its expression also depends on year conditions, amount of fertilizers and parent variety, whether this is in itself dense- or lax-eared.

In 1947 a definite erectoid mutant was isolated from Bonus. It was later denoted $ert\text{-}k^{32}$. Its history has been reviewed in another article (Gustafsson 1969). Together with other Bonus erectoids, especially $ert\text{-}a^{23}$ and $ert\text{-}a^{28}$, it was further studied with regard to suitability for large area cultivation. All three mutants were definitely more lodging-resistant than Bonus. Under ordinary conditions they gave high yields. Careful comparisons and extensive tests indicated a definite superiority of $ert\text{-}k^{32}$ over the other erectoids and over Bonus. Especially under good soil and fertility conditions it was superior ("a nitrogen ecotype"). Finally, in 1958, this mutant was approved by the official State Seed Board as a new variety under the name of Pallas (Borg *et al.* 1958, Borg 1959). It was released to growers in 1960. In the next few years it gained a fairly wide market in Sweden but became especially popular in Denmark and England. In Denmark, Pallas has been ranking very high for many years, cultivated on hundreds of thousands of hectares, and is still the barley standard in the official yield testing of new and old strains. "Pallas is a fodder barley, but very robust and secure in cultivation "(Thögersen 1970). In England, Pallas was said to break the national yield record (Gustafsson 1963), and was also cultivated over large areas in Scotland and Ireland. The Bonus parent was never recommended for use in Great Britain. However, the mutant, as well as its parent, was not sufficiently disease-resistant, especially against mildew, and its cultivation in Great Britain decreased. It has been replaced by other varieties, mostly mildew-resistant, among them a widespread mutant cross Midas, produced in England, and another mutant, Betina, recently isolated and released in France. Another barley mutant variety which became popular in Britain, was Milns Golden Promise, which was bred following gamma-ray treatments. In Sweden, $ert\text{-}k^{32}$ (Pallas) was earlier used as a crossing parent.

Further improvement of lodging resistance: Hellas barley

Partner in the recombination work involving Pallas was the prominent Weibullsholm variety Herta (Wålstedt *et al.* 1970). A population of the cross Pallas × Herta was sent to the breeding station Linköping in Middle Sweden (58° N.L., Fig. 1). In 1955 the first plant selection was carried out, giving a strain named Ög 56316. The selected strain was characterized by its superior stem quality — it was very straw-

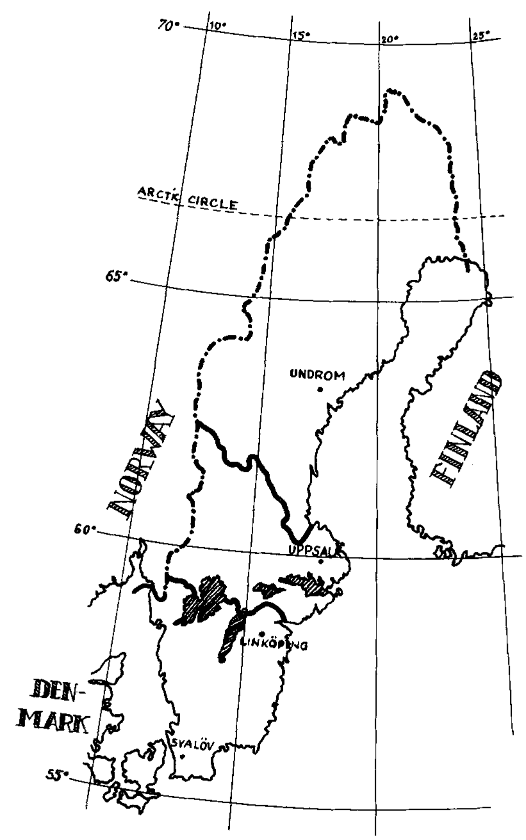

Fig. 1. Sweden divided into three regions of cereal cultivation. The Swedish Seed Association has its chief station at Svalöv and three out of several more branch stations at Linköping, Uppsala (Ultuna) and Undrom

stiff from heading until maturity, and also possessed a high resistance to straw breakage at and after maturity. Pallas fails to some extent in this respect. Ög 56316 was approved as a commercial variety in 1967 under the name of Hellas, after six years of local and official testing. It was released to growers in 1969.

Hellas is superior in yield to the official standard variety Ingrid by 1—3%. According to the authors quoted, it has an "unsurpassed" lodging resistance; in fact, it has a relative figure of 86 as compared to 54 for Ingrid in South Sweden and Denmark; 100 being fully erect, O fully lodging. Its straw breakage is decidedly decreased. In addition, it possesses a high sprouting resistance at harvest time, as determined in the field, in fog chambers and by the α-amylase test. Like Pallas it has little open-flowering, less than Herta and Ingrid, and in consequence, it is rarely infected by *Ustilago* (smut). It is less attacked by mildew than Pallas, and seems to have a fair field resistance.

In Hellas, the prominent agronomic characters of Pallas have been further accentuated by gene recombination. The erectoid appearance still prevails. The variety has a wide adaptability and a high yielding ability. It is in the top range of Scandinavian varieties, especially suited to good soils, and in Sweden it is a definite improvement over its parents Pallas and Herta.

Making Pallas and Hellas mildew-resistant: Visir barley and still newer strains

A year ago there was no mildew-resistant Swedish barley in the national list of approved varieties. The introduced Danish variety Bomi was resistant to approximately half of the number of races occurring in Sweden. In this respect, the new Visir barley is an improvement, since its resistance is broad enough to cover all known races except one. The resistance was obtained after crossing and back-crossing Pallas with the primitive "Long glumes" barley, which has the same type of resistance as the German variety Amsel. Average yielding ability is increased in comparison to the standard (Ingrid) by a couple of per cent, but is influenced by the occurrence and degree of mildew attacks. The lodging resistance is fairly good (equal to the standard), the time of maturity slightly shorter, and its α-amylase activity in fog chamber experiments lower than in the standard (which means less field sprouting). However, its degree of straw breakage is somewhat higher. Visir is recommended for cultivation in high-fertile regions carrying risks of heavy mildew attacks.

Visir barley was approved as a commercial variety (for export) in 1970 and constitutes an important step in Swedish barley breeding. It is already followed by several new varieties, maintaining many of the original Bonus-Pallas features (genes) while containing valuable agronomic characteristics from Herta and other varieties with genes for resistance to mildew and nematodes, incorporating spontaneous as well as induced sources of resistance (*cf.* p. 244, the mutant Refoma).

The mentioned steps of improvement do not imply an end stage, rather a passage *en route*. It has taken 12 years from the approval of Pallas and 23 years from the isolation of *ert-k*^{32}. In the beginning, there was even in Sweden an official attitude of hesitation towards the use of induced mutations in breeding, which led to a delay in releasing the mutant varieties. To some extent, a conservative attitude still exists towards the use of the new barley type represented by the "erectoides" character.

The isolation of Mari barley, an early mutant

The *erectoides* mutants of Gull and Maja formed the theoretical background for the induction of similar mutations in Bonus, the final selection of *ert-k*^{32} and its further transformations. An exactly parallel procedure was initiated with regard to earliness. At the beginning of the '40s, a series of high-productive mutants were induced in Maja barley. Some were tested on a fairly wide scale. One was the erectoid mutation *ert-o*^{16}, which besides its dense ear character, which was not very pronounced, had a strikingly stiffer straw and a remarkably early maturity. In spite of a decrease in yield, it was proposed for further propagation and testing. It possessed, however, some beauty spots from the agronomic point of view (for instance, discoloured lemmas at the time of ripening). However, what was obtained in Maja could, we considered, also, and with better success, be obtained in Bonus.

In fact, a set of early mutants were soon isolated (*v.* Gustafsson 1969). "Early 2" from Bonus was interesting: it was earlier by a couple of days, had improved standing ability and high yield. However, some drastic mutations, later identified with distinct gene loci: *mat-a*, *mat-b*, *mat-c*, etc., became of greater interest, especially mutants of locus *a* (early 8, 11, 12), where mutant early 8 (*mat-a*^{8}) was soon propagated and included in official tests. *Ert-o*^{16} and *mat-a*^{8} were found to be allelic with regard to the feature "earliness", but early 8 was comparatively not as dense-eared as *erectoides* 16 (possibly connected with a different origin: Bonus *versus* Maja). Early 8 was isolated in 1950. It was approved as a commercial variety by the State Board in 1960 under the name of Mari barley (from Latin for *ma*tura=early and *ri*gidus=stiff).

The reason why this mutant was received with so much enthusiasm was that besides its increase in earliness (7—10 days over Bonus) and its consequent use in South Sweden as a pre-crop to winter rape, it had a semi-dwarf habit with a profound lodging resistance. It also lacked the beauty spots of *erectoides* 16. The existing Swedish early varieties, mostly of the six-row type, could not match Mari in tillering capacity, productivity and lodging resistance. Under appropriate conditions it yields extremely well; it is on par with the medium-late varieties. After overcoming difficulties caused by insufficient knowledge of suitable cultivation techniques, Mari became a wide-spread variety, also outside Sweden. It certainly has some negative sides. The feature "semidwarf habit" leads in some seasons, especially in dry and warm early summers, to very short straw and decreased tillering. Like its parent Bonus it is characterized by a certain mildew susceptibility and a tendency to straw breakage. On the whole, however, it is an interesting and valuable acquisition to the variety collection of Northern Europe.

The original Mari mutation, as well as the allelic mutants of the *a*-locus, are photopheriodically insensitive or, rather, less photoperiod-sensitive than Bonus and the other northern two-row varieties tested. In phytotron cultivation, Mari sets seed with as little as 8 hours of artificial light, when Bonus, and others, grow only vegetatively and form no seed. At 14 hours of artificial light the difference in heading between Bonus and Mari has already increased from 7—10 days (in field cultivation) to ca. three weeks. Bonus, on the whole, is a long-day plant, suited for fairly low day/night temperatures (Dormling *et al.* 1966, 1969, in phytotron studies). A definite interaction of thermo- and photoperiod sensitivity often occurs, which is especially noticeable in the locus *mat-c*-mutants (Dormling and Gustafsson 1969). However, compared to Bonus, Mari has a wider photo- and thermoperiod tolerance. Consequently, Mari can be cultivated in extraneous regions and countries, where Bonus and other northern varieties fail.

Improved malting quality, lodging resistance, yield: Kristina barley

The Norwegian variety Domen is characterized by its high malting quality. In its genotype it contains an allele of the gene locus *ert-k* which in Bonus has mutated to produce Pallas. Domen itself is a cross between two- and six-rowed barleys (with genes for dense-ear, in this case *ert-k*). Bonus, Pallas and Mari may be considered medium-good malting barleys. The cross Domen × Mari turned out especially interesting. One line of this cross SvÅ 61726 was selected and tested for the first time in 1961. The official standard variety in this testing was again Ingrid barley. Kristina has, according to official reports, a yield superior by some 4—6% and is, most definitely, widely superior in lodging resistance, and also has a notably better resistance to straw breakage. It is later than Ingrid by two or three days and thus a week to ten days later than Mari; to some extent it has maintained the straw properties and general appearance of Mari.

Of special importance in this interesting variety is its high enzymatic potential leading to a fast germination of economic consequence for malting and brewing. Kristina has high α-amylase activity, diastatic power and Kolbach index. In addition, the grain flour possesses a low grade of viscosity, depending on the rapid breakdown of glukane. It is therefore better suited for poultry (broiler) feeding than other Scandinavian varieties.

The high enzymatic activity results in a certain inferior property. The mature crop rapidly germinates (sprouts) under wet and rainy harvest conditions. This hampers the cultivation in humid regions. Nevertheless, Kristina is one of the best malting barleys existing. Experiments, both including crossings as well as mutagenic treatments, are in progress for its further improvement. The mutagenic treatments consist of gamma- and neutron irradiation as well as treatments with ethyl methane sulfonate (EMS), methyl nitroso urea (MNU) and ethylene imine (EI). They have led to the isolation of lines with improved dormancy. Selection was carried out with the aid of fog chambers. In the mutation experiments, EMS, EI and neutron treatments were especially efficient. Field analysis and yield trials will elucidate the detail properties of the isolated mutant lines.

Kristina barley was approved as a commercial variety (for export) in 1969.

Mildew resistance introduced into the Mari genotype: Mona barley

The line Sv 65505 was isolated after the crossing of Mari with the primitive variety 'Monte Cristo', with two subsequent back-crossings to Mari, leaving the background genotype of Mari intact, while introducing from Monte Cristo a complete resistance to all known and tested mildew races occurring in Sweden (*cf.* the Visir variety).

The yield data show Mona barley to be about 7% higher than Mari. It is, on the average, equal in yield to the much later Ingrid, which is also inferior in high-yielding districts, where mildew attacks are common. The official tests in Sweden cover fifteen districts. In seven districts with high yields Mona is superior to Mari by six per cent and equal or slightly better than Ingrid. In the remaining districts with rather low average yields, Mona is still superior to Mari but inferior to Ingrid.

Like Mari, Mona barley, with its fairly high yields, is intended, in the south of Sweden, as a pre-crop to winter oil-rape. For this reason a pronounced earliness is of importance. Mona barley is somewhat later than Mari (one or two days) but definitely earlier than Ingrid (three to four days). Mona has better lodging resistance than both varieties and has better resistance to straw breakage than Mari. Its straw is longer than in Mari and thus it is better in competition with noxious weeds.

Mona barley was approved as a commercial variety in 1970.

It is appropriate to add here that, although mildew resistance can be rather easily transferred from a known primitive source to a modern cultivar, by applying repeated back-crossing, it may be preferable in many instances to induce resistance directly in a well-adapted high-yielding variety, using the mutation method. The most recent complications on this subject have been made by Jörgensen (1969 and 1971) and Favret (1963 and 1971, in press). Recently, a panel of experts convened by the Joint FAO/IAEA Division, dealt with problems of disease resistance, the proceedings of which will appear in 1971.

Mutations giving resistance to mildew have been repeatedly obtained. Jörgensen has shown that a series of recessive mutants with specific properties of resistance and necrosis were allelic. In contrast to most spontaneous genes and alleles, generally dominant, it is not situated in chromosome 5. The Swedish mutant Refoma originated from the variety Foma and is inferior by some 5—10%. Unfortunately, Foma (and automatically its mutant Refoma) is susceptible to attacks by *Helminthosporium sativum* (spot blotch). Owing to this, the parent has lost most of its former area of cultivation. This difficulty also influences the use of the mutant in crosses. However, many promising varieties carrying the Refoma-gene are now being tested. The resistance gene in Refoma represents a new type of resistance not found to occur spontaneously and so may prove quite useful in the continued efforts to protect the barley crop against mildew.

In 1964—1966 Mari barley was treated for three years with chronic gamma irradiation (from sowing to harvest) in the caesium-137 field of the Royal College of Forestry near Stockholm. The population method devised by Gustafsson (1951) was applied. A reasonably high level of productivity was obtained by the group threshing of irradiated plants. One "sterility" group and one "fertility" group were isolated. Resistant barleys had never been used or cultivated in this region of Sweden.

In later generations, plant progenies from individual $\gamma_2, \gamma_3, \gamma_4$ plants and spikes were tested for mildew resistance, in greenhouse inoculations 1968—1971, and in field cultures 1968—1970. The repeated chronic irradiation gave rise to numerous chlorophyll and sterility mutants, dwarfs of various kinds, translocations and other chromosomal rearrangements, *densinodosum*, *erectoides* and *eceriferum* mutants, in addition to reversions in straw-height and lateness. Moreover, at least 15 original spike progenies showed partial or complete resistance to almost all tested mildew races of Sweden (31 races). These fifteen spike progenies correspond to seven original plant progenies. It is evident that two (or more) spike progenies of an original γ_2 plant may show different reaction types of resistance, also including full immunity.

The theoretical analysis is under way. It is certain, however, that the chronic irradiation has led to several cases of resistance (perhaps multiplied by the use of the population method). It is unfortunate that such irradiation and screening programs were not previously adopted, for instance in the case of Pallas, which may have prolonged its wide use considerably.

Yield tests of these Mari mutants have not yet been carried out. If not immediately releasable — owing partially to the recent release of varieties like Visir, Mona and others — the mutant genes and alleles will certainly be useful in further recombination work, combined with the valuable gene background of Bonus/Mari.

The mentioned steps of improvement Bonus-Mari-Kristina-Mona have taken twenty years from the isolation of early 8 (*mat-a*^{8}) and ten years from the release of Mari, an advance in time as compared to the Pallas events.

The case of an inferior mutant of Gull barley and its improvement: Gunilla barley

Gustafsson (1947, p. 33) described the quoted mutant in some detail: "It differs much from all barley lines growing at present at Svalöf. The seedlings early become very bushy and at the same time extremely waxy . . . A most remarkable feature is the extreme straw-strength . . . The extreme straw-strength is counteracted by two bad properties, a low 1000-grain weight and an inferior malting quality . . . This interesting mutant reveals one of the disadvantages of the X-ray method: while good "progressive" properties can certainly be induced, changes often arise that reduce the value of the mutant". The mutant arose 1939 and was denoted 44/3. In spite of the low grain weight it had an almost normal yield, compared to Gull itself.

The variety "Gunilla", officially approved as a commercial variety in 1970, was the outcome of the cross Birgitta × Å 56888. This latter component was constructed by a cross of (Opal × Vega) × the blue, lodging-resistant mutant 44/3. Opal is a southern medium-late two-row variety. Vega is an early northern six-row type. The cross of these two varieties gave, after intense selection, rise to some early two-row types which, however, lacked several desired two-row characters. To reach this goal, the F_1 of the Opal × Vega strain and the blue mutant was once more back-crossed to the mutant, leading to the selected line Å 56888.

The variety Birgitta, taking part in the final production of Gunilla, also contains genes from Opal × Vega but further recombined with the southern variety Maja, and then Birgitta was selected. Gunilla, therefore, has genes from six-row Vega, two-row Opal, Maja, Gull (both Opal and Maja are Gull crosses), loaded with even more Gull genes through the mutant, and further back-crossing, adding the required

Table 1. *Agronomic properties of the Gunilla barley compared to five prominent Swedish strains (1964—1969)*

	Ingrid	Gunilla	Birgitta	Gunilla	Mari	Gunilla	Arla	Gunilla	Edda II	Gunilla
I. *The four northern-most districts*										
Relative yield	100	*106*	100	*105*	100	*108*	100	*114*	100	*110*
Earliness, days	102	*97*	99	*96*	102	*98*	95	98	88	97
Lodging resistance*	53	*72*	72	*80*	84	*90*	71	*90*	55	*87*
II. *Four more southern districts of North Sweden*										
Relative yield	100	97	100	98	100	*104*	100	*105*	100	*128*
Earliness, days	98	*94*	95	*94*	94	94	93	94	92	95
III. *South and central parts of Sweden where variety was tested*										
Relative yield	100	94	100	97	100	*101*	100	*113*	100	*140*
Earliness, days	105	*101*	103	*101*	101	101	98	101	89	90
II. + III.										
Lodging resistance*	81	*83*	83	83	76	*82*	68	*82*	47	*82*

* 100 = fully erect, 0 = fully lodging

characters of the mutant (lodging resistance, blue bushy habit, wide adaptability).

The mutant was indeed a "useful" ingredient, not least owing to the adaptability to the North-Swedish climate. Selection was carried out for the obvious phenotypical characters of the original mutant. By the cross with Birgitta, a large-kernelled variety, the small grain size and weight was finally overcome.

Gunilla has been tested since 1964/66. Judging from the trials, it is especially adapted for the North-Swedish area of cultivation. From the yield point-of-view it is superior to all other strains cultivated there. Here it is compared to one medium-late variety (Ingrid), one medium-early (Birgitta), one early (Mari) and two very early varieties (Arla and Edda II; the latter one a six-row barley).

Earliness, lodging resistance and relative yields for the years 1964—1969 are found in Table 1.

The very high productivity, the favorable earliness data, the outstanding lodging resistance are obvious.

This further stresses the point that, by well-planned and careful experimentation, a mutant with favorable *and* unfavorable (even disastrous) properties can be transferred into a most valuable agronomic cultivar. In this special case the favorable and the unfavorable properties were so closely connected (by pleiotropism or linked genes) that they could not be separated until the cross with the large-kernelled Birgitta overcame the striking, negative character of small seed size. The rich tillering, the blue bushy habit, and the lodging resistance were the favorable properties kept.

This new variety also has potentialities for further improvement. It is rather easily attacked by mildew

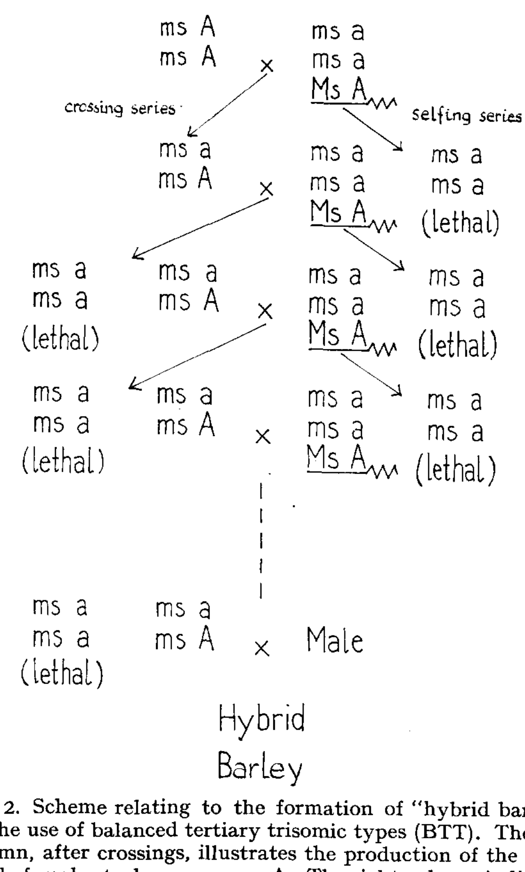

Fig. 2. Scheme relating to the formation of "hybrid barley" by the use of balanced tertiary trisomic types (BTT). The left column, after crossings, illustrates the production of the male sterile female stock — ms a ms A. The right column indicates the multiplication of the balanced tertiary trisomic (BTT) after continued selfing — ms a ms a Ms A. At the extreme bottom: the final production of hybrid seed by using a suitable male partner

in southern regions. Resistance can be introduced by back-crossing with old or new sources of resistance (immunity). In northern parts of Sweden the mildew attacks are generally not severe.

Chromosome engineering, a tool in the production of hybrid barley

In order to produce hybrid barley Ramage (1965), and Wiebe and Ramage (1969) have devised ingenious methods of using mutants with male sterility (*ms*), chlorophyll deficiency (*a*) and chromosome translocations. Most of these translocations are induced and isolated in the Bonus variety.

The principle is simply, as described by the authors, to use balanced tertiary trisomics (BTT), with the breakage point closely linked to genes for male sterility and chlorophyll deficiency. The dominant alleles are carried by the extra interchange chromosome, the recessive alleles by the normal chromosomes.

BTT plants, constituted in this way, produce only one kind of functional pollen: ms a, but mainly two kinds of egg cells: ms a and Ms A . ms a[1], because of a preferential disjunction of the two normal chromosomes. The gametes receiving only the interchange chromosome ( ) abort.

Selfed BTT plants, ms a ms a Ms A , produce the following progeny (*l. c.*; Fig. 2):

Pollen	Egg cells	Progeny	Frequency	Viability
ms a	ms a	ms a ms a	70%	Die (homozygous aa)
ms a	Ms A ms a	ms a ms a Ms A	30%	Viable (A covers aa)

Consequently, the progeny will consist of only BTT. The BTT plants may successively be multiplied as far as required by the repetition of the same procedure (Fig. 2, to the right). BTT is used as male in the recurrent production of the female stock, with the constitution ms a ms A, male-sterile but normally green (A covers a). This stock is obtained in the following way (Fig. 2, to the left): ms A ms A (male-sterile, normal) × BTT (ms a ms a Ms A) gives rise to ms a ms A only.

The female stock ms a ms A is then finally, after sufficient multiplication, crossed to a suitable male partner specially selected to produce the superior hybrid seed (Fig. 2, extreme bottom). "The experienced hybrid seed producer who uses the best scientific knowledge available, will contribute his skills in devising methods and procedures for maximizing crossed seed production, for finding efficient ways of growing and maintaining parental seed stocks; and for developing adapted high-yielding, high-quality hybrids for each barley-growing area" (Wiebe and Ramage *l. c.*).

Interchange chromosomes (reciprocal translocations) have been induced and isolated in great numbers, especially in the Bonus barley, parallel to other mutation studies (Hagberg and his coworkers; *v.* for instance Hagberg and Hagberg 1968). They have then been utilized in Swedish-American analyses of breakage points and their distribution to different chromosomes, building up entirely new chromosomes (also with a new basic chromosome number $x = 8$) and special tester sets for gene localization. In the hybrid barley, synthesized in the way described (or in similar, even more advanced ways), the Bonus translocations form an essential ingredient. The translocation line T2-7d, with the breakage point closely linked to the gene for "lemma-like glumes" (macrolepis) was the source of Ramage's initial BTT-type now used commercially.

Translocation lines, when appropriately selected and crossed, will give rise to duplications for definite genes and gene sequences. This is another sort of chromosome engineering which will successively turn out useful in diploid crop plants, also for the mapping of gene sequences in the individual chromosomes.

Here it may be added that translocation lines, when heterozygous, generally decrease fertility. In the homozygous condition, on the contrary, they are usually equal to their parent lines in productivity (*v.* for instance Gustafsson *et al.* 1966). Chromosome breakage, formerly considered a deleterious phenomenon, is, in the form of reciprocal translocation, mostly insignificant with respect to viability. Position effects are not specially prominent in barley. Chromosome rearrangements of the translocation or inversion type do not greatly influence morphology, physiology and viability. The phenotypical expression is more or less of a modifier character. When the break takes place in (or close to) a mutated gene locus, the breakage event gives rise to a mutant character absolutely linked to the translocation. Such is the case with *ert-c*[1], *ert-c*[14], *ert-c*[59], *ert-d*[7], *mat*[6], and others.

Conclusions

The following evidence is apparent, judging from the reported cases of mutants and mutant crosses: (1) Mutants, including those of a discrete nature, have been released directly as new varieties. Some have gained a wide distribution. (2) They have been used, with great advantage, in hybridization work (including back-crossing), as readily or, rather, more readily than ordinary varieties. (3) Unfavorable

[1] The sign ______ below ms a denotes a normal chromosome, the sign ____~~~ below Ms A an interchange chromosome.

traits have been separated from the favorable ones by well-planned hybridization procedures. Mutant characters, even of the harmful kind, are counteracted (or covered), using strains strictly contrary to the mutant (or mutant cross) in the harmful character involved. (4) After the induction of numerous chromosome translocations, preferably by irradiation, well-analyzed translocated chromosomes constitute a useful genetical tool for engineering purposes, leading to, *inter alia*, duplications of genes and gene segments, the origin of trisomics, valuable in hybrid barley production, profound changes of linkage patterns, etc.

The mutant examples, presented in this treatise, deal with qualities like lodging resistance, semi-dwarf habit (straw height), nitrogen preference, earliness, day neutrality, wide adaptability, and high productivity. Properties like malting quality and disease resistance have also been considered, likewise changed ecological (climatical) preferences. High increases in protein content resulted from the mutation work of Scholz (1960 and later), in barley. Recent data by Doll (1970) on protein and amino acid balance in induced mutants suggest possibilities for further improvement, especially with regard to lysine content.

It has been a question over the years whether mutations with discrete, qualitative effects (critomutations, Gustafsson 1970) or small, quantitative effects (acritomutations), are the most important ones in mutation breeding. The question is meaningless. Critomutations have given rise directly to new released strains of wide-spread use and importance. They have also been useful in cross-breeding programs, involving hundreds of genes; most of these having small effects. Moreover, there is, as pointed out repeatedly, no distinct line between the two groups of mutations. Both types of effects will turn out useful, depending on breeding aims, varieties used, kinds of environment. Pallas and Mari imply morphological changes of a rather conspicuous character, but involve also effects of a more physiological kind. The mutant 44/3 from Gull, although striking phenotypically, was classified as a physiological mutant. The first mutant released in a self-fertilizing crop, Strålärt in *Pisum* (Gelin 1954), produces in dry seasons a higher branching than the parent variety and is of a strictly quantitative type.

The mentioned cases may be considered "factor" mutations. Such are in general fully viable and fertile in the heterozygous condition, if freed from chromosome rearrangements, but decrease productivity in the homozygous state. Translocations, on the other hand, decrease fertility in the heterozygous state (owing to meiotic disturbances) but are generally completely fertile and productive as homozygotes. In an article of 1965 it was considered that approximately 80% of homozygous translocations were of this high-productive kind (Gustafsson 1965; cf. Gustafsson *et al.* 1966). This contrast is well worth pointing out in the present lively discussion of so-called adaptively neutral traits (King and Jukes 1969, Dobzhansky 1970). If translocations (and inversions), in a diploid like barley, are not accompanied by deficiencies at the points of breakage, they are in fact "neutral", with some narrow variation to the plus and to the minus side. On the other hand, they may exert some slight phenotypical effects, not easily detected or defined. Moreover, they certainly are effective in breaking and changing gene linkages. This may have a great operational value with regard to crossing-over in gene segments of heterozygotes. It is our opinion that the full use of translocations (and transpositions) in plant breeding has not yet been realized, in spite of the ingenious procedures worked out by Sears (1956 and later), by Ramage and Wiebe (in papers cited), and Hagberg and Hagberg (l. c. and previously), as well as by scientists not quoted here.

Returning to the starting point, the positive or the negative attitude to the use of induced mutations in plant breeding, we can now definitely assert that the negative attitude has failed (or was based on false premises). Nilsson-Ehle, a successful breeder of wheats, oats and barleys, often used the expression "It is as unscientific to be too critical as too little critical." Plant breeding, comprising many fields of applied genetics, is an undoctrinary discipline, requiring talent, experience and optimism. In addition to the mutation method, now fully appreciated, covering both induced and spontaneous mutations, we have to include successively new aspects of genetics and agrotechniques: transformation of chromosome DNA, genetic alterations of the cytoplasm (involving plastid, mitochondrial, ribosomal DNA and RNA), and a series of biotechnical mass screening methods. The green evolution, in a modern sense, has just begun.

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