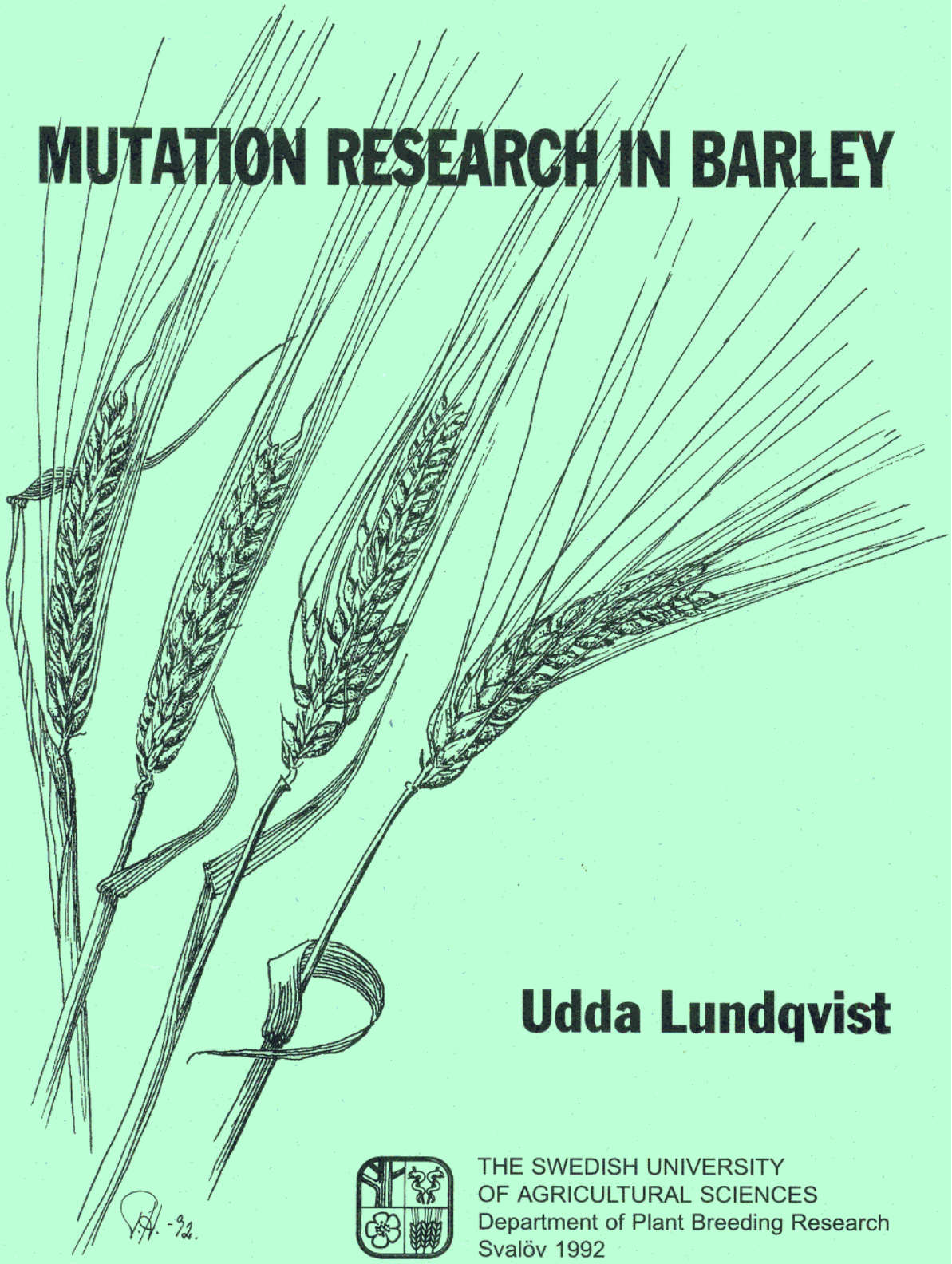
MUTATION RESEARCH IN BARLEY



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A. DEVELOPMENT OF SWEDISH MUTATION RESEARCH

1. Historical background

Swedish research on induced mutations started in a small scale at Svalöf already in 1928, more than 60 years ago, at the initiation of Herman Nilsson-Ehle and Åke Gustafsson. In 1927 the eminent American geneticist and, later, Nobel laureate H.J. Muller, using ingenious experiments, had shown that ionizing irradiation could increase the mutation frequency in the fruit fly *Drosophila*. New, large vistas for future research were opened (Muller, 1927, 1928).

Muller was hoping that induced mutations were similar to spontaneous mutations, which form the basis for natural selection and evolution. But already in 1930, the equally eminent L.J. Stadler, the famous American geneticist and plant breeder, published data on induced mutations in several species of cultivated plants, which he interpreted with much pessimism. In his opinion, no practical progress could be expected from artificial gene changes. This strong pessimism was based on three considerations. First, a great wealth of germinal variation is already available in nature. These genes have survived severe selection pressure. Secondly, induced mutations are in most cases unfavourable. The rare favourable mutation is likely to be accompanied by unfavourable mutations. Thirdly, characters of value in breeding are more likely to be found in the varietal collection than in the progeny of X-rayed plants. (Stadler, 1930).

Nilsson-Ehle and Gustafsson did not share this pessimism. On Gustafsson's suggestion, experiments were initiated with induced mutations in plant material.

The first irradiations

At the beginning of the thirties the first treatments with X-rays and ultraviolet irradiation commenced in barley, using the Svalöf variety 'Gull'. For the first X-ray irradiations a Coolidge tube belonging to the Geological Institute of the University of

Lund was employed (P.A. Herrlin). Different durations of irradiation were applied instead of measuring exact dosages. For the main experiments, during the late 30s the equipment at the Radiological Institute of the University of Lund was used (L. Edling) (Gustafsson, 1936, 1940).

Already at that time different types of treatments had been tested. Since Stadler's investigations (1928) it was known that the mutation frequency increases considerably if the seeds are soaked in water before being irradiated. Accordingly, the following methods were tried: (1) Seeds were dried to a lower water content; (2) seeds were presoaked; (3) seeds were kept under conditions where they were not able to germinate or absorb water. The first chromosome aberrations were studied; mainly chromosome fragmentations, fusions and translocations occurred. In the same experiments sensitivity to exterior agencies was studied, and the highest rate of mortality was found in those cells which are the first to divide, when germination sets in, and which also have the highest water content. Also the first genotypical changes in the seedlings, chlorophyll mutations, occurred. In most cases these mutants were sublethal, and because of their distinct colour changes, they were very useful material for laboratory studies (Gustafsson, 1947).

The chlorophyll mutations

Already at that time distinct categories could be established to form a practical system that was very valuable for future mutation research. Chlorophyll mutations were the most common mutations after different types of irradiation, and were always the first indications of how successful the treatment was. (cf. figure 1).

The different categories could be classified into the following types. [a] *albina*: white seedlings without carotinoids or chlorophyll, [b] *viridis* (lutescens, virescens and chlorina): this is a very heterogenous group characterized by yellowish-green or light-green types at the seedling stage. The chlorina, lutescens and virescens are viable, all the others lethal, [c] *xantha*: light yellow coloured where the carotinoids prevail or the chlorophylls are not even produced, [d] the two-coloured chlorophyll mutations: *alboxantha* (tip white, base yellow), *xanthalba* (tip yellow, base white or faintly coloured), *alboviridis* (tip white, base green or yellowish-green), *viridо-albina* (tip more or less greenish or yellowish-green, base white), [e] *tigrina*: transverse

destruction of pigments, the transverse stripes are usually brown or yellow, narrow and pinched, [f] *striata*: longitudinal stripes of white or yellow colours, [g] *maculata*: chlorophyll and carotin destruction in the form of dots distributed over the leaf, [h] *zonata*: dark and light coloured bands like distinct zones are spread over the leaf (Gustafsson, 1940) (cf. figures 17 and 18, p. 77).

Figure 1. Barley spikes planted for greenhouse analyses of chlorophyll mutation frequencies in second generation seedlings.

Frequencies of mutations

The mutation frequency or rate was calculated according to the "spike progeny method", as the number of segregating spike progenies divided by the total number of spike progenies. This method was introduced by Gustafsson (1940) and has served as the standard method for measuring induced mutagenic effects.

The frequencies of the different chlorophyll mutation types at this early stage varied according to the different irradiation doses and also depended on the water content in the seeds, whether seeds are dried or water-soaked in connection with X-raying. The frequency of *albina* mutations compared to the other mutants is much higher, and they arise more readily at low dosages and in water-soaked progenies. *Xantha* mutations are most easily produced at relatively high dosages. *Viridis* mutations are most easily induced by increasing the dosages and soaking in water. Most of the two-coloured chlorophyll mutations are produced as easily in dry materials as in wet. Finally, *tigrina* mutations are more rare and arise exclusively by irradiation of dry seeds. Already at that early stage of the mutation research it is possible to talk about directing the mutation process (Gustafsson, 1940, 1941a).

The sterility phenomena

A very important character was studied very intensively: the sterility in the X_1-generation (X is used for generations derived from irradiated seeds). It was very soon noticed that after X-raying of seeds a certain sterility was induced in the X_1-plants, varying according to the X-ray dosage and the physiological state of the cytoplasm. The sterility of X_1-plants increases with the dosage and the physiological activity of the seeds. This primary effect was to be a very important criterium for all different mutagenic treatments. The sterility of all spikes of the individual plants was evaluated, and four, later five, different fertility classes were established. It could also be determined that there was a connection between sterility and chromosome aberrations. A very high sterility rate (0% - 70% fertility) indicated that all or most of the spikes showed irregularities at meiosis, a high to full fertility (90% - 100%) in the spikes was associated with a more or less regular meiosis. This result was very important for later investigations, especially when isolating translocation lines.

It could also be shown that there is a conspicuous parallelism between sterility and mutation rate, even for the different mutation types. Mutation frequency and degree of sterility are roughly proportional. A high fertility rate is connected to low mutability. When increasing the dosage of X-rays, the X_1-fertility is affected greatly. The increased mutation rate is limited to the offspring of sterile X_1-plants, while the mutation rates of fertile X_1-plants remain constant (Gustafsson, 1940).

The sterility of individual spikes and plants is connected with chlorophyll mutating in a special way. *Albina* mutations, for example, are randomly distributed, arising independently of the X_1-sterility. They occur abundantly even in the offspring of 100% fertile X_1-plants and spikes. *Xantha* mutations do not arise, or only with difficulty, in the offspring of completely fertile X_1-plants. They accumulate in the offspring of slightly sterile X_1-plants, and they are most easily produced at relatively high dosages (the range of irradiations applied, 500 - 25 000 r). *Viridis* mutations are most easily induced in material with greatly increased sterility and then mainly in the offspring of highly sterile plants, where translocations and other chromosomal aberrations are accumulated. The two-coloured mutants are chiefly produced in the progeny of sterile plants.

Finally it can be concluded that the X_1-sterility and the mutation frequencies, as well as the rate of chromosome disturbances, serve as a measure of the sensitivity to X-irradiation (Gustafsson, 1940, 1941a).

The first viable mutations

Already in the mid thirties the first "vital" mutations appeared. It was possible already at that time to distinguish two sub-groups: [1] *Morphological mutations*, in which morphological characters are altered; as a rule they segregate sharply as qualitative characters in intercrossings or in crossings with the original line. [2] *Physiological mutations*, which can be clearly distinguished from the original ones, but only in properties with quantitative variation relating to straw-length, straw-stiffness, lateness, early maturity, tillering capacity, seed-size, seed-colour, and others. There is hardly any well-defined boundary between these two sub-groups, as all mutations observed were pleiotropic (Gustafsson, 1941a).

The most common group of vital mutations at that time consists of the so-called *erectoides* mutations. They are easily distinguished from the original variety 'Gull', which has a typical *nutans* spike, by *compact* or *dense* spikes. Morphologically they resemble the *erectum* barleys. The spike of the erectoides mutants, besides being more compact, is also erect, and the straw is often shorter and stiffer. Some of them have also an increased wax coating. The number of induced *erectoides* mutations in comparison to chlorophyll mutations seems to be much more limited. In later

investigations, during the forties and fifties it could be calculated that they arise at a rate of one erectoides per 25 chlorophyll mutations (cf. figure 2).

Figure 2. Two different *erectoides* mutants compared with the mother variety 'Gull' and a lax spike. From left to right: a most drastic erectoid spike, a normal erectoid spike, 'Gull', and a laxatum spike. (By courtesy of Arne Hagberg).

Besides these erectoides mutants other important morphological and physiological mutants could also be isolated. Some of them are worth-while mentioning here, as they have been important in the future mutation research. They are: *six-row* mutants, changed glumes to a lemma-like shape (*lemmalike glumes or macrolepis*), "*winter-like*" barley forms, mutants with reduced or increased straw-length, strawstiff mutants, early maturity (*praematurum*), late maturity mutants, dwarf mutants, semi-naked caryopsis mutants, changed flower construction mutants

(triaristatum), and others. Some of them are completely sterile and have to be kept alive year after year through the heterozygotes. Of course it soon could be stated that most of the mutations are detrimental, but, nevertheless, they were preserved for theoretical studies in the future, as will be shown later (Gustafsson and Åberg, 1940; Gustafsson, 1941b) (figure 3).

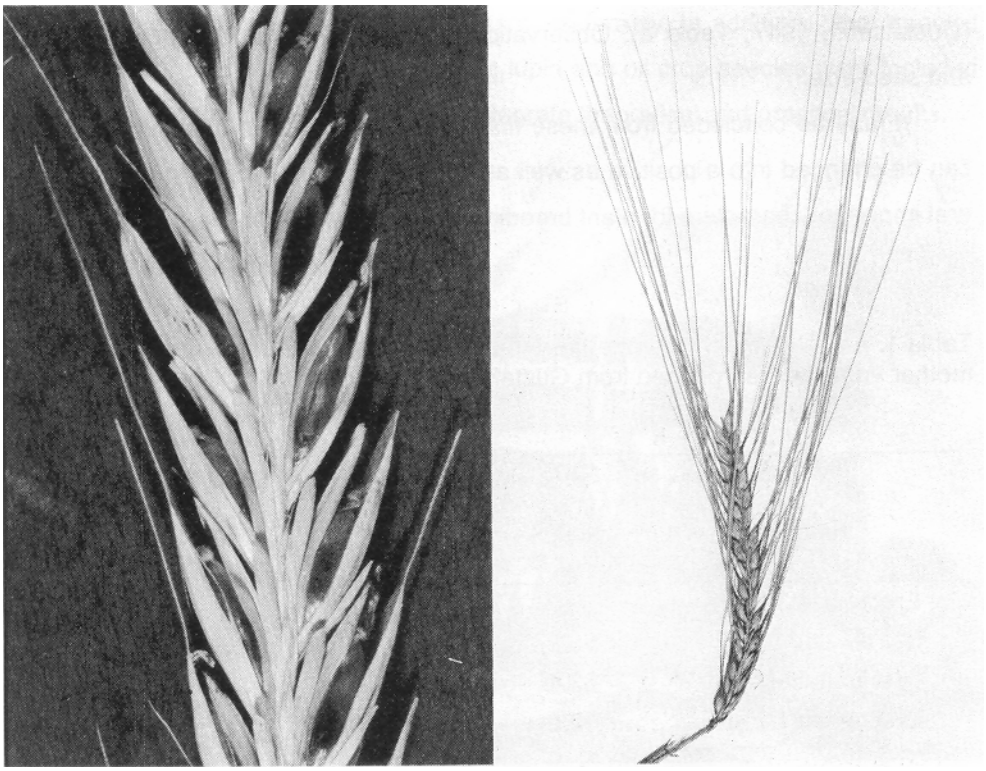

Figure 3. Two among the first isolated morphological mutants: to the left 'semi-naked caryopsis'; to the right 'triaristatum'.

Preliminary yield experiments

In the beginning of the forties preliminary yield experiments were started with the most promising mutants being compared with the mother variety 'Gull' (released in 1913) and also 'Maja' barley, the latter being one of the highest-yielding varieties at that time (released in 1927) cultivated in Scandinavia. It could be shown that at least

3 mutants (2 erectoides and 1 late mutant) equalled or surpassed the mother variety in yield; one of them attained the level of Maja barley. Several of the mutants decreased yield considerably. The 1000-grain weight could be altered in both directions. Also protein and starch contents were examined. There was a pronounced negative correlation between yield and protein content. Some results are shown in Table 1. Malting properties were studied; some of the mutants exceeded the mother variety 'Gull' in single characters of importance for a good malting barley (Gustafsson, 1947, Table 8). Observations were also made for lodging, earliness and seed-size.

It may be concluded from these first yield trials that by mutations 'Gull' barley can be changed into a positive as well as into a negative direction in regard to several important characters for plant breeding (Gustafsson, 1941c).

Table 1. A survey of three properties tested in eight mutants in comparison to the mother varieties (reproduced from Gustafsson, 1941c, Table 2)

Mutants and varieties	1000-grain weight gr	Protein content %	Starch %
Erectoides 3	58.2	11,48	60,4
'Maja'	55.2	11,97	59,03
Late mutation	53.4	12,3	59,52
Erectoides 1	50.0	13,8	56,75
'Gull'	50.9	13,8	57.80
Erectoides 2	49.5	14,58	55,4
Erectoides 5	46.5	14,95	54,98
Erectoides 4	46.9	15,15	54,58
Lemma-like glumes	51.3	15,65	56,53
Strawstiff mutant 1	45.2	16,03	56,35

2. The Group for Theoretical and Applied Mutation Research

The results from these experiments looked so promising, even for plant breeding that, in 1940, the Swedish Seed Association at Svalöf started sponsoring this new research, with financial support from the milling industry by the initiation of the Head of the Swedish Seed Association, Åke Åkerman (Gustafsson,1954). This rendered it possible to extend the experiments considerably. In addition, other species such as wheat, oats, soybeans, flax, sweet lupin and oil crop species were included in the programme. It became possible to integrate theoretical and practical results.

Figure 4. The Group for Theoretical and Applied Mutation Research, meeting at the Weibullsholm Plant Breeding Institute in the mid 50s.

In 1948, the Wallenberg Foundation incorporated mutation activities into its research programme, thereby permitting Åke Gustafsson to gather a group of specialists to carry on the research work on a wider front. Finally, in 1953, at the instigation of the Swedish Government, the "Group for Theoretical and Applied Mutation Research" was established, with the aim of studying basic research problems in order to influence and improve the methods for breeding cultivated plants (Lundqvist, 1991) (cf. figure 4).

The mutation group comprised scientists of several institutions and organizations, performing investigations and studies in basic as well as applied research. The institutes which have been involved are listed below:

[1] The Forest Research Institute of Sweden, Genetics Department, Stockholm (Å. Gustafsson, D. von Wettstein, M. Simak, G. Eriksson, I. Ekberg).

[2] Institute of Organic Chemistry and Biochemistry, University of Stockholm (L. Ehrenberg and co-workers).

[3] Institute of Radiobiology, University of Stockholm (L. Ehrenberg and co-workers).

[4] The Swedish Seed Association, Svalöv, later Svalöf AB (Å. Åkerman, O. Tedin, G. Andersson, K. Fröier, E. Åkerberg, A. Hagberg, J. Mac Key, G. Olsson, G. Persson, J. Sjödin, I. Törnqvist, K. Wiklund, A. Wiberg).

[5] Institute of Genetics, University of Lund (A. Müntzing, A. Levan, N. Nybom, G. Holm and co-workers).

[6] Weibullsholm Plant Breeding Institute, Landskrona (O. Gelin, S. Blixt).

[7] Fruit Breeding Institute, Balsgård, Fjälkestad (I. Granhall, N. Nybom and co-workers).

[8] Institute of Plant Breeding, University of Agriculture, Uppsala (G. Turesson, J. Mac Key, T. Hermelin and co-workers).

[9] Institute of Genetics, University of Copenhagen (M. Westergaard, D. von Wettstein, O. Frydenberg, B. Søgaard).

[10] Carlsberg Laboratory, Physiological Department, Copenhagen (D. von Wettstein, P. von Wettstein-Knowles, B. Søgaard, B. Jende-Strid).

In addition, the following institutes have been in co-operation with the above mentioned institutes, with facilities generously placed at their disposal:

[1] The Nobel Institute of Physics, Stockholm (M. Siegbahn).
[2] The Radiophysics Institute, Stockholm (R. Sievert, A. Forssberg).
[3] Gustaf Werner Institute, University of Uppsala (T. Svedberg).
[4] Institute of Plant Physiology, University of Agriculture, Uppsala (H.Lundegårdh, I. Ekdahl).
[5] The Royal Veterinary College, Stockholm.
[6] The Isotope Department, Joint Establishment for Nuclear Energy Research, Kjeller, Norway (E. Saeland).

The Agricultural Research Council had provided the financial support for most of the Mutation Group's scientific activities, from special funds appropriated by the Swedish Parliament (Gustafsson, 1954.)

3. Comparison of variatious types of radiation

Ionizing radiations

As described above, X-irradiation on dry seeds was used as a standard method for studying the mutation process. As soon as different research groups were established, new types of irradiation such as γ -rays (acute and chronic), neutrons (fast and thermal), electrons, protons, α-rays from radon, β-rays from Phosphorus 32 and Sulphur 35 have been included in the experiments. Also the application of different pre-treatments with different soaking times of the seeds, both before and after irradiation was studied. Other modifications of the irradiation in connection with nitrogen, oxygen and colchicine were introduced. For all the different experiments, the best yielding barley cultivars have always been used. From now on, the Svalöf variety 'Bonus' (released in 1952) was used as experimental material.

Very soon, in the early 50s, during the different series of experimental treatments (it being difficult to reproduce an experiment from one year to another) it

could be stated that the water content of the seeds was an important trait in relation to radiation sensitivity. The sensitivity turned out to be high at very low water contents, decreasing to a minimum at 12 per cent water (concerning barley), and increasing again at still higher water contents. The physiological state of so-called dormant seeds is rather undefined, being influenced by their water content, storage temperature and age. For a standardization of the material seed samples were equilibrated for a certain time period at different relative humidities (Ehrenberg, 1955; Ehrenberg et al. 1956). Concerning the action of ionizing radiations, both the chemical and the physical actions are of importance. Many different experiments, measurements and calculations have been done in co-operation with radiophysicists and radiobiochemists (Ehrenberg, 1954). In this presentation I will concentrate on studies obtained on immediate effects, later effects and induced mutations by different types of irradiation and their modifications. Not only is the water content of the seeds of importance, but also different environmental conditions.

Two types of ionizing radiations differing in ion density produced and the distribution of the ionizing rays are to be distinguished:

a) sparsely ionizing radiation (X-rays, γ-rays)

b) densely ionizing radiation (fast and thermal neutrons, electrons, protons, α- and β-rays from isotopes).

Many different experiments with these two types of ionizing radiation have been compared where seeds of different metabolic activity (dry dormant seeds with a water content of about 10 - 12 per cent as well as presoaked germinating seeds) were treated in various ways. The following properties have been analyzed more or less as a standard method: [1] the number of chromosome disturbances in the germinating seeds immediately after irradiation, [2] the field germination, determined about 3 - 4 weeks after the time of sowing, [3] the number of mature plants at the time of harvest, [4] the average plant and spike fertilities of the mature plants, [5] the mutation rate determined from the number of various chlorophyll-deficient mutants of different kinds arising from the ripe spikes when planted in moist sand, and [6] the different types and frequencies of vital mutants determined on field material in the second generation after the treatments. Experiences from these experiments

(Ehrenberg et al., 1952a; Ehrenberg and Nybom, 1954; Ehrenberg and Gustafsson, 1954) may be summarized as follows:

The use of X-rays on *dry dormant seeds* showed a rapid increase of cells with chromosome disturbances within the dose range used. The number of mature plants in relation to the number of seeds sown, rapidly decreases in the higher dose range. The fertility of the harvested X_1 plants is on average fairly high, it rarely gets reduced to below 60 or 70 per cent, and the mutation rate decreases after high X-ray treatments (Ehrenberg et al., 1952a; Ehrenberg and Nybom, 1954).

On the contrary, when neutrons are used on *dry dormant seeds* the frequency of chromosome disturbances immediately after irradiation is only about ten times as high as in the case of X-rays. There is a high number of surviving plants even in the upper dose range. The plant fertility approaches zero within the region of 100 - 1000 rep, the mutation rate increases more or less proportionally to the dose and the distribution of the mutation types differs from that of the X-rays (Ehrenberg et al., 1952a; Ehrenberg and Nybom, 1954).

When comparing the two irradiation types in the experiments with *presoaked seeds*, X-rays behave in most respects like the treatments with dry dormant seeds. The frequencies of chromosome disturbances is about five times higher. The number of surviving plants is greatly decreased. The plant fertilities and mutation rates correspond to those of the dry seed series. However, when using neutron irradiation the results markedly differ from those with the dry dormant seeds. There are about five times as many cells with chromosome disturbances, the plant mortality is greatly increased within the dose range of 100 to 1 000 rep, leading to a low DL_{50}. Plant fertilities decrease rapidly, the mutation rate reaches a very high level, and a relatively high per cent of *albina* chlorophyll mutations is found. Among the morphological mutations there are also some mutagen specificities to be noticed, e.g., the *erectoides* (dense spike) mutants, which are much more frequently induced after neutron- than after X-irradiation, and within the *eceriferum* (waxless) mutation group there is one locus specialized on neutron-induced mutants (see chapter B.1) (Ehrenberg et al., 1952a; Ehrenberg and Nybom, 1954).

When some of the isotopes are used as mutagenic agents, e.g., radon (α-rays), the effects resemble the neutron action on the same type of material. For Phosphorus 32 and Sulphur 35 (β-rays), the effects have to be compared with the X-ray treatments of presoaked seeds. It can be said that their mode of action is intermediate between X-rays and neutrons (Ehrenberg et al., 1952a; Ehrenberg and Nybom, 1954).

In summary, it can be concluded that the injurious action of neutrons differs from that of X-rays in several respects: a) the seeds are 20 -30 times more sensitive to neutrons than to X-rays, when equal doses are given, and b) germinating seeds are only 2 - 3 times more sensitive to neutrons than dormant seeds. The differences between neutrons and X-rays are due to the difference in ion density produced; neutrons are approximately 10 times as effective as X-rays of equivalent energy dissipation in producing chromosome disturbances, and about 50 to 100 times as effective in producing first generation sterility and increasing the mutation rate in the second generation. The neutrons produce relatively more chlorophyll mutations than X-rays do. It is also observed that, while X-irradiated seeds, (except those that survive) die at a very early stage of development, the neutron-treated seeds which have got a lethal dose often start their germination, not dying until cell divisions become of critical importance for their further growth. Generally stated: The number of mutants increases in proportion to irradiation doses within the low and medium range but causes a considerable drop or stop at high dosages depending on various elimination processes.

Modifying treatments in connection with irradiation

Several experiments with sparsely and densely ionizing irradiation have been tried with different oxygen pressures, nitrogen atmosphere, variation in water content, protection with hydrogen sulphide and different temperatures, especially low ones, during irradiation, both on dry dormant and pre-soaked seeds. The same properties as mentioned above have been studied. Summarizing the results after these modifying treatments, it can be stated that a change in oxygen pressure at the time of

irradiation has a profound effect on all properties examined, and that it exerts a much smaller influence on the sensitivity of germinating seeds, but increases the irradiation damage (Ehrenberg and Gustafsson, 1954).

On the other hand in treatments with nitrogen the X_1 damage is less pronounced, there is a better germination in the field, a larger number of harvested plants, a higher average plant and spike fertility, and a lower mutation rate at the lower dose rates when compared with seeds irradiated in the normal manner. Nitrogen and hydrogen have a distinct protective influence. When equilibrating the seeds to water contents ranging from 7 to 20 per cent, the driest seeds were the most sensitive ones to sparsely ionizing radiations, but not when the irradiation is performed with neutrons. There is a striking change of the mutation spectrum, e.g., a pronounced increase of viridis mutations. Judging from chromosome disturbances, germination conditions, and mutation rates, irradiation at low temperatures (-190^{o}C) is less injurious. The low temperature acts as a protection against physiological as well as genetical X-ray damage. It eliminates most or part of the indirect radiation effects.

All these modifying influences on the radiation sensitivity are to be described as after-effects. In conclusion, after all the different modifications of treatments - chemical protection, varying oxygen tension, or by a variation of the physiological conditions of the cell - the neutron effect cannot be influenced, in contrast to the effect of sparsely ionizing irradiations (Ehrenberg et al. 1952b, 1953; Nybom et al. 1952, 1953; Ehrenberg and Gustafsson, 1954; Gustafsson et al. 1956/1957).

So far, all the mutations had primarily been induced by acute irradiations of seeds. As a means of completing, improving and modifying the methods of irradiation, a ^{60}Co-source (chronic γ-rays) was used to induce genetical changes during different development stages of the plants, and in order to detect specially sensitive or insensitive stages during the vegetation period. The plants were either irradiated continuously or were protected by means of movable lead and iron-concrete shields during certain parts of the vegetation period.

The conclusions from this series of studies are as follows: (1) with the lowest dose-rates used per day the genetic effects of chronic irradiation are truly additive, both with regard to chromosomal re-arrangements leading to sterility in the second

generation as well as to recessive mutations appearing in the third generation; (2) there seems to be no threshold below which the irradiation becomes ineffective, when barley is exposed to chronic irradiation; (3) acute and chronic irradiations produce similar mutation rates; (4) no special sensitive period could be detected in barley, only the period from germination to spike appearance is sensitive for the induction of sterility; and (5) when growing plants are irradiated, the mode of appearance in the offspring and perhaps also the type of the mutations vary with the stage of origin during the life cycle (Nybom et al., 1956).

4. Chemical mutagenesis

The beginnings

Already in the mid forties the following chemicals were included in experiments: Colchicine, Potassium cyanide, Hydrogen peroxide, Butter-yellow, Uranyl nitrate and Ferri sulphate. The seeds were pre-treated with these different chemicals and then irradiated with 7 500 r. The idea was to influence not only the mutation rate, but also the types of mutations. The following properties were studied: 1) the percentage of mature X_1 plants, 2) the average fertility of the X_1 plants, 3) the mutation rate, and 4) the types of mutations.

In general, the pre-treated series showed a more intense irradiation effect than the dry series, the mutation rates were generally increased, and the mutation types were not markedly changed, except with colchicine. This chemical distinctly widens the range of mutability and also induces other chlorophyll mutant types, which do not normally arise in dry series. Viridis mutants become rarer, which deviates from results obtained for other chemical mutagens in later years, where rare or very rare types become more frequent. Some at that time unknown mutants were also discovered (D'Amato and Gustafsson, 1948; Gustafsson and Nybom, 1949).

Application of mustard gas substances

The real work on chemical mutagenesis in crop plants began with studies on the effects of mustards. The studies with mustards were centered on a special water-soluble nitrogen mustard, dichlorodiethylmethylamine hydrochloride or methylbis(2-chloroethyl)amine hydrochloride), $HCl.CH_3N(CH_2CH_2Cl)_2$ on dormant seeds. These treatments give few translocations, whereas lethals and chlorophyll deficiencies are common. These treatments also indicate a slow and parallel decrease in the number of mature plants and average plant fertility. Germination experiments sown indoors differ from those under unfavourable environmental conditions in the field. The mutation rate shows a slight increase compared to the control material. The chlorophyll mutant types appearing are predominantly of the viridis type, while albinas are rare and the rare types are very few. To sum up: By facilitating a diffusion of the mustard from the treated seed the effect may be greatly diminished. Mustards are less active on cells in a resting stage, the toxicity of the mustards nearly completely overshadows their ability to induce heritable mutations. The mutation spectrum obtained is clearly different from that induced by ionizing radiations (Gustafsson and Mac Key, 1948; Ehrenberg et al. 1956b).

Organic chemical mutagens

Various chemical compounds were tried as mutagens and used all over the world. The following subdivisions based on their expected mode of action can be made

(a) Epoxides and epimines
(b) Various alkylating and oxidizing compounds
(c) Purine and purine derivatives
(d) Alkanesulphonic esters

When dealing with chemical mutagens, the letter M = mutagenic is used instead of X (rays).

The exact ranging of these chemical mutagens has changed several times as the investigations have proceeded, so it is not certain that it is the definite one (Ehrenberg et al. 1956a; Ehrenberg, 1960; Gustafsson, 1960a). In the description of the different chemical mutagens used by the Swedish mutation group, the scheme

listed here will not be followed precisely. The mutagens will be discussed in the order they have been used during the experiments. The different chemicals tested during the fifties were chosen according to their chemical and biochemical characteristics.

(1) 8-Ethoxycaffeine, a purine derivative, shows a distinct dependence on oxygen tension and temperature. It causes a good deal of chromosome breakages, in this case leading to plant sterility, whereas it does not produce a distinct rise in the rate of chlorophyll mutations. The behaviour of offspring after these treatments indicates that point mutations and chromosome disturbances causing sterility are non-correlated. In other words: the data for 8-Ethoxycaffeine show that chromosome breakage and M_1-sterility can be induced by distinct chemicals, at least in barley, although visible chlorophyll mutations do not appear. It is worth while mentioning that 8-ethoxycaffeine breaks the chromosomes in quite a distinct manner. The two satellite chromosomes 6 and 7 are relatively susceptible to breakage. Here the breaks are concentrated to the regions around the centromeres, the satellite necks, and the tertiary constrictions (Ehrenberg et al. 1956b; Gustafsson, 1960a and 1960b).

(2) Nebularine, another purine derivate, which is isolated from the mushroom species *Agaricus nebularis* and has a high antibiotic activity. It is a nucleoside not occurring in nucleic acids of higher plants. Both series with dry dormant seeds and with pre-soaked seeds were carried out. A sterility effect was not detectable in any of the treatments. On the other hand, there is an indication of a higher average fertility than in the control material. Also the germination capacity is intact. The effect on mutation rate is quite conspicuous, and in well performed treatments it was up to ten times higher than the spontaneous rate. It changes the mutation spectrum fundamentally by a considerable increase of viridis mutants.

Another chemical mutagen Di(beta-chloroethyl)phenylalanine (CB-3025) reacts similarly to Nebularine and induces almost only viridis mutations (Ehrenberg et al. 1956a and 1956b; Gustafsson and von Wettstein, 1956-1957; von Wettstein et al. 1959; Gustafsson, 1960a).

(3) Among various alkylating and oxidizing compounds several organic and inorganic chemicals as Iodine, Peroxide, Chlorine, Dioxan, Formaldehyde, the latter attacking amino- and other groups, and others have been included in different experiments. Most of them are rather inefficient mutagens, i.e., their effects on the biological material are very unspecific. The series of treatments have been quite small, and consequently no conclusions can be drawn (Ehrenberg et al. 1956a).

(4) Diethyl sulfate is an alkylating compound and highly mutagenic. It causes mutation rates twice as high as those obtained with sparsely ionizing radiation. Rates up to about 10 per cent have been produced in the Swedish barley experiments. These rates are below the glycidol rates (see below) but above those of neutrons and X-rays (Gustafsson, 1960a; Ehrenberg et al. 1961).

(5) Ethylene oxide, an epoxide, has been applied either in gas phase or solution. Treatments with the compound in the gas phase seem to be especially effective. Immediate seed mortality occurs first in the higher concentrations, and the degree of sterility is an exponential function of the concentration; the results resemble those obtained in treatments with sparsely ionizing radiations. Plant mortality and sterility have similar rates at low concentrations. Therefore, it can be justified to conclude that the decrease of plant survival has to be ascribed to initial chemical actions of the same kind as those causing sterility. The maximum rates of "translocation sterility" reach values of around 40 per cent, which is less than when ionizing radiations have been used. The maximum rates of chlorophyll mutations are almost equal to those induced by X-rays and neutrons. Even slightly higher values are reached. The mutation rate is practically rectilinear with the concentration. The same distribution of chlorophyll deficient mutants as produced by X-rays is obtained with a surplus of viridis types and a slight decrease of rare types. Field experiments showed that viable mutations are considerably more numerous after particular Ethylene oxide treatments than those obtained after ionizing radiation. It can be concluded that more visible mutations (point mutations) and less chromosome aberrations are induced by this chemical. Ethylene oxide turned out to be at least as efficient as mutagenic agents (Ehrenberg et al. 1956a and 1956b; Ehrenberg and

Gustafsson, 1957; von Wettstein et al. 1959).

Glycidol, a derivative of ethylene oxide, induces mutations at rates considerably higher (up to 22%) than those obtained when neutrons or X-rays are used. Glycidol does not induce as many rare mutants as does, e.g., Ethylene imine, but the rare mutation rate is about the same as with ionizing radiation. It is the most efficient epoxide to date (Gustafsson, 1960a; Ehrenberg et al. 1961).

(6) Diepoxybutane, a double epoxide. This compound is very toxic to the barley plant, and it is much more effective in causing lethality and sterility than ethylene oxide, which might be ascribed to its double epoxide character. Obviously, growth inhibition is due to an interference with nuclear division. Diepoxybutane seems to produce sterility in proportion to the concentration used, but does not produce as many mutations compared to the above described mutagen, probably due to its high toxicity. According to these results, it was stated that the polyfunctional compounds are less efficient in higher organisms like barley (Ehrenberg and Gustafsson, 1957).

(7) Ethylene imine, an epimine, in particular has a genetically devastating effect: it simply leads to an "explosion" of the barley genome. This nitrogen analogue of ethylene oxide is a strong base, and is practically exclusively ionized in a neutral environment. At that time (1957) it was found to be the most efficient mutagenic agent, at least in barley. Series with both dry dormant and pre-soaked seeds were investigated, and the influence of the pH value and the temperature during the treatment is of great importance. The pre-soaked seeds were less sensitive in the toxicity test, and it was possible to treat them with somewhat higher concentrations. Low temperature at the treatment of seeds leads to less damage than room temperature does. Also the mode of treatment considerably affects the degree of lethality and fertility, as well as the mutation rate. If the seeds are sown immediately after a treatment, lethality, sterility, and mutation rates will become decidedly lower than where treated seeds are stored for 24 hours previous to sowing. The mutation rate of the chlorophyll mutations is three to four times higher than those obtained with ionizing radiations. Rates of 25 - 30 per cent were reached, and in some cases rates

of 33 per cent were obtained in the most favourable treatments. The mutation spectrum differs widely, where the rare mutation types appear to be more frequent after the use of ethylene compounds than after ionizing radiations. There is also a decrease of *albina* mutations and an increase of *viridis* mutations, but not so pronounced as for example with Nebularine and CB-3025.

Two types of induced sterility occur: a) slight sterility, with a reduction of 5 to 20 per cent and chiefly depending on chromosome translocations; it is called "translocation sterility"; b) more profound sterility, a reduction of 50 per cent, or more, and is usually due to changes in the genes themselves. Translocation sterility is less frequent, and it does not exceed the maximum rate of 40 per cent. It can be concluded that the ethylene compounds, compared to ionizing radiations, increase the rates of chlorophyll mutation relative to the rates of translocation sterility. The sterility exhibits an exponential dependence on concentration. The induced M_1 sterility is more or less pronounced. With ethylene imine there is a considerable amount of chromosomal aberrations in the roots of seeds treated at high doses. When comparing the M_1 sterilities and the M_2 mutation rates, the contrast between ionizing radiations and ethylene imine becomes apparent. The increase of sterility is generally to a certain extent correlated to the average mutation rate. However, with ethylene imine, in contrast to ionizing radiations, mutations occur in the offspring of M_1 plants without any detectable decrease in fertility. Highest mutation rates cause around 50 per cent sterility, and the effect is about the same as with X-rays. An increase of mutation rates above 25 per cent causes increased M_1 seed lethality. At a certain mutation rate chromosomal disturbances are less frequent when treating with ethylene imine than with X-rays.

Similar results, which were received from the chlorophyll mutation tests, are also valid for the viable mutations, especially those of potential value in plant breeding, including the so-called erectoides mutants. The viable mutants comprise a variation of morphological and physiological changes. The highest mutation rate obtained with ethylene imine is 18 per cent or more, 5 per cent with X-ray, 4 per cent with neutrons and 9 per cent with ethylene oxide.

The contrast in the distribution of the aberration types becomes very striking when compared with the earlier described mutagens. The mutation spectrum is

markedly different. Rare mutants, more or less apparent, even drastic ones, were isolated in these series. In fact, they appear at a rate twice as high as that obtained after irradiation. Some of them have not been detected previously in Swedish radiation experiments. The high mutagenic efficiency of the epoxides and epimines has to be ascribed to alkylations of biologically important compounds: proteins or nucleic acids (Ehrenberg et al., 1958, 1959; Gustafsson and Ehrenberg, 1959).

(8) The sulfonates are alkanesulfonic esters and comprise many different derivatives. The most important and mostly used one is Ethyl methanesulfonate (EMS). The group can be divided into two subgroups: (a) monofunctional and (b) di- and trifunctional. (cf. Table 2.)

The *monofunctional* compounds have in common that they are active at much higher concentrations and have a much narrower concentration range before general cell toxicity sets in. Several analyses, investigations and treatments have been done from the chemical and biochemical points of view with these compounds (Ehrenberg, 1960; Gustafsson, 1960a). However, in this context the effects of the mutation process and the reactions in plant materials, especially in barley will be treated.

Ethyl methanesulfonate (EMS)

Dormant seeds as well as seeds pre-soaked for 16 or 24 hours have been treated with ethyl methanesulfonate usually at room temperature. Seed germination immediately after sowing in the field is an indication of the effect of this treatment. The highest concentrations (0.25% - 0.33% - 0.50%) result in 50% lethality. The sensitivity depends upon the treatment, i.e., whether the seeds have been dry or pre-soaked. The pre-soaked seeds take up the substance much more effectively than dry seeds. Regarding immediate effects of treatment, chromosomal aberrations are rare or absent, at least at low and medium concentrations, and they usually first

Table 2. Survey of the alkanesulfonic esters used as mutagens

Chemical name	Abbreviation
I. *Monofunctional*:	
methyl methanesulfonate	MMS
methyl ethanesulfonate	MES
ethyl methanesulfonate	EMS
ethyl ethanesulfonate	EES
n-propyl methanesulfonate	PMS
isopropyl methanesulfonate	iPMS
n-butyl methanesulfonate	BMS
sec-butyl methanesulfonate	sBMS
isobutyl methanesulfonate	iBMS
tert-butyl methanesulfonate	tBMS
neopentyl methanesulfonate	NeoMS
allyl methanesulfonate	AMS
2-chloroethyl methanesulfonate	ClEMS
2-methoxyethyl methanesulfonate	MOEMS
II. *Di- and trifunctional*:	
1,4-bis(methanesulfonyloxy)butane	Myleran
diethyl 1,3-propanedisulfonate	DEPD
1,1,1-tris(methanesulfonyloxy-methyl)propane	111

appear in the second or later cycle of mitosis. M_1 sterilities reached very high values. The mutation rate for chlorophyll mutations obtained after greenhouse studies is 50 - 60% per spike progeny. The highest percentages are generally reached in experiments using fairly sterile M_1 offspring. However, even in offspring from fully fertile M_1 plants, EMS produces twice as high rates of mutation as do the ionizing radiations.

The mutation spectrum also shows variations. A lower frequency of albina mutants are produced in comparison to viridis and rare mutants, the latter ones are not as pronounced as with ethylene imine. When the fertility is decreasing, there is an increase of albinas; at low fertility this type of mutants predominates. The viable mutations obtained are on average very numerous, and more or less all different types of morphologically, physiological and semilethal mutations can be isolated. The rare mutations are decidely not so frequent as they are with ethylene imine.

Partial sterility ("semisterility or translocation sterility") occurs also quite frequently with EMS in the second generation after the treatments, but progenies with "translocation sterility" are less abundant. By that time it was known that partial sterility, induced by ionizing radiations, is mainly conditioned by chromosomal irregularities in the X_2 meiosis, commonly translocations. In the case of EMS-treatment, the partial sterility depends primarily on gene mutations (or deficiencies) and to a minor degree on chromosome alterations.

The mutagenic efficiency of EMS is fairly independent of the conditions for treatment: Shortening of the treatment time, lowering of the treatment temperature, re-drying and storage before sowing of the treated seeds, variation of the temperature during treatment, and presoaking of the seeds, all give about the same maximum mutation rate expressed as per cent per spike progeny. It has also been demonstrated experimentally that the pH of the treatment solution affects the ratio of mutation to other effects, a high pH favouring a high mutagenic efficiency (Ehrenberg 1960; Gustafsson 1960a; Ehrenberg et al. 1961, 1966).

Many other compounds of this sub-group have been tested on biological material. They will not be described in detail, but only the most important differences and effects will be mentioned. The mutation rates are given in % per spike progeny.

Commonly it must be stated that most of the compounds are rather ineffective in inducing chromosomal aberrations. Methyl methanesulfonate (MMS) and methyl ethanesulfonate (MES) are appreciably more toxic, and the killing of seeds already at low concentrations is probably the factor limiting the maximum mutation rates (max 16%). They also induce chromosomal aberrations in high frequencies in contrast to other homologues.

The ethanesulfonic esters, as in ethyl ethanesulfonate (EES), are slightly less effective than the corresponding methanesulfonates, with a maximum mutation rate of about 40%.

n-Propyl methanesulfonate (PMS) exhibits a biological reaction pattern very similar to that of ethyl methanesulfonate. It is generally somewhat less effective with respect to lethality, sterility, and mutation. PMS is thus the least toxic compound of the homologous series of methyl, ethyl, propyl and butyl methanesulfonates. The mutagenic efficiency of PMS is, however, about the same as that of EMS, in that the same high rates of chlorophyll mutations are obtained. The reaction rates seem to be intermediate between those of EMS and BMS.

Isopropyl methanesulfonate (iPMS) reacts so fast that biological experiments without after-effects caused by slow reaction of absorbed ester can easily be arranged. It is a highly efficient mutagen, giving around 20 per cent chlorophyll mutations per spike progeny. In repeated experiments a linear mutation rate versus concentration curve was obtained.

n-Butyl methanesulfonate n(BMS) reacted somewhat more slowly than EMS, exhibiting a mutagenic efficiency somewhat lower than that of EMS, about 20 - 30%. It is more toxic than PMS. Nevertheless, it is a good mutagen.

see-Butyl methanesulfonate (sBMS) produced in a single treatment about 3 - 5 per cent mutations, which distinctly demonstrates the mutagenicity of this compound.

Isobutyl methanesulfonate (iBMS) and neopentyl methanesulfonate (NeoMS) are fatal to the treated seeds at about the same concentrations as nBMS, but mutations are practically absent. The experiments have been carried out on a large scale for two years, and would certainly have detected mutation rates of 10 per cent as for BMS.

Allyl methanesulfonate (AMS) represents the first case of a monofunctional methanesulfonic ester capable of inducing tumors. AMS is highly toxic, but there is only a slight mutagenic action. Only 4 cases of *viridis* mutations were obtained in 578 analysed spike progenies, but this mutation rate is significantly higher than the spontaneous rate at the one per cent level. AMS did not produce any significant sterility.

2-methoxyethyl methanesulfonate (MOEMS). The slow reaction rates of this compound leads to less than 20 per cent lethality, and consequently the maximum mutation rate was not reached, within a 24 h period of treatment of dry seeds.

2-chloroethyl methanesulfonate (ClEMS) is a very toxic compound and produces a high degree of sterility; these effects render the compound mutagenically inefficient. The mutation rate is about 5%.

In conclusion: When comparing experiments carried out in different years with the forementioned compounds, the effects are astonishingly reproducible in view of the variability of experimental conditions. Some factors are worth mentioning: a) a slight variation of the time between the end of the treatment and sowing; b) different conditions such as temperature and soil moisture for leakage from seeds of unreacted substance; c) different conditions for later consequences of different types of alkylation. The great reproducibility of effect of alkanesulfonic esters increases the usefulness of these compounds in plant breeding work (Ehrenberg et al. 1966).

Of the *di- and trifunctional alkanesulfonic esters* two agents were used in the mutation experiments. Myleran has a very low solubility in water and therefore it was tested in a dose series obtained by the variation of treatment time with nearly saturated solutions. It has one of the most pronounced delayed effects on the growing plants among all the different compounds tested. Whereas germination and

sprouting are normal, a clear retardation of growth is observed at the two-leaf stage. It seems, however, that the reaction of seeds to Myleran treatment, for unknown reasons, is very variable. The fertility and survival are strongly affected, and of all the methanesulfonates tested it is distinctly the one which induces chromosome aberrations most effectively. It was suggested that at least a part of the sterility may be due to translocations, similar to the effect of ionizing radiations. Nevertheless, no translocations were formed. The maximum mutation rates obtained are in the range 1 - 5 per cent.

The other agent which has been tested was 1,1,1-tris(methanesulfonyloxymethyl)propane (111). This is a trifunctional Myleran analogue. It reacts very slowly and induces chromosomal aberrations. It reacted as completely non-mutagenic but did not cause any sterility.

In summary: among the number of alkyl alkanesulfonates studied, most of the compounds have some influence on the mutation process. The factors limiting the mutation rates are either the induced sterility, giving high mutation rates, or the killing of the material, giving low rates of mutation. In fact, the inefficient mutagens of the investigated series all produce low rates of sterility.

Ethyl methanesulfonate (EMS) and n-propyl methanesulfonate exhibit the highest mutagenic efficiency compared to other effects. In the case of EMS, the high rate of sterility induced, limits the mutation rates, rather than decreased survival. Most alkanesulfonic esters and especially EMS produce only very low frequencies of chromosomal aberrations. The sterility, measured as empty spikelets in the treated generation, may be chiefly due to lethal mutations. It is interesting to note that some of the mutagens described above which are highly effective in viruses and microorganisms, and which may be assumed to produce point mutations, are very or completely ineffective in barley.

It seems hardly possible to find monofunctional alkanesulfonic esters which induce mutations but not sterility or which give rise to an entirely deviating mutation spectrum. EMS and compounds with a similar action induce mutations in so high frequencies that, in the chlorophyll mutation test, every offspring plant may carry several mutations (Ehrenberg et al., 1966).

(9) N-alkyl-N-nitrosamides. The following compounds with their abbreviations are included in this group of mutagens.

N-methyl-N-nitrosourea	MNU
N-ethyl-N-nitrosourea	ENU
N-methyl-N-nitrosourethane	MNC
N-ethyl-N-nitrosourethane	ENC
N-methyl-N-nitroso-N'-nitroguanidine	MNG
N-ethyl-N-nitroso-N'-nitroguanidine	ENG

All the above listed compounds are strongly toxic to seed material, and indeed more toxic than most monofunctional alkyl alkanesulfonates. In many higher plants studied, all these tested compounds were highly mutagenic. But that is not the case for barley. Three of the compounds, MNU, ENU and ENC were shown to be strong mutagens, whereas the three other compounds, MNC, MNG and ENG were practically non-mutagenic. Therefore the list can be divided into two groups.

All treatments were done on dormant seeds with a treatment temperature of 20^{o}C in the first series. Later temperatures of 18^{o}C and 24^{o}C were compared. At the beginning of the treatment, the pH was 4.5 - 5.5 and then was increased to 5.5 - 6 during the treatment. Generally, the seeds were sown in the field within a few hours after the end of the treatment. One replication was stored at +5^{o}C overnight before sowing. But no deviating effects could be observed. As usual, field germination, survival at maturity, sterility, and chlorophyll mutations were determined. Some series were analysed for chromosome aberrations.

The results of the first group (MNU, ENU and ENC) are as follows: Death of plants characteristically occurs at later stages after germination. At the concentration (CL_{50}) giving 50 per cent survival at maturity, the compounds induce appreciable sterility, and the maximum mutation rates are obtained in the group of approx. 50 per cent fertility. If the sterility is assumed to be partly caused by lethal mutations acting on different stages of embryonal development, the similarity in ratio of mutation rate compared to sterility may be taken as an indication of similarity in mutation spectra.

The mutagenic efficiencies of the three compounds giving mutations seem to be

approximately the same, which indicates that the frequencies of around 40 per cent obtained with the two ethylnitrosamides are somewhat higher than for methylnitrosourea. The mutation rates are nearly as high as those obtained after treatment with mutagenically highly efficient alkanesulfonic esters, and definitly higher than those induced by ionizing radiations. The spectra of mutation types induced seem to be similar for the three compounds; there are no significant differences found from the mutation spectra following treatment with alkyl alkanesulfonates. MNU is able to induce chromosomal aberrations in the form of fragments and bridges.

By using these different N-alkyl-N-nitrosamides, the idea was to induce higher mutation rates than those obtained by, e.g., ethyl methanesulfonate and to change the mutation spectrum. But these compounds did not present possibilities of inducing variation in other properties than those already available for mutation by, e.g., EMS. Nevertheless, they have certain advantages with respect to reproducibility of treatment. The slow reaction of EMS and certain other compounds leads to a situation where the reactions proceed for a long time after treatment, which may be preferred by the plant breeder.

Within the non-mutagenic group (MNC, MNG, and ENG) death is an immediate all-or-nothing phenomenon after treatment, where all seeds which have germinated give rise to mature plants. These compounds also induce a significant rate of sterility, especially at concentrations higher than CL_{50}. Since toxic compounds may induce sterility by mechanisms other than mutation and translocation types of chromosomal aberrations, it is not known if this sterility, unaccompanied by chlorophyll mutations, indicates a spectrum of genetical effects different from that given by MNU, ENU and ENC. MNG is also able to induce chromosomal aberrations such as fragments and bridges (Ehrenberg and Gichner, 1967).

Inorganic chemical mutagen

Sodium azide; NaN_3

In the mid sixties the first treatments and experiments were already started with this inorganic chemical mutagen. Sodium azide is a common laboratory chemical and is probably best known as a respiration inhibitor and has been extensively used in

the laboratory in that capacity. This research was not done by the Swedish group, but at Washington State University, Pullman, USA, which is another centre for mutation research and genetics in barley (Sideris et al. 1969). Being a respiration inhibitor, Sodium azide, was initially employed as a tool to determine how chromosomes break and how mutations are induced and/or repaired in cells of irradiated seeds. The first more concrete results were reported in 1969.

The first application of sodium azide was made in combination with irradiation in order to test reduction of seedling heights on the sixth day after planting and the chromosome aberrations at the first mitotic anaphase. After these preliminary tests, large series of experiments were carried out with all kinds of different variations of the treatments.

Inorganic azides can be grouped into 5 classes (Kleinhofs et al. 1978) but only the normal azides, which include simple metallic azides such as sodium azide, potassium azide and barium azide are discussed here, particularly sodium azide. After many series of investigations it was noticed that pH values of the azide solutions are of greatest importance; the lower the pH value the greater the effects. Also the conditions of the seeds and the temperature during the treatments have a great influence. The presoaked seeds are more efficient in producing mutations than dried seeds; the efficiency is strongly depending on the time of soaking or the stage of germination. The increase of the mutagenic effect was even greater if the seeds were presoaked in oxygen.

The results can be summarized as follows: the survival of M_1 plants is in the region of 80% to 90 %, the effect of sodium azide treatment on the growth of seedlings is highly pH dependent: the seedling height is strongly reduced from seeds treated with the inhibitor at pH = 3.0, while pH = 11.0 is ineffective. The maximum chlorophyll mutant rates occur in seeds soaked for 8 and 16 hours at pH = 3 (62% and 64 % respectively), and under optimum conditions of treatments. The series with presoaked seeds exhibit the lowest number of seedlings per spike, indicating a high degree of lethality. This lethality was probably induced during meiosis of either the male or female organs. Azide induces very low frequencies of chromosome breakage. The relatively high degree of sterility in the M_1 barley plants must be caused at least in part by mutations and not chromosome aberrations in the male or

female gametophytes (Nilan et al. 1973; Nilan and Sander, 1974; Kleinhofs et al. 1974; Nilan and Pearson, 1975).

Striped or striata-like leaves occur occasionally among M_1 plants after treatments with several physical and chemical mutagens. Compared to other mutagens, sodium azide was found by the Pullman group to induce a much higher frequency of such changes. In the best treatments, the frequencies of M_1 striped plants have been more than 10 %. Concerning the chlorophyll mutation spectrum the same proportions of *albina* : *viridis* were found, and less of "others"; (the figures are 35, 36 and 16% respectively). This azide spectrum has been more closely compared with those of the diethyl sulfate and other alkylating agents by the Pullman group. Also relatively high frequencies of viable morphological mutants, e.g., erectoides, dwarfs, and male steriles have been observed in mature M_2 plants (Nilan et al. 1975).

The high frequencies of mutations and negligible frequencies of chromosome aberrations demonstrate that sodium azide is an unusually efficient mutagen. Compared to other potent mutagens, it is also relatively safe to handle, non-persistent and inexpensive. Therefore, sodium azide may be a very useful mutagen for practical plant breeding purposes.

Azide as the sodium salt is extensively used by numerous groups of workers in various fields (Kleinhofs et al. 1978; Nilan, 1981). It is intensively used as a herbicide, nematocide, and a fungicide, as well as in clinical and analytical laboratories as a preservative. Its use as a nitrogen gas generator in automobile safety air bags will tremendously increase in the production of this compound and also the exposure of the general public to it.

The mutagenic action of the inorganic azide is rather surprising. It has turned out, however, that it is mutagenic through an organic moiety (Nilan, 1981). This metabolite is an organic azide, beta-azidoalanine, which barley is able to produce. Its action as well as its metabolite in barley appear to be unique among the presently known mutagens (Rosichan et al. 1983; Nilan, 1986;).

Also the Swedish mutation research group used sodium azide. The first treatments were done in 1973, mostly with presoaked seeds and with two different

pH values, pH = 4 and 7.7. Different series of concentrations were at all times tested. Chlorophyll mutation tests were performed, but mutation rates as high as for the Pullman group were not obtained. The maximum rate under our optimum conditions was 53% during eight years of experiments (Lundqvist, unpublished). No treatments of presoaked seeds in oxygen were made. The lower mutation rate may be due to the genetic background, as another variety, 'Sv Bonus', has been used. An intensive research on sodium azide in comparison with other physical and chemical mutagens has not been done in Sweden. A lethality in the M_1 spikes could be also observed as a direct effect of the treatments. In the second generation semisterile plants (so-called "translocation sterility") could be isolated, but translocation lines were very rare, with about 2 cases among several isolated M_2 plants (Prina et al. 1983).

Most of the sodium azide treated material in Sweden was used for isolation of viable mutants for practical agronomical purposes. Some specificities may be mentioned here. Sodium azide has proved to be substantially more efficient in inducing mutants for powdery mildew resistance, it induces easily early mutants, but is less efficient in producing early mutants with day-length neutrality, and finally high rates of eceriferum (waxless) mutants are induced.

5. Summarizing experiences of mutagenic research

The Swedish mutation research was non-commercial, despite that some mutants have been used in practice - directly or after recombination breeding. It brought a wealth of observations of general biological importance: Chronology of chromosome reproduction, irradiation sensitivity of different mitotic stages, the importance of the heterochromatin, mutations in polyploids, the variation of irradiation sensitivity in different plant species, competition between various elements in plant tissues (Gustafsson, 1969 and 1986; Lundqvist, 1991).

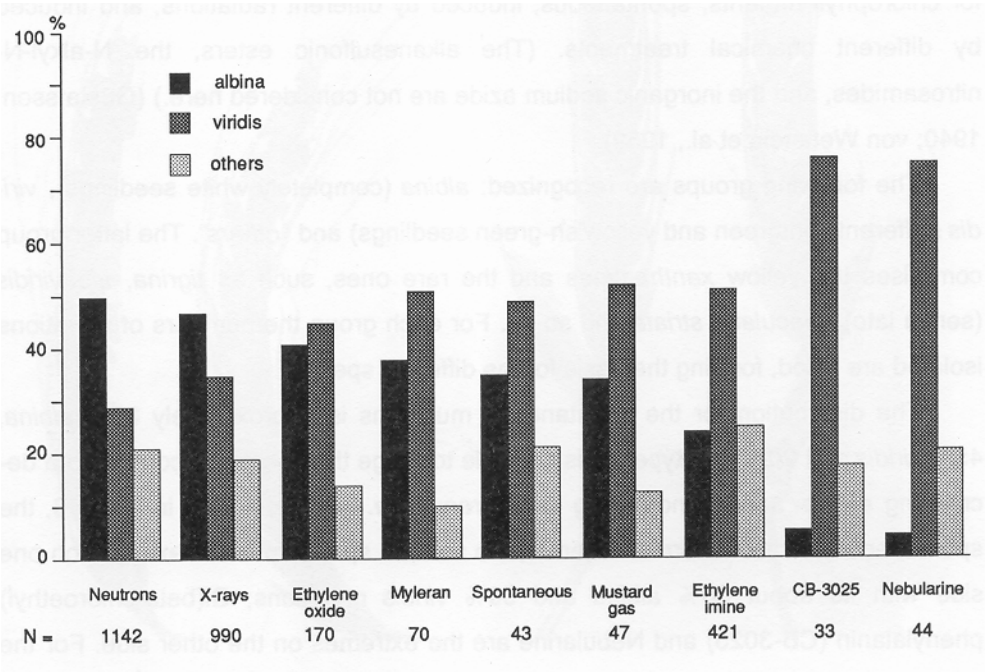

Figure 5. Distribution of chlorophyll mutation types, spontaneous and induced by different mutagens (after von Wettstein et al., 1959).

The intention was to direct the mutagenesis. Evident genetic differences

between the action of ionizing radiations and of chemical mutagens could be demonstrated after more than three decades of intensive research. For chemical mutagens the mutation frequency increased rapidly up to 80%; they were 20 times more effective than irradiation. Neutrons and sodium azide formed two extremes, neutrons inducing a relatively large number of chromosome and chromatid changes, sodium azide primarily causing gene mutations at the nucleotide level (Nilan et al., 1976; Nilan, 1981). Differences in the mutation spectrum could be noticed, first of all for chlorophyll mutations, which were the most frequent ones. However, these were lethal. Secondly, for some viable morphological mutant groups, various gene loci reacted in specific ways.

The chlorophyll mutants, which were studied most intensively, are particularly informative with regard to mutagen specific reactions. Figure 5 illustrates the spectra for chlorophyll mutants, spontaneous, induced by different radiations, and induced by different chemical treatments. (The alkanesulfonic esters, the N-alkyl-N-nitrosamides, and the inorganic sodium azide are not considered here.) (Gustafsson, 1940; von Wettstein et al., 1959).

The following groups are recognized: *albina* (completely white seedlings), *viridis* (different lightgreen and yellowish-green seedlings) and "*others*". The latter group comprises the yellow *xantha*-types and the rare ones, such as *tigrina*, *alboviridis* (sensu lato), *maculata*, *striata* and so on. For each group the numbers of mutations isolated are listed, forming the basis for the different spectra.

The distribution for the spontaneous mutations is approximately 35% *albina*, 45% *viridis* and 20% rare types. It is possible to range the spectra according to a decreasing *albina*- and an increasing *viridis*-frequency. In the diagram in figure 6, the spontaneous mutations form the centre; the neutron spectrum is the extreme on one side with its about 50% *albina* and 30% *viridis* mutations; Di(beta-chloroethyl) phenylalanin (CB-3025) and Nebularine are the extremes on the other side. For the latter ones, most of the mutations are found in the classes *viridis* (80%) and "the others", the *albina*-types being in minority; probably they are only spontaneous mutation cases. X-rays and ethylene oxide also show a distribution of mutations which deviates from the spectrum of spontaneous mutations. The spectra for Myleran and mustard gas are quite similar to that of the spontaneous mutations. It is possible

to distinguish differences for the rare types. Many more chlorophyll mutations of the rare types are induced by neutrons and ethylene imine.

Differing mutation spectra can be due not only to different mutagens, but also to different kinds of treatments involving the mutagen. Thus, by using high doses of X-rays and a low water content in the seeds the *viridis* mutants can be increased to more than 60%, at the cost of *albina*-types. Low X-ray doses do not induce any change of the spectrum. Also the ion density is of importance, as already reported above.

Figure 6. Two drastic morphological mutants included in the genetically investigated mutant groups: to the left 'Calcaroides', to the right 'Bracteatum or third outer glume'. (cf. Table 3)

Also for the viable mutants and the mutants useful in plant breeding, it is possible to observe some mutagen specificity. Under certain conditions, every fifth spike progeny may be expected to carry a morphological drastic mutation of potential value for plant breeding. The erectoides mutants form one example. They are among those most commonly induced by irradiation, about 20% of all viable, morphological and physiological mutants induced with sparsely ionizing radiation being erectoides. With densely ionizing radiation the frequency is 50%, but with ethylene compounds the frequency is only about 12% (von Wettstein et al., 1959).

Figure 7. Two other important morphological mutants: to the left six-row mutant spikes, to the right a short-awned Breviaristatum mutant. (cf. Table 3).

Since most mutations act primarily in a detrimental direction, it will be difficult to detect and isolate positive and useful mutations. It is therefore not always self-evident that the practical work with plant breeding aspects should start with a material previously treated under conditions optimal for mutation induction. In practical work it is recommended to utilize a series of concentrations, and to choose,

for studies in later generations, those that appear appropriate in the light of the value of CL_{50} found (Ehrenberg et al. 1966).

Moreover, it has to be stressed that ionizing irradiations and chemical mutagens have different modes of action, depending on the type both of radiation and of the chemical compounds. In the future this will be profitable for the practical plant breeding work. Being able to separate gene mutation and chromosome breakage, the plant breeder will have great possibilities to induce productive mutants. More visible mutations (point mutations) and less chromosome aberrations are induced by ethylene imine than by the ionizing radiations. Already in the late 50s, the Swedish mutation research could show that there are mutagens which do not lead to chromosome breakage but cause a rise in the mutation frequency (Ehrenberg et al. 1958 and 1959; Gustafsson and Ehrenberg, 1959).

Genetic diversity is an important feature in plant breeding research and the plant breeder cannot neglect the artificially induced mutants for the further improvement of his varieties. A methodical work will sooner or later lead to positive results.

Over the years, with a variety of research branches, a rather large collection of morphological mutations, with a broad variation range, has successfully been brought together. About 10 000 different mutants have been genetically and agronomically studied. They consist of the following ten main categories with 95 different types of mutants:

1. Changes in spike and spikelets
2. Changes in culm length and culm composition
3. Changes in growth type
4. Physiological mutants
5. Changes in awns
6. Changes in seed size and shape
7. Changes in leaf blades
8. Changes in anthocyanin and colour
9. Resistance to barley powdery mildew
10. Translocation lines

So far, about half of the 10 000 mutants have been analysed genetically in more or less great detail, but these mutants form only a minor part of the range of mutant types. They are all incorporated into the Nordic Gene Bank and documented in various databases (Lundqvist, 1991).

The mutant groups shown in Table 3 have been studied in more detail genetically and with regard to mutagen specificity and increased our knowledge of the mutation process and the genetic architecture of the different characters.

The collection forms an outstanding material for investigations within radiobiology, genecology, gene physiology, ultrastructural research, and plant biochemistry and -physiology. Barley has become one of the few higher plants in which biochemical genetics and molecular biological studies are now feasible.

Table 3. Survey of the 11 genetically investigated mutant groups (cf. figures 6 and 7)

	Number of	
Mutant group	Mutants	Loci
Praematurum (Early heading)	172	9
Erectoides	205	26
Breviaristatum (Short awns)	140	17
Eceriferum (Waxless)	1580	79
Intermedium	103	11
Hexastichon (Six-row)	41	1
Macrolepis (Lemmalike glumes)	40	1
Bracteatum (Third outer glume)	28	4
Calcaroides	18	4
Mildew resistance	77	several
Exrubrum (Anthocyanin-less)	approx. 500	approx.27

B. GENETICAL ANALYSES OF MUTANT GROUPS

1. Eceriferum (waxless) mutants

Introduction

Many plants, for instance cereals, are covered with epicuticular waxes on their outer cuticular surfaces. The presence of the wax coating and its compostion is of great importance for plant survival. The wax coating may act as a protection against evaporation, and other climatic factors. Plants with a wax coating survive more easily at high altitudes, while plants without a wax coating survive more easily in moist regions at sea-level. This surface wax may be of importance for moisture run-off from the leaf blade (from rainfall or dew), depending on the type of wax coating. On the other hand, sometimes water may be gathered by special wax receptacles on the plant.

Barley with a reduced wax coating retain water droplets longer, which may result in loss of nutritional substances, leading to reduced growth. The wax coating on the barley plant functions like a raincoat.

Several mutations affecting epicuticular waxes have been reported in barley, where epicuticular waxes on the surfaces of the plant are controlled by *eceriferum (cer)* genes.

The chemical composition of barley waxes is rather complex and probably common to many grass species. The most important wax constituents are alkanes, primary alcohols, free fatty acids, aldehydes, beta-diketones, hydroxy-beta-diketones, esters and hydrocarbons (von Wettstein-Knowles, 1971, 1979, 1982 and 1987; Simpson and von Wettstein-Knowles, 1980; Mikkelsen and von Wettstein-Knowles, 1984). There is a definite organ specificity with regard to the occurrence and amount of different wax constituents. Therefore the eceriferum mutants are of special interest for studies of the chemical wax composition and its biosynthesis. The complex genetic architecture, which the analysis of the eceriferum mutants has

revealed, corresponds to the complexity of the enzymatic activities that causes the fatty acids synthesis.

Already in 1968, results showed that some of the *eceriferum* loci control the synthesis of the wax constituents which are responsible for the amount and morphology of the wax coating. Since there are many lipid classes involved, consisting together of something like 50 different long chained aliphatic compounds, it is not surprising, that already in the first investigations, 44 different *eceriferum* loci had been identified (Lundqvist et al., 1968 [paper II]). Because of genetic control of organ specificity and the genetic control of the structural and biochemical apparatus for secretion, even a higher number of *eceriferum* loci could be expected (Lundqvist et al. 1968 [paper II]). Today, 79 *eceriferum* loci are identified (Lundqvist and Lundqvist, 1988 [paper III]).

The biochemical research on the wax compostion was studied in great detail by P. von Wettstein-Knowles and coworkers (von Wettstein-Knowles, 1971, 1979, 1982 and 1987; Simpson and von Wettstein-Knowles, 1980; Mikkelsen and von Wettstein-Knowles, 1984; and references therein.)

Description of eceriferum mutants

The *eceriferum* mutants have a reduced or completely absent coating of wax on various plant organs such as spikes, leaf sheaths and leaf blades. Such mutants are among the most frequently induced morphological mutants along with the other type of viable morphological mutants: the *erectoides.* The reduction or the absence of the wax coating has been described under various names: "klargrön", bright green, waxless, or "wachsfrei" (Gustafsson, 1946, 1947 and 1951; Nybom, 1954; Scholz and Lehmann, 1958, 1959 and 1961) or as bloomless, glaucous ear, glaucous sheath and leaf blade, waxless head, glossy, glossy leaf, glossy spike, glossy sheath and glossy seedling (cf. Smith, 1951; Nilan, 1964).

The following gene symbols have been used for this character: *Ge* = *wh* (glaucous ear, waxless head), *gl* = *wl* (glossy seedling or leaf), *gs* (glossy sheath and spike), *gs3* = *vs3* (glossy sheath), *gs4* = *wh1* = *wh2* (waxy bloom on leaf and leaf sheath), *Gle* (glossy spike) and *cer* (eceriferum), (Smith, 1951; Lundqvist and von

Wettstein, 1962 [paper I]; Nilan 1964; Søgaard and von Wettstein-Knowles, 1987a).

In order to avoid all the confusing names and symbols used over the years, a more convenient name "*eceriferum*" was given to the whole Swedish group of mutations which affected wax coating on different plant organs. The gene symbol, "*cer*" is used instead of all the confusing designations used previously (Lundqvist and von Wettstein, 1962 [paper I]). Three of the original names and their symbols *glossy sheath*/*spike* (*gs*), *glossy seedling*/*leaf* (*gl*), and *glossy spike* (*Gle*) are still in use. This is due to the fact that the original barley marker stocks at the Main Stock Center, Fort Collins, USA, are still maintained. They are used in different linkage group studies by different barley research workers (Søgaard and von Wettstein-Knowles, 1987a).

Phenotypically three different organs of the barley plant were studied in regard to wax coating and composition (cf. figures 19 and 20, p. 78): the spike, the leaf sheath, and the leaf blade (lamina) (Lundqvist and von Wettstein, 1962 [paper I]). Five phenotype categories were distinguished:

[1] spike and leaf sheath
[2] spike and leaf sheath, partially
[3] spike
[4] leaf blade
[5] spike, leaf sheath and leaf blade

Special signs have been used to classify the type of the wax coating:
- = the wax coating is absent
+ = the wax coating is reduced
++ = the wax coating is normal

The wax coating is determined separately on the spike, on the leaf sheath and stem, and on the leaf blades respectively. Thus the formula for the wild type is ++ ++ ++, for a spike and leaf sheath mutant - - ++, for a spike and leaf sheath mutant partially + + ++, for a spike mutant - ++ ++, and for a leaf blade mutant ++ ++ -.

Over the years, a total of 1890 *eceriferum* mutants were isolated. Different kinds of mutagenic treatments were used, and according to the mode of origin the mutants fall into the following groups:

[1] sparsely ionizing radiations
[2] densely ionizing radiations
[3] ethylene oxide
[4] ethylene imine
[5] sulfonates (methyl, ethyl, propyl, butyl, hydroxy ethyl, metoxy ethyl, hydroxy propyl, and isopropyl methanesulfonate; propane disulfonic acid diethylester; ethyl ethanesulfonate; and ethylhydroxy ethanesulfonate).
[6] combined treatments of neutrons and ethyl methanesulfonate
[7] combined treatments of γ-rays and ethyl methanesulfonate
[8] combined treatments of γ-rays and diethyl sulfate
[9] other organic mutagens (diethyl sulfate, epichlorhydrine, glycidol, myleran, N-methyl-N-nitrosourea and N-ethyl-N-nitrosourea.
[10] other mutagens: UV
[11] sodium azide

The genetical analyses of the eceriferum mutants

A total of 1580 such *eceriferum* mutants, have been localized to 79 *cer* loci (Lundqvist and Lundqvist, 1988 [paper III]). 78 of these loci are recessive and one is dominant (*Cer-yy*). The contributions to the five phenotype categories among the loci are given in Table 4.

The great number of gene loci necessitated a simplified procedure for gene localization of mutants (Lundqvist and von Wettstein, 1962 [paper I]). When a new *eceriferum* mutant is being tested, it is initially crossed with representatives of the *cer* loci that are characterized by the particular mutant's own phenotype (Table 4). Thus, a "spike" mutant will be tested against the 23 known loci for this phenotype category. Two representative alleles at each of these loci have been used for optimal safety. "Spike and leaf sheath" mutants also have been tested against the 19 loci within category + + ++, and the "Partial" mutants also have been tested against the 8 loci within the category - - ++, because of accidental phenotypic overlapping between

these two categories. Two mutants are considered allelic, when the F_1 has an *eceriferum* phenotype. But in case of a dominant mutant gene, the analysis has to be extended to the F_2 generation.

Table 4. The five phenotype categories (- = absent, + = reduced, and ++ = normal wax coating), with their numbers of loci and mutants

Spike	Leaf sheath	Leaf blade	Category of mutant	No. of loci	No. of mutants
-	-	++	"Spike and leaf sheath" mutants	8	533
+	+	++	"Partial" mutants	19	339
-	+	++			
+	-	++			
-	++	++	"Spike" mutants	23	294
+	++	++			
++	++	-	"Leaf blade" mutants	25	390
++	++	+			
-	-	-	"Spike, leaf sheath, leaf blade" mutants	4	24
+	+	+			

Considering the distribution of the 79 *cer* loci on the five phenotypic classes commented upon in Table 4, the majority of the loci belong to the three classes "partial", "spike", and "leaf blade" mutants. The class "spike and leaf sheath" mutants has only 8 loci. The smallest class, with only 4 loci, has its wax deposition affected on all organs, spike, leaf sheath, and leaf blade. In Lundqvist and Lundqvist (1988, Table 4 [paper III]) the 79 *cer* loci have been grouped on the five *cer* phenotypes.

The majority of *cer* loci mutate only sporadically, 44 loci having mutated between 1 and 5 times. On the other hand, 16 of the loci comprise no less than 70% of the mutants. The class "spike and leaf sheath" mutants (only 8 loci) has the highest number of mutations, depending on the loci *cer-c* and *-q*, with altogether 382 mutations.

The *cer* loci are widely distributed in the barley genome, as shown in intensive linkage studies by the Danish barley research group at the Carlsberg Laboratory, Copenhagen. Up to date, to my knowledge, 26 different *cer* loci are mapped on the seven chromosomes, apparently well distributed among them, apart from chromosome 6, where only one *cer* gene could be localized (Søgaard and von Wettstein-Knowles, 1987; von Wettstein-Knowles, 1992a and b; Lundqvist, 1990.)

The cer mutants in relation to the various mutagens

The following seven mutagens will be considered for the distribution of the 1580 *cer* mutants and the five *eceriferum* phenotypes:

[1] X-rays
[2] Neutrons
[3] Ethylene imine
[4] Sulfonates
[5] Other organics
[6] Sulfonates + X- or γ-rays
[7] Sodium azide (NaN_3)

In Table 5, the mutations within the five phenotypic classes are arranged according to the different kinds of mutagenic treatments. The seven mutagenic treatments gave significantly different numbers of mutants, but do they display similar patterns of their distribution of mutants?

Different loci may show markedly differing mutagen specific reactions. (1) There are especially large mutagenic differences between chemicals and ionizing radiation, particularly neutrons. (2) No significant differences among various kinds of organic chemicals can be established. (3) There are significant differences between organic chemicals and sodium azide. (4) The combined treatment (sulfonate + X- or γ -rays) does not differ from treatment with sulfonates alone, but differs from the treatment with X-rays alone. No difference to sodium azide can be demonstrated. (5) Sodium azide differs strongly from X-rays; and still more from neutrons. (6) There are clear differences between the two kinds of treatments with ionizing radiation, as expected, sparsely ionizing X-rays and densely ionizing neutrons having different effects on the target DNA molecule.

Table 5. Distribution of 1580 cer mutants among the five phenotypes for various mutagens (number of loci is shown in brackets)

Mutagens	Spike & leaf sheath	Partial Spike & leaf sheath	Spike	Leaf blade	Spike, leaf sheath & blade	Total
X-rays	47 (7)	30 (8)	44 (15)	30 (14)	5 (3)	156
Neutrons	53 (6)	52 (8)	101 (13)	64 (12)	13 (1)	283
Ethylene imine	74 (7)	43 (10)	41 (14)	46 (11)	1 (1)	205
Sulfonates	195 (7)	130 (13)	63 (18)	129 (17)	3 (3)	520
Other organics	47 (6)	34 (6)	15 (9)	26 (9)	1 (1)	123
Sulf.+ X- or γ-rays	21 (4)	6 (3)	9 (6)	17 (5)	1 (1)	54
Sodium azide	96 (4)	44 (10)	21 (9)	78 (10)	0	239
Total	533	339	294	390	24	1580

The response in individual gene loci, to the different mutagenic treatments, can be summarized as follows (combined treatments with radiation and chemicals being excluded):

[1] The phenotype category "spike and leaf sheath"

This discussion is based on paper III, tables 7 and 8.

The loci *cer-c* and *-q* have the highest number of mutants, with a total of 364 mutations. It is obvious that the organic mutagens and sodium azide are superior in producing mutants in these loci than treatments with radiations. Thus, the sulfonates are especially efficient for obtaining alleles at the loci *cer-c* and *cer-q.* Locus *cer-b* with 37 alleles mutates more easily with radiation than with organic mutagens, which in turn, are more efficient than sodium azide. Also the loci *cer-a* with 62 alleles and *cer-x* with 33 alleles have a higher proportion of mutants induced by sulfonates and sodium azide. Pooling the three loci with a low number of mutants, (totally 16 alleles), they show a tendency to a stronger reaction to radiation than to chemical mutagens. No sodium azide induced mutant has been found.

[2] The phenotype category "partial, spike and leaf sheath"

This discussion is based on paper III, tables 9 and 10.

Locus *cer-u* has the highest number of mutants with 156 cases. It mutates much more frequently when exposed to organic mutagens than to radiation and sodium azide. Locus *cer-g* with 40 alleles and locus *cer-n* with 55 alleles mutate much more readily for radiation than for organic mutagens and chemicals, respectively. Within the group of 14 pooled loci, with a total number of 45 cases, there is a strong tendency to react more favourably both to radiation and to organic chemicals than to sodium azide.

[3] The phenotype category "spike"

This discussion is based on paper III, tables 11 and 12.

Locus *cer-i* with 67 alleles mutates preferentially with radiation compared with organic chemicals, neutrons being clearly superior, also when compared with sodium azide. Also locus *cer-t* with 50 alleles reacts more favourably to neutrons than to organic chemicals. For locus *cer-e* with 42 alleles, sodium azide is the superior mutagen, particularly in comparison to radiation, and also organic chemicals show a

clear tendency to be superior to neutrons. Loci *cer-w* with 18 alleles and *cer-zc* [1] *with 14 alleles give a stronger reaction to organic chemicals than to neutrons, and also X-rays tend to be less effective. Locus cer-d*, also with 14 alleles tends to react more strongly to sodium azide than to organic chemicals. Most of the alleles of this locus are connected with early heading. Locus *Cer-yy* with 18 cases, where all the alleles are dominant, does not show any pronounced mutagen specific reaction. When the remaining 16 loci with only a few alleles each (a total number of 62 cases) are pooled together, there is a significant tendency to react more strongly to organic chemicals and X-rays than to neutrons; also sodium azide tends to be less effective than organic chemicals.

[4] The phenotype category "leaf blade"

This discussion is based on paper III, tables 13 and 14.

Locus *cer-za* with 77 alleles mutates much more readily for organic chemicals than for radiation, with a tendency that sodium azide also is inferior to organic chemicals; locus *cer-ze* with 68 alleles reacts much more strongly to both sodium azide and radiation, with the main emphasis on sodium azide. For locus *cer-j* with 61 alleles, chemicals are much more efficient than radiation, with a tendency that sodium azide is superior to organic chemicals. Not a single mutant was induced by neutrons and only one mutant by sparsely ionizing radiation in this locus. Locus *cer-zj* with 55 alleles shows a clear tendency for radiation to be more efficient than sodium azide, and a tendency for sodium azide also to be inferior to organic chemicals. Locus *cer-p* with 37 alleles does not show pronounced mutagen specificity. Possibly fewer mutants are induced by neutrons. Within the group of 20 pooled loci with a total number of 75 alleles, there is a significant tendency to react more strongly to radiation than to chemicals.

[1]It should be noted that in Lundqvist and Lundqvist (1988) [paper III], *zd* in Tables 11, 12 and 15 and in Fig. 1 and in the text on page 9, should read *zc.*

[5] The phenotype category "spike, leaf sheath, and leaf blade"

This discussion is based on paper III, table 1.

This category comprises only 4 *cer* loci and a total number of 24 alleles. It shows a significant number of mutants after treatment with radiation. Neutrons are most efficient in producing mutants at these loci. No mutants induced by sodium azide have been found.

One drawback with the presentation above is that quite different numbers of mutations have been isolated from the various kinds of mutagenic treatment. One way to compensate for these differences is to normalize the numbers of mutants within a particular locus in relation to the total number of mutants for the particular mutagen. An example will be taken from Lundqvist and Lundqvist (1988, Table 1 [paper III]). For locus *cer-c,* under X-rays there are 13 out of 156 mutants; under neutrons there are 18 out of 283 mutants and so on, and, finally, under sodium azide, there are 41 out of 239 mutants. The individual proportions of mutants in relation to the sum of proportions will then constitute a measure of the efficiency of the different mutagens. This is demonstrated in Figure 8. The diagram shows the mutation numbers normalized within the 16 "largest" *cer* loci and two classes of pooled loci with 19 down to 4, and 3 down to 1 mutations, respectively, for sodium azide, organic chemicals, neutrons, and X-rays.

Some features are evident: (1) Locus *cer-i* is predominantly affected by radiation, particularly by densely ionizing neutrons, and locus *cer-t* has high frequencies of radiation induced mutants; both these two loci affect the wax coating on the spike. (2) Locus *cer-j* has a high mutation frequency for treatment with chemicals, both organics and sodium azide, and no mutant isolated with neutrons has been received; locus *cer-ze* has a high frequency of sodium azide induced mutants; and locus *cer-zj* mutates with a large number of neutron induced mutants. These three loci all affect the wax coating on the leaf blade. (3) Locus *cer-b* is lacking mutations after treatment with sodium azide; and the loci *cer-c, cer-q,* and *cer-u* have a high number of mutations after treatment with chemicals, sodium azide included. These four loci are affecting the wax coating on the spike and leaf sheath. On the whole the diagram supports the conclusions in the previous sections.

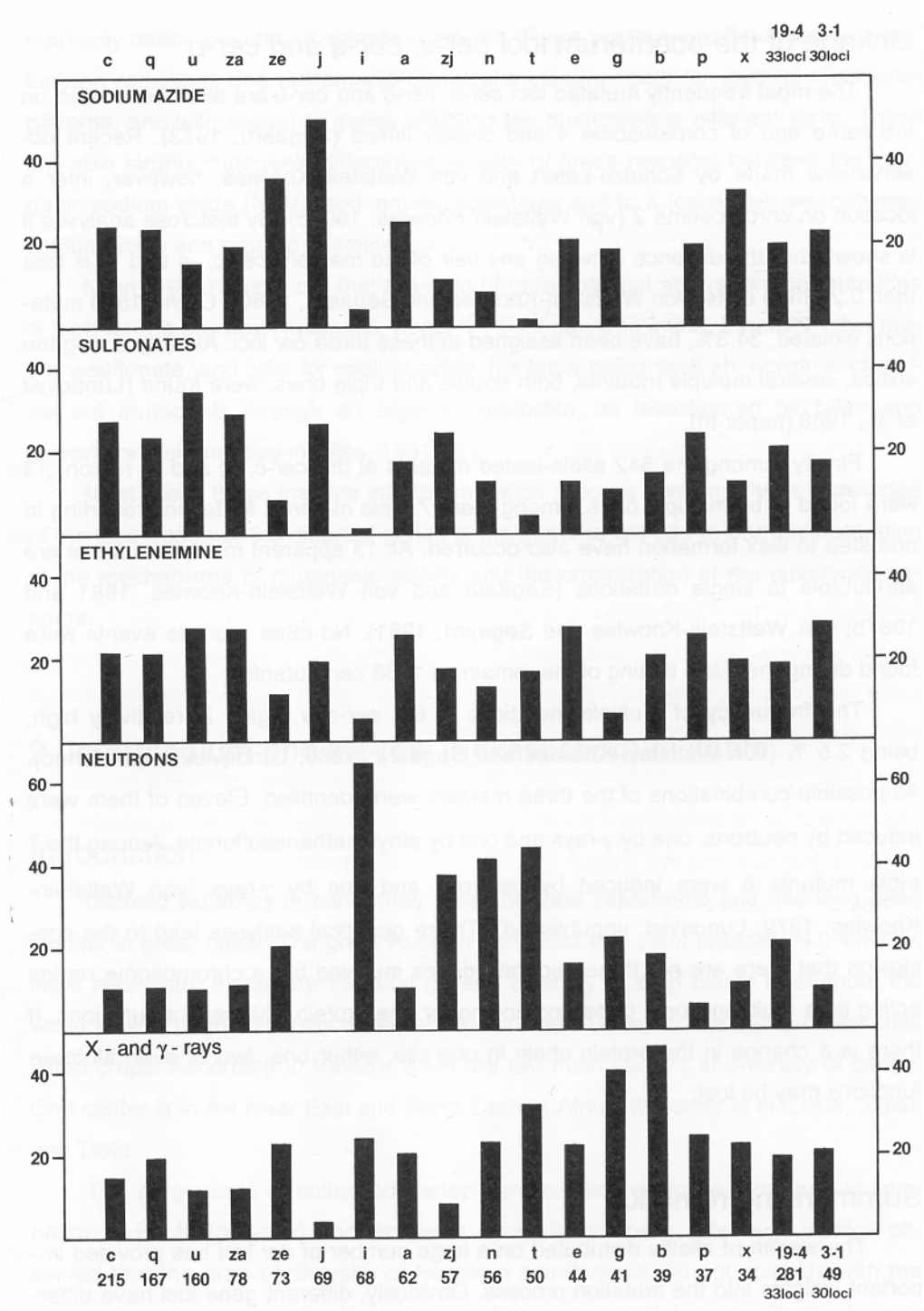

Figure 8. Percent of *cer* mutants at each locus, normalized for mutagens.

Linkage of the eceriferum loci *cer-c, cer-q and cer-u*

The most frequently mutated loci *cer-c, cer-q* and *cer-u* are all three located on the same end of chromosome 4 and closely linked (Søgaard, 1973). Recent observations made by Schulze-Lefert and von Wettstein-Knowles, however, infer a location on chromosome 2 (von Wettstein-Knowles, 1992b). By testcross analyses it is shown that the distance between any pair of the markers *cer-c, -q* and *-u* is less than 0.25 map units (von Wettstein-Knowles and Søgaard, 1980). Of the 1580 mutations isolated, 34.3%, have been assigned to these three *cer* loci. Already during the sixties, several multiple mutants, both double and triple ones, were found (Lundqvist et al., 1968 [paper II]).

Finally, among the 542 allele-tested mutants at the *cer-c, -q* and *-u* region, 13 were found to be multiple ones, among them *7 triple mutants.* Mutations reverting in one step to wax formation have also occurred. All 13 apparent multiple mutants are attributable to single mutations (Søgaard and von Wettstein-Knowles, 1981 and 1987b; von Wettstein-Knowles and Søgaard, 1981). No other multiple events were found during the allele testing of the remaining 1038 *cer* mutants.

The frequency of multiple mutations in the *cer-cqu* region is relatively high, being 2.5 % (von Wettstein-Knowles and Søgaard, 1980; Lundqvist, unpublished). All possible combinations of the three markers were identified. Eleven of them were induced by neutrons, one by γ-rays and one by ethyl methanesulfonate. Among the 7 triple mutants 6 were induced by neutrons and one by γ-rays (von Wettstein-Knowles, 1979; Lundqvist, unpublished). These genetical analyses lead to the conclusion that there are not three separate genes involved but a chromosome region acting as a multifunctional gene and coding for one protein with several functions. If there is a change in the protein chain in one site, either one, two or even all three functions may be lost.

Summarizing remarks

The wealth of alleles distributed on a large number of *cer* loci has provided important insights into the mutation process. Obviously, different gene loci have different mutabilities, irrespective of mutagen. It is equally obvious that different loci show markedly differing mutagen specific reactions. There are strong differences

between ionizing radiations and the organic-chemical mutagens, both for general mutational patterns, and with regard to genes affecting the phenotype in different ways. There are also strong mutagenic differences in both of these respects between the inorganic sodium azide (NaN_3) and ionizing radiations and to a lesser degree, between sodium azide and organic chemicals.

Nilan (1981) points out that the ratio of chromosomal aberrations to mutations is high for the physical mutagens, about 1 : 1 for ethylene imine, lower for ethyl methanesulfonate, and zero for sodium azide, the latter being itself an inorganic chemical but mutagenic through an organic metabolite, as investigated by Nilan and coworkers (summarized in Nilan, 1981).

Most likely, these insights into the mutation process combined with knowledge of the localization of the different genes in the genome will add to our understanding of the mechanisms of mutagenic activity and the organization of the eukaryotic genome.

2. Intermedium and six-row (hexastichion) mutants

Introduction

Genetic variability in barley has been of great importance and has long been studied in great detail. The great Russian geneticist and plant breeder, N.I. Vavilov, felt it necessary to explore the total genetic diversity of crop plants throughout the world as well as the diversity of related wild species. Barley is one of the oldest cultivated crops. According to Vavilov, there are two main centers of diversity of barley. One center is in the Near East and North-Eastern Africa, the other is in China, Japan and Tibet.

The progenitors of cultivated barley were considered to be *Hordeum spontaneum* C. Koch, (two-row) and *Hordeum agriocrithon* Åberg, (six-row). Vavilov observed that the area of diversity of *Hordeum spontaneum* did not coincide with the two centers of diversity of cultivated types, but corresponded more with the African. The wild and weedy races are usually designated *H*. *spontaneum* but, biologically,

they belong to the same species as the cultivated races, i.e., *H*. *vulgare ssp*. *spontaneum* (C.Koch) Thell (Smith, 1951; Nilan, 1964; Harlan, 1979).

All the truly wild forms of the genus *Hordeum* are two-rowed, that is, of the three spikelets at each node of the spike, the two lateral ones are rudimentary (female-sterile) and only the central one develops a grain. Under domestication, six-rowed races appeared where all three spikelets produce grains. In the spontaneous two-rowed forms, the spikes disarticulate at maturity and the entire spikelets fall to the ground, while in domesticated races, the rachis is tough and the grains persistent. Six-rowed genotypes with fragile spikes are known, but do not appear to be truly wild plants. They should be regarded as secondary mutation and hybridization products. They are probably derived from six-rowed cultivars (Harlan, 1979).

The problem regarding the progenitor of cultivated barley has been discussed for at least one hundred years: was it a two-rowed *spontaneum* type or an extinct, brittle six-rowed form? During the continuing debate two different proponents were established: One represented the two-rowed progenitor-hypothesis (Körnicke & Werner, Covas, Helback and Zohary), the other one represented the six-rowed progenitor-hypothesis (Schiemann, Åberg, Nevski, Parodi, Kamm, and Takahashi) (for references, see von Bothmer et al., 1991). After many years of discussion of how barley had developed and of the relationships between the different species, the accumulated evidence indicates that in fact we are dealing with one single species. A three flowered triplet with sterile lateral florets being more efficient for seed dispersal, the original form of *Hordeum vulgare* is judged to be two-rowed (von Bothmer et al., 1991).

Classification of the two- and six-rowed barleys

The characters of these types are described as kernel rows and fertility of lateral spikelets. First Harlan (1918), later Mansfeld (1950, 1959) and Hoffmann (1959) used a system, involving the following taxa of primitive and cultivated barleys:

[1] *Hordeum vulgare* L. convar. *hexastichon* Alef.
Six-rowed barley with all rows similar in fertility and development of awns or hoods.

[2] *Hordeum vulgare* L. convar. *intermedium* (Körn.) Mansf.
Intermediate barley, with partial fertility of lateral spikelets, though they are irregular in awn formation. Already Vavilov and Orlov used "*intermedium*" for forms with partly developed and partially fertile lateral spikelets, without taking into consideration awn development of the lateral spikelets. However, Körnicke (1885) [cit. Stubbe and Bandlow, 1946/1947] described many-rowed types with awnless lateral spikelets as *intermedium*. Stubbe and Bandlow (1946/1947) mentioned four types of "interjectum" mutations: (1) *divisa*, being many-rowed only in the upper part of the spike, (2) *incomposita*, irregularly many-rowed, (3) *sola*, with occasional fertile lateral spikelets, and (4) *partita*, only the upper lateral spikelets fertile, the basal ones sterile (Nybom, 1954 and references therein).

[3] *Hordeum vulgare* L. convar. *distichon* Alef.
Two-rowed barley with completely sterile lateral spikelets.

[4] *Hordeum vulgare* L. convar. *deficiens* (Steud.) Mansf.
Two-rowed barley, with rudimentary lateral spikelets, missing the sex organs.

[5] *Hordeum vulgare* L. convar. *labile* (Schiem.) Mansf.
Spikes are irregularly formed and the fertility of the spikelets varies considerably.

The above described system has been used in the analysis of the six-row and the intermedium types in the Swedish mutation research (Stubbe and Bandlow, 1946/1947; Mansfeld, 1950 and 1959; Gustafsson and Lundqvist, 1980 [paper IV] and references therein). Referring to convar. *labile*, it is systematically close to the species called *irregulare* (Åberg and Wiebe, 1945). In fact, for many reasons the designation *irregulare* (in English *irregular*) is much more suitable than the expression "labile". The "labile" mutants in the Swedish material range from *slightly irregular* to typically *irregular* and finally, *highly deformed* and *abnormal* types and mutants. In this survey no consideration will be taken of the so-called deficiens character, though the occurrence of deficiens mutants is not especially rare.

Induction of six-row and intermedium mutants and its genetical studies

These mutants affect the development of the lateral spikelets. Normal two-row barley carries, on opposite sides of the spike, central spikelets with two reduced, sterile and awnless lateral spikelets. This two-row barley is able to produce six-row barley in a single mutational step. These mutants have well developed lateral spikelets, fully fertile and with long awns. All the 41 isolated cases have been localized to one and the same locus, *hex-v*, located on the long arm of chromosome 2 (Nilan 1964; Gustafsson et al., 1969; Lundqvist and Lundqvist, 1987a).

Two-row barley may also produce mutants with spike development intermediate between the two-row and the six-row states. These mutants have enlarged lateral spikelets, which vary in characteristic ways with regard to awn development, fertility and kernel development, not only among mutants, but also depending on environmental conditions. Some of them may sometimes look somewhat hexastichon-like. A total of 126 such *intermedium* mutants have been isolated, and 103 of them have been localized to 11 different *int* loci by means of diallelic crosses; both the F_1 and F_2 generations have been studied, in some cases, individual F_2 plants have been followed into the F_3 generation. Two mutants are considered as non-allelic when the F_1 between them is of more or less normal phenotype and with an evident and regular Mendelian segregation in the F_2. When two mutants are supposed to be allelic, the F_1 shows mutant phenotype. Among these mutants, 69 were studied in more details.

Their distribution to the 9 *int* loci is as follows:

Locus *int*	*-a*	*-b*	*-c*	*-d*	*-e*	*-f*	*-h*	*-i*	*-k*
Frequency	23	2	18	13	7	1	3	1	1

The two loci *int-l* and *int-m* are represented by a single mutant each, but no further studies have been made on these.

The tests for inheritance of the *intermedium* mutants demonstrated, in backcrosses to the mother varieties, that 8 of the above mentioned *int* loci are

recessive, and only independent inheritance has been established. The mutants belonging to the locus *Int-d* all show a more or less clear tendency to be dominant to the two-row normal state. One of the alleles of this locus seems to be completely dominant. Also the six-row locus *hex-v* is semidominant. In addition, the two loci, *hex-v* and *Int-d* are closely linked to each other. All the semidominant alleles of these two loci give in F_1 combinations with mother varieties heterozygotes that have lateral spikelets with pointed lemma (the so-called S-plants) (Gustafsson and Lundqvist, 1980 [paper IV]; Lundqvist and Lundqvist, 1988a [paper VI]).

All the intermedium mutants tested here, have been induced in the three previously used outstanding commercial varieties of two-row barley, Sv Bonus, Sv Foma and Sv Kristina. Different kinds of mutagenic treatments have been used: sparsely and densely ionizing radiations, ethylene imine, most of the organic sulfonates and also the inorganic sodium azide.

Of the 88 mutants, induced in the three above mentioned varieties (69 of them more thoroughly investigated), no less than 67 (= 76.1%) have arisen after chemical mutagen treatments, 11 cases (= 12.5%) when using sparsely ionizing radiation and 8 cases (= 9.1%) when using densely ionizing radiation. Two cases (= 2.3%) were induced, when combined treatment (radiation + chemicals) was applied. No definite gene preference to the type of mutagen applied has been found. But the number of investigated mutants is too small for any conclusions (Gustafsson and Lundqvist, 1980 [paper IV]; Table 1 in Lundqvist and Lundqvist, 1988a [paper VI]).

Morphological description of the intermedium mutants

The nine intermedium loci found for this character form rather natural morphological groups with similar, however, particular traits; some of these traits are shared. The characteristics of the lateral spikelets for the 69 mutants are based on the scheme in Lundqvist and Lundqvist, 1988a, Fig.1 [paper VI]. The normal two-row barley has the values 0, 0, and 0 for awn development, fertility, and seed development, respectively, in lateral spikelets (Lundqvist and Lundqvist, 1988a, Table 1 [paper VI]).

The majority of the mutants belong to *int-a*, *-c*, *-d* and *-e*. A short description of the 9 most investigated *intermedium* loci will be presented as follows:

[1] *int-a*: The lateral spikelets are characteristically enlarged with seed set in the upper two-thirds of the spike. The central spikelets often have double awns, one on the lemma and one on the palea (cf. figure 9).

Figure 9. Intermedium mutant in the locus *int-a* (to the right) compared with the two-rowed spike of the mother variety 'Bonus' (to the left).

[2] *int-b*: The spikes are of a rather irregular shape, the lateral spikelets are conspicuously enlarged with partial seed set. The plant is tall and tillers poorly.

[3] *int-c*: The lateral spikelets are fairly large and broad, the lemma is often rounded or weakly pointed at the apex. Seed development is rather variable, both among mutants, among parts of the spike, and among different years (cf. figure 10).

Figure 10. Intermedium mutant in locus *int-c* to the left and intermedium mutant in locus *Int-d* to the right.

[4] *Int-d*: This locus is marked by fairly large and distinctly pointed lateral spikelets, with short or long awns of variable length, but rarely reaching the lengths of the central spikelet awns. The seed set of the lateral spikelets is very variable, and in some mutants the laterals are completely sterile, in other mutants they are partly or wholly filled with seeds, although these are never as large as six-row mutants or especially varieties. There is a great variation among different years (cf. figure 10).

[5] *int-e*: Only the lateral spikelets in the upper third of the spike are enlarged, with seeds; these lateral spikelets have pointed tips. In the lower part of the spike, the lemma of the lateral spikelets are somewhat rounded at apex (cf. figure 11).

Figure 11. Intermedium mutant in locus *int-e* to the left and intermedium mutant in locus *int-f* to the right.

[6] *int-f*: This locus presents only one single mutant, with a typical dense erectoides-like base. All the lateral spikelets are pointed, sometimes with short awns. The lateral spikelets of the upper part of the spike have a partial seed set (cf. figure 11).

[7] *int-h*: The lateral spikelets are strongly enlarged, inconspicuously pointed at apex, mostly sterile with occasional awns. The spike is of lax type, and all three alleles are associated with early heading.

[8] *int-i*: This locus is represented by a single mutant. The lateral spikelets are enlarged and partially pointed at the apex. The tip of the spike has dense rachis internodes and due to this character the spike tip is of a very dense erectoides type.

[9] *int-k*: The lateral spikelets are enlarged, pointed and completely sterile. The spike is short and dense. It is also covered with more wax than the normal barley plant. It is not known yet whether this is a case of pleiotropic effect.

In the *hexastichon* mutants, the lateral spikelets have a good seed set, but they are not as large as in the cultivated six-row barley varieties. The awns of the lateral spikelets do not reach the length of the central spikelet awns.

The Swedish *hex* and *int* genes in relation to earlier known six-row genes

Studies on kernel rows and fertility of lateral spikelets have been reported several times (e.g., Smith, 1951; Nilan, 1964). According to these reports, the allelic series V^{t}, V^{d}, *V*, V^{m20}, *v-1*, *v-2*; I^{h}, *I*, *i-4*; I_1*-4* are controlling this character. This statement could be supported by the fact that the row number on the spike is controlled by a single locus, with two-row being dominant over six-row. The other gene locus *I* was considered to be responsible for occasional fertility of lateral spikelets in two-row types, and was also described as "Infertile and Fertile intermedium" (Smith, 1951; Nilan, 1964). The locus *v* got renamed *hex-v* for the six-row mutants, and the gene symbol *int* was assigned for the intermedium mutants in the Swedish mutant collection (Gustafsson et al., 1969).

During the seventies the Japanese group at Okayama University studied several induced so-called "six-row" mutants. They also described at an early stage that some of them have spikes that are somewhat different in appearance, with less fertility in the lower and upper lateral spikelets and with relatively short awns or with undeveloped lateral spikelets (Fukuyama et al., 1972 and 1975). As a result of their genetical and linkage studies, they assigned to their mutants the gene symbols *v* - v_5. They were able to localize the genes v_2, v_3, v_4 and v_5 on the chromosomes 7, 5, 3 and 4, respectively. All of these genes were recessive (Fukuyama et al., 1972, 1975, and 1982).

In the Swedish mutation research, studies were conducted on the relationships of intermedium mutants in barley during the 60s. It was possible to localize three *int* genes: *int-a*, *int-c* and *int-e* to the chromosomes 5, 4, and 3, respectively (Persson, 1969). Furthermore, Persson (op. cit.) localized the *int-c* gene to the short arm of chromosome 4, and suggested probable allelism with I^h, I and *i* series located in the same region. This got verified later (Gymer, 1977; Lundqvist, unpublished).

In further studies at Svalöv, allelism tests were carried out between the Swedish *int* loci and the 5 Japanese *v* genes. The most mutable *int* genes, plus *int-f* with only one mutant, were used. Both the F_1 and the F_2 generations were studied. The results of these tests can be summarized as follows: both v_1 lines are allelic to *hex-v* on chromosome 2; v_3 is allelic to *int-a* on chromosome 5; v_4 is allelic to *int-e* on chromosome 3; and v_5 is allelic to *int-c* on chromosome 4. v_2 is not allelic to any of the 5 *int* and *hex-v* loci tested (Lundqvist, 1990, and unpublished).

Inheritance of int loci

The isolated *intermedium* mutants have continually been intercrossed. The non-allelic crosses between different loci have been studied in the F_2 generation in more detail. The segregations have been clearly dihybrid, with independent inheritance. It was striking to find as double-recessives, later called double mutants, types of six-rowed, irregular, deformed or highly deformed spikes (Gustafsson and Lundqvist, 1980 [paper IV]). A more detailed description of the double mutant combinations will be discussed in a later section.

Crosses among recessive *int* loci

This discussion is based on paper VI, tables 4 - 13.

These crosses have largely involved all alleles of the loci. In Tables 4 - 8 the χ^2 components (= diff^2/expected) for individual alleles in combination with the other loci are demonstrated for the class of normal plants (N) with independent segregation expected. The χ^2 components do not in any case give a clear indication of the recombinant class N being under-represented. Crosses involving *int-c* alleles obtained from the variety Sv Kristina (it has a tendency to have smaller lateral spikelets than for instance Sv Bonus) may have a misclassification of the class N,

which may go in both directions, forming a surplus or a deficit to the N class.

From the complete χ^2 analysis of linkage, the corresponding χ^2_L values (test for free segregation) are shown in Tables 9 - 13. Here, only the "negative" deviations, for the recombinant classes N and double mutant *int/int* respectively, are of interest.

Summarizing the results of this χ^2 analysis among crosses of the recessive *int* loci: *int-c* alleles may show tendencies to "negative" values for χ^2_L, which indicates a deficit of the recombinant classes N and double mutants. It may be suggested that the double homozygous mutants tend to be classified as single mutants, e.g., *ccee* as int e; *ccff* as int f, or *cchh* as int c. The double mutants may sometimes be subviable and therefore get eliminated in the field (*ccii*). Besides, mutants derived from the barley variety Sv Kristina are frequently associated with an overlap between the N and the int c classes. The crosses between the *int-e* alleles and *int-f* locus might indicate a slight linkage, because of a deficit of N plants. There are fairly great "negative" χ^2_L values in crosses between *int-h* alleles and the *int-i* locus; this is probably due to a tendency for *hhii* plants to be classified as *int-i* plants.

Crosses between *Int-d* and the recessive *int* loci

This discussion is based on paper VI, tables 14 - 21.

All 13 alleles of the *Int-d* locus have been crossed to the majority of the recessive *int* loci. Each table shows the F_2 segregations of the different alleles of the *Int-d* locus in crosses to the pooled alleles of each recessive *int* locus. With independent inheritance, the four classes N + S, int rec., Int d, and double mutant in the F_2 are expected to form 9/16, 3/16, 3/16, and 1/16, respectively (S-plants are the heterozygotes with pointed lemma on the lateral spikelets, which arise in combinations with *Int-d*).

The analysis of the gene locus *Int-d*, showed two different tendencies among the *Int-d* alleles. Some alleles are able to form a double mutant phenotype or the Int d phenotype even in the heterozygous *Dd* state; this is especially the case with the mutants *d* 36 and *d* 40. Here the class N + S tends to be under-represented. Other alleles, especially *d* 11, *d* 12, *d* 22, *d* 24, *d* 28, and *d* 69, seem to form inconspicuous double mutant phenotypes, the genotype $ddint_Xint_X$ apparently becoming classified as Int d or sometimes as int x, and the double mutant class will be underrepresented.

In both cases linkage is simulated by increasing "negative" χ^2_L values. Disturbing effects on the classification ratios may also result when recessive *int* genes, even in single doses already, enhance an effect on lateral spikelet development initiated by another gene (Lundqvist and Lundqvist, 1987a).

Summarizing conclusions: cases of synteny should be unavoidable when only 7 pairs of chromosomes are at hand for 9 *intermedium* loci. Nevertheless, no case of undisputable linkage has been established among these loci. Disturbing factors include recessive genes in repulsion phase forming a blunt tool for an F_2 analysis of linkage. There are also signs of disturbing phenotypic overlapping. On the other hand, the variable dominance, which characterizes locus *Int-d*, causes a considerable blurring in the demarcation of the phenotypic classes.

Double mutant combinations of *int* genes

Interaction between the *int*-loci resulting in a further enhanced development of the lateral spikelets has been observed at an early stage of these investigations (Gustafsson and Lundqvist, 1980 [paper IV]). As already mentioned, double mutants were found in studies of F_2 generations from interlocus cross combinations. Combinations of *int* genes in double homozygous state have frequently resulted in typical six-rowed spikes, whereas other double mutant combinations have given rise to irregular or deformed and even highly deformed spikes. The competence of *int* genes to interact efficiently, and its dependence on the interaction of particular loci and alleles have been investigated on a rather large material of double mutant combinations.

The 69 first isolated *int* mutants, which have been localized to 9 different *int* loci, have continually been crossed with one another. Only one combination *int-b x int-f* among 36 possible combinations is missing. As a whole, there are 1879 possible combinations among non-allelic mutants. 1384 of them have been investigated in the F_2 generation, and individual plants classified as double mutants have been followed into the F_3 generation. The gaps in the scheme are mainly due to the combinations among the first isolated mutants, before the surprising spectrum of the double mutant phenotypes had been discovered (Lundqvist and Lundqvist, 1988b, Figure 1 [paper VII]).

The ranking of the double mutant F_2 segregants into the three groups in their spike development does not show clear-cut borders among the different types. There is a continuous transition between irregular and deformed types of spike, and the six-rowed spikes may sometimes look somewhat irregular regarding lateral spikelet fertility. Therefore, the demarcation is probably in both cases more or less artificial and must be regarded with some caution. It is, however, perfectly clear that typical six-rowed and irregular spikes always arise in different progenies. The final classification of the types of spike development uses a scale with 9 steps, steps 1 - 3 denoting six-rowed types with gradually decreasing regularity, and the steps 4 - 6

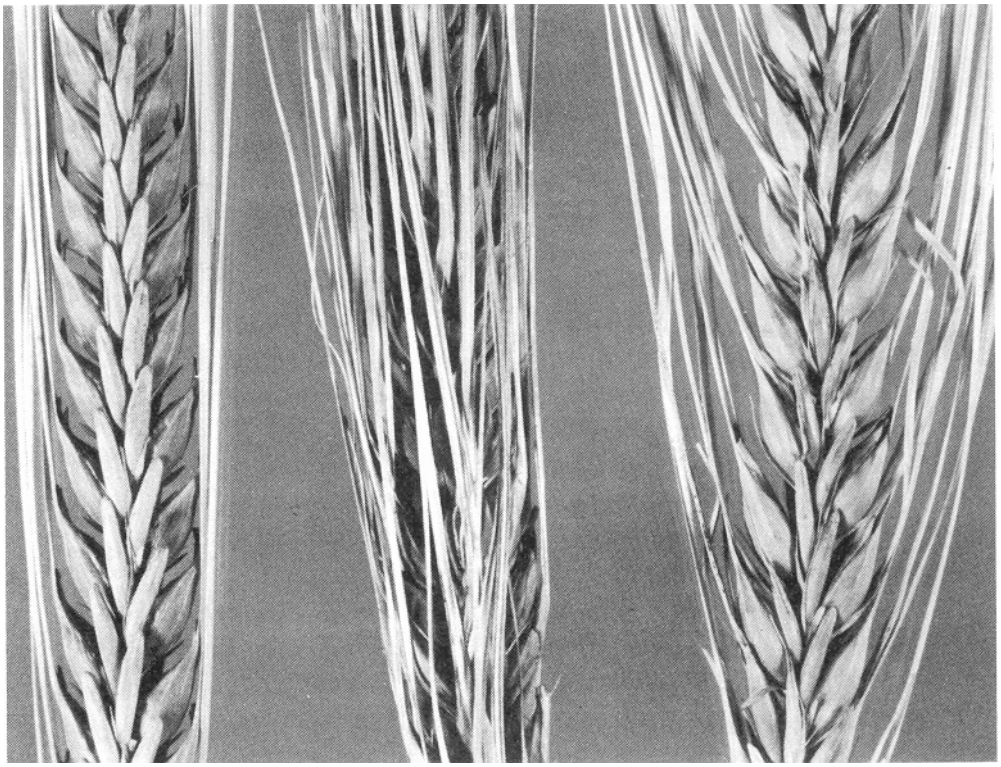

Figure 12. Six-rowed double mutant F_2-segregant compared with the parents. From left to right: *int-c*, double mutant, *int-a*.

and 7 - 9 corresponding to irregular and deformed spikes of increasingly extreme shapes respectively (Gustafsson and Lundqvist, 1980 [paper IV]; Lundqvist and Lundqvist, 1987a and 1988b [paper VII]) (cf. figures 12 and 13).

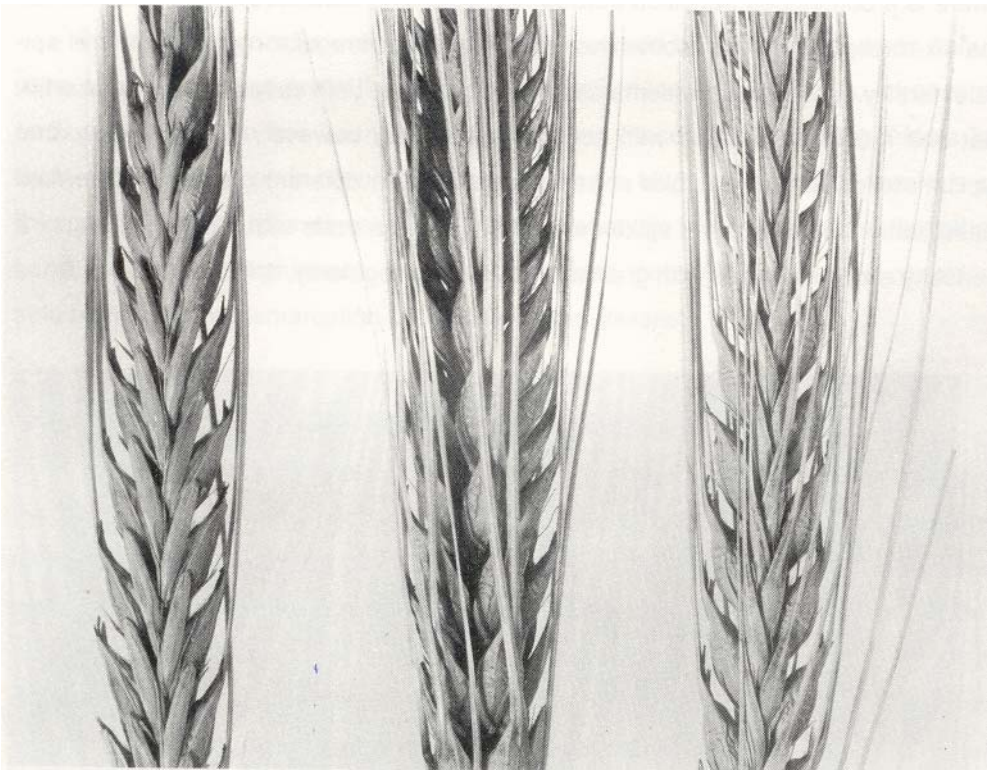

Figure 13. Six-rowed double mutant F_2- segregant from the most efficient combiners. From left to right: *int-c*, double mutant, *Int-d*.

The means of the spike phenotypes of available double mutants show that there are apparent differences among *int* loci in their ability to co-operate in the formation of six-rowed spikes in double mutants. The most efficient combiners are *Int-d* and *int-c*. It is striking that the two loci *int-a* and *int-e* both interact so successfully with *int-c* and *Int-d*, and at the same time are quite inefficient partners to one another (Lundqvist and Lundqvist, 1988b, Table 1 [paper VII]) (cf. figure 14).

When studying the four most frequently mutated *int* loci (*int-a*, *int-c*, *Int-d*, and *int-e*), apparent differences are revealed not only among loci, but also among their alleles in ability to co-operate in the formation of six-rowed spikes in double mutants; in fact a bimodal distribution of spike development values is apparent for each of the six combinations among the alleles of the four *int* loci (Lundqvist and Lundqvist, 1988b, Tables 2, 4 - 7 [paper VII]). With the exception of the combinations between *int-a* and *int-e*, the majority of double mutants have a more or less regular six-rowed phenotype. Among the remaining double mutant combinations of alleles a very wide range of phenotypes is covered.

Figure 14. Deformed double mutant F_2-segregant from poor combining partners. From left to right: *int-e*, double mutant, *int-a*.

When comparing the distributions of spike phenotypes for the 23 *int-a* alleles with members of the other three main *int* loci, *int-c*, *Int-d*, and *int-e*, the arrays of double mutant combinations do not reveal any significant tendencies to heterogeneity. For the 18 *int-c* alleles, the arrays of double mutant combinations with *int-a* show strong tendencies to heterogeneity, but with *Int-d* and *int-e* they do not show any significant tendencies to heterogeneity among the *int-c* alleles; for the 13 *Int-d* alleles, the arrays of double mutant combinations with *int-a* are the only ones showing tendencies to heterogeneity. Some *Int-d* alleles are particularly efficient, others clearly less efficient. Finally, for the 7 *int-e* alleles, the arrays of double mutant combinations both with *int-a* and *int-c* show very strong tendencies to heterogeneity. We can conclude, thus, that *int* alleles differ in their efficiency to produce six-rowed double mutant derivatives, but that, in order to reveal such differences, particular *int* loci are required as partners.

Irrespective of the phenotypic outcome of the double mutants *int/int*, whether six-rowed, irregular, or more or less deformed, the phenotypes display a surprisingly strong degree of synergistic interaction between mutant genes which by themselves only cause rather modest changes in the normal two-row spike. No exception to the synergistic interaction is shown by double mutants obtained among the 9 *int* loci. In spite of apparent differences among *int* loci in their ability to co-operate in the formation of six-rowed spikes in double mutants, bimodal distributions of spike development values indicate clear differences also among alleles. This bimodality may indicate thresholds on the way to the six-rowed double mutant state. Another indication of the existence of such thresholds is the environmental influence on the development of lateral spikelets.

As mentioned before, *int-c* and *Int-d* interact with one another with outstanding success, whereas *int-a* and *int-e* are poor partners to one another. Both *int-a* and *int-e* in combination with *int-c* and *Int-d* show good co-operation. One can speculate that the alleles of *int-c* and *Int-d* are more flexible to the development of a regular six-rowed double mutant. The alleles of *int-a* and *int-e*, on the other hand, may be looked upon as more rigid. As a matter of fact, *int-a*, as a partner to form double mutants, has revealed differences within series of alleles at the other three main loci (*int-c*, *Int-d*, and *int-e*). A rigid state within *int-a* would, so to say, force the alleles of the other

locus to show their efficiency: more rigid alleles become less successful, more flexible alleles are more capable of making a six-rowed double mutant (Lundqvist and Lundqvist, 1988b [paper VII]).

Interaction of *int* genes and *hex-v* genes

The inheritance of seven *int* loci (*int-a*, -*b*, -*c*, -*e*, -*f,* -*h*, and -*i*) in relation to the *hex-v* locus has been studied in crosses by using two alleles at *hex-v*, viz. the six-row mutant *hex* 3, isolated from the two-row variety Svalöf's 'Bonus', and the allele carried by the six-row variety Svalöf's 'Agneta'. The majority of alleles that are included in the test belong to *int-a*, -*c*, and -*e*, and the segregation studies have been focussed on the F_2 generation. There are no clear indications of failure to interact with *hex* 3 in forming regular six-rowed spikes in double mutants *intint vv* for any of these *int* loci studied (Lundqvist et al., 1988a; Abebe et al., 1990).

For independent inheritance in a dihybrid segregation, N plants (N = normal) + S plants (S = plants with sterile lateral spikelets mostly without awns, but with pointed lemma) should be expected in the frequency 9/16, the remaining 7/16 representing the *six-row* homozygote, the *intermedium* homozygote, and the double homozygote *hex-v*/*int*, respectively. The expected frequency 9/16 appears very nicely in all the crosses with *hex* 3 (Lundqvist and Lundqvist, 1987b, Tables 1 and 2 [paper V]). But there are some indications from a limited material that *int* genes (*int-a*, -*c*, -*e*) are more efficient as enhancers of lateral spikelet fertility in the double mutant *int*/*int* state than in double mutant combinations with the *hex-v*^{3} gene (Lundqvist et al., 1989b [paper IX]). On the other hand, an important enhancing interaction between the *int-c* and *hex-v* loci has been recognized in six-row barley varieties already in early inheritance studies (Robertsson, 1933; Leonard, 1942; Robertson et al. 1955; Gymer, 1978).

When analysing the F_2 segregations of the crosses with 'Agneta', the class N + S shows a more or less pronounced deficit of plants, with particular classification difficulties appearing for the crosses to alleles of locus *int-c* (Lundqvist and Lundqvist, 1987b, Tables 1 and 2 [paper V]). Very soon the 'Agneta' was also suspected of carrying an *int* gene (viz. *int-c*) along with the *hex* gene for enhanced development of lateral spikelets (Lundqvist and Lundqvist, 1987b, Tables 3 and 4 [paper V]). This

conclusion is also supported by an analysis of data from a cross between the Svalöf variety 'Mona' (normal two-row barley) and 'Agneta' (Lundqvist and Lundqvist, 1987b, Table 5 [paper V]).

In crosses between representatives of *int-c* and 'Agneta', F_2 plants classed as six-row occur much more frequently relative to the ordinary 1/4 ratio expected for -- *vv* plants. Obviously, *cc Vv* plants have to a large extent been classified as six-row. The ability among the 18 *int-c* alleles studied in these crosses, differed in assigning a six-row phenotype to *cc Vv*, with a single *hex-v* allele from 'Agneta'. From 17 different mutant *int-c* alleles tested, six alleles gave a significant surplus of six-row plants, and they showed an interesting agreement with the hypothesis of 6/16 six-row F_2 plants. The *int-c* allele originally carried by 'Agneta' is not among the "strong" alleles. The enhancing *int-c* alleles seem to express their influence only in the homozygous state, the heterozygotes with the c_{Agn} allele apparently being inefficient (Lundqvist and Lundqvist, 1987b, Tables 10 and 11 [paper V]).

A closer investigation of the 'Agneta' *int-c* allele and its interactive competence together with three other alleles of the *int-c* locus was performed (Lundqvist and Lundqvist, 1989). The three mutants, c^5 from the variety 'Bonus', and c^{13} and c^{29} from the variety 'Foma' were used. It may be mentioned that both c^5 and c^{13} have not been very strong *int-c* alleles, but c^{29} has not been studied in that connection (Lundqvist and Lundqvist, 1987b [paper V]). In the cross, there is no dominant normal allele at *int-c* present. The following characters were studied on the main spike and on an adequate number of plants: awn development, fertility and kernel development of lateral spikelets according to the scheme for numerical classification of lateral spikelet characters (Lundqvist and Lundqvist, 1989, Figure 1 [paper VIII]).

The F_2 genotypic constituents may contribute to the phenotypic outcome according to five different models, A - E (Lundqvist and Lundqvist, 1989, Table 2 [paper VIII]), where the first four, A - D, in a rising series, assume that [A] none; [B] the *cc* homozygotes; [C] the c_Ac heterozygotes, too; and finally, [D] all *Vv* plants, respectively, are raised to the six-row (*hex-v*^{3}) phenotypic level. For model E, it is expected that *Vv cc* plants reach the 'Agneta' six-row level. The analyses of observed data in relation to expectations on various hypotheses for interaction between *hex-v* and *int-c* showed some relevant trends (Lundqvist and Lundqvist, 1989, Tables 3 and

4 [paper VIII]). Hypotheses C - E have been less adequate for awn length, and fit well for fertility, while the seed size character shows a more heterogeneous pattern. When looking at the χ^2 values, the conclusions can be summarized as follows:

For the awn length character and the fertility, all the present three *int-c* alleles reacted in fairly similar ways. The seed size character revealed clear heterogeneity among the present *int-c* alleles, *int-c*^{5} being the most efficient enhancer, with a preference for hypotheses D and E; *int-c*^{13} fitted best to the hypotheses A and B; and *int-c*^{29} showed agreement with hypothesis C.

Thus the degrees of phenotypic enhancement may differ among characters of lateral spikelets development and among *int-c* mutant genes. There are no indications that the *int-c* allele present in 'Agneta' is the superior enhancer of the *hex-v* gene in this variety. Nor are there indications that this *hex-v* gene is a superior one. It is, however, of interest that whereas the *int-c* allele of 'Agneta' has poor phenotypic expression of the awn length character, it has a stronger influence on fertility and seed size of lateral spikelets. Most probably, it would pay, in a plant-breeding programme with six-row barley to try various constellations of *hex-v* and *int-c* mutants.

Triple mutant combinations with *hex-v*

The question arose whether more complex gene systems would show a progressive promotion of lateral spikelet development, or whether there is an optimum indicated for the number of genes influencing the development of lateral spikelets. Cross combinations of double mutants *int/int* and the *hex-v* gene have produced beautiful six-row types with conspicuous large spikes and thick culms, to such a degree that we denoted them as 'King-size' (Lundqvist and Lundqvist, 1987a) (cf. figures 15 and 16). Such six-rowed segregants had not been observed before in crosses leading to double mutants with *int* genes and the *hex-v* gene in various two-gene combinations. No strict genetical verification of their triple mutant state could be done at that time, but there were interesting indications of a connection between the efficiency of the *int* genes to produce the supposed triple mutant type in combinations with *hex-v*, and their efficiency to produce double mutants *int/int* with regular six-rowed spikes. The 'King-size' phenotype apparently depended on particular types of

gene interaction, certain *int* loci being more competent than others, and the particular *int* allele being competent in relation to the constellation of loci.

Figure 15. Triple mutant combination from the six-rowed double mutant [*int-c*^{5} + *int-a*^{34}] combined with the six-row mutant *hex-v*^{3}. From left to right: [*int-c*^{5} + *int-a*^{34}], triple mutant of six-row type denoted as 'King-size', *hex-v*^{3}.

Therefore further investigations have been initiated to study the effects of the accumulation of genes promoting lateral spikelet development in a number of genotypically verified *int/int/hex-v* recombinants (Lundqvist et al., 1989b [paper IX]). As a whole, 24 recessive intermedium mutants belonging to the four loci *int-a*, *int-b*, *int-c*, and *int-e* and two *hex-v* alleles, *hex-v*^{3} and the allele present in the six-row variety 'Agneta' (*hex-v*$_{A}$) have been involved in producing the triple constellations *int/int/hex-v*. The 19 investigated triple combinations (six with *hex-v*^{3}, and 13 with

hex-v$_A$) were based on 15 different *int/int* double mutant combinations of six-rowed type, and all of them build upon a representative of *int-c*, this locus being outstanding among the recessive *int* loci as a component in regular six-rowed *int/int* double mutants (Lundqvist and Lundqvist, 1988b [paper VII]).

Figure 16. Triple mutant combination from the six-rowed double mutant [*int-c*^{5} + *int-a*^{34}] combined with the six-row variety 'Agneta'. From left to right: [*int-c*^{5} + *int-a*^{34}], triple mutant of six-row type denoted as 'King-size', 'Agneta'.

Altogether, there are six systems of comparisons, and within each individual system, *int* genes and *hex-v* genes are added or subtracted in a systematical way (triple x hex-v^3; triple x 'Agneta'; triple x double; Lundqvist et al., 1989b, Table 1 [paper IX]).

The statistical analyses of the effects, when *hex-v* genes or *int* genes or both of them are added to or withdrawn from a set of genes with influence on lateral spikelet development, can be summarized as follows:

[1] The present *int* genes are more efficient as enhancers of lateral spikelet fertility in the double mutant (*int/int*) state than in double mutant combinations (*int/hex-v*^{3}) with the *hex-v*^{3} gene (Lundqvist et al., 1989b, Tables 2 and 8 [paper IX]).

[2] With the present *int* genes, an enhancement of lateral spikelet fertility is likely when the *hex-v*^{3} gene is added to a double mutant (*int/int*) basis. When *hex-v*$_A$ is added, this one tends to be the more efficient enhancer (Lundqvist et al., 1989b, Tables 3 and 8 [paper IX]).

[3]The subtraction of one *hex-v*^{3} gene from the triple *int/int/hex-v*^{3} combinations has caused slightly significant reduction in awn length and seed size of lateral spikelets in the F_1's (2 2 1 for promotive genes at *int-c*, the second *int* locus, and *hex-v*). No positive effects could be demonstrated for the addition of one *hex-v*^{3} gene to the double mutant (*int/int*) basis (Lundqvist et al., 1989b, Tables 4 and 8 [paper IX]).

[4] The subtraction of one *hex-v*$_A$ gene from the triple *int/int/hex-v*$_A$ combinations, in the F_1's (2 2 1 for promotive genes at *int-c*, the second *int* locus, and *hex-v*), has caused clearly significant reductions for all three lateral spikelet characters. No positive effects could be demonstrated for the addition of one *hex-v*$_A$ gene to the double mutant (*int/int*) basis (Lundqvist et al., 1989b, Tables 5 and 8 [paper IX]).

[5] The subtraction of 1 + 1 *int* genes from the triple *int/int/hex-v*^{3} combinations, in the F_1's (1 1 2 for promotive genes at *int-c*, the second *int* locus, and *hex-v*), is likely to have caused some deterioration in all three lateral spikelet characters. The addition of 1 + 1 *int* genes to the *hex-v*^{3} mutant has caused an increase in awn length (Lundqvist et al., 1989b, Tables 6 and 8 [paper IX]).

[6] The highly developed 'Agneta' variety (2 0 2 promotive genes at *int-c*, the second *int* locus, and *hex-v*), has reached a level of six-row performance that is largely maintained in the F_1 from crosses to *int/int/hex-v*$_A$ triple combinations, but seems to have been partly lost in the latter after some generations of recombination in the genic background (Lundqvist et al., 1989b, Tables 7 and 8 [paper IX]).

The addition of the *hex-v* gene to *int/int* double mutants, in the *int/int/hex-v* constellation promoted lateral spikelet development. Probably, *hex-v* should form the fundamental constituent in the synthesis of gene systems with the most efficient promotion of lateral spikelet development in barley. Interaction between *hex-v* and the *intermedium* gene *int-c* has been recognized earlier (Robertsson, 1933; Leonard, 1942; Robertson et al., 1955; Gymer, 1978; Lundqvist and Lundqvist, 1987b [paper V]).

Progressive promotion of lateral spikelet development

More complex crosses for a systematical synthesis of triple or quadruple combinations of *intermedium* genes have been tried. The analyses are based on four independently inherited loci, the recessive *int-a*, *int-c*, and *int-e*, and the semidominant *Int-d*, represented by a total of 7 alleles (*Int-d* by the weakly semidominant *Int-d*^{12} and the more strongly semidominat *Int-d*^{36}). These seven single mutant genes were deliberately selected, being reliably efficient to produce double mutants of regular six-rowed phenotype. This was considered as an adequate platform for the study of lateral spikelet development, and to look for evidence of progressive promotion or for indications of an optimum in the number of *intermedium* genes involved in lateral spikelet development.

In order to obtain three-gene segregations, double mutants with one of their intermedium genes in common were combined in adequate crosses (Lundqvist and Lundqvist, 1991). Out of 96 possible combinations, 20 combinations were used. For four-gene segregations two double mutants, with none of their *intermedium* genes in common, were combined in a cross. Only one combination was analyzed. Sets of standards consisting of the two-rowed mother variety, single mutants, and double

mutants, were compared with the range of phenotypes expected to be due to a segregation with up to triple or quadruple mutants obtained in the F_2, when double mutants with one or none of their *intermedium* genes in common had been combined in crosses.

Standards are applied to these data in the proportions expected from a Mendelian segregation of independently inherited genes. Then, any statistically significant incremental difference between data expected from the standards and from F_2 data observed, should indicate the presence of enhancing genes. The three lateral spikelet characters showed enhancement effects over the whole range of character grades. The enhancement effects differed among the 20 combinations in the three-gene segregations, different *intermedium* genes giving different enhancement effects. An apparent further progressive enhancement for lateral spikelet characters was indicated when a fourth *intermedium* gene was introduced, in the four-gene cross, particularly for fertility and seed size.

For further evidence of a progressive promotion of lateral spikelet development or, alternatively, for indications of an optimum in the number of *intermedium* genes involved, the F_3-offspring derived from segregants selected in the F_2-generation have been investigated in more detail. In order to analyze the three-gene segregants, 111 phenotypically prominent F_2-plants have been brought to the F_3-generation and F_3-families were grown individually. For the four-gene segregants, 109 phenotypically prominent plants were selected in the F_2-material to form the F_3-families, which were grown individually in the same way as the three-gene segregants (Lundqvist and Lundqvist, 1990 [paper X]).

Offspring from selected three-gene segregants displayed a clear superiority over the pooled corresponding three lines with the genes in the double mutant state. Among the 51 comparisons in the three characters (awn length, fertility and seed size), 36 indicated significant superiority for three-gene segregants, and in only 2 comparisons was there a slight superiority for the genes in the double mutant state. The superiority of the three-gene segregant state, relative to the double mutant state, was much more pronounced for the fertility and seed size character than for the awn length. The sometimes rather wide range of variation in the F_3 data may indicate that the prominence of selected three-gene segregants in the F_2-generation was not

necessarily bound to triple homozygosity for the *intermedium* genes involved (Lundqvist and Lundqvist, 1990, Tables 1 - 3 [paper X]).

When a fourth *intermedium* gene was introduced into the segregation, the progressive promotion continued at an increased rate. The progressive promotion of lateral spikelet development was particularly apparent for the fertility and seed size character (Lundqvist and Lundqvist, 1990, Table 7 [paper X]).

The progressive promotion of lateral spikelet development being particularly apparent for the fertility and seed size characters may have practically important implications for plant breeding in six-rowed barley, when especially efficient constellations of alleles belonging to different *intermedium* loci have been attempted. It should, however, be observed that our investigations have not yet been able to definitely pinpoint individual plants homozygous at three or four *intermedium* loci.

Summarizing points of view

The use of clearly defined mutants evaluated against one another in allelism tests has demonstrated that there are many degrees in the development of lateral spikelets in cultivated barley, both among loci and among alleles, and that genes promoting lateral spikelet development may interact in unexpected reinforcing or disturbing manners. Such a complex genetic situation gives a key to the somewhat confusing literature with conflicting reports on the genetics of kernel rows in barley, and may explain the deviating and deformed offspring often arising in crosses between primitive or advanced six-rowed and two-rowed barley strains.

By themselves the *intermedium* genes may cause rather modest changes in the normal two-row spike. Nevertheless, they are, without exception, capable of a surprisingly strong degree of synergistic interaction when they are brought together in double mutant plants. Attention to this co-operative ability was first drawn by Gustafsson and Lundqvist (1980) [paper IV], observing that *intermedium* genes in double mutant state frequently produce typical six-row spikes. Even in the heterozygous state, recessive *int* alleles have turned out to act as enhancers if a promotion of lateral spikelet development has been initiated in a recessive *int* homozygote (Lundqvist et al., 1988b; Abebe, 1990a) or by the semidominant *Int-d* locus (Lundqvist et al., 1989a; Abebe, 1990b). There may, however, be a

fundamental difference between the enhancement caused by an additional *int* gene in the heterozygous state and, on the other hand, the complementary interaction when the two *intermedium* loci are, by themselves, both capable of a promotion of lateral spikelet development.

Similar types of enhancing interaction can be established between heterozygous recessive *int* genes and the weakly semidominant *hex-v* gene (Lundqvist et al., 1988a; Abebe et al., 1990). The interaction between locus *int-c* and the *hex-v* gene is a matter of specific interest, since *int-c* has proved to be particularly efficient as an enhancer in interactions promoting lateral spikelet development, in double mutants *int/int*, together with *Int-d*, and with locus *hex-v*. It is, therefore, not unexpected that the enhancing interaction between *int-c* genes and the *hex-v* locus has long been recognized, and that alleles of *int-c* have been suggested to be important in the control of the six-row character and to occur frequently among cultivated six-rowed barleys (Robertson, 1933; Leonard, 1942; Robertsson et al. 1955; Gymer, 1978).

Figure 17. Greenhouse material of M2 generation seedlings representing different chlorophyll mutants.

Figure 18. Barley spike segregating for two chlorophyll mutations: normal green seedlings, albina and xantha mutants.

Figure 19. Eceriferum leaf blade (*cer-j*) mutant to the right and the normal variety “Bonus”.

Figure 20. Eceriferum leaf sheath/spike (*cer-c*) mutant to the left and the normal variety “Bonus”.

Figure 21. Variation of praematurum mutants in yield trials.

3. PRAEMATURUM (EARLY MATURITY) MUTANTS

Introduction

The demand for early varieties has grown and has therefore become an important goal in plant breeding. On one hand, the farmer wants to spread out his harvest season by growing crops that mature at different times, and he also wants an early crop for his crop rotation programme. On the other hand, in some areas, e.g., northern Scandinavia, the short vegetation period requires an early maturing crop. The importance of earliness was already discovered in the 30s by Russian plant breeders (Smith, 1951). Earliness is an important feature also under natural conditions.

Already in the 40s it was established that maturity in barley can easily be changed by X-ray mutation, in either direction, although changes to increased lateness occur more readily. However, screening for early mutants was much easier. It was also found that high-yielding mutations for earliness were not very uncommon (Gustafsson, 1942) (cf. figure 21, p. 78). For plant breeding purposes the following questions were raised: Which mutants have the greatest practical value: those which alter the earliness only slightly as well as other properties of the mother variety, or those which alter the earliness drastically? To what extent can results from one variety be extrapolated to other varieties?

The time of heading was chosen as a safe character for the selection of the induced early mutants. The time of ripening was difficult to use for the selection in the M_2 and M_3 generations. But there is a distinct correlation between heading time and the time of ripening, and consequently the former character was used for the selection. However, early heading and early ripening are characters where environmental influences, especially climatic conditions of the year, may hamper a safe classification.

Several varieties have been used for the induction of agriculturally useful mutants. Results obtained in Sv Gull and Sv Maja barley during the 40s were fundamental to the successful extraction of highly productive mutations in the

continuing experiments. In fact, in 1940, an erectoides mutant, called erectoides 16, with a slightly more dense spike than the mother variety Maja, was the first extreme early mutant isolated. It ripened on average six to ten days before the mother variety. It also combined high stiffness of the straw, high yield, increased protein content and a changed mode of tillering and growth. It also had a pronounced anthocyanin coloring of the sterile lemmas. When used in breeding work, this mutant very soon showed an inferior combining ability, and the resultant lines were often susceptible to *Helminthosporium gramineum* (Gustafsson, 1951 and 1969).

Genetical studies

Concerning the nomenclature of early maturity it was described as *earliness* with the gene symbol *Ea* with the alleles *Ea-1*; *Ea2 - 5*; and *ea*- 3 (Smith, 1951). One of the first genetic studies of early maturity indicated that earliness was recessive, although the classification into early and late plants was not distinct. However, dominant earliness was also found. Several studies in this respect have been reported. Concerning the localization on chromosomes, two of the dominant genes belong to the linkage groups I and IV and the recessive one to linkage group V (Smith, 1951). The gene locations have been described on chromosomes 1, 2, 4, 6 and 7 (Nilan, 1964), to-day on chromosomes 4, 5, 6 and 7 (Søgaard and von Wettstein-Knowles, 1987).

In German mutation experiments the early maturity mutants were called *matura* with the gene symbol *ma* (Stubbe and Bandlow, 1946/1947; Bandlow, 1959). Some years later the gene symbol *mat* was proposed (Mettin, 1961), but it was suggested that the symbol *mat* should not be accepted until the relationship between the alleles of *ea* and *mat* had been determined.

In the Swedish mutation collection early maturity was described as *early* with the gene symbol *ea* in the beginning of the studies (Gustafsson et al., 1960 [paper XI]). In the late 60s, when the establishment of a proposed system of symbols for the Swedish barley mutation collection was attempted at, the early maturity mutants were designated as *praematurum* with the symbol *mat* (Gustafsson et al., 1969). Subsequently, *praematurum* and *mat* was used for the Swedish early maturity mutants.

Induction of praematurum mutants

During the 50s when the first early mutants with normal nutans spikes were induced, the barley variety, 'Sv Bonus', was used. It is extremely high-yielding and also has a high tillering ability and is a reasonably good malting barley. It has, however, one drawback: in certain years it shows a tendency to lodging. Since then, various other varieties have been used for induction of mutants: 'Sv Foma', 'Sv Kristina', 'Sv 79353', 'Sv Semira', 'Sv Frida'. 'Bonus', 'Foma', and 'Kristina' have been used most frequently (Lundqvist, unpublished).

Over the many years, about 1100 different early mutants have been isolated after various mutagenic treatments. The whole spectrum of mutagens, both types of ionizing radiations, different kinds of organic chemicals, and also the inorganic sodium azide, have been applied. The mutants can be grouped into three different categories according to their heading time with a variation between one and ten days:

[1] drastically altered earliness
[2] medium increase of earliness
[3] slightly modified earliness

Very soon it could be stated that an increase of earliness by means of mutation most frequently leads to a lower grain-, straw- or total yield. The early mutations often show a weak development of the vegetative parts, for instance the height of the straw or the number of the tillers. The former property leads to less lodging, the latter one to less production (Gustafsson, 1958; Gustafsson et al., 1960 [paper XI]).

When the first 20 different early maturity or praematurum mutants in the Swedish collection had been collected, intensive studies were started to define the different groups of earliness and their genetic relationships. Very soon the three above-mentioned categories appeared: 8 mutants belonging to the drastic group, 3 to the intermediate earliness, and the rest were only slightly changed in heading-time. At least 4 different gene loci could be established. Three of them were found for the drastic early mutants and the fourth belonged to the intermediate group. For the rest of the mutants, no loci, for purely technical reasons, could be established. Further studies during the following decades made it possible to establish 9 different *mat* loci among 172 localized mutants. Two of them belong to the medium increased

earliness. The allelism tests were conducted with the drastic praematurum mutants only. For the slightly modifying types, it was difficult to make an exact comparison of the heading time from one year to the other, even within one and the same year. But there are, without doubt, numerous genes involved in earliness (Lundqvist, 1991 and unpublished).

The distribution of the mutants localized to the 9 *mat* loci is as follows:

Locus *mat*	-a	*-b*	*-c*	*-d*	*-e*	*-f*	*-g*	*-h*	*-i*	Total
Frequency	62	49	31	2	9	7	2	2	6	172

The crosses within the loci produce early F_1 and F_2 offspring, and crosses between the loci lead to late F_1 and are segregating for earliness in the F_2. However, earliness may not be entirely recessive, since some F_1's of the mutants with the mother variety are not as late as the mother variety. Erectoides 16 (*ert-o*^{16}), described as the first isolated extreme early mutant, was localized to locus *mat-a*. Early 14, which is characterized by a medium earliness, in the locus *mat-d*, showed partial dominance. It reacted in a specific way with different alleles of the drastic early loci. Since the property of "slightly enhanced earliness" is rather complex in character and depends on many morphological, including anatomical and physiological changes, a fairly large number of independent early mutations can be expected to appear, representing numerous gene loci. It was also striking to notice that not all of the early heading mutants ripened as fast as other allelic mutants for the same locus (Gustafsson et al., 1960 [paper XI]).

Praematurum mutants in relation to culm length

As already mentioned above, mutations selected for earliness also change other properties of agricultural value. Lodging is an important feature in plant breeding and varies greatly from year to year. It depends primarily on an interaction between the variety and the amount of rainfall during the growing season, and indeed

also the amount of fertilizer and the type of soil used. Lodging resistance is affected by a great number of morphological and anatomical properties, such as total straw (culm + spike) length, number and the length of the internodes, cross-sectional area of the stem, sclerenchyma formation in various parts of the stem, type of the root system, and the yield capacity of the mutants (Gustafsson et al., 1960 [paper XI]).

In this context only straw and culm length, and internode construction will be discussed. Several mutants of the drastic group have a total straw length equalling that of Bonus barley. Significantly shorter straw is found in some extreme early mutants, namely *mat-b*^{7}, *mat-a*^{8}, *mat-b*^{13}, *mat-c*^{16}, and *mat-c*^{19}. All these mutants have a reduced culm length, and generally the spike length is proportional to the total straw length, with the exception of the two mutants in locus *mat-c*, which have a more reduced length of the spike (Lundqvist, unpublished).

The early mutants also have an altered number of internodes (Ehrenberg et al., 1956). Bonus barley under average field conditions produces plants where the main straw has 5, 6 or 7 internodes; in most plants the internode number is six. The extreme early mutants, especially in locus *mat-a* and *mat-b*, have approximately one internode less per culm than Bonus barley and never possess seven internodes.

There is a wide variation among the early mutants as to internode length. Most of the drastic early maturity mutants have a longer upper first internode than Bonus. Almost all early mutants decrease their basal internode length in relation to culm length. The shortest basal internodes are met with in *praematurum* 5, *mat-b*^{7} and *mat-a*^{8}. Another interesting mutant is *praematurum* 2, which is about 2 - 3 days earlier in heading, higher in yield and more resistant to lodging. Its number of internodes is reduced, and it has a shorter basal internode. It never became locus identified (Gustafsson et al., 1960 [paper XI]; Gustafsson, 1969).

Lodging properties may be summarized as follows: The properties mentioned above - reduced straw (and culm) length, lower internode number, longer upper first internode, shorter basal internodes - promote lodging resistance. Thus enhanced earliness is automatically accompanied by such changes, which in turn leads to an increase of lodging resistance. Mutants of locus *mat-a* are generally more resistant to lodging than mutants in locus *mat-b*.

The Praematurum 8 mutant (*mat-a*^{8}).

This is a drastic early maturity mutant, 8 to 10 days earlier than the mother variety Bonus. It has arisen as a one-step mutation induced by X-rays in 1950. It can also be considered a "climate ecotype" of Bonus barley. Of all early mutants tested, it is the stiffest mutant, has the shortest straw, the lowest internode number, a relatively long upper first internode, the shortest basal internode and the highest internode index (the relationship between upper first and most basal internodes). The *mat-a*^{8} is also characterized by an altered root system, which develops very quickly after seed germination and consequently penetrates deeply into the soil. In this way it utilizes to its advantage water availability during its first period of development. It is less affected by summer drought than other mutants and varieties. It is also characterized by light- or yellow-green seedlings at an early stage of germination and is thus readily distinguished from other early mutants (Gustafsson et al., 1960 [paper XI]; Hagberg, 1961; Takahashi and Yasuda, 1971; Gustafsson et al., 1982).

Yield is certainly a complex character and difficult to improve. All the induced early mutants were tested for their yield capacity in quite extensive trials. Generally, the slightly changed early mutants retain the production capacity of the mother strain, or even exceed it. The extreme early mutants, on the other hand, decrease yield considerably. Most of the modifying early mutants tested are equal or slightly inferior to the mother variety Sv Bonus in respect to yield capacity, and they surpass the variety Ws Herta, which was used as standard variety in all the official Swedish yield tests. When the five drastic early mutants are tested, they are on an average 8 per cent inferior to their mother variety Sv Bonus and 3 per cent inferior to Ws Herta. Nevertheless, one of the extreme early mutants *early* 8, in the *mat-a* locus, (assigned with the registration number "Sv 04080") immediately attracted a good deal of interest and has been extensively tested in yield trials in various parts of Sweden. Soon it was found to maintain its relative earliness in the whole of Sweden, being characterized as "a new type" of two-row strain in the Swedish barley assortment. It is remarkable that a mutant, arisen from a South-Swedish variety, can do well even in very northerly provinces. In South Sweden, Sv 04080 is inferior in production, and in Central Sweden this inferiority is less marked. In North Sweden Sv 04080 is equal or even superior to the compared varieties, which mostly are early six-rowed.

Compared to the six-row varieties Edda II and Åsa, the two-rowed early 8 was superior in yield.

In 1960, this mutant was approved as a commercial Swedish variety under the name of *Mari* barley (from Latin: *ma*tura = early and *ri*gidus = stiff) and was thought to replace the early six-row varieties (Hagberg, 1961; Gustafsson et al., 1971). It was released on the market two years later (Hagberg and Persson, 1964). *Mari* barley has become a wide-spread variety, and it is to-day still grown in Iceland as the only barley variety which consistently gives good economic yields (Sigurbjörnsson, 1976). Mari certainly has some negative sides also. It is characterized by a certain mildew susceptibility. Some years later, a line was isolated after the crossing of Mari with the primitive variety 'Monte Cristo', with two subsequent back-crossings to Mari. This left the background genotype of Mari essentially intact, while introducing from Monte Cristo complete resistance to all known and tested mildew races occurring in Sweden during the 60s. Also the yield of this line showed to be superior to Mari barley. This line was approved as a commercial variety under the name of *Mona* barley in 1970. Mona is somewhat later than Mari (one or two days), it has longer straw, better lodging resistance and has better resistance to straw breakage than Mari (Gustafsson et al., 1971).

Praematurum mutants and day-length reactions

During the 60s, the phytotron of the Royal College of Forestry in Stockholm, Sweden was built and inaugurated. During the first years of operation, general studies relating to cultivation methods of different plant species were undertaken. The first experiments covered, among other crop species, also the barley varieties Sv Bonus and its mutant varieties Sv Pallas and Sv Mari. For a couple of years several experiments were carried out under different photoperiod conditions. The climate conditions consisted of 24, 16, and 8 hours of light from Sylvania lamps giving a light intensity of 20 000 lux, with different temperatures during the light and dark conditions and with a relative air humidity between 75 and 80 per cent (Dormling et al., 1966).

The two barley varieties, Bonus and Mari, were strikingly different regarding growth and spike formation. The most important results from the above-mentioned experiments will be described here.

24 hours of continuous light**.** Under extreme long-day conditions and at high temperatures (25^{o}C) the number of heading spikes is higher in Bonus barley than in Mari. At lower temperatures (15 - 20^{o}C and 10 -15^{o}C, respectively) Mari gains more and more in spike number, relatively seen, and may even surpass its mother variety. Bonus has a good seed set at low and medium temperatures. Also under this light condition Mari is more flexible. The superiority is especially noticeable at high temperatures (25^{o}C). On the whole, Mari performs much more efficiently than Bonus.

16 hours of light**.** At high temperatures Bonus develops a low number of spikes per plant, Mari still heads well under 16 hours light, and does not show any special preference to either high, medium or low temperature ranges. Mari is definitely superior. Bonus shows full fertility at the low temperature, while Mari shows good fertility at the low and medium temperatures.

8 hours of light**.** Bonus does not form a single heading tiller, it remains purely in the vegetative stage. It behaves as a long-day plant, with its light optimum somewhere between 16 and 24 hours. Mari heads well, but at high temperatures tillering is decreased. Fertility is almost complete at the medium and low temperatures. Mari also develops better generatively at low than at high temperatures.

Other properties of importance are culm and spike lengths as well as the internode number. (1) *At 24 hours of continuous light,* culm lengths are greater in Bonus than in Mari; at low temperatures the culm lengths are greater for both of the varieties. However, the spikes are larger in Mari than in Bonus, independently of temperature conditions. (2) *At 16 hours of light*, Mari is superior in culm and spike length, moreover Mari is less variable than Bonus. The internode number is higher in both varieties, and even somewhat higher in Bonus than in Mari. In this respect, Mari is more stable. (3) *At 8 hours of light* culm length and spikes are greater in Mari under all temperature conditions (Bonus never heads under this light condition).

Concerning the number of internodes, Mari has a small variation in internode

numbers at all three light conditions, but there are differences under various temperature conditions. High temperatures give a low number of internodes in both varieties. Bonus seems to be more sensitive to the very low temperatures, the number of internodes is decreased, while Mari has an increased internode number even at the lowest temperature.

Under extreme long day length conditions the time differences in heading between Mari and Bonus amount to about one week, whereas under conditions of moderate day length the heading differences reach values of three or more weeks (Dormling et al., 1966).

In summary, Mari, a mutant of the *mat-a* locus, has a special property definitely distinguishing it from the Bonus parent, namely a profound change in photo- and thermoperiod reaction, making it heading and seed fertile also at 8 hours of daylight. It is denoted as "photoperiod insensitive" (or short-day tolerant), heading and forming ample amounts of seeds at short-day conditions.

In the following years several other mutants, especially in the loci *mat-a*, *mat-b* and *mat-c*, were examined in phytotron cultivations for photoperiod reactions. All of them show rather drastic earliness in field cultivation. These loci have special phenotypical characteristics. Compared with locus *mat-a*, locus *b*-mutants are generally more productive in the field, but less lodging resistant, partially owing to a different culm and internode structure. Locus *c*-mutants differ strikingly in morphological respects, especially with regard to spike length and spikelet number (Dormling and Gustafsson, 1969; Lundqvist, unpublished).

The results from these extended experiments showed the same photoperiod insensitivity for the tested alleles from locus *mat-a* as for Mari. The locus *b*- and *c*-mutants are photoperiod sensitive, and they behave similar in this respect to Bonus. They produce some tillers at low temperature, but none or almost none at medium temperatures (Dormling and Gustafsson, 1969).

For many years several hundred *praematurum* mutants originating from different parent varieties were isolated. A complete phytotron test would have been too space-consuming and too expensive. Therefore, a simple manual darkening arrangement, using a special black plastic tissue, was installed in ordinary greenhouse rooms, permitting natural light lasting for 8 hours (the normal working-

time for one day and thereby lowering the costs of the staff). 8 hours is an extreme photoperiod change and gives very drastic differences. No special temperature control was installed. Measurements of the greenhouse conditions of day and night temperatures were automatically carried out (Gustafsson and Lundqvist, 1976 [paper XII]). This simple type of arrangement served quite adaquately for identifying short-day neutral mutants.

During 1974 and 1975 different commercial varieties (in several replications) and 59 different more or less drastic early mutants were tested for their short-day reactions using the arrangement described previously. At that time the genetical analysis of these mutants was far from completed. It was possible to distinguish three genotype categories under extreme short-day conditions of 8 hours light (Gustafsson and Lundqvist, 1976 [paper XII]):

1. Genotypes with complete and early heading and good seed set.

2. Genotypes with incomplete and late heading and seed set.

3. Genotypes that never head, remaining in a purely vegetative stage, often luxurious. Consequently, they never set seed.

In 1974 about 500 early mutants of the Svalöf collection were grown in the field at Ciano, Ciudad Obregon, the winter experimental station of CIMMYT, Mexico, under the short-day conditions prevailing there. Both Mari and Mona and other mutants of the *mat-a* locus became early-heading at Ciano. By contrast, mutants of loci *mat-b* and *mat-c* and others displayed incomplete heading or headed up to three weeks later than the *mat-a* mutants. The agreement between the greenhouse cultivations in Sweden and the Mexico results is remarkable.

Investigations in later years showed that extreme tolerance to short-day treatment is displayed by 59 among the 62 *mat-a* mutants. Data for 3 of them have been insufficient for a definite decision. In respect of the mutants in *mat-c* and *mat-e*, they are characterized by delayed heading and, thus, less pronounced short-day neutrality. The mutants of all the other loci *mat-b*, *mat-d*, *mat-f*, *mat-g*, *mat-h* and *mat-i* are long-day adapted like the parents Bonus, Foma and Kristina (Gustafsson et al., 1982; Lundqvist, 1991, and unpublished).

No definite instance of a short-day tolerant locus-*b* mutant was found, although all the mutants of this locus are on an average as early in field tests as are the locus-

a and -*c* mutants. All of them are concentrated to group 3. The locus-*c* mutants show a varied reaction, 2 of them with slight or delayed heading and 2 others with no heading at all. Also among the locus-*a* mutants there are differences in respect to short-day tolerance.

Other properties of the praematurum mutants, especially of the *mat-a* locus

Concerning the mutagenic treatments applied, there is some concentration of short-day adapted mutants under sulfonate treatments, whereas the long-day adapted cases seem preferably to accumulate when ethylene imine is applied. Recent observations indicate that sodium azide is less efficient in producing mutants in locus *mat-a* (Lundqvist, 1991 and unpublished).

It has been known for several years that one of the early genes is located on chromosome 5, but it has not been explored whether this gene is photoperiod insensitive (Smith, 1951). During the 60s, an allelism test between *mat-a*^{8} (Mari) and the Japanese variety *Kinai 5* showed homozygosity for earliness in an F_1 generation (Favret and Frecha, 1967). In Kinai 5, the early gene was found as a single recessive early gene with photoperiod neutrality and with the yellow-green seedlings at an early stage of germination. The gene symbol *ea*$_k$ was allotted to this early gene (Takahashi and Yasuda, 1971). Linkage studies showed that *ea*$_k$ is situated close to *B* (black lemma and pericarp), *trd* (third outer glume) and *f-7* (chlorina-type) at the end of the long arm of chromosome 5 (Takahashi and Yasuda, 1971; Jensen, 1981). In a cross between the Swedish *mat-a*^{8} (Mari) gene and Kinai 5, all the F_2 plants proved, without exception, to be of the yellow-green seedling type. In later experiments F_1 plants showed early heading, and it could be confirmed that the *mat-a* gene is allelic to *ea*$_k$ (Takahashi and Yasuda, 1971; Yasuda, 1977).

The Cimmyt organization in Mexico incorporated the Mari and Mona gene in their breeding programme. Several recombinants were tested, and a special Mona strain was tested at several Mexican stations, on Cyprus, in Tunisia, South Korea, Iran, Iraq, Jordan, Turkey, Peru, and Spain. Apart from its earliness, it had a high yielding ability, was superior in lodging resistance and was satisfactory in disease resistance.

Owing to these properties and the wide photoperiod adaptability, both Mari and Mona are cultivated over large areas. They have also been used in the Swedish recombination breeding work with great advantage. Especially the Mari mutant turned out to be the best recombiner in all diverse exterior conditions possible. Six different varieties with Mari as recombiner were released in Sweden, which will be demonstrated in more detail in chapter C. Nothing is known about the remaining *a*-mutant cases, apart from erectoides 16, regarding recombining ability, and few adequate data on yield performance have been recorded.

Two other alleles of the *mat-a* locus, *mat-a*^{11} and *mat-a*^{12}, have successfully been used in plant breeding in Great Britain. British plant breeders have found a favourable ear emergence and recommended the mutant *mat-a*^{12} as a good source of new genetic material for improved earliness in barley (Gustafsson and Lundqvist, 1976 [paper XII]; Gustafsson et al., 1982).

C. BREEDING ASPECTS

Gene mutations enhance variation in natural populations. This is a process by which novel variation is formed and new characters are found. Mutation is a spontaneous phenomenon and occurs at a certain rate for each locus in each organism. There is also a mutation pressure on each generation, and there is no reason to believe that the mutation rate is different for wild populations than for evolution under domestication.

Mutations constitute the raw material for evolution. An inferior phenotype is mostly due to a mutant gene. However, in a new environment - outer or genetic - the mutant gene can be of advantage. Here is certainly the key to biological evolution: Genes mutate at a low rate to new genes, these genes become recombined in various ways, and new recombinants are subject to natural selection. Without this interaction between mutation, recombination, and selection, life would sooner or later have disappeared through the changing fortunes of our earth.

The spreading or disappearance of genes relate to past and present conditions of climates and soils and to interactions with other organisms. Tolerant demands from an environment with a wide range of variation open the door to a wide range of genic variation. Severe demands from the environment may in marginal areas imply a hard selection. Also in cultivated plants, spontaneous mutations will have a direct as well as an indirect importance. In combination breeding, the breeder unconsciously often exploits spontaneous mutations, particularly the ones with minor effects. A new successful variety may in many cases be due to mutation instead of, or in conjunction with, recombination.

Plants under domestication may face a more or less major change of ecological conditions. A radically new environment with new demands on adaptations may arise. It is exactly in this situation that *induced* mutations give the plant breeder much larger possibilities with a broad spectrum of new genetic variation when trying to create desired agro-ecotypes. Plant breeders have to develop crops with increased yield and high quality adapting to a variety of soils, climate, and daylength conditions. These crops should also be resistant to pests and diseases and able to grow with reduced fertilizer and pesticide inputs.

Induced mutations applied

Since work with artificial induction of mutations began, it was evident that mutation programmes should be regularly included in breeding programmes of crop plants. The practical application of mutation research in plant breeding has been the most important stimulus. It was also stated that the mutation method, in accordance with Muller's view but in contrast to Stadler's arguments, is a method which under defined conditions, depending on the particular crop plant, the characters to be improved, and the available mass screening techniques, may turn out not only useful but in special cases will be the most appropriate or the only method possible (Muller, 1927, 1928; Stadler, 1930; Gustafsson et al., 1971).

Barley has been used as a model crop in mutation research programmes in Sweden. There are several reasons for this: Barley is a diploid self-fertilizer, it is rather easy to handle, gives a sufficently large progeny from a single plant, and outcrosses only rarely. Moreover, a great amount of variation can easily be induced by various mutagens.

It was shown already in the fifties and sixties that the barley breeding work at Svalöf should be used as an example how mutation breeding can be employed in a crop improvement programme. On one hand, the direct use of induced mutations has been tried. In this attempt, the main interest was focussed on macromutations. Simple or rather complex characters such as strength of the straw, earliness, disease resistance and others are of importance. In this technique the great advantage is that the new genes are produced in a genotype already selected for high yield and for important agronomic and qualitative characters. However, on the other hand the indirect use of induced mutations has been important. In this case breeding work tried to change modifying systems by crossing mutants with various well established varieties and selecting the best recombinants homozygous for the mutations. In the Swedish breeding work, this use of the macromutations in conventional crossbreeding programmes has proved to be more successful than recurrent mutagenic treatment and selection for micromutations (Hagberg et al., 1963; Hagberg and Persson, 1964).

Mutant varieties in Swedish agriculture

The first official releases of induced mutant barley varieties in Sweden occurred in 1958 and 1960, as selections from carefully tested X-ray material. However, during these years, it was not the purpose primarily to breed new barley varieties in the mutation work, but to analyse the mutation process itself and to study various properties and physiological reactions of induced mutations.

Through the joint work with several Swedish barley breeders (A. Hagberg, G. Persson, K. Wiklund) and other scientists at Svalöf, a rather large number of mutant varieties of two-row barley were registered as originals and commercially released (Gustafsson, 1969; Gustafsson et al., 1971). Some of them have been of distinct importance to Swedish barley cultivation. Two of these varieties, 'Pallas', a strawstiff, lodging resistant and high-yielding erectoides mutant, and 'Mari', an extremely early, photoperiod insensitive mutant barley, were produced directly by X-irradiation.

All the other varieties listed in the Table 6 derive from crossings, where the original breeding material was based on three primary high-yielding Swedish X-ray mutant varieties: 'Pallas', 'Sv 44/3', both with extreme lodging resistance, and 'Mari', which is extremely early and short-day tolerant. These series of varieties were tested and adapted agriculturally to various parts of Scandinavia and have also been grown in other parts of Europe. The aim of this work was to demonstrate that original mutant materials can be used successfully in a breeding programme, when crossed and backcrossed in a planned way, and the offspring selected and tested repeatedly in suitable regions and soils.

The Pallas gene *(ert-k*^{32}*)* used in combination breeding

For further improvement of lodging resistance as one partner the Weibullsholm variety 'Herta' was used as a partner in combination work. A selected strain out of the cross Pallas $\times$ Herta, was characterized by its superior stem quality - it was very strawstiff from heading until maturity, and also possessed a high resistance to straw breakage at and after maturity. This line was approved as a commercial variety in 1967 under the name of 'Hellas', and was released to farmers in 1969. Besides the superior characters mentioned above, Hellas possesses a high sprouting resistance at harvest time, as determined in the field, in fog chambers and by α-amylase tests.

The flowers hardly open at anthesis, like Pallas, and Hellas is therefore rarely infected by smut. The prominent agronomic characters of Pallas have been further accentuated by gene recombination. The erectoid appearance still prevails.

Examples of mutants in complex crosses, where Pallas was used as basic breeding material, are as follows: Pallas was crossed and backcrossed with the primitive 'Long Glumes' barley which is an important source of complex resistance to powdery mildew. This strain was approved under the name of 'Visir' as a commercial variety for export in 1970. It had a fairly good lodging resistance, the time of maturity was slightly shorter, and it gave less field sprouting than the standards. Visir was recommended for cultivation in high-fertile regions when there was risk of heavy mildew attack.

The variety 'Visir' was soon followed by several new varieties, maintaining many of the original traits of Bonus - Pallas features (genes) while containing valuable agronomic characteristics from Herta and other varieties with genes for resistance to mildew and nematodes. Varieties such as 'Senat' and 'Jenny' deserve to be mentioned. The forementioned steps of improvement do not imply that development has reached an end, rather a passage *en route*. The varieties have taken 12 years to develop from the approval of Pallas and 23 years from the isolation of erectoides 32 (*ert-k*^{32}) (Table 6).

The early Mari (*mat-a*^{8}) gene used in combination breeding

A parallel procedure was initiated with regard to earliness, and it could be soon demonstrated that this early gene, *mat-a*^{8}, can be used successfully in crossing and backcrossing programmes in a planned way. As one partner the Norwegian variety 'Domen', which is characterized by its high malting quality and carrying an allele of the gene locus *ert-k*, was used in the combination work. The cross Domen × Mari turned out to be of special interest. The selected line was superior in yield tests in comparison with the standard varieties, it was widely superior in lodging resistance, and had a higher resistance to straw breakage. But it was seven to ten days later than Mari; to some extent it has maintained the straw properties and general appearance of Mari. This line was approved as a commercial variety (for export) in 1969 under the name of 'Kristina'. Of special importance in this variety is its high

enzymatic potential, leading to a fast germination of economic consequence for malting and brewing. Kristina has a high α-amylase activity, diastatic power and Kolbach index. This high enzymatic activity results in a certain inferior property. The mature crop rapidly germinates under wet and rainy harvest conditions and this hampers the cultivation in humid regions. In addition, the grain flour possesses a low grade of viscosity, depending on the breakdown of glukane. It is therefore better suited for broiler feeding than other Scandinavian varieties.

Another example of the utilization of mutants is the introduction of mildew resistance into the Mari genotype (cf. figure 22). The cross of Mari with the primitive variety 'Monte Cristo', with two subsequent back-crossings to Mari, left the background genotype of Mari intact and introduced complete resistance to all known and tested mildew races occurring in Sweden during the 60s. This cross was approved as a commercial variety in 1970 under the name of 'Mona'. The yield showed to be superior to Mari, Mona is one or two days later than Mari, has a better lodging resistance, has better resistance to straw breakage and has a longer straw than Mari. The steps mentioned to improvement of 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, quite a timesaving compared to the use of Pallas in recombination breeding.

As seen from Table 6, other varieties derived from mutant crosses and complex mutant crosses where the genes of Pallas and Mari have been introduced, have been approved over the years. The varieties 'Eva' and 'Salve' were released in the years 1973 and 1974, and both of them have a resistance to lodging which is introduced from Mari, and a high yielding capacity. The variety 'Lina' is a complex mutant outcross with the Mari gene involved and has been introduced in 1982. It is today still grown in Scandinavia. Lina has a high yielding capacity, has a very good lodging resistance, and has mildew resistance incorporated from the Indian primitive barley variety Multan with the resistance gene *Ml-a7*.

Table 6. Survey of induced barley mutants
and their derivatives, approved and released at Svalöf
(after Gustafsson, 1986)

Parent strains	Primary mutant varieties
Gull	44/3 (extreme lodging resistance)
Bonus	Pallas (*ert-k*^{32}), approved 1958
Bonus	Mari (*mat-a*^{8}), approved 1960

Mutant crosses	Varieties approved
Herta × Pallas	Hellas 1967
Domen × Mari	Kristina 1969
Mari × Monte Cristo	Mona 1970
Birgitta × Mari	Eva and Salve 1973, 1974
44/3 × Birgitta	Gunilla 1970
(Birgitta × Mari) × Gunilla	Pernilla 1979

Complex mutant crosses	Varieties approved
(Pallas × Triple awn lemma) × Pallas^{bc}	Visir 1970
(Triple awn lemma × Pallas^{3}) × Hellas	Senat 1974
Å 61657 × (Mari^{5} × Triple awn lemma)	Troja, withdrawn 1981
Kristina × [Hellas^{2} × (Pallas^{5} × Rupee)]	Jenny 1980
Lofa × [Å 6564 × (Mari^{bc} × Multan)]	Lina 1982

Figure 22. Mildew resistant mutants used in breeding work to the right compared with susceptible material to the left.

The lodging resistant mutant 44/3 in combination breeding

The mutant 44/3 was isolated in 'Gull' barley already in 1939 and was described as very bushy and extremely waxy at seedling stage. But the most pronounced feature was its extreme straw-strength. This character is unfortunately counteracted by a low 1000-grain weight and an inferior malting quality (Gustafsson, 1947).

In 1970, the variety 'Gunilla' was officially approved as a commercial variety. It is an outcome of a complicated cross starting with the cross 'Birgitta' × Å 56888. This latter component was constructed by a cross of ('Opal' × 'Vega') and the blue, lodging-resistant mutant 44/3. After several backcrosses in order to obtain the

desired two-row character (Vega is an early six-row type), the strain was once more backcrossed to the blue mutant 44/3 and led to the selected line Å 56888. The variety Birgitta contributed in the final production of Gunilla, and the small grain size and weight were overcome. Gunilla is characterized by very high productivity, favourable earliness and outstanding lodging resistance. It is especially adapted for the North-Swedish area of farming (Gustafsson et al., 1971).

'Pernilla' is another variety where the mutant 44/3 has been used. This variety is a result of the cross (Birgitta × Mari) × Gunilla. Early maturity is incorporated from Gunilla, and good straw characters are inherited from Birgitta. Of special importance in Pernilla is its resistance to late green tillering, which can be of importance in those parts of Sweden with severe droughts in the early summer. It also possesses a high resistance to straw breakage at and after maturity. Pernilla was released on the market in 1979 and is grown in the southern and central parts of Sweden in particular.

Introduction of mutations in combination with well-planned crossing work and in the hands of skilful breeders, has given a succesive positive trend, in fact as positive and progressive as any other method of plant breeding.

The successful strains of barley as well as other crop plants which have resulted from mutation breeding, attest to the correctness of the view first deduced by Åke Gustafsson. The induced mutations are not different from spontaneous ones, and all mutation breeding does is to speed up natural evolution of crop plants by a factor of between one hundred and one thousand.

Induced mutations: Is their practical value over- or underestimated?

In one of his conclusions, Stadler (1930) pointed out that favourable breeding types and characters with a greater potential will be discovered in collections of natural strains and varieties rather than in progenies of X-irradiated plants. But in direct opposition, different views were advanced by several researchers (see Gustafsson, 1986 and references therein).

Favourable mutations, in no way associated with unfavourable side effects, do occur. Admittedly, favourable mutations are rare, and most conspicuous mutations result in decrease of viability or may be deleterious in other ways. The same is true with spontaneous mutations. Induced mutations will be an important future tool in progressive plant breeding. This will be even more so when the chemistry of the gene has been studied more thoroughly. Genetic instruments of artificial selection will increase the power and capacity of the plant breeder. It seems rather strange that also today there is a certain negative attitude towards the use of mutations in plant breeding or in most experiments concerning general evolutionary theory. Such negative ideas are often connected with the view that mutationists ignore the natural source of genetic variability and oppose the breeding value of primitive biotype collections (summarized from Åke Gustafsson's last paper, 1986).

At present natural sources of variation are being replaced by artificial mutations. The value of induced mutations is increasing, to the same degree as natural sources of variation unfortunately disappear. We also know that induced variation may in the future fill and replace the loss or lack of natural variability, or may even extend the limits of variation.

The Swedish collection of barley mutant genes is now incorporated into the Nordic Gene Bank, and about 10 000 different accessions are being documented in databases. This collection, with a large number of different types of mutants induced by different types of mutagenic treatments, is one example of the large diversity existing in barley. In other parts of the world large collections of barley have also been gathered, and with a high degree of duplication amongst them. Thus, in several meetings it has been discussed to introduce the core concept in barley, a network approach for conservation and utilization of genetic resources. It is important to co-

ordinate the different collections to make them usefully available for barley researchers and plant breeders.

Useful mutations in barley include a wide range of economically important characters: disease resistance, low- and high-temperature tolerance, photo- and thermo-period adaptation, earliness, grain weight and -size, protein content, improved amino acid compostion, good brewing properties, and improved straw morphology and anatomy in relation to superior lodging resistance.

No doubt, the collections of barley mutants, with their well defined origins of mutant genes, will form a major input for future gene mapping and be immensely valuable for molecular genetical analyses of cloned mutant genes. Mutation research, now well established, will provide a detailed understanding of the genetic composition of the barley genome. Increased knowledge of chromosomes and genes combined with studies of DNA constitution and amino acid composition of proteins will lead to continued progress in barley breeding.

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B. GENETICAL ANALYSES OF MUTANT GROUPS

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