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SEMI-DWARF CEREAL MUTANTS AND THEIR USE IN CROSS-BREEDING III

PROCEEDINGS OF THE FINAL RESEARCH CO-ORDINATION MEETING
ON EVALUATION OF SEMI-DWARF CEREAL MUTANTS
FOR CROSS-BREEDING
ORGANIZED BY THE
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FOREWORD

A Co-ordinated Research Programme on the "Evaluation of semi-dwarf mutants as cross-breeding material in cereals" was initiated in 1980, with the main objective to provide cereal breeders with new, alternative sources of genes for semi-dwarf, lodging resistant plant types. In the early 50's it became obvious that lodging, caused by long, weak straw and leading to a low response to nitrogen fertilization, was the main reason which limited the yield potential of cereals, particularly in good agro-ecological conditions. This problem has increased drastically with the use of higher doses of fertilizer. Introduction of genes responsible for short stature plant type from certain local forms to wheat and rice cultivars solved the problem and led to better utilization of water and nitrogen input. By transferring such genes new, intensive cultivars were developed - which initiated the agro-economical changes now described as the "Green Revolution".

Unfortunately, only a few semi-dwarfing gene sources, useful for cross breeding, were found in germplasm collections around the world. The very narrow genetic variation for this character has generated a risk of genetic vulnerability towards pests, diseases and other hazards. On the other hand, short stature forms of cereals are frequently selected after mutagenic treatment with the use of radiation or chemical mutagens. A number of modern semi-dwarf cereal varieties, directly developed from selected mutants, were released in various countries, but a long list of other short stature mutants induced in different laboratories needed evaluation for use in cross-breeding. It was the ultimate aim to identify and make available new sources of semi-dwarf plant types and in this way open possibilities for varietal improvement beyond the limits set by the presently used gene sources and by the same means reduce the genetic vulnerability.

The present publication includes papers presented at the the final Research Co-ordination Meeting. Papers presented by participants in the Co-ordinated Research Programme (see "Project Reports") demonstrate that these objectives were successfully achieved. As an additional result of this programme more improved genotypes of cereals with other desirable characters as earliness, better harvest index and improved plant architecture have become available for practical breeding.

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INTRODUCTORY PAPERS

BIOTECHNOLOGY AND MUTATION BREEDING

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Abstract

Mutation breeding has been successful. New biotechnological techniques such as in vitro mutagenesis, in vitro selection, and haploidy may assist in, or improve mutation breeding where applicable. In vitro mutagenesis will increase the specificity of the mutation process. In vitro selection and haploidy will improve mutation identification and selection capabilities. These techniques, their uses, and their limitations are discussed.

Mutation breeding has been unattractive to many plant breeders because many of the techniques for inducing and selecting useful mutations involve random mutations for qualitative traits coupled with large screens of the mutated plants and/or their progeny. Most breeders prefer to work with predictable variation by hybridizing parents with known genetic qualities. Mutation breeding has been suggested where there is little known variation for a trait of interest or where an otherwise excellent cultivar is missing a single trait that may be introduced by mutation. In the latter case, mutation breeding replaces backcrossing. The promise and successes of mutation breeding in seed-propagated crops have been recently reviewed by Konzak, et al (1984). Mutation breeding has provided both novel genes and alleles with previously known phenotypes, but in better adapted backgrounds.

The impact of biotechnology on mutation breeding will be significant if biotechnology can alleviate some of the constraints and limitations of mutation breeding. Three areas where biotechnology may improve mutation breeding are: (1) in vitro mutagenesis; (2) in vitro selection; and (3) the use of haploidy to aid in the identification of mutations.

The methods and applications of in vitro mutagenesis, for this paper narrowly defined as mutations of DNA outside of a cell or organism, have recently been comprehensively reviewed by Botstein and Shortle (1985). In this technique, a gene is cloned and then is mutated (usually on a small cloning vector in *Escherichia coli*) either by restructuring segments of DNA, localized random mutagenesis, or oligonucleotide-directed mutagensis. In the first type of mutagensis, the gene is usually mutated by systematically deleting portions of the DNA and then assaying the gene to see if it still functions. In this method the functional aspects of the gene can be delineated In the second type of mutagenesis, a small fragment of the cloned DNA is isolated, exposed to the mutagenic treatment, and then allowed to recombine with the intact, larger cloned gene. The value of this method is that only a relatively small portion of the DNA is exposed to the mutagenic treatment so that unwanted, secondary mutations in the DNA may be limited. In the third type of mutagenesis, the mutation of interest is synthesized *de novo* in as a single stranded DNA oligonucleotide and allowed to reanneal to a wild type gene or the oligonucleotide is synthesized *de novo* as double stranded DNA and then inserted into a larger DNA sequence containing the remaining parts of the gene of interest (both methods giving a precisely mutated gene).

The advantage of *in vitro* mutagenesis is that relatively small portions of the genome are exposed to the mutagen. The disadvantages are that (1) the DNA sequence for the gene of interest must be known; (2) the mutations will need to be assayed in an expression system (often not the crop in which the gene was identified) to determine their efficacy, which can be laborious, and, (3) to be useful for some applications in modifying the organism of interest, the *in vitro* mutation should be inserted to replace the wild type allele (Botstein and Shortle, 1985) If the desired mutation is recessive, then it will have to be inserted to replace the wild type allele or the wild type allele will have to be inactivated. A dominant or co-dominant mutation will be expressed regardless of where it is inserted and need not replace the wild type allele to have the desired phenotype.

In cereals, most genes of interest are unknown and very difficult to identify. Transposon mutagenesis where the transposon sequence is known has been used in corn to identify and clone genes (Fedoroff et al, 1983; Fedoroff et al, 1984; O'Reilly et al, 1985). Transposon mutagenesis is particularly useful when only the desired phenotype is known. Biochemical knowledge of the trait is not necessary as the transposon sequence can be used to probe for the gene of interest in lines having the desired phenotype due to transposon mutatagenesis Species with smaller genomes and low copy number genes (i.e *Arabidopsis thaliana*) may provide probes for useful genes in cereals (Meyerowitz and Pruitt, 1985). In cereals, where useful transformation systems are still being developed (Fromm et al, 1985), the mutant genes will have to be expressed in other host organisms, such as *E. coli*, yeast *(Saccharomyces cerevisiae)*, dicots, and *Xenopus laevis* (Smith et al, 1985; Taylor et al, 1985). Development of technology for replacing the wild type allele with a mutant allele will have to wait for effective transformation technology. However, even with the disadvantages and technical hurdles described above, having a better understanding of the gene and the type of mutation needed, as well as the resultant protein, may lead to more efficient whole plant or tissue mutation strategies and better phenotypic screens. For example, if it is known that the desired mutation is a single amino acid change in the protein which is coded by a single nucleotide change from the wildtype DNA sequence, it should be possible to better choose the mutagen

The value of *in vitro* selection using callus, suspension, or protoplast cultures is in its ability to select variants from large numbers of potentially totipotent cells in small culture flasks with relatively uniform conditions. Its efficiency is derived from its ability to select cells that then are able to regenerate plants rather than select at the whole plant level.

For example, 5,000,000 plant cells can be challenged with a toxic chemical such as a herbicide in a single flask. It would require many hectares of land to grow five million corn or tobacco plants and it would be impossible to uniformly spray them with a chemical. In some ways in vitro selection can be considered an extension of the seed or embryo selective assays for useful mutations. Additionally, the culturing techniques are often mutagenic and by themselves provide sufficient variation for the in vitro selection to be effective (Larkin and Scowcroft, 1981). Mutagens also can be added to the medium to further enhance the genetic variation available for selection (for example, Chaleff and Ray, 1984). As such, in vitro selection of cultured cells is best viewed as a selection method for mutations.

Meredith (1984) reviewed the subject of selecting better crops from cultured cells and has described the challenges, opportunities, and pitfalls of these techniques. The main challenges are to reduce complex agricultural problems to a meaningful and relevant selection medium, have the cellular phenotype expressed in culture so it can be selected, and to have the selected cellular phenotype translate to a useful whole plant phenotype. Obviously, these limitations will greatly restrict the type of mutations that can be selected, but as Meredith suggested, phenotypes that involve cellular processes should be more amenable to in vitro selection. As salinity, mineral stresses, herbicide tolerances, and disease toxins all involve cellular processes, they should be amenable to in vitro selection (Meredith, 1984; Chaleff and Ray, 1984).

The last area of biotechnology to be discussed as a possible method to improve mutation breeding is haploidy. Haploidy and doubled haploidy have been reviewed by many authors (Collins and Genovesi, 1982; Maheshwari et al, 1982; Baenziger et al, 1984, Choo et al, 1985). The significance it has to mutation breeding is two-fold. Firstly, a haploid contains only the gametic number of chromosomes so that both dominant and recessive gene mutations will be expressed and can be identified in the mutagenized haploid cell line or plant. The haploid plant can then have its chromosome number doubled and the gene will be homozygous in the doubled haploid plant. For example, anthers from tobacco have been treated with mutagens to produce highly variable, haploid plants. These haploid plants were then successfully selected for high photosynthetic efficiency prior to chromosome doubling (Medrano and Primo-Millo, 1985). In a mutagenized diploid plant, a recessive muation would be expressed only if a double mutation or a mutation and a deletion occurred (unlikely events). Identification of recessive mutations in diploid crops usually requires progeny selection in segregating generations. Similarly a dominant mutation will normally be heterozygous in the mutagenized plant which will require selfing to obtain homozygosity. Secondly, because dominant and recessive mutations are expressed in the haploid plant and the resulting doubled haploids, any other mutations inadvertently produced in addition to the mutation of interest in the selected lines will be expressed. Hence, it should be possible to select mutant lines having only the phenotype of interest from those lines having the phenotype of interest and secondary mutations that may be deleterious. In a mutagenized diploid line, many of the secondary mutations would be recessive, hence masked in many of the selected lines. Selfing the lines with desired phenotype would be necessary to discover these secondary mutations. Also, deleterious recessive alleles are lost slowly from a population.

Combining *in vitro* selection for screening and haploidy for expressing mutations, will provide powerful methods for mutation breeding. However, there are a number of drawbacks to using haploid cell cultures in cereals. Firstly, many of the cereals do not have good cell culture technologies. For example, anther culture in wheat is genotype specific (Bullock et al, 1982; Lazar et al, 1984). While haploid plants of cereals can be obtained through a number of other methods and could be used for explant tissue sources, the cell cultures from these plant tissue are poorly developed. Secondly, haploid cultures often undergo spontaneous doubling (Chaleff and Ray, 1984; Kudirka et al, 1983) thus defeating the purpose of using "haploid" cultures.

In this brief discussion, three technologies have been discussed that will be useful in mutation breeding. A question that may be asked, is will they become a dominant technology or replace some of the existing mutation methods. In general, the methods described here will continue to have major limitations and will be used to solve specific problems in cereals until more is understood about cereal physiology, biochemistry, genome structure, tissue culture, and transformation. Though these techniques are fledgling, significant progress has been made with biochemical selection. However, the more classical techniques described by Konzak et al (1984) have broader usage and will remain for the near future the more widely used and generally more successful mutation breeding tools.

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ACTIVITY OF THE INSTITUTE OF EXPERIMENTAL RESEARCH FOR CEREAL CROPS, ROME

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Abstract

The achievements of the Instituto Sperimentale per la Cerealicoltura are briefly summarized in the presented paper. Between them the semi dwarf wheat varieties bred by this Institute like Ardito, Villa Glori, Mentana, Damiano, Balilla and for durum species Senatore Cappelli. The present main activity of the Institute is devoted to the design of experiments, applied genetics, agronomic techniques and technology of cereal products.

This institute was named the Institute of Genetics of Cereals by its founder Nazareno Strampelli, and in 1968 the name was changed to the above. In fact Nazareno Strampelli realized the first Green Revolution in the 1920's, this means after two decades of his first cross breeding activities. He put to work Mendel's laws exactly at the time when they were rediscovered by using recombination for breeding purposes. Indeed, he crossed the wheat varieties Noé and Rieti in order to combine the resistance to lodging of the former with the resistance to rust of the later. But the reminiscence of the genius of Strampelli is specifically appropriate in the context of this meeting in connection with his use of the short Japanese variety Akagomughi, very early, even if of poor agronomic value, under our conditions. Strampelli in 1906 had crossed the Italian variety Rieti with Dutch Wilhelmine Tarwe, obtaining a type with high yielding potential, but very late. In 1913 this was crossed with Akagomughi and from the segregating generation, in 1920, the famous Ardito variety was obtained. This variety matured 15-20 days earlier than the other contemporary varieties and was resistant to lodging because of its height, only 80 cm. By its high yield Ardito gave a first, clear demonstration that it was not an alternative to earliness. The spreading of Ardito into Italian agriculture induced also several indirect benefits. A harvest of wheat 2-3 weeks earlier than usual allowed a reasonably advantageous second crop on the same field. This allowed people to escape the advancing worst phase of mosquito populations in the areas afflicted with malaria. The resistance to lodging allowed farmers to use more fertilizer and consequently additional yield was gained as a result of this improvement.

In a few years other successful Stampelli varieties invaded Italy. There were well known early varieties Villa Glori, Mentana, Damiano and the earliest, Balilla. Among the late types was spread Virgilio and for durum species Senatore Cappelli (still largely cultivated and still one of the best for quality). The last of Strampelli's achievements was the variety S. Pastore, a well known bread wheat very successful in Italy and many other countries for more than 40 years.

The Istituto Sperimentale per la Cerealicoltura carries out studies and research dealing with cereal genetics, breeding of wheat, maize, rice and minor cereals as well as with agronomic techniques of cultivation of these cereals. This Experimental Institute for Cereal Research succeeds with its activity and to a large extent with its properties in general, to institutions working in the field of cereal research, especially in aspects of genetics, agronomy and technology, such as The National Institute of Genetics for Cereal Research "N. Strampelli", Rome; the Experimental Station for Wheat Research, Rieti; the Plant Breeding Institute, Bologna; the Experimental Station for Maize Research, Bergamo; and the Experimental Station for Rice Research and Irrigated Crops, Vercelli, where great breeders such as Strampelli, Todaro, Zapparoli, Novelli and Bonvicini have been active in the field of plant improvement. The Institute consists of four central and seven peripheral sections plus the Morando Bolognini Foundation. The central sections are devoted to: design of experiments; applied genetics; agronomic techniques; and technology of cereal products. The peripheral sections are specialized as follows: Vercelli, in genetic improvement and agronomic techniques of rice; S. Angelo Lodigiano (Milan), genetic improvement of wheat and rye; Bergamo, genetic improvement and agronomic techniques of maize and sorghum; Badia Polesine (Rovigo) and Fiorenzuola d'Arda (Piacenza), genetic improvement and agronomic techniques of cereal crops; Foggia, genetic improvement and agronomic techniques of wheat, barley, sorghum, oat and maize; Catania, genetic improvement and agronomic techniques of wheat, barley and sorghum.

Bread wheat is considered the most important cereal crop in Italy, and it has held this position for the last centuries. During the last few years its area has been surpassed by durum wheat. At present the bread wheat area is about 1.400.000 h, being half of the area on which bread wheat was grown a few decades ago. The reduction is the consequence of the relatively scarce economic competitiveness of the yield and quality of Italian bread wheat: the national yield is below 4 t/ha, this means below the level of average yield of several European countries. The bread making quality is not the best, and Italy is forced to import bread wheat from North America. For this reason, the Institute chose two main breeding objectives: to improve the quality in a relatively short time, which appears the most realistic goal. Even small improvements of quality can solve the problem, considering that for practical purposes a mixture of flours is utilized. It appears that it will be difficult to obtain varieties with high yield in Italy, similar to the level in Central Europe and England, because of the unfavourable growing conditions in most areas. The very high temperature in June, together with the usual lack of rain, accounts for the very early ending of the wheat plant cycle. For this reason, since Strampelli's time, the breeding for earliness is the second important objective of our Institute. The irrigation of cereals does not appear promising under our conditions if the absolute cost of the operation is considered. The use of water for other crops is more profitable. Under these circumstances we believe that hybrid plants can be another possibility for our conditions. The physiology, resistance to disease as well as to other stresses are the main topics of experimental activity to cope with the difficult task presented.

As mentioned, during the last few years, durum wheat has become the largest cultivated crop of Italy, with an area of about 1.600,000 ha. This crop is significantly favoured by European Community policy. At present the greatest producers are Italy and Greece. However, the yield is lower than bread wheat and the national average is higher than 2.5 t/ha only in very favourable years. Great handicaps for better results are: poor rainfall, concentrated in the winter months; dry winds in May and June that cause early maturing of the plant associated with severe shrivelling of kernels; the yellow berry; sensitivity to rusts, mildew and *Fusarium*. During the past two decades *durum* wheat has greatly increased total production thanks to new higher yielding varieties such as those bred by ENEA. Other breeding activities of this Institute, made possible the expansion of *durum* cultivation in Central and Northern Italy where new genotypes with cold resistance finally did well. As everyone knows, one of the most important destinations of *durum* grain in this country is pasta making with special emphasis on spagetti. The quality of Italian products in this sector is well known and the increase of pasta consumption worldwide is largely accredited to the successful export of Italian specialities. For this reason the improvement of *durum* wheat quality is imperative for Italian breeding and this Institute makes no exception. Even if some industries assert that with modern technology it is possible to obtain a good quality of pasta, the Institute stresses the importance of the quality improvement of *durum* grain for nutritional as well as for technological reasons. The situation will become more critical when several countries of Central Europe should begin or expand their *durum* area cultivation. In this respect it should be mentioned that in spite of the fact that Italy is exporter of *durum* wheat products, the import of *durum* grain is necessary not only in years of scarce production but also to meet external demands for products from Italy's specialized industry, considered one of the best in this specific sector.

Maize, also third in the acreage list for Italian cereals, with its 1,000,000 hectares, supplies the highest number of calories, as a consequence of its very high average yield of about 7 t/ha (not to mention on additional 350,000 ha cultivated as silage crop). In Nothern Italy there are provinces with yields of over 8 t/ha and farms that consider 10 t/ha as a normal result. However, the problems are numerous also under such circumstances. The cost of maize grain production under Italian conditions, with the reduced size of farm, artificial irrigation and chemical and mechanical input, is unbearable for yields below a given level. This is the reason for which the Institute co-ordinates a programme of co-operative trials with the best hybrids developed by the main seed companies. In this context the specific contribution of the Institute to maize improvement is mainly the breeding via improvement of hybrid components, and in Southern Italy, analysis of irrigation aspects. Nevertheless, the study of genetic basis of protein quality, breeding of valid opaque lines, chemical expression of glossy genotypes in epicuticular wax, and the analysis of controlling elements through genetic, in-vitro and molecular experiments, are other topics of the Institute's activities.

The fourth cereal of Italian agriculture is barley, the area and yield of which has grown continuously and significantly during the last 20 years. During this period the area of cultivation was doubled and total production was quadrupled in the same period. This has been caused mainly by the growing request for fodder barley. Such increase in barley production was chiefly possible by introducing high yielding varieties bred in central Europe. However, the spreading of similar genotypes on a large scale in several regions of North Italy was soon followed by parallel spreading of serious epidemics. The very poor lodging resistance of introduced varieties under Italian conditions was connected with these varieties as well. This situation has promoted a programme which has led to the first promising results in breeding varieties for Northern Italy.

New types of barley are also developed for Southern Italy where barley is also an important crop in many hilly areas. This activity was developed on the basis of studies on irrigation and drought stress problems which are carried out for this region.

In spite of the fact that rice is cultivated only on about 200,000 ha it is an important and interesting crop in Italy for several regions: Italy is by far the largest producer of rice in the European Community. More than 50% of the production is exported mainly to the Common Market countries. The cultivation is concentrated in a few provinces of North Italy, mainly, between the cities of Turin and Milan, but also in districts of the Po Valley near the Adriatic Sea and in Sardinia where the dry conditions favour a superb seed production. Because of the special climatic conditions provided by the Alps and Appenines, Po Valley, with its more than 45^{o} of North latitude is the largest area of rice cultivation in similar geographical locations. Mountain chains protect the rice cultivation area from critical transpiration and fall of temperature during the night. The problems with which we have to cope are various: earliness coupled with yield in connection with the specific pedoclimatic situation, resistance to diseases and lodging, pollution of irrigation water and completely mechanized monocultures. Under such conditions it should not be surprising to learn that although the yield is one of the highest in the world (5.5-6 t/ha of paddy crops) it has changed slightly as compared to other crops where the productivity has more than doubled since World War II. On the other hand the hours of work that were needed to obtain a quintal of product have decreased manifold. 45 hours of work is estimated at present to produce 6 t/ha while 30 years ago one hectare needed about 700 work hours with a production of 5.5 tons.

The breeding and agrotechnical activity is oriented to keep the cost of production per unit to a minimum level and to improve grain quality. The so called japonica type is preferred for internal consumption of rice whereas indica type has preference for export to most markets. For various reasons the indica type of rice in Italy is less productive.

Other cereals cultivated in Italy are oat, rye and sorghum; whereas the first two crops have unfortunately scarcely been a subject of systematic experimental activity, sorghum is a subject of much research in several institutes. Investigations are concentrated on the improvement of adaptability, agronomic techniques, quality improvement with special emphasis on tannin content of grain to upgrade socalled "bird resistance" while retaining the nutritional value for use in animal feeding.

The Institute closely collaborates with the Morando Bolognini Foundation. This Institution is responsible for multiplication of seeds of newly developed varieties and distribution to seed companies, not only in Italy but in various countries as well. The promotion of new, improved genotypes as appears from promising results of maize lines and cultivars of wheat and barley recently released, is another activity of this foundation.

GENE MANIPULATION BY MUTATION TECHNIQUES

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Abstract

The most important results of cereal mutation breeding are presented as examples of the usefulness of techniques for crop improvement. These economically valuable results were obtained with very limited input. It was demonstrated that the mutation method, in comparison to conventional plant breeding, involved only a small number of breeders with limited facilities and financial support. The introduction of biotechnology methods in plant breeding provided an opportunity for wider implementation of mutants and mutation techniques.

Many terms are used in relation to biotechnology. Recent advances in recombinant-DNA technology or more general, in genetic engineering, fully justify a very wide definition: Biotechnology includes the utilization of a biological system or process of microbial, plant or animal cells and their constituents in organized human activities. Laskin (1983) clarified this in a simple manner: "it is an integrated multi-disciplinary field that utilizes many different technologies and impacts on a wide variety of areas". In his opinion the core disciplines of biotechnology are microbiology, biochemistry, enzymology and molecular genetics. This core is surrounded by such disciplines as molecular and cell biology, immunology, organic chemistry and the applied sciences of agricultural and food technology. Although industries are increasingly utilizing biotechnological processes, agriculture is without a doubt one of the most important impact areas for biotechnology.

Simmonds (1983) discussed the complicated relationships between basic research, some areas of agricultural biotechnology and plant breeding. An interdisciplinary approach to plant breeding includes such fundamental disciplines as cytogenetics, chemistry, biomolecular chemistry, plant physiology, cellular physiology and of course, genetic engineering. These disciplines, together with other methods of biotechnology help solve the main task of plant breeding: gene manipulation for crop improvement. The main aspects involved in this area of biotechnology are: embryo-culture, shoot tip culture, clonal multiplication, disease resistance, genetic resource conservation, somaclonal variation, haploids, mutation induction, stress selection, in-vitro hybridization and industrial products.

New combinations of genes can be created by crossing two parent plants possessing gene sets capable of improving desirable characters in the progeny. The main problem of this conventional breeding technique evolves from the parallel transfer from one genotype to another unwanted gene(s). The required generations of backcrossing drastically decrease the efficiency of this gene manipulation technique. Nevertheless, during the very long history of this method, many spectacular successes were obtained and cross breeding is now practically the main way to improve germplasm of currently cultivated varieties. Such spectacular success using cross breeding techniques was observed in the improvement of rice cultivars. The breeding programme of one of the developing countries could be used as an example, but similar conclusions can be drawn if highly industrialized countries are taken into consideration.

In Japan the story of rice cultivar improvement can be described as follows. The average yield has increased from 3.0 t/ha in 1955 to about 5.0 in 1979. At the turn of the 60's there was a breakthrough from a 0.5 million ton shortage of rice to overproduction of about 1 million tons. Parallel to these changes there was an increased tendency toward mechanization in rice cultivation in the early 70's. Harvesting machines were used - 27% in 1970, 59% in 1973, 87% in 1976 and 95% in 1979 (Sato and Kaneda, 1981). It should be noted that a different type of rice plant architecture and physiology (stiff, lodging resistant straw) is needed for mechanical harvesting. The introduction of new mechanical techniques in rice cultivation was mainly possible because of the development of new types of rice varieties which directly initiated the "Green Revolution" in Asia and other regions of the world.

Two main characters limiting rice yield, particularly in good agro-ecological conditions are: lodging (caused by long, weak straw) and low response to nitrogen fertilization (Fig. 1). It should be noted that lodging can not only decrease grain yields and make mechanized harvest more difficult but can as well reduce the grain quality. These problems will increase drastically with a significant increase of the use of fertilizer. It was feasible to solve this technological problem by rather simple gene manipulations. The transfer of desirable genes was possible in this case because of the existance of the Dee-geo-woo-gen (DGWG) in a Chinese variety. This gene manipulation was done by its introduction to a genotype of the tall local Taiwanese variety, Tsai-yuan-chung. This cross resulted in the release in Taiwan (1956) of the semi-dwarf rice variety

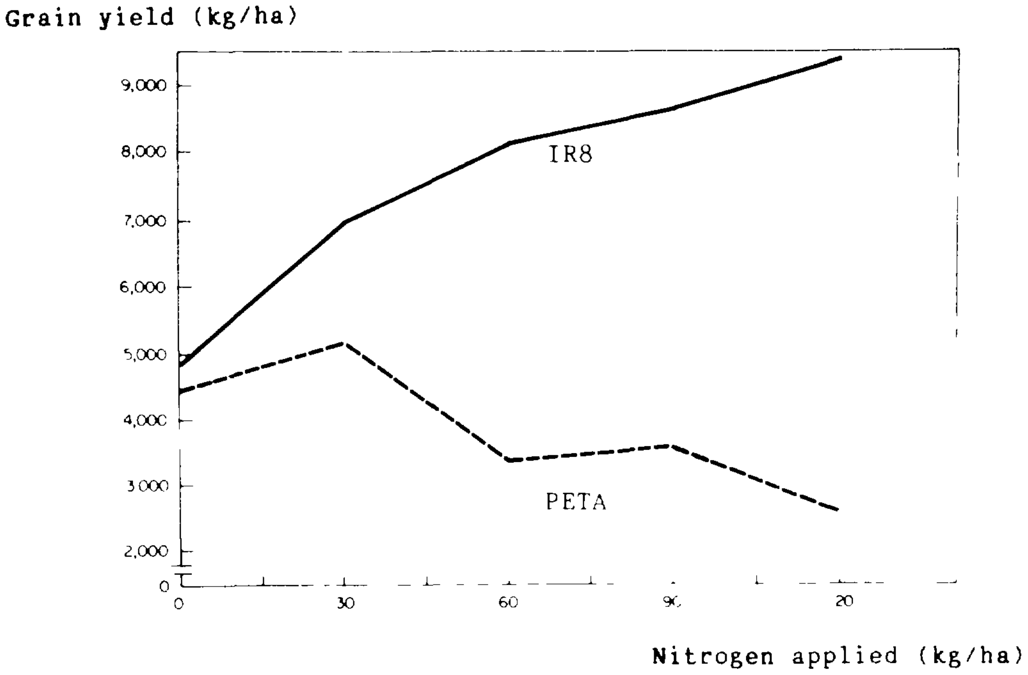

Figure 1: Nitrogen response of Peta and IR8 during 1966 dry season at IRRI (after Khush, 1984)

Taichung Native 1 (TN1). This new variety type was used as a parent in many crosses in different countries. As a result of another transfer of the semi-dwarfness gene from DGWG to an Indonesian tall indica variety Peta in 1966, a new semi-dwarf variety IR8 was developed. The cross was made at the International Rice Research Institute and this variety, together with its descendants, brought the "Green Revolution" to many countries in the tropical zone of Asia (Khush, 1984).

A gene similar to DGWG was found in a native, semi-dwarf japonica type, variety Jikkoku. Three new, high-yielding varieties (Hoyoku, Kokumasari, Shirani) were released in southern Japan in the early 1960's as a result of transfer of this character by crossing with variety Zensho 26 (Kikuchi and Ikehashi, 1983).

Another approach to manipulation of gene(s) responsible for semi-dwarf character was demonstrated by Futsuhara et al., (1967). 20 kR and 30 kR gamma-rays from a ^{60}Co source were used for irradiation in 1959 of 2500 dry seeds of a promising line, Fukei No. 47, later known as variety Fujiminori. The frequency of short-culm plants detected in the second generation (about 50,000 plants) after treatment was relatively high and reached a value between 4.3 - 9.5%. Of the 112 observed plants only 46 were selected for observation of this character in the next progeny. Most of these selected plants gave true breeding lines in the F_3 progeny. This presented an opportunity to evaluate of their yield capability. From 13 lines chosen for M_4 progeny, 4 have shown higher yields than the parent variety.

Two promising lines (Fukei No. 70 and Fukei No. 71) selected the fourth year after mutagenic treatment, practically completed the breeding procedure. During three year performance trials test Fukei No. 70 was recognized as being more suitable for the northeastern part of Japan and was officially released as a new variety named Reimei in 1966 (Futsuhara, 1968). Reimei was cultivated over wide areas during the early 1970's, ranging from 3rd-5th place, and helped to overcome the shortage of rice in Japan - due to its lodging resistance and high yielding ability. Because of these characters Reimei was used as well in cross breeding programmes and as a result the variety Akihikari (Norin No. 238) was released in 1976. Since 1979 it has ranked 4th or 5th on the list of planted rice varieties in Japan with a cultivated area of about 120,000 ha. This variety (Toyonishiki x Reimei) inherited both of the most important characters of Reimei: resistance to lodging with high utilization of nitrogenous fertilizer and high yield.

Eradication of the rice deficit by introducing high yielding varieties, together with a declining tendency in rice consumption, has drastically decreased its cultivated area. In 1981 only 79% of the 1965 lowland area and 22% of the 1969 upland fields were cultivated with rice (Sato and Kaneda, 1981).

The importance of mutation techniques as a tool for gene manipulation in modern plant breeding can be demonstrated using rice production in the USA as an example. This country's contribution to the total world production is only 1.6%, or 6.2 million tons with an average yield of about 5 t/ha. This average yield placed the USA in the 6th position in the world - after Oceanic region (6.3 t/ha), Japan (5.8 t/ha), South Korea (5.7 t/ha), North Korea (5.6 t/ha) and North Africa region (5.6 t/ha). This high production, together with comparatively low rice consumption, of yield accounts for the fact that the USA is the world's largest exporter of rice.

The average yield of rice cultures in California is about 50% higher than in the other rice-producing states in the USA. Basically, a cold-tolerant japonica type of rice with short to medium grain is grown. This is in contrast to the southern US where more indica or indica/japonica type with long-grain are cultivated. The production of rice in the temperate climate of the Sacramento Valley in California is one of the most highly sophisticated agricultural battles in the world - using laser beams for precise levelling of rice fields, airplanes to sow seeds and dispense granular herbicides and special combines that will not bog down in mud. Under these conditions the average yield in California in 1979 was 50% higher than the average in the other states, reaching about 7.2 t/ha (Rutger and Brandon, 1981).

To properly evaluate the excellent results of plant breeding and other agricultural sciences it should be noted that rice became a commercial crop in California only about 1912. Plant breeders and geneticists had established a programme for consequent improvement of rice cultivars. The breeding objective, similar to the Japanese one, was defined as follows: to develop short-stature types of rice plants which will be highly resistant to lodging or falling over, especially during maturity time. Currently available methods of gene manipulation were used to realize this task. Lodging resistant varieties with high response to nitrogen fertilization were developed by mutations, hybridization and by immediate integration of induced mutants into hybridization programmes. McKenzie and Rutger (1984) report that since 1970, 19 commercial rice cultivars were released in California. Three of them were directly selected induced mutants and seven were recombinants from crosses where mutants were used as parents. This success in utilizing mutation techniques in California has a short history. Calrose, (released in 1948), tall (120-130 cm), medium-grain variety was irradiated with 20 kR and 25 kR gamma-rays from ^{60}Co at the University of California in 1969. Only 2,050 seeds were used for each dose. In 1972, after selection in early progenies, 11 more promising mutants were investigated in a small-plot replicated M_4 generation yield trial.

Already in 1974 three short-stature mutants (D7, D51 and D24) were included in multi-location, large-plot yield trials in California. In 1976 the mutant D7 was registered as a new variety "Calrose 76" (Reg. No. 45). Calrose 76 is similar to the parent variety except for a 25% plant height reduction. During 3 years of observation its average mature height was 87 cm. but most important, the panicle length remained unchanged. Parallel to testing procedures of selected mutants for agronomical characters, two of them (mutants D7 and D51) were used as parents for hybridization with Calrose and a tall check cultivar CS-M3 already in 1972. (Rutger et al., 1976 and Rutger et al., 1977). Immediate transfer of the induced semi-dwarfness gene to another genotype of cultivated varieties gave six additional cultivars in the years 1977-81. This was possible, because of the collaboration of Dr. Rutger's team at the University of California with the California Co-operative Rice Research Foundation Inc. at Biggs. In 1981, 12 years after irradiation of seeds of the old variety Calrose, 7 new varieties with the Calrose 76 gene of semi-dwarfness were cultivated on about 59% of the 245,000 ha rice area in California. New semi-dwarf varieties, as indicated by cumulative evidence, brought a yield increase of about 15% and in farms with intensified cultural practices of about 25% This means about 1 metric ton more per hectare in comparison to California's 6 metric ton/ha base yield level (Rutger, 1983). This is an example of the economic importance of this method. Rutger's work demonstrates that mutation techniques provided a promising opportunity for

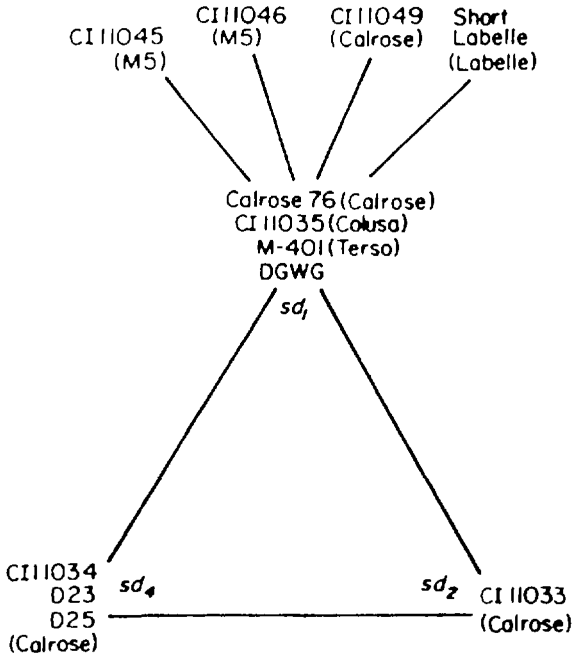

Figure 2 Allelic relationships of induced semi-dwarf mutants and DGWG Gentotypes of the same corner of the triangle are allelic· those at different corners are nonallelic Genotypes in the "fan' at top are nonallelic to the $\underline{sd}_1$ locus but their relationships to other loci are unknown Parent cultivars are shown in brackets (after Rutger et al , 1985)

plant genetic engineering. His team found that semi dwarfness can be induced in several independant, non allelic loci (Fig. 2). The allelism test of different semi-dwarf mutants brought one unexpected result that tall plant type can be inherited recessively to semi-dwarfs The "elongated uppermost internode" gene (eui) results in extreme elongation of the first internode under the panicle. This mutant can help in production of hybrid seeds which will result in F_1 plants with heterosis effect on yield. The eui gene can be transferred to pollen fertility restoring parents, which would be especially useful when a semi dwarf type of F_1 plants is wanted. The tall plant, as donor of pollen for a cytoplasmic male sterile parent, will be a more effective pollinator which is extremely important for seed set and finally for the price of hybrid seeds (Rutger et al., 1985).

Rice is not the only example among cereals for successful manipulation of genes by mutation techniques. The technology for breeding barley with shorter straw was developed in England during the last 80 years. Released in 1900, the spring barley variety Plumage reached a final plant height of about 112 cm. Triumph, released in 1980, grows only up to 70 cm. Together with this tendency to decreasing plant height, it is possible to observe the increase of yield capability (Fig. 3). As presented in the cited paper (Riggs et al., 1981) data from plants with a high level of nitrogen fertilization and support treatments for all considered varieties gave an excellent opportunity to compare the yield potential between old-tall and modern-short stature spring barley varieties. In a more drastic case, the yield difference is about 2 t/ha (Chevalier 6.46 t/ha and Egmont, Korn, Triumph with mean yields 8.45 t/ha) in these same preferable growth conditions.

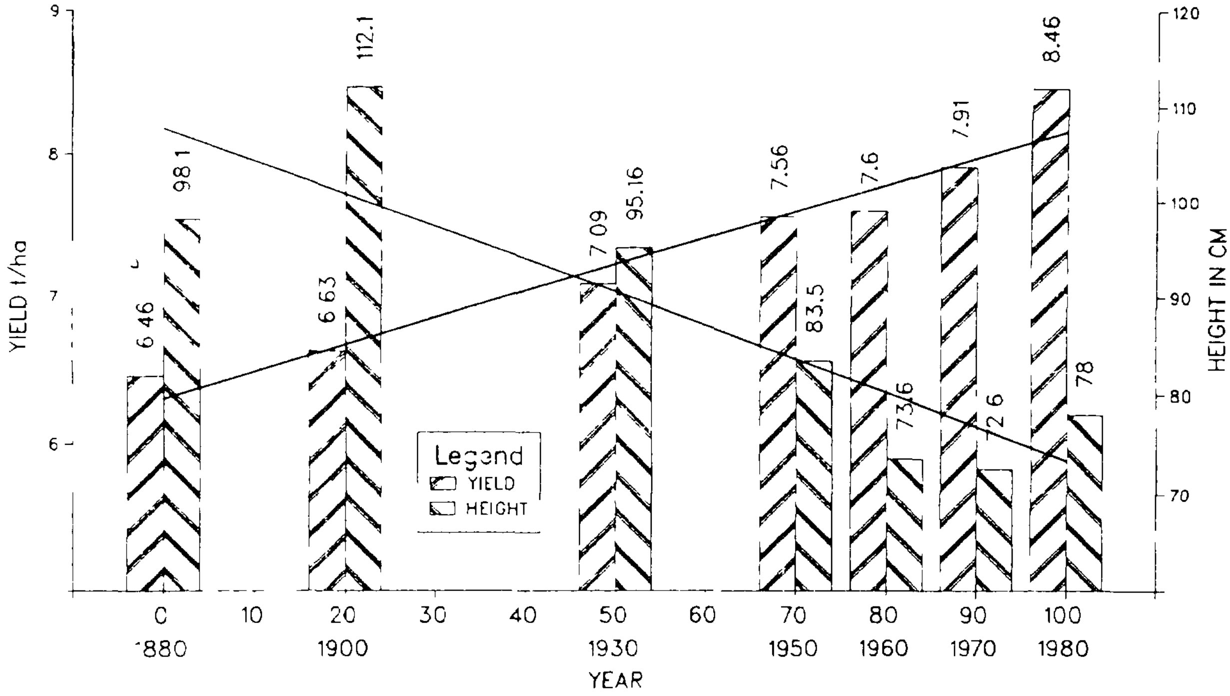

Figure 3: Yield potential of spring barley varieties released during the last 100 years in UK in relation to final plant height (from data presented by Riggs et al., 1981)

Of the 8 spring barley varieties now grown in England and Wales, 3 are semi-dwarf mutant varieties. On the basis of data on seed multiplication area presented by Lidgate (1982) it is possible to calculate that 41.6% is covered by mutant varieties, which allows us to presume that these varieties are cultivated on about 450,000 ha in this region of the UK.

The breeding objective of plant breeders in Czechoslovakia was to obtain spring barley with shorter and stiffer straw. Seeds of the old Moravian land variety Valticky were irradiated with 10 krad in 1956. Nine years later the mutant line VR_2 was registered as a new Czechoslovakian improved variety under the name Diamant (FAO/IAEA MBNL, 1976). After a few years it became the leading barley variety in all regions of Czechoslovakia. This variety, which is well adapted for Czechoslovakian agro-climatic conditions with good grain malting quality, has been used as a popular parent variety for different cross-breeding programmes. Altogether 11 varieties (progenies of crosses with Diamant) were released - not only in Czechoslovakia but in neighbouring countries such as the DDR, USSR and Poland as well. More than 96% of the spring barley areas in Czechoslovakia are sown with varieties of the "Diamant series" (Bouma, personal communication). Trumph, one of these varieties released in the DDR in 1973, is cultivated in this country and in Poland as the best brewery spring barley and moreover is utilized as parent variety in new breeding programmes.

These economically valuable results of the use of mutation techniques were obtained with very limited input. In comparison to conventional plant breeding, the mutation method usually involved only a small number of scientific teams with limited facilities and financial support. It should be considered that all the mutant varieties presented above were selected in the second generation after mutation treatment as individual plants from a comparatively small population of about 50,000 plants.This successful selection of cereals was carried out on a 2,000 m^2 experimental field. The scale of this activity is practically uncomparable to any other plant breeding programme. It should be noted for example, that the British Association of Plant Breeders estimated the number of F_2 cereal plants grown in the breeding programme fields in the UK in 1980 as close to 10 million (Foster, 1982). Very high efficiency must have led to the recent successes of gene manipulation using induced mutations.

The International Atomic Energy Agency, jointly with FAO, has supported activity on the use of the above discussed techniques in many countries around the world. The Green Revolution flourished due to introduction of short statured varieties of rice and wheat with high response to nitrogenous fertilization, thus solving many problems of food supply in developing countries. Nevertheless, it should be considered that modern rice varieties, for example, cover about 42% of the rice area in 11 countries of Asia (Pinstrup-Andersen and Hazell, 1985). The rest is still cultivated by traditional local varieties which very often represent a special value for inhabitants of this area (Table 1). The cooking quality or the specific aroma are usually the most important characters prefered by people and for this reason traditional varieties often win in competition with modern varieties on local markets, even though their yield potential is much lower.

Table 1: Area Grown with Modern Rice Varieties in 11 Asian Countries

Country	Year	1000 ha	Rice Area %
Bangladesh	1981	2,325	22
India	1980	18,495	47
Nepal	1981	326	26
Pakistan	1978	1,015	50
Sri Lanka	1980	612	71
Burma	1980	1,502	29
Indonesia	1980	5,416	60
Malaysia W	1977	316	44
Philippines	1980	2,710	78
Thailand	1979	800	9
South Korea	1981	321	26

(after Pinstrup-Andersen and Hazell, 1985)

The genetic constitution of these local varieties can usually be improved by changing one or two main characters limiting their yield capacity. This should be done in such a way that the genetic background responsible for their valuable traits is left unchanged. In this case the manipulation of genes by cross breeding is very difficult and time consuming. Mutation techniques offer a simple and efficient solution.

There are many examples of successful use of mutagens for improvement of rice cultivars (Micke et al., 1985), e.g.:

India:	variety Jagannath, released in 1969, after x-rays of plant variety T141, direct selection in M_2 progeny. Important traits: medium slender grain, good cooking quality. It is grown in lowland coastal areas of peninsular India with a yield of 6-7 t/ha.
Pakistan:	variety Kashmir Basmati, released in 1977, after gamma-rays of aromatic variety Basmati 370. Important traits: early maturity, higher yield potential, aromatic. It is cultivated in Northern areas of Pakistan (Azad Kashmir) at an altitude of about 560-1400 m above sea level.
Thailand:	variety RD6, released in 1977, after gamma irradiation of variety Khao Dawk Mali 105. Important traits: glutinous endosperm and improved blast resistance, very popular in the northern part of Thailand.
Japan:	variety Miyuki-mochi, released in 1979, after gamma irradiation of parent variety Toyonishiki. Important traits: glutinous endosperm, other characters same as parent. It is locally distributed in Nakano prefecture, Central Japan.
China:	variety Yuan Fengzao, released 1975, after gamma irradiation of parent variety Kezi No. 6. Important traits: 45 days earlier, yield 10% higher than local commercial variety, 8-14% higher lysine content. It is cultivated on about 1 million ha.

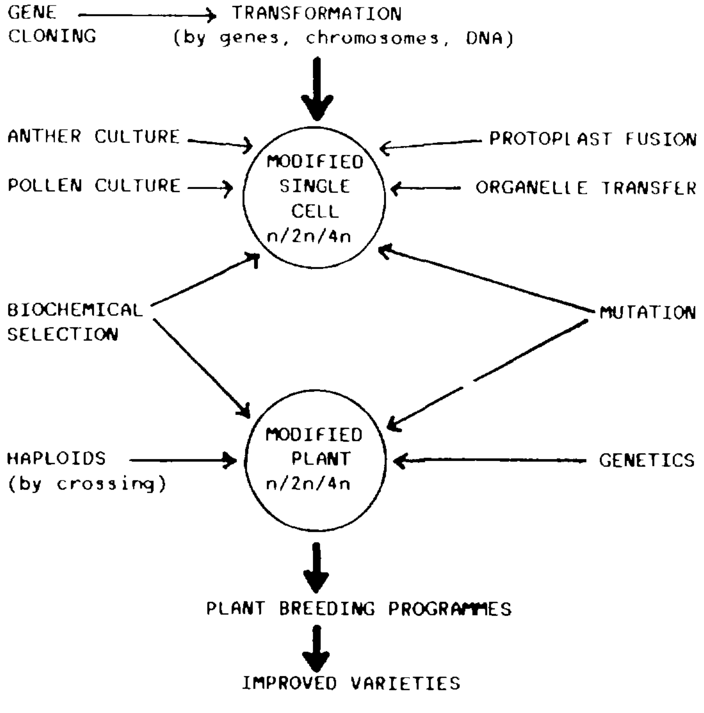

Fig 4. New inputs to plant breeding (Bright et al., 1982)

It was possible as well to solve some biotechnological problems of beer production by using induced mutagenesis. The precipitation of polyphenols is the reason for the so-called chill haze. Proanthocyanidins of barley are considered a main source of the polyphenols in beer. To obtain colloidal stability and brilliance of beer it is necessary to remove the polyphenols by chemical treatment. Von Wettstein and his co-workers already solved this problem. After mutagenic treatment of spring and winter barley they obtained many mutants free of proanthocyanidines. The mutation frequency of mutants with this character averages 0.003% on an M_2-plant-basis but reached 0.011% in the variety Ark Royal. 116 mutants of spring barley and 11 of winter barley were found (Larsen, 1981). It should be emphasized that beer brewed from proanthocyanadin-free mutants had good quality and brilliance stability without any chemical treatment.

Cereal improvement was used as an example in the above deliberations on the possibilities of gene manipulations by induced mutation for two reasons. The first because of its importance for the world food supply and the second because in such species as rice or barley conventional breeding is so sophisticated and has such a high rate of success that special techniques are needed to give superior results. For the last reason more examples were taken from developed countries where plant breeding is carried out on an extremely high standard. The results of crop improvement by the use of mutation techniques were recently summarized in other papers (Kawai, 1983; Sigurbjörnsson, 1983; Konzak, 1984; Micke et al., 1987).

The introduction of biotechnology methods to plant breeding provide opportunity for wider implementation of mutants and mutation techniques.

Plants regenerated from in-vitro cultures may differ from the original in many characters. This kind of variation is known as "somaclonal variation". Different variants obtained in this way can potentially be utilized as new genetic sources for crop improvement. Unfortunately, regeneration of plants from isolated cells of common growing cereals has so far been unsuccessful. But even in the future - after such promising techniques as gene manipulation by protoplast fusion are available for wheat, rice or barley - the interest will be in transfering only a small part of the donor genome to the improved genotype. Theoretically this partial genome transfer can be carried out with the help of fragmentation of the donor genome by radiation. The efficiency of this method is already evaluated with the use of potato protoplast culture where plant regeneration is possible (Jones et al., 1984).

Doubled haploids (DH) methods became more readily available in cereal breeding after improvement and development of general methods. The main breeding advantages of these techniques are rapid shortening of the breeding cycle, need for derivation of homozygous lines and new possibility of selection for desired characters in the absence of dominant variation in the genotype. Swaminathan (1984) recognized these techniques as the first of many genetic engineering techniques which may offer new ways to improve cultivar yields. He calculated "the DH technique reduced the time needed to get a new variety from 10 generations with conventional breeding methods to as few as three generations".

The other advantages in using doubled haploids were discussed by Baenziger and Schaeffer (1984). In the haploid level, selection can be made not only for dominant but for recessive genes as well. Doubling of selected haploid genotypes, changed previously by mutagens, makes possible the agrobotanical evaluation of the next progeny on a plot basis in the field trials. Such practically homozygous progeny makes selection more efficient by decreasing the time needed for genetic stabilization of evaluated stocks.

Mutation techniques (gamma irradiation) can be used as well for stimulation of callus initiation and shoot differentiation in wheat in-vitro culture (Gao Mingwei et al., 1985).

Mutation techniques can be used with success in F_1 hybrid cereal programmes. Similar to results obtained on sugar beet (Kinoshita et al., 1982), mutagens can be used for induction of cytoplasmic sterility or for male sterility genes in cereals as well (Foster and Fothergill, 1982). Mutants of crop plants can be produced on F_1 hybrid plants with high vigor and high yield potential in crosses with other mutants or even the parent variety. These phenomenons were observed in sweet clover (Micke, 1976), barley (Maluszynski, 1982) and recently in maize (Dollinger, 1985). Results published in these papers indicated the next possibility for gene manipulation.

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PROJECT REPORTS

EXAMINATION OF DWARF WHEATS PRODUCED BY MUTATION, CROSSING AND PHYSIOLOGICAL PROCEDURES

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Abstract

By induced mutations more than 2000 dwarf lines were produced and 1600 were examined in ear-to-row experiments in 1984-85. The new dwarf forms showed a reduction in height of 11-28 per cent as compared to the parental cultivars.

By "cleaning up" the genetic background euplasmic new dwarf forms can be produced and the productivity of which reaches that of the tall ones, possibly because of the elimination of chromosome and cytoplasm aberrations.

Genetic analysis of dwarf wheats verified, that in combining ability for height Mini Manó proved to be the best, reducing the height of its hybrids by more than 7 cm. As Mini Manó has a number of excellent characteristics it seems to be very promising both as a cultivar and as a crossing parent.

The internode length of 45 F_1 hybrids, along with their parents, was measured in the field. Three of the parents involved /Mv 3, Gödöllő 1 and Gödöllő 2/ were of mutation origin. The behaviour of Apollo cv. was very favourable. In almost all cross combinations it reduced the length of the first internodes and increased the peduncle length.

Using tissue culture methods somaclonal variants in a number of characteristics have been produced. For improving the characteristics of dwarf lines, progenies of their F_1, F_2 and F_3 generations were included in the haploid breeding program. A number of androgenetic doubled haploids have been improved. More than 5.000 gametoclone and/or somaclone lines are under tests in field nurseries.

Reduction of the influence of gravitation results as well in a reduction in height.

1. Introduction

The average yield of wheat on a country-wide scale is close to 5.0 t/ha on an acreage of 1.3 million hectares which represents one fifth of the total lands in Hungary. The top yield on the best fields reached 10 t/ha and in experimental conditions 11-12 t/ha are not exceptional. On this level of production the strong dwarf strow of the cereals is a crucial requirement. Most of the Hungarian wheats are semi-dwarfs and some newer ones are dwarfs /60-70 cm in height/ such as Mv 13 and GK Mini Manó which are being introduced on a large farm scale. Our mutation work started about ten years ago /Barabás, 1977/ but the yield of dwarfs obtained so far did not reach that of the parentals. However, Micke et al. recently /1985/ reported on more than a dosen productive dwarf cultivars of mutant origin.

Since 1980 the results in this field have been summarized in 1982, 1983 and 1984 in Vienna, Davis, and Ciudad Obregon, respectively. Now we would like only briefly to dwell upon the conclusions of earlier reports, focusing on the new facets of the methods, results and problems of the dwarfism in wheat.

2. Induction and selection of dwarf mutants

Seeds of cultivars Partizanka and Jubilejnaya 50 were exposed to gamma radiation of 5, 10 and 15 kR. After growing up the single seed M_1 generation, a selection of semi-dwarf and dwarf variants was carried out from different M_2 and M_3 populations in two successive seasons. Altogether 2000 semi-dwarf variants were selected so far /Barabás & Kertész, 1982/.

In 1985 1600 putative dwarf mutants, selected earlier were grown and evaluated in an ear-to-row nursery. Each progeny represented one row 1.2 m long with a distance of 20 cm between the rows, resulting in a density of about 400 seedlings per m^2. This density is close to that used in the farm practice. According to our methodological experiment /G. Erdei, 1985/ it provides a reliable information about their real productivity. Each sixth plot /row/ was used as a control and compised nontreated cultivars. The newly obtained induced dwarf mutants on the mutant genetic background and cytoplasm proved to be of poorer performance compared to the original cultivars /Table 1/. However, there were some exceptions. The best dwarf mutant of Partizanka reached the yield of the parental line and the best Jubilejnaya mutant dwarf showed only 10% yield reduction as compared to the original form.

From this reselected mutant lines some 800 dwarfs were planted along with the parents in an ear-to-row experiment in the fall of 1985. Besides this, 50 lines will be tested in a drill plot screening nursery and 5 in a replicated yield trial.

TABLE I. Height and yield reduction of dwarf mutants /on mutant genetic background and treated cytoplasm/

	No. of mutant families	Average height reduction cm	%	Average yield reduction %
Partizánka	31	10-25	11-29	30-50
Jubilejnaya 50	30	10-25	11-28	30-40

3. Back-cross program for "cleaning up" the genetic background of the mutants

Although the yield of the best mutants is similar or, in some cases, equal to that of the parental cultivars, they are by no means competitive with the best cross lines registered. It is well known that mutagen treated cytoplasm /and genetical background/ cause a setback in productivity. Gustafsson /1961/ reaches the conclusion that the nucleus is relatively radio-resistant and the injuries are, in general, of cytoplasmic origin. In Sorghum the exchange of the mutant cytoplasm with a normal one resulted a significant increase in the yield /in: Barabás, 1964/. This change can be achieved by back-crossing, by gametic selections using ms lines, by Chase /1963/ androgenetic method, as well as by the up to date protoplast culture methods.

Cleaning up the genetic background of the dwarf M_3-M_5 mutants is being carried out since 1983. At present 44 mutant lines of 26 mutant families and 34 mutant lines of 20 mutant families are in different stage of back-cross /BC_1-BC_2/ generations from Partizanka and Jubilejnaya 50, respectively. The M330 mutant on the original montreated/ background has a reduced height and a better vitality than that on the mutant background. Similar tendency was found in case of the M 66 Partizanka mutant etc.

4. Combining ability of dwarfs for height

The behaviour of a series of dwarf wheats was studied in a diallel crossing program analysing F_1 and F_2 generations. The set of wheat lines studied was as follows: Krasnodar 1, Karcagi AM 522, Tom Thumb, Bulgarian dwarf, Apo dwarf and Mv C_4. As a tall tester the well adapted cultivar Jubilejnaya 50 was used. According to the general combining ability of the lines /Table 2/, the "Apo dwarf" is worth of attention.

TABLE II. General combining ability for height in dwarf wheat lines

Lines	General combining ability values /cm/
1. Jubilejnaya 50 /tall tester/	6.89
2. Krasnodar 1	1.76
3. Karcagi AM 522	2.22
4. Tom Thumb	0.46
5. Bulgarian dwarf	0.44
6. Apo dwarf	-12.00
7. Mv C_4	0.23

It reduced the height of its F_1 progenies by an average of 12 cm. This result also indicated that dominant dwarf gene/s/ controls the height of this particular line. Segregation test in F_2 confirmed that the dwarf carries one single gene for dwarfness and shows complets dominance on the basis of a 3:1 - short : tall segregation ratio in the Jubilejnaya x Apo dwarf crossing. In spite of the favourable properties of this line difficulties arised in fixing its height. It shows sometimes a random segregation ratio /perhaps chromosomal aberrations on 4A/ in spite of 10 generation selfing.

In a top-cross series including more than 100 F_1 hybrids of 31 parents, Mini Manó is being tested and evaluated as a parent. On the basis of the F_1 analysis, the general combining ability of the parents for height and some other agronomic traits is being tested under greenhouse conditions.

In this trial, Mini Manó /Mx 158/ produced excellent F_1 progenies, and proved to be the best among the lines. It reduced the height of its hybrids by 7.3 cm in one series of tests and by 5.9 cm in another compared to the overall average of the hybrids /Table 3/. Besides the very good agronomic characteristics of this line Mini Manó seems to be very promising in producing SD hybrid populations and finally SD cultivars.

Both in our performance nurseries and in state yield trials Mini Manó was equal or superior in yield as compared to the other cultivars with normal height although Mini Manó is just half as tall as Jubilejnaya 50 /65 vs 120 cm/. Compared to the released dwarf feed-wheat /Mv 13/ its yielding ability is the same, but it has a better resistance to stem rust, leaf rust, mildew, and shows higher tolerance against aphid and Barley Yellow Dwarf Virus as well. Its drought

TABLE III. General combining ability for height in cultivars and new candidates

Entries	Combining ability values /cm/
GK Mini Manó	- 7,31
Zld - Ttj	3,35
8001 - Zld	1,49
Pdj 2 - Sv	2,45
Mv 11	- 3,75
Rsk-Rn 12 x Rna 3	3,84
Csg-Zg 4364	- 0,05
234.13 - Bzt 1	- 2,04
Tpr 349 x 8001 - Sv	0,72
GT-Pdj 2 x Hs 2	- 3,78
Jbj-Sdv "S"	6,07
453.602 - Prtk	- 1,05
Rsk-Rn 12 x Rbn	0,05

tolerance and baking quality is excellent, compared to the other dwarf and tall wheats. On the basis of these, Mini Manó is very promising both as a cultivar and as a crossing parent. It is kindly suggested to winter wheat breeders. It might be released next year in Hungary. /Kertész & Barabás, 1984/.

5. GA-insensitivity test of Hungarian SD wheat lines

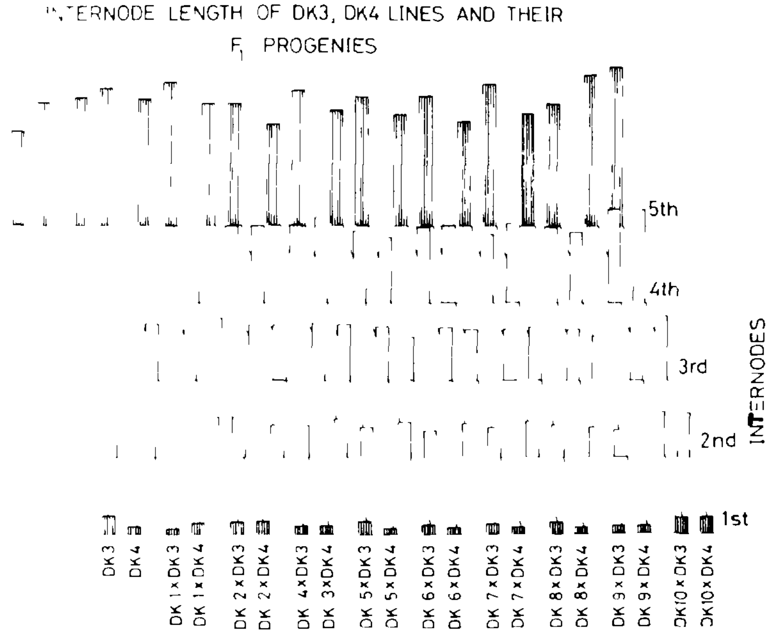
Of 20 semi-dwarf and dwarf lines widely used in crossing programs, the following proved to be insensitive: Gödöllő 2, Rf Super 2, Mini Manó, Apo dwarf, Apolló and Mv 5 /Table 4/.

6. Inheritance of the internode length of the mutants

The internode length of 45 F_1 hybrids, along with their parents, was measured in the field. Three of the parents involved /DK1, DK3 and DK9, Mv 8, Gödöllő 1 and Gödöllő 2, respectively/ were of mutation origin. Although Gödöllő 1 had a short culm its first and second internodes were extremly long and the peduncle was relatively short. This characteristic was

TABLE IV. GA insensitive lines and their culm length

Entry	Culm length /cm/
Godöllő 2	56,5
Rf Super 2	60,0
Mini Manó	60,0
Apo	45,0
Apolló	69,8
Mv 5	67,3



Abbreviations

Actual	previous
DK1	Mv 8
DK2	Mv 4
DK3	Godollo 1
DK4	Apollo
DK5	Szeged
DK6	Mv 5
DK7	Mv 103W
DK8	Szil. 2
DK9	Godollo 2
DK10	Mrn 808

* mutants underlined

Figure 1 Internode length of DK3, DK4 lines and their F_1 progenies rather disadvantageous for producing hybrids and lines with an ideal culm structure. On the other hand the behaviour of Apollo cv. was very favourable. In almost all cross combinations it reduced the length of the first internodes and increased the peduncle length /Fig. 1/.

7. Mutants obtained by means of biotechnological procedures

In the last two years a relatively large number of somaclones and gametoclones were produced from bread wheat, durum wheat and some interspecific hybrids. At present /1985 winter/ some 1500 plots of SC /somaclone/ progenies and some 4000 plots of gametoclones /androgenetic doubled haploids/ are planted in the nursery. About 50 of the SC and GC progeny generations were planted in a replicated yield trial.

A distinct reduction in height was found in the SC_1 progenies of the Mini Manó somaclones: the SC_1 was almost 50% shorter than the parental form. In the next SC_2 generation the reduction in height was minimal only, 7-8% of the original Mini Manó, which grew 47 cm high under greenhouse conditions. Among the 18 second SC generation progenies of 2 lines proved to be significantly shorter and 2 taller than the parental line. None of the SC_2 families showed significant increase in kernels /head, and Harvest index/. Only one family had higher seed-weight/head value. Otherwise in 6 cases the SC_2 families surpassed the control Mini Manó in 1000 kernel weight.

In another experiment with another cultivars: 17 from 37 SC_2 families of GK Kincső and GK Csongor showed differences in various morphological traits as compared to the parents. Changes in height were observed and the obtained reduction varied from 14-35% as compared to the original cultivars. These changes were inherited in SC_2 and SC_3 generations as well. /Galiba & Kertész et al. 1985/. No doubt, these types of the dwarfs seem to be promising for practical breeding purposes.

One of the most remarkable biotechnological experiments is the application of normally reproduced wheat x barley hybrids in the breeding practice alongwith biotechnological methods. By producing somaclones and possibly gametoclones from steril wheat x barley hybrids we hope to transfer the dwarfness and standing ability of wheat to barley plants, and vice versa - the character of Fusarium nivale resistance from barley to wheat.

8. Induction of dwarf modifications by changing the influence of gravitation

The height of wheat plant can be considerably reduced by laying down the pot horizontally and daily turning it twice 180-180°. Turning the pots round the vertical axis causes no modification. The horizontal treatment reduces the longitudinal growth of the plant by 20-40%, presumably by the alteration of the influence of gravitation. This effect causes further height reduction in the dwarf Mini Manó. As side effects heading difficulties and other teratological symptoms also occur in some cases /Sági et al. 1985/. (Fig. 2)

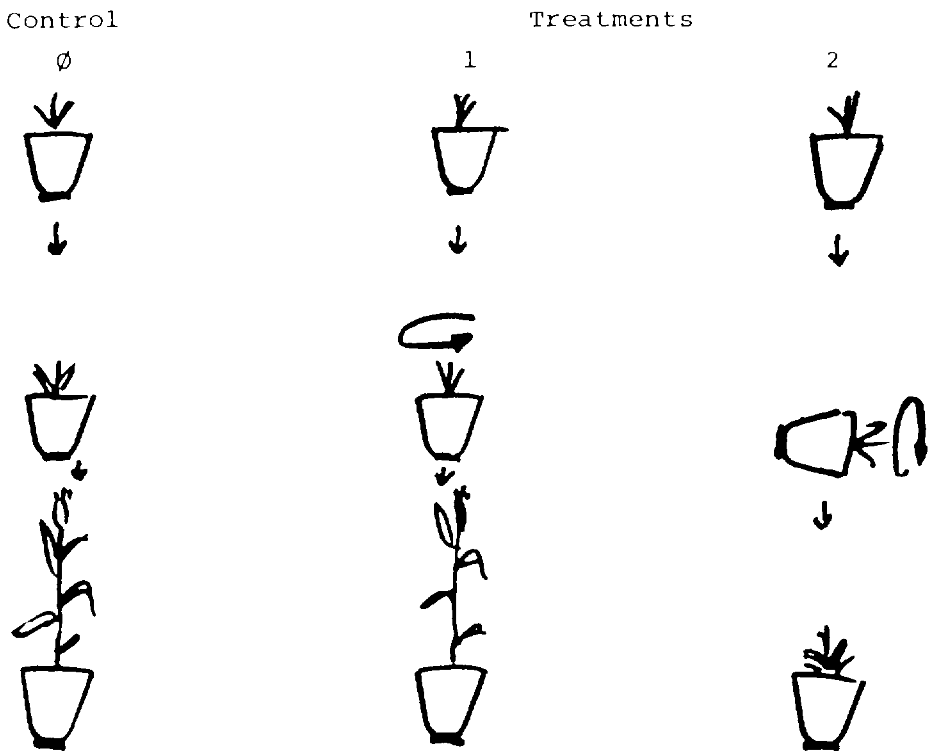


Figure 2. Influence of gravitation

The growth inhibition affected by turning the pots round the horizontal axis not necessarily causes other stresses. E.g. in one of our experiments resistant and moderately resistant wheat varieties /Mini Manó and Mv 10/ were artificially infected by powdery mildew. The epidemic level of mildew was similar in both the horizontally and the vertically treated /by turning/ as well as in nontreated plants. Naturally, the dwarf modification by horizontal turning continues only as far as the effect exists, and changes as a function of the time and treatment. We think that the so called gravitation experiments will bring us closer to better knowledge of the physiological background of dwarf growth.

Acknowledgement

Appreciation is expressed to Dr. J. Sutka who provided the set of diallele F_1-s and parents for internode-studies.

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EVALUATION OF SEMI-DWARF MUTANTS IN TRITICALE AND WHEAT BREEDING

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Abstract

Two triticale lines, Beagle and DR - IRA were subjected to either X-rays or E.M S and later generation derivatives scored for height and yield. 219 single M_2 plant selections were reduced to 18 lines for replicated yield analysis, on the basis of yield in the intervening generations. Higher yielding selections appear to have been isolated; however, they are not notably shorter than the controls.

Wheat Composite Crosses have been established based on the X-ray induced nuclear male-sterility mutant 'Cornerstone'. Derivatives, following three rounds of obligatory outcrossing, include high yielding lines. Composite Crosses appear to be more successful if they are homogeneous for a semi-dwarf allele.

I. Induced Mutations for Short Stature in Triticale

Triticale production is continuing to increase in Australia with 163,000 ha. grown in 1985 which is a 627% increase since 1980. The most successful genotypes in Australia are the long headed types that contain all seven rye chromosomes. This type of plant is represented by the CIMMYT selections Beagle and DR - IRA. These lines were 120 cms and 110 cms tall, respectively, in good field growing conditions in 1983 and 100 cms and 85 cms in poorer field growing conditions in 1984.

Dormant seed of the two lines were subjected to mutagen treatment in 1977 in order to induce height variants. Two separate treatments were applied to both lines, viz., 25 K X-rays and 0.5% E.M.S.

219 single M_2 plant selections were made and 47 of these were taken to M_9 replicated yield plots in 1984 (3 replications and 1 location). The selection method employed before and after the M_9 placed major emphasis on the yielding ability of the selections rather than the retention of various height classes.

The 1984 report detailed the height distributions of the 49 selections in the 1984 trials. The 1984 yield data have been analysed and 18 lines were selected for inclusion in the 1985 yield test (3 replications at each of 4 locations). The 1984 yield performance of the lines retained in 1985 are shown in Tables I and II. Two of the four 1985 sites had been harvested by mid-December and the mean uncorrected weight of the three replications of the two sites are shown in Tables I and II.

Two points emerge from these observations. One, although no higher-yielding Beagle selections have been isolated, higher-yielding lines of DR - IRA appear to have been isolated. For example, DR - IRA Irrad-129 and Irrad-61 have consistently yielded highly.

The second point is the high yielding selections are not noticably reduced in height. The ten DR - IRA selections range from 94 to 119% of the height of the DR - IRA control and the 8 Beagle selections vary from 95 to 105% of the Beagle control.

TABLE I. 1985 uncorrected yields and 1984 corrected yields and heights of the ten DR - IRA selections and control.

	1985 Yield (% Control)	1984 Yield (%Control)	Height (% Control)
Irrad - 129	157	146	106
Irrad - 61	126	130	94
EMS - 64	117	127	119
EMS - 20	115	182	100
Irrad - 87	111	145	94
Irrad - 54	105	184	119
EMS - 41	105	134	106
*DR - IRA Control	100	100	100
EMS - 24	98	115	106
Irrad - 45	97	146	112
EMS - 11	47	132	100

*DR - IRA Control : 1985 uncorrected yield (average of 3 replications at each of two sites) 1.9 t ha $^{-1}$,
1984 NN ANAL corrected yield 1.0 t ha $^{-1}$; 85 cms.

TABLE II. 1985 uncorrectred yields and 1984 corrected yields and heights of the eight Beagle selections and control.

	1985 Yield (% Control)	1984 Yield (% Control)	Height (% Control)
* Beagle control	100	100	100
EMS - 36	94	98	100
EMS - 41	93	91	100
EMS - 63	87	92	95
EMS - 50	85	104	110
EMS - 3	78	93	100
EMS - 20	77	96	100
EMS - 39	73	120	95
EMS - 54	73	98	105

* Beagle Control : 1985 uncorrected yields (average of 3 replications at each of two sites) 2.8 t ha $^{-1}$.
1984 NN ANAL corrected yield 2.1 t ha $^{-1}$; 100 cms.

Potentially useful improved DR - IRA genotypes for crossbreeding and have already been used for this purpose in the general breeding program. They would not be directly useful as varieties as the recently released variety "Currency" in the same 1984 yield trial yielded 310% of the DR - IRA control.

II. Composite Crosses and *Gai/Rht 1* in Wheat

Wheat breeding often involves crossing two parents and then selfing to homozygosity accompanied by evaluation of yield, agronomic and other traits. Variation on this theme involves crossing F_1's to a third parent, and recycling F_6's, for example, as parents. In general, the heterozygous phase is limited.

Composite Crosses based on the nuclear male sterility mutant 'Cornerstone' extends the heterozygous phase by enforcing outcrossing. Composite Crosses have been established by crossing Cornerstone with a number of wheat lines, and growing a mixed F_2 usually of 1,000 plants. The F_2 segregates fertiles: steriles and the latter are pollinated by fertiles at random. Ten seeds from each of 100 selected steriles are used to produce the next generation. This procedure is repeated until there has been at least three rounds of random outcrossing. [1-3]

Following this, homozygous fertile selections are made and subjected to yield tests. Four Composite Crosses (CC I, II, III and V) have been set up with yield increase as the main objective. The male parents used in these Composites are shown in Table III. Each Composite Cross appears to be an improvement on the one that was established earlier than it. There are two

Table III. Male parents used in the Composite Crosses I, II, III and V.

CC I	CC II
Condor	Condor x Warimba
Gamenya	Egret x Warimba
Gamset	Gabo x Warimba
Kite	Halberd x Warimba
Pitic	Kite x Warimba
Timgalen	(M * K * R / 111 / 8) x Warimba
WW15	Olympic * 8156 / 20 / 9 x Warimba
Zenith	Oxley x Warimba
	RAC - 177 x Warimba
	WW 15 X Warimba
CC III	CC V
Aroona	Aroona
Avocet	Banks
Banks	Cook
Matipo	Oxley
Oxley	Suneca
Toquifen	Warimba
Warigal	Warigal

reasons for this. The male-sterile female parent has been continually improved, and it normally contributes 50% of the alleles to the Composite. The pedigrees of the female parents are shown in Table IV.

Table IV. Pedigrees of the male-sterile female parent of Composite Crosses I, II, III and IV.

CCI	Federation /2 * Pitic.
CCII	Federation /2 * Pitic //3 * Gamenya / 3 / Warimba.
CCIII	Federation / 2 * Pitic //3 * WW15.
CCV	Federation /2 * Pitic //3 * WW15 / 3 / Oxley.

Table V. High yielding lines derived from CCII in 1984.

Rank	Entry	Corrected Weight
1	CCII - 213	442
2	FW CCII - 51/1	439
3	FW CCII - 49/1	431
4	FW CCII - 82/1	424
5	Aroona Control	413
6	FW CCII - 73/1	412
7	CCII - 179	378
8	FW CCII - 4/4	375
9	FW CCII - 12/1	374
10	FW CCII - 46/1	369
11	FW CCII - 50/1	368
12	FW CCII - 33/2	364
13	FW CCII - 113/1	366
14	CCII - 166	361
15	CCII - 80	360
16	Condor Control	358
17	CCII - 18	355
18	CCII - 142	355
19	FW CCII - 47/2	354
20	CCII - 153	353
21	CCII - 204	351
22	CCII - 289	350

The second reason is the reduction of the number of tall plants in later Composite Crosses. The earlier composites included tall pollen parents and the heterozygosity generated by intercrossing resulted in a large proportion of tall wheats. The male-sterile stock of CC III and CC V were homozygous *Gai/Rht 1* and all pollen parents of each were homozygous *Gai/Rht 1* Hence, excessively tall wheats were not a feature of CC III and V and this is reflected in lines that have been extracted from CC III.

CC I produced high yielding lines [5], however all except one was found to have flour quality problems. The one line that has been retained (CC I 61 S/5) has been re-selected and 60 sub-lines of it are in the process of being yield evaluated

CC II also produced a number of high yielding lines (see Table V). These have not as yet been subjected to quality evaluation These high yielding lines are in replicated, multi-location yield trials in 1985.

However, CC I and II were disadvantaged by segregation of dwarfing alleles, in that many of the selections were excessively tall. The CC III composite has not had this problem and the derived selections are all of reasonable height Of 1,080 CC III derived rows grown in 1985, 306 homozygous fertile rows have been selected for replicated yield trials in 1986

The other problem with CC I was the inferior flour quality of the derived high yielding lines. CC V has been set up with good flour quality lines only With the restriction of good quality and homogeneity of the *Gai/Rht 1* allele, CC V should produce a large proportion of acceptable derivatives. The danger is excessive restriction on entries could result in insufficient heterozygosity

Success of Composite Cross breeding requires, one, an improved genotype of the male-sterile parent and, two, a restriction(s) on the pollen parents introduced into the composite The X-ray induced nuclear male sterility line 'Cornerstone' is suitable for Composite Cross breeding and the effectiveness of this breeding method depends upon the two points that have been emphasised[4].

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EVALUATION OF MUTANT STOCKS FOR SEMI-DWARF PLANT TYPE AS MATERIAL FOR CROSS-BREEDING IN DURUM WHEAT

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Abstract

Mutation breeding has been an important part of the durum wheat breeding programme for reduced height, lodging and cold resistance. A scheme has been tested, which makes it possible to speed up the plant breeding period and create valuable semi-dwarf durum wheat lines or cultivars. A close correlation was found between: plant height and length of the last internode, weight of grain of main spike and yield per plant, tillering and yield per plant, and number of grain of main spike and grain weight of main spike. The obtained results indicated that short stature mutant lines with different semi-dwarf genes and GA response have been created, characterized by cold and lodging resistance, high yielding potential and protein content. Some of them proved to be a valuable cross-breeding material in durum wheat, others for example, Losen 76, were directly released as a new cultivar.

INTRODUCTION

The most important aim of the durum wheat breeding programme of the Institute of Genetics in Sofia and the Institute of Cotton in Chirpan is to obtain cultivars with semi-dwarf type, cold and lodging resistance, early maturity, high yield potential with good technological characters by using radiation and chemical mutagenic agents. This report gives data about the more valuable durum wheat mutants and the methods of their creation.

Materials and methods

In our investigations local cultivars or lines and Italian cultivars Castelporziano and Creso were used for the creation of new mutant lines by mutagenesis and hybridization [1,3,4,5]. Agronomic trials have been carried out in four replications, with the size of every plot being 10 m^2. The protein content was calculated by estimating nitrogen content, e.g. by the Kjeldahl method. For the estimation of yield components, 100 plants of each line and cultivar were analysed. The method to analyse GA response was given in previous publications [5].

Results and discussion

We used mutation, hybridization and individual selection methods for improvement of durum wheat. The scheme of the current investigation is shown in Figure 1. A great genetic recombination was noted after irradiation of hybrids. Progeny lines selected up to $BC_1M_4F_4$ generations growing under stress conditions, showed a great frequency of

1. Crossing after irradiation of the P_1 and P_2 gametophyte or only of P_2 spikes, emasculation of P_1 flowers and pollination with the irradiated pollen (1500 - 2000 rads)	P_1 x P_2
2. Backcross with P_2 or P_3 pollen irradiated with 1000 - 1500 rads with a view to receive compound hybrids.	M_1P_1 x $P_2(P_3)$
3. Individual sowing	$BC_1M_1F_1$
4. Individual selection (1) of semi-dwarf, cold resistant progenies	$BC_1M_2F_2$
5. Individual selection (2) comparison with the standard.	$BC_1M_3F_3$
6. Microtrial, individual selection (3) semi-dwarf plant height, yield, cold resistance and protein content.	$BC_1M_4F_4$
7. Preliminary trial in 4 replications in 5 m^2	$BC_1M_5F_5$
8. Strain trial in 4 replications in 10 m^2 simultaneously at the Institute and the National Strain Testing Board.	$BC_1M_6F_6$

Fig. 1. Scheme proposed to incorporate some promising characters of P_1 (cold resistant, good quality but tall and lodging) to P_2 genotype (semi-dwarf gene scource) using gametophyte irradiation and crossing in durum wheat

plants with such characteristics as semi-dwarfness, cold resistance and high protein content with improved yield.

The results of three years research on yield components of several durum wheat mutants are shown on Table 1. The mutants lines are considerably shorter (38.8 - 50.5%) when compared with the parent cultivar N788. The lines 15/4-2 and Zeverjana are shorter by 9.8 and 7.8% than the semi-dwarf cultivar Creso. The number of tillers is close to that of cultivar N788, but the most important yield components as the number of spikelets per spike, the number of grains in the main spike, the weight of the main spike grains and the weight of the whole plant grains, were found to exceed the parent cultivars [2].

Table 1. Yield components of durum wheat mutants (3 year average)

Mutants	Plant height (cm)			Tillering (No.)			No of productive spikelets per spike			Grains per main spike			Weight of grain/ main spike (g)			Weight of grain/ plant (g)		
	M	±m	SD	M	±m	SD	M	±m	SD	M	±m	SD	M	±m	SD	M	±m	SD
Ambra	77,5	1,2	6,1	4,5	0,3	1,5	15,2	0,3	1,5	46,4	1,8	9,3	2,4	0,1	0,6	11,6	0,8	4,0
15/4-2	61,7	1,0	5,0	4,6	0,3	1,4	18,0	0,3	1,4	39,9	1,4	7,5	2,0	0,1	0,5	8,8	0,5	2,8
159	78,8	1,2	6,0	4,0	0,2	1,1	17,8	0,3	1,5	44,0	1,3	6,7	2,6	0,1	0,6	10,4	0,5	2,8
Losen 76	81,3	1,2	6,1	4,2	0,3	1,3	19,4	0,3	1,7	54,6	2,0	10,4	3,0	0,1	0,7	12,2	0,7	3,6
720-C	73,7	1,3	6,9	3,7	0,2	1,0	18,7	0,3	1,4	47,4	2,0	10,6	2,5	0,1	0,6	10,3	0,4	2,5
Zeverjana	63,1	0,9	4,5	4,6	0,4	1,9	17,8	0,3	1,6	45,4	1,8	9,3	2,2	0,1	0,5	11,2	0,8	4,0
N788	124,7	1,1	6,1	4,3	0,3	1,6	16,6	0,2	1,2	29,8	1,0	5,6	1,6	1,0	0,4	9,1	0,5	3,0
Creso	68,4	1,2	5,0	3,9	0,1	0,8	17,0	1,7	2,8	37,3	2,2	9,3	1,8	0,5	0,3	8,9	0,4	2,3
Castelporziano	73,2	0,6	2,0	2,6	0,2	0,8	18,2	0,3	1,0	46,2	2,9	9,4	2,4	0,2	0,6	9,0	0,6	2,0

The stem of durum wheat is mainly composed of 5-6 internodes. The last (1st uppermost) internode of durum wheat is longer than that of ordinary wheat and constitutes from 39 to 54% of the total stem length of various cultivars, mutants and lines. A positive correlation between the plant height and length of the last internode (r=0.98***) was found. Figure 2 and 3 illustrate that the length of the last internode decreases with the decrease of plant height.

As seen in Figure 2, eight lodging resistant mutants possess the same number of internodes. It is clear that shortness is not necessarily correlated with reduction in the number of internodes. In fact the shortening of the II, IV and V internodes concomitant to the lengthening of the uppermost ones, which is associated with straw stiffness. Data on the number of internodes shows that the stem structure of the majority of mutants is not different from that of N788.

The results regarding the yield components show that there is a possibility for plant height reduction in durum wheat without interferring negatively on other agronomically important traits. Our results had reconfirmed the previous one of Scarascia-Mugnozza and Bozzini [8].

Figure 4 illustrates the relationship between the weight of the main spike grains and the yield per plant. There is a positive correlation between these two traits (r = 0.92***). The results illustrating the correlation between the number of productive tillers and yield per plant are given on Figure 5. From the diagram it is clear that the increase in the number of productive tillers entails the increase of the yield per plants in durum wheat.

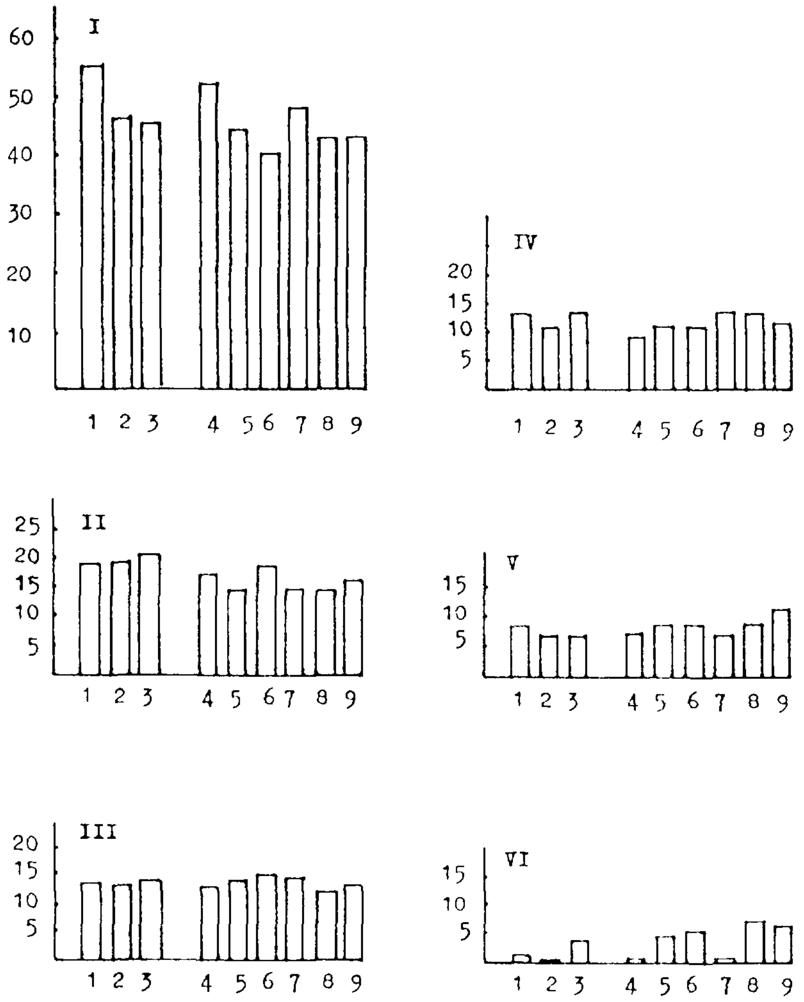

Fig. 2. Number and relative length of internodes of control N788 (1) and mutants or mutant cultivars: Castelporziano (2), Cresso (3), 15/4-2 (4), Zeverjana (5), 720-C (6), Ambra (7), Losen 76 (8) and 159 (9), (1-st uppermost internode).

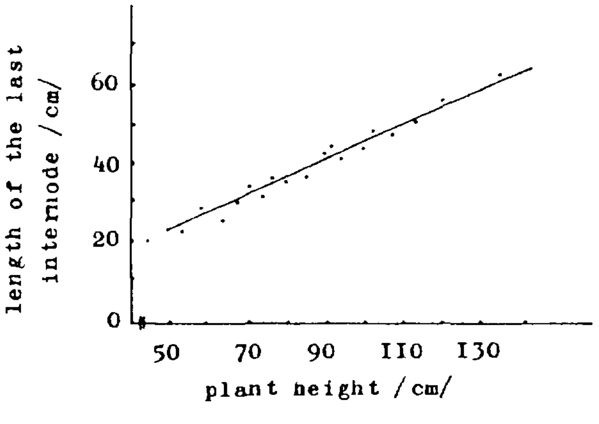

Fig.3 **The relationship between plant height and length of the last internode /$r=0,98^{***}$/**

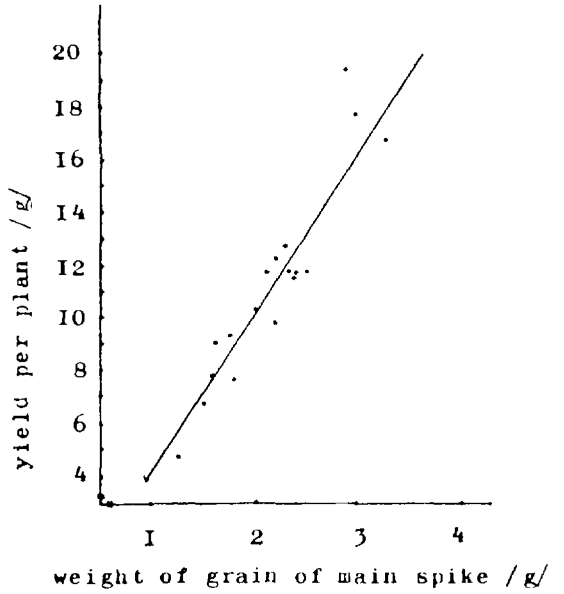

Fig.4 The relationship between weight of grain of main spike and yield per plant /r=0,92***/

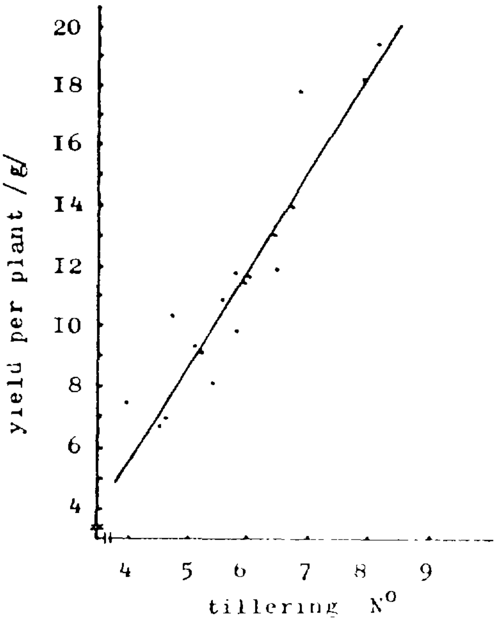

Fig.5 The relationship between tillering and yield per plant /r=0,85***/

The increase of the main spike grains number results in the increase of its total grain weight per plant. This is illustrated in Figure 6.

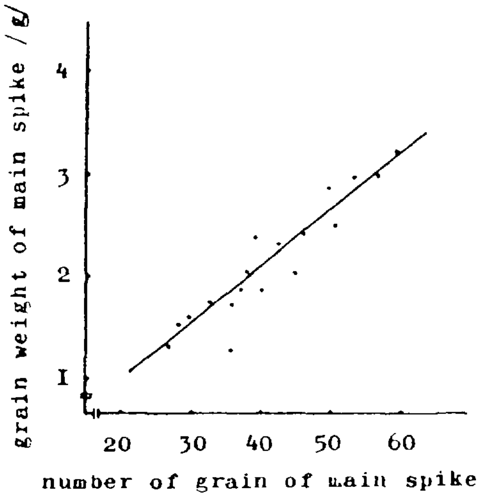

Fig.6. The relationship between number of grain of main spike and grain weight of main spike /r=0,87***/

From 1981 to 1984 comparative trials of mutant lines were carried out in 4 replications in 10 m^2. The results are listed in Table 2.

Table 2. Grain yield of semi-dwarf durum mutants for the period 1981-1984 (yield in kg/ha and % of control)

Mutants	1981		1982		1983		1984		Average for 4 years	
	kg/ha	%	kg/ha	%	kg/ha	%	kg/ha	%	kg/ha	%
Lozen 76	5565	120,3**	5447	110,0**	4580	119,6**	4830	116,1**	5106	116,3**
Zeverjana	5045	109,1**	5720	115,5**	3470	90,6	4090	98,3	4582	104,3*
Ambra	5144	111,2**	5447	110,0**	4461	116,5**	3980	95,7	4758	108,3*
15/4-2	4650	100,5	4510	91,1	2534	66,2	4060	97,6	3939	89,7
720-C	5011	108,3*	5940	120,0**	4970	129,8**	4940	118,7**	5215	118,7**
159	5043	109,0**	5320	107,4*	3643	95,1	4020	96,6	4507	102,7
Control	4625	100,0	4953	100,0	3830	100,0	4160	100,0	4392	100,0

*P ∠ 0,05 **P ∠ 0,01 ***P ∠ 0,001

The average yield of all lines are given in percent in relation to the control. The results obtained indicate that only the mutant line 15/4-2 is characterized by a lower yielding potential, the average 4 year yield is 10.3% lower than that of the control. The other lines exceed the control by 2.7% to 18.7%. This indicates that lines with very high yielding potential had been created. Among these lines 720-C is characterized by extremely high yield (5,215 kg/ha), which exceeds the control by 18.7%. Lozen 76 variety (5,106 kg/ha) which exceeds the control by 16.3% and Ambra (4,758 kg/ha) which exceeds the control by 8.3%. In 1982 the Lozen 76 mutant was approved by the State Commision as a variety. Mutant line 720-C is now undergoing a preliminary trial at the State Commission.

The protein content analyses were carried out from 1981 to 1983. The results, as listed in Table 3, indicate that only Ambra is characterized by lower protein content - 3.1% as compared to the control. The rest of the mutant lines exceed the control by 3.1 to 8.8%, which indicates that mutants with a higher protein content have been obtained (16.60% and 16.46%).

The results of the trials on resistance to cold of <u>durum</u> wheat mutants under controlled conditions are listed in Table 4. It can be seen that <u>durum</u> wheat mutants Lozen 76 and Ambra are characterized with a considerably higher level of cold tolerance with respect to the control - Zagorka (hybrid of <u>durum</u> wheat with <u>Triticum</u> <u>aestivum</u>), which is a winter type. The mutant line Zeverjana, one of the earlist maturing <u>durum</u> wheats, is also characterized by a high level of cold resistance.

The results obtained indicated that semi-dwarf mutant lines have been created which are cold and lodging resistant and with a high yielding potential and protein content. The mutant lines Zeverjana, Ambra, 159 and 720-C have proven to be valuable cross breeding materials in <u>durum</u> wheat.

Table 3. Protein content of durum wheat mutant lines for the period 1981-1983

Mutant lines	1981		1982		1983		Average for 3 years	
	Protein content (%)	Relation to control (%)	Protein content (%)	Relation to control (%)	Protein content (%)	Relation to control (%)	Protein content (%)	Relation to control (%)
Losen 76	17,72*	105,9	14,43*	107,9	14,96	95,7	15,70*	103,1
Zeverjana	17,60*	105,4	14,30*	106,9	15,50	99,2	15,80*	103,7
Ambra	15,82	94,6	13,40	100,2	15,08	96,5	14,76	96,9
15/4-2	18,90**	113,2	14,90**	111,4	16,01	102,4	16,60*	108,9
720 - C	19,68**	117,4	14,76**	110,4	14,92	95,5	16,40*	108,8
159	16,83	100,6	14,65**	109,6	16,30*	104,3	15,93*	104,6
Control	16,70	100,0	13,37	100,0	15,63	100,0	15,24	100,0

* P ∠ 0,05 ** P ∠ 0,01 *** P ∠ 0,001

Table 4. Cold resistance of durum wheat mutants tested in a cold chamber

Mutants and cultivars	Surviving plants (%)			
	-11°C	-14°C	-17°C	-20°C
Zagorka-control	96	41	4	0
Ambra	97	94	30	14
Losen 76	89	74	31	11
Zeverjana	97	86	20	0
720 - C	87	85	17	0
159	100	57	8	0
15/4-2	94	73	26	4

They have very good combining ability and will be used for crossing with other high quality lines from Italy, France, Canada and with the best lines from Prof. Konzak, USA. Hybrids obtained in F_1 and F_2 progenies will be investigated for agronomic characters.

To increase the number of our semi-dwarf mutant stock of wheat, seeds of local, tall, cold resistant durum wheat cultivars have been treated with NaN_3, EMS and gamma rays separately and in different combinations. It was found that the most effective chemical mutagens are NaN_3 and EMS 2%, when used in combination.

Four mutants and eleven cultivars of different plant height were analysed for GA response. Two semi-dwarf *durum* wheat cultivars (Faia 54 cm and Zeverjana - 63 cm) were found to possess different GA sensitivity [7]. The results of the genetic analysis showed that the semi-dwarfing genes of varieties Faia and Zeverjana were allelic while mutants 15/4-2 and Ambra possess a different one, non-allelic, GA sensitive [5,6]. Genetic analysis showed that semi-dwarf mutant is independently segregated with locus.

Finally, our studies with induced semi-dwaff mutants of *durum* wheat indicated also, that some height reducing genes have good potential application in breeding.

The results show that if the methods of experimental mutagenesis are combined with those of classical breeding a large variability of *durum* wheat can be more effectively and more rapidly created.

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THE EXPLOITATION OF THE TOM THUMB DWARFING GENE, *Rht3*, IN F_1 HYBRID WHEATS

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Abstract

Nine F_1 hybrids, all with an Rht3 dwarf female parent, were produced using the Shell chemical hybridising agent, WL 84811. Yield trials show that on average the yield potential of the F_1s exceeded that of the high yielding parent by 17 per cent. The increase in yield potential in the Rht3/rht heterozygote was brought about by an increase in grain number of 32%. The increased yields in the intervarietal F_1s were obtained by improved filling at these extra grain sites. No evidence for an effect on tiller density was found.

A close correlation between heterosis for plant height and heterosis for grain yield was found between the hybrids which may provide a screen for rapid identification of high yielding F_1 combinations.

A cytological examination of tall 'off-types' among the F_1s indicated the presence of a high level of aneuploidy in Rht3/rht hybrids. The causes and consequences of this chromosomal instability are discussed.

INTRODUCTION

With the advent of efficient chemical hybridizing agents (CHAs), it is now possible to produce sufficient F_1 hybrid wheat grain for large scale yield trials from any parental combination. Therefore, in addition to being able to approach the question of whether F_1 hybrids are a realistic commercial proposition, it is possible to explore the use of specific major genes which are of little value in pure line breeding programmes.

The major dwarfing genes in wheat, some of which are now known to have dramatic effects on yield components (review, Gale and Youssefian 1985), present many such possibilities in F_1 hybrids. One such gene is Rht3, which is usually considered to have too extreme an effect on plant height as a homozygote. However, it offers a number of features of potential value to the production of F_1 hybrids. As a female parent, the yield of F_1 hybrid seed may be improved because of its high spikelet fertility (Gale and Flintham 1983) and the better pollination achieved by being surrounded by taller male parents. In the hybrid, the known additive effect of Rht3 on reducing plant height will give semi-dwarf phenotypes with good lodging resistance. But perhaps of greater importance is the possibility that the effect of Rht3 in increasing spikelet fertility is dominant so that the hybrid will have increased numbers of grain sites at which any hybrid vigour for improved grain size might be expressed.

For these reasons, a number of crosses involving an isogenic line of Rht3 in the variety Maris Huntsman as a female parent were made. A hybrid with Maris Huntsman was made to establish the effects of Rht3 itself in the heterozygous condition. Hybrids with a selection of other tall (rht) and semi-dwarf varieties were made to indicate what levels of heterosis could be expected when the effects of Rht3 and other sources of heterosis were combined.

MATERIALS AND METHODS

Genotypes

The female parent in the F_1 hybrid production plots was Huntsman Rht3 (HRht3), an isogenic line derived at the PBI by six backcrosses to the tall parent from an F_1 between Maris Huntsman and Minister Dwarf, a source of Rht3.

The male parents were all winter wheat varieties and included Maris Huntsman, a UK variety bred at the Plant Breeding Institute (PBI); Stuart, Banner, Aquila and Ambassador, UK varieties bred at Nickerson RPB Ltd., (NRPB); and Merkur, Vuka, Nimbus and Oberst, German varieties. All except Ambassador, which carries Rht2, are tall, rht, varieties.

F_1 grain production

The F_1 seed production blocks, which were grown at NRPB in 1984, consisted of a number of females sown in plots 24 m long by 1.1 m wide between drills of the same male parent. Each male block was surrounded by 12 m of the male parent and separated from other male blocks by at least 24 m. The females were sprayed with the Shell chemical hybridizing agent (CHA) WL 84811, azetidine-3-carboxylic acid, at the optimum growth stage and dose rate. Yields of F_1 grain were about 12 kg per plot. The absence of grain set on ears within the HRht3 plots bagged before male pollen was available and the absence of Rht3 homozygotes in the F_1 yield trials indicated that the degree of selfing was essentially zero.

F_1 yield trials

Four trials were carried out in 1985, two at the PBI and two at NRPB. At each location one trial was given the complete current NIAB/ADAS recommended prophylactic fungicide, herbicide and insecticide treatment (sprayed) and the other was untreated (unsprayed).

All the trials employed lattice designs with three replications at the PBI and two at NRPB. All plots were approximately 6 m x 1.16 m and the grain yields obtained are expressed as percentages relative to the means of the control varieties Aquila, Avalon and Norman.

At the PBI the plots were scored for plant height (cm to tip of the ear), tiller density (no. fertile ears in a 30 cm quadrant across the drill), anthesis time (estimated as 50 per cent anther extrusion in the plot), lodging (on a scale 0-9 one week before harvest), 1000 grain weight (estimated from the weight of 250 grains) and α-amylase levels (extracted from wholemeal flour and expressed in mU/g by calibration with the Phadebas (Pharmacia) method). From six random single leading tillers per plot, estimates were obtained for tiller yield, spikelet number per ear, grain number per ear, total tiller mass, non-productive (vegetative) tiller mass and tiller harvest index.

RESULTS

The 'sprayed' trials were carried out to estimate the potential grain yield of the F_1 hybrids, while the 'unsprayed' trials estimated performance when disease was not controlled. Both sites showed high levels of mildew, *Erysiphe graminis* and *Septoria tritici*, attack. At the PBI a late attack of brown rust, *Puccinia recondata*, may also have reduced the 'unsprayed' yields.

The F_1 combinations tested allow (i) observation of the effects of *Rht3*, as a heterozygote, unconfounded by other sources of heterosis in the isogenic HRht3 x Maris Huntsman F_1, (ii) observation of the levels of heterosis obtained over a sample of seven different tall parents in the presence of *Rht3/rht*, and (iii) an indication of how *Rht3/rht*, *rht/Rht2* varietal hybrid F_1s might perform from the HRht3 x Ambassador F_1.

Yield in the 'sprayed' trials

The results are shown in detail in Tables 1 and 2 and estimates of heterosis are shown in Table 3. The yield data for the parents and F_1 hybrids is also shown in Figure 1.

The grain yields indicate that the *Rht3* allele as a heterozygote may directly effect yields. The net effect of 3.7 per cent grain yield advantage over Maris Huntsman seen in the HRht3 x Huntsman F_1, although nonsignificant, was derived from major changes in ear conformation, i.e. an increase in grain number per ear of 44% with a concurrent decrease in grain weight of 18%. No effect on tiller density was evident.

The F_1s between HRht3 and the seven tall varieties all produced higher yields than either parent, even when the yield potential of HRht3 is considered to be that of Huntsman itself (Table 1, Figure 1). This was necessary to avoid comparisons involving HRht3 which performed extremely poorly in 1985. The heterosis figures range from 10% to 35% relative to 4% for HRht3 x Huntsman F_1. The yield component analysis on tiller samples from the same plots clearly shows the source of these increases in yield. Increases in grain numbers are similar for all F_1s, including HRht3 x H, indicating that this is most likely due to the effects of *Rht3*. Grain size, however behaves quite differently in the intervarietal hybrids. The heterosis values for grain size are -18% for the isogenic cross and -5% over the intervarietal HRht3 x *rht* F_1s, with the result that the mean heterosis for single ear grain yield is increased to 31%.

The biomass data indicates that heterosis for grain size is associated with heterosis for plant size (Table 1). The intervarietal F_1 hybrids are taller, relative to the mid-parent, and have increased straw

Table 1. Yield and yield related characters in parental and F_1 hybrid 'sprayed' trials

	Mean plot yield % controls[1]	Height cm[2]	Tiller yield (g)	Vegetative tiller yield	Grain No. (ear)	1000 grain weight (g)	α-Amylase level (mU/g)[3]
Huntsman	89.8	112.0	2.53	2.62	41.0	57.8	61.5
F_1 HRht3 x H	93.1	84.7	3.06	2.43	58.9	47.5	19.5
Merkur	92.1	116.7	2.58	2.80	44.5	52.1	19.3
F_1 HRht3 x M	112.7	91.7	3.34	2.69	52.0	52.0	39.0
Aquila	100.8	112.7	2.07	2.41	39.3	50.0	4.6
F_1 HRht3 x Aq	109.9	90.7	3.05	2.46	52.3	51.6	9.0
Vuka	87.9	133.0	2.76	3.47	47.0	53.6	2.7
F_1 HRht3 x V	106.1	94.0	3.48	2.94	61.3	54.2	14.5
Banner	78.3	97.3	2.05	2.01	49.5	37.0	2.4
F_1 HRht3 x B	110.2	88.3	3.14	2.63	63.9	46.6	12.1
Stuart	88.5	109.0	2.75	2.91	53.2	50.0	6.1
F_1 HRht3 x S	116.2	90.7	3.16	2.62	56.5	52.4	18.3
Nimbus	81.6	114.3	1.94	2.42	40.3	46.4	3.5
F_1 HRht3 x N	98.4	92.0	3.02	2.73	56.3	49.1	12.4
Oberst	81.8	118.3	2.19	2.70	44.5	44.1	2.6
F_1 HRht3 x O	94.7	90.7	3.16	2.62	62.2	45.8	6.7
Ambassador	95.8	91.7	2.43	2.13	54.3	42.5	29.0
F_1 HRht3 x Am	96.4	73.3	2.81	2.16	56.6	46.3	42.3
HRht3	64.3	59.7	2.55	2.06	52.5	46.9	41.5
SED	4.36	204	0.182	0.272	3.49	1.53	e^x (.44)
rht parents (mean)[4]	87.2	114.5	2.33	2.67	45.8	47.6	5.9
HRht3 x rht F_1s (mean)	106.9	91.2	3.19	2.67	58.9	50.2	16.0
SED	2.12	.77	0.069	0.103	1.32	0.58	e^x (.17)

Notes

1 Yield figures are means over 3 replicates at PBI and 2 replicates at NRPB, relative to the means of Norman, Avalon and Aquila checks at both sites. 100 = 8.46 t h^{-1} at PBI and 7.03 t h^{-1} at NRPB.

2 All remaining data from PBI trial only.

3 α-Amylase analysis carried out on $\log_e$ transformed data.

4 The mean figures are obtained from the seven intervarietal rht parents and F_1s, i.e. excluding Huntsman and Ambassador.

Table 2. Tiller density, anthesis date and spikelet number per ear in parents and F_1 hybrids in fungicide treated trial

	Tiller No. (m^{-2})	Anthesis date (days)	Spikelet No. (ear)
Huntsman	472	2.0	19.3
F_1 HRht3 x H	469	1.0	19.8
HRht3	403	0.0	19.0
Other parents (mean)[1]	532	4.8	21.8
F_1 (mean)[1]	460	1.3	21.0
SED[2]	38.3	.70	.54

Notes

1 Parental and F_1 means obtained from all intervarietal hybrids.

2 'SED' applies to differences between means and single genotypes.

Table 3. Estimates of heterosis in F_1 hybrids in fungicide treated trials

F_1 combination HRht3 x ...	Mean plot[1] yield[1]	Height[2]	Tiller yield	Vegetative tiller mass	Grain No. per ear	1000 grain weight
Huntsman	+3.7	-4.6	+21.0	-7.3	+43.7	-17.7
Merkur	+23.9	+12.3	+30.7	-0.7	+39.4	-5.3
Aquila	+15.3	+17.0	+32.6	-2.2	+30.3	-4.2
Vuka	+19.4	-6.4	+31.6	-3.4	+39.3	-2.6
Banner	+31.1	+52.2	+37.1	+13.6	+41.2	-1.7
Stuart	+30.3	+25.8	+19.7	-5.2	+20.0	-2.8
Nimbus	+14.8	+18.4	+35.1	+8.32	+38.5	-5.6
Oberst	+10.4	+5.8	+33.9	-1.5	+45.5	-11.1
Ambassador	+3.9	-15.0	+13.3	-10.03	+18.8	-7.6
SED[3]	4.60[4]	5.28	6.59	10.73	6.56	3.13
F_1 HRht3 x <u>rht</u>	+20.7	+17.9	+31.5	+1.37	+36.3	-4.8
SED[4]	3.48[4]	3.93	4.90	8.11	4.96	2.37

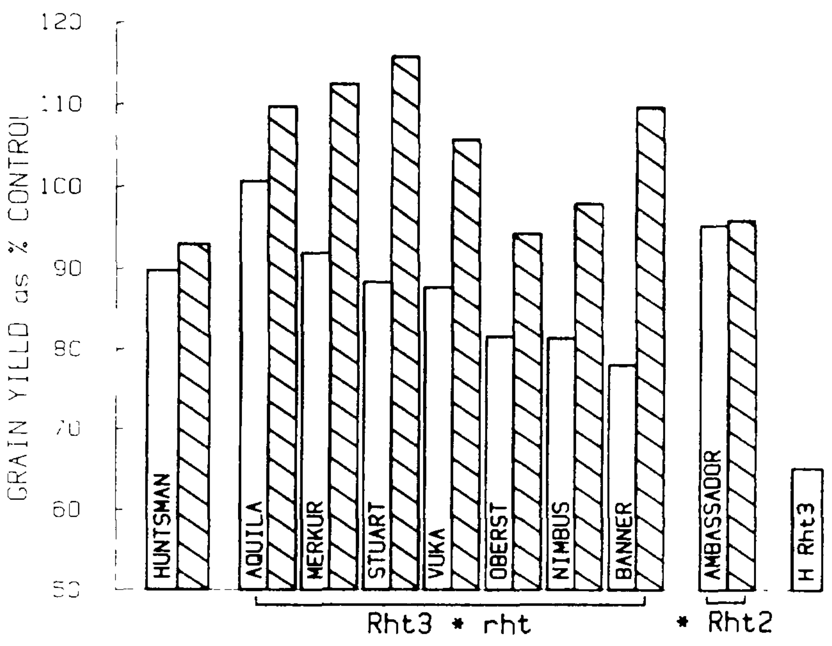
Notes

1 All values, except those for plant height, calculated as F_1/((Huntsman + male parent)/2)%. Thus the values for the HRht3 x Huntsman hybrid measure the effects of Rht3 in the heterozygous state, while in the other hybrids the values measure the heterosis in a Huntsman x P2 hybrid in the presence of Rht3/rht.

2 The values for plant height have been obtained as deviations about the midparent (=0) on a scale of HRht3 = -100 and P2 = +100.

3 SED (calculated as $\sqrt{(EMS/n_1 + EMS/n_2) \cdot 10^4/\bar{x}^2}$ where n_1 and n_2 are the number of replications in the means and $\bar{x}$ = grand mean) applies to differences in heterosis estimates between hybrid combinations.

4 SED applies to differences from the mean midparent value (see notes 1 and 2).



Note. The open bars are pure line varieties and the hatched bars the F_1 hybrid of that variety with HRht3. The LSD 5% for parental v F_1 hybrid differences is 8.6%.

Figure 1. Parental and F_1 hybrid yields.

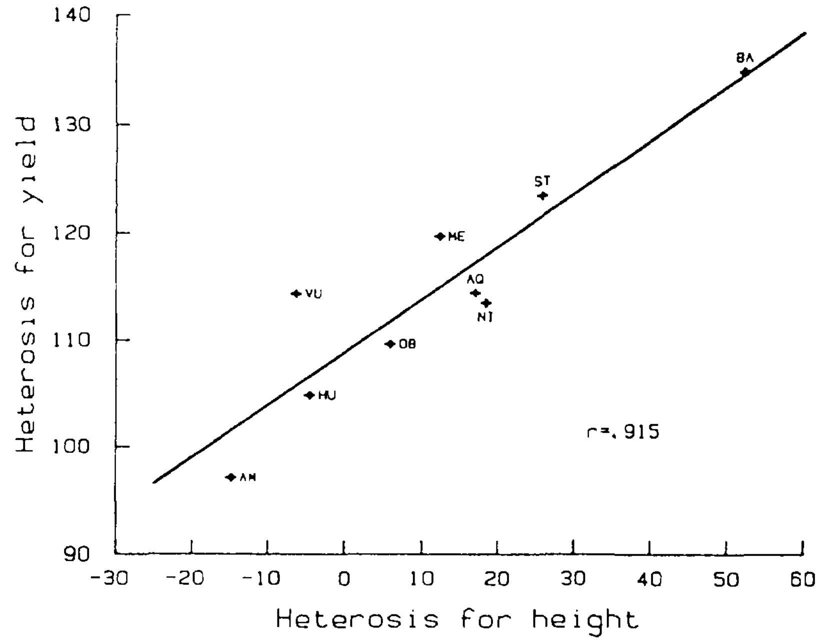
weights relative to the means of Huntsman and the male parent even though plant heights are reduced by 14% overall due to Rht3/rht. Observation of tiller density data indicates that this is not a factor involved in the heterosis for yield. Indeed, the means shown in Table 2 indicate that high tiller number is an almost completely recessive character in the limited number of hybrids included in this investigation.

Anthesis data shows simple dominance for earliness and spikelet number per ear shows no dominance. Neither of these characters appear to be contributory factors to the increased yields demonstrated by the F_1 hybrids.

The hybrid involving the semi-dwarf male parent Ambassador showed little promise, possibly because the spike fertility was already high due to Rht2 and because the vegetative biomass, although not further reduced in the short F_1, may not have been adequate to support the grain filling found in the HRht3 x rht hybrids.

The association between plant size and grain yield in F_1s

As noted above, the increased grain yields in the hybrids are obtained on relatively larger and taller plants. Reasonable positive correlations were obtained between heterosis for yield and heterosis for vegetative tiller weight ($r = .68$, $p = .063$) and grain size ($r = .75$, $p = .034$), however the closest relationship was achieved using the plant height data. Figure 2 shows the relationship between heterosis for height (as calculated and shown in Table 1) and heterosis for yield. The correlation is $r = .91$, $p = <.001$.



Note. Heterosis calculated as described in Table 3. Yield and height data taken from PBI sprayed trial only.

Figure 2. The relationship between heterosis for plant height and heterosis for yield among nine F_1 hybrids.

α-Amylase levels

The α-amylase levels were generally high in the 1985 UK harvest because of the cool, wet, grain ripening conditions. In these trials the the preponderance of Huntsman 'genes' increased levels still further, Huntsman, and some other UK varieties, characteristically producing α-amylase before maturity even in the absence of visible preharvest sprouting (Gale, Flintham and Arthur, 1983).

The Rht3 allele, however, tends to reduce α-amylase in germinating grain because it confers relative insensitivity to gibberellin upon the aleurone cells (Gale and Marshall, 1975). The allele can, as a homozygote, reduce enzyme levels by 77% in a sprouted crop (Flintham and Gale, 1982). Of course, the grain on an F_1 crop will segregate as an F_2 for endosperm characters, and the Rht3 allele will be present in the ratio 3 doses; 2 doses: 1 dose: 0 dose in the triploid aleurone tissue.

The enzyme levels shown in Table 1 demonstrate that the F_1s generally fall below the midparent calculated from Huntsman and the male parent, and are related to the male parent level. This may reflect an effect of Rht3, however the advantages in terms of breadmaking quality would be slight.

The levels found in HRht3 (41 m U/g) are unexpectedly high compared with those found in the taller heterozygous genotypes. One explanation may be that the moisture levels in the HRht3 stand were even higher than elsewhere, because the ears were close to the ground and were protected from any drying wind by the surrounding taller plots.

Yield in the unsprayed plots

The mean values and levels of heterosis for grain yield and yield components are shown in Tables 4 and 5. The yield data are summarised in Figure 3.

Table 4.

Yield and yield related characters in F_1 hybrids between HRht3 and Huntsman, seven tall varieties and Ambassador, an Rht2 semi-dwarf in unsprayed trials[1]

	Mean plot yield % controls	Tiller yield (g)	Vegetative tiller yield (g)	Grain no. (ear)	50 grain weight (g)[2]
Huntsman	101.2	2.18	2.35	44.8	2.44
F_1 HRht3 x H	84.2	2.17	2.10	58.1	1.87
Tall parents (mean)	98.5	1.72	2.26	43.7	1.96
HRht3 x F_1s (mean)	94.3	2.35	2.48	58.7	1.99
Ambassador	106.9	1.78	1.84	56.3	1.59
F_1 HRht3 x Am	82.3	2.45	2.18	59.8	2.06
HRht3	51.3	1.89	1.84	50.3	1.88
SED (i)[3]	5.55	0.210	.167	3.57	0.141
SED (ii)	4.19	0.159	0.128	2.74	0.108

Notes

1 As in Table 1, the yield figures obtained from both PBI and NRPB trials and the component data from PBI trial only. % Yield figures relative to 'unsprayed' treated control means.

2 Grain weights calculated from sampled ears.

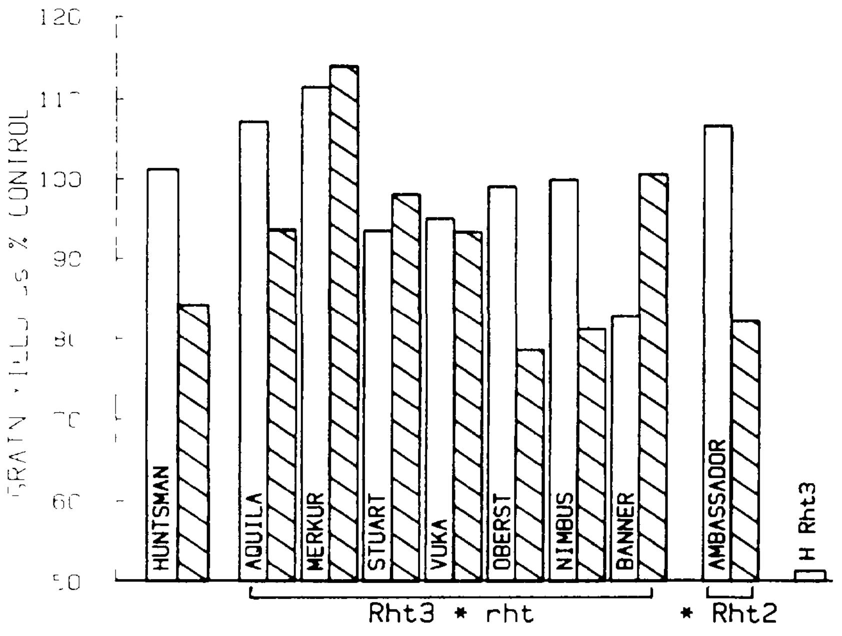
3 SED (i) applies to differences between single genotypes, (ii) applies to differences between means and single genotypes.

Table 5. Estimates of heterosis in F_1 hybrids in unsprayed trials

F_1 combination HRht3 x ...	Mean plot yield	Tiller yield	Vegetative tiller yield	Grain No.	50 grain weight
Huntsman	-16.8	-0.5	-10.6	29.7	-23.4
rht Parents (mean)	-5.4	+19.3	+7.8	+32.7	+9.0
Ambassador	-20.9	+23.7	+4.1	+18.3	+2.2
SED	6.34	+7.62	+5.55	+5.11	+5.3

Note

Calculations and SEDs as in Table 3.



Note. As Figure 1. LSD 5% = 12.7%.

Figure 3. Parental and F_1 hybrid yields in unsprayed trials.

In those conditions the hybrids are generally less productive than the pure lines although the intervarietal F_1s are relatively higher yielding than the HRht3 x Huntsman F_1. The yield components and heterosis values rank similarly to those obtained in the sprayed trials, but all at less productive levels.

This may not reflect the performance of all F_1s under these conditions and, indeed, conflicts with results obtained with other F_1s trialled at the PBI in 1985 where levels of heterosis were generally higher in the unsprayed plots (J. Bingham, pers. comm.). The male parents were not selected for their levels of resistance to disease and were particularly susceptible to mildew. In addition, the common female parent has little to offer in terms of dominant genes for resistance to any of the diseases encountered. In addition the short statured *Rht3/rht* and *Rht3/rht*, *rht/Rht2* F_1 may have succumbed more rapidly to the severe attack of the splash transmitted *Septoria tritici* than the taller pure lines.

Chromosome stability

A feature of the *Rht3* hybrid trial was the presence in all the F_1 plots of between three and 20 tall plants, at least 20-30 cm taller than the bulk of the stand.

Ears from these plants were harvested separately, the grains germinated and the root tips scored for chromosome number. All the plants scored were shown to be monosomic, i.e. to have 41 rather than the euploid 42 chromosomes. The explanation for the tall plants must be that they represent monosomics for chromosome 4A, the location of the *Rht3* gene. Monosomics which have lost the homologue derived from the tall parent will still be dwarf, although probably a few centimetres taller than euploid plants, but the half which have lost the 4A chromosome derived from HRht3 will be tall, taller in fact, than a euploid rht/rht.

The conclusion that the monosomy occurred due to instability in the developing F_1 grain, is supported by the observation that two height classes of tall monosomic plants were observed in the HRht3 x Ambassador F_1. The shorter class is consistent with monosomy for the 4D chromosome carrying Rht2 derived from Ambassador while the taller class were presumably monosomics for the 4A derived from HRht3.

As yet it is not clear whether all chromosomes are as unstable as 4A and 4D in Rht3/rht hybrids. In some Rht2 semi-dwarf pure line varieties where similar tall monosomics for chromosome 4D mar the appearance of the stand, there is some evidence that this single chromosome is that most likely to be lost. However the levels of monosomy for 4D at 0.131% (Worland and Law, 1985) are some ten times lower than observed for 4A here.

CONCLUSION

The yield levels of the seven intervarietal F_1s incorporating the Rht3 gene in the heterozygous state are encouraging. The mean figures of 20.7% over the midparent, or 16.7% over the highest yielding parent, were obtained in trial conditions which are likely to give a reflection of yield potential. The actual levels of yield were high (9.04 tonnes h^{-1}) and higher than the check varieties, all of which were on the National Institute of Agricultural Botany recommended list of varieties for use by farmers in 1985 (Anon, 1985). This is not withstanding the fact that many of the varieties used in the F_1 combinations were either rather old, such as Maris Huntsman which was released in 1971, or not bred and adapted for UK conditions as is the case for all the German varieties. It seems likely therefore that, with more modern parents, especially those with improved levels of disease resistance, even greater yields could be obtained. It should be said, of course, that such tall parents may have to be bred especially for an Rht3/rht F_1 programme because most of the UK varieties released since the mid-1970s carry the Rht2 semi-dwarfing gene.

The source of the yield increases were similar in all the Rht3/rht F_1s. Increased spikelet fertility (+36% over the midparent) was introduced by the single Rht3 allele and then the generally improved vigour derived from heterozygosity elsewhere, reflected in improved size and mass in the hybrids, was available to fill the extra grains set. Thus there is no evidence here that developmental events or timing are affected in F_1s. All improvements are consistent with faster growth rates throughout the plant.

This use of Rht3 demonstrates that parents may have to be designed and bred especially for use in F_1 hybrids. Here we have specifically improved one yield component, i.e. the number of grain sites, so that the hybrid vigour may be channeled into grain growth rather than vegetative growth. Other major genes which effect development may have similar potential.

The effects of Rht3 on plant height represent another 'design feature' in these F_1s. Hybrids are often relatively tall because minor genes effecting plant height are usually dominant for tallness, and as seen here in the HRht3 x Banner F_1, the highest yielding combinations will produce the tallest F_1 relative to the parents. Thus, hybrids between *Rht1* or *Rht2* semi-dwarfs and *rht* genotypes will usually be too tall and prone to lodging. Even homozygous *Rht1* or *Rht2* (or the double heterozygote *Rht1/rht*, *rht2/Rht2* which has the same effect) produced by crossing two semi-dwarf varieties will often be too tall for the high input husbandry employed on much of the UK acreage. Three allele hybrids, e.g. *Rht1/Rht1*, *rht2/Rht2*, provide a similar solution to that reached with *Rht3*, however the breeding of suitable two gene dwarfs as parents may present more difficulties than the incorporation of a single *Rht3* gene.

The hybrids tested here provide little information on the breadmaking quality to be expected in F_1s. The use of the Huntsman varietal background has raised levels of α-amylase in the hybrids so that any adverse effects of 'vigour' on this character may have been obscured. For the same reason any beneficial effects of the GA-insensitivity introduced with *Rht3* may also have been obscured, however the fact that the grain on the F_1 crop was segregating for *Rht3* will reduce its efficiency by at least 50% anyway.

Among the correlations between plant characters and the expression of heterosis for yield, the relationship with plant height (relative to the parents) is particularly encouraging and may provide the basis of an early screen for high yielding combinations. This character can, of course, be gathered from only a few plants, which could be grown in a glasshouse out of season. Work is in progress to assess the value of this relationship in other, non-*Rht3*, hybrids.

The major disappointment surrounding these experiments, with a view to the commercial exploitation of *Rht3* in F_1s, is the presence of tall monosomics in the stands, at levels much higher than allowed by the EEC standards of purity for conventional varieties. More work is required to determine the source of these aneuploids, but at present the possibility that a direct effect of the GA-insensitive dwarfing gene on chromosome loss in F_1s cannot be ruled out.

We conclude, however, that the high levels of heterosis for yield combined with an ideal plant height to provide lodging resistance make *Rht3* a realistic commercial proposition for hybrid wheat. The gene must first be incorporated in high yielding tall backgrounds with improved quality and disease resistance characteristics. These hybrids made with modern tall genotypes, again probably bred especially for this purpose, will demonstrate the full extent of the yield advantages to be gained in *Rht3/rht* F_1 hybrids.

ACKNOWLEDGEMENTS

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COMPARISON OF TWO SEMI-DWARFING GENES ON YIELD PERFORMANCE OF DURUM WHEAT LINES*

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Abstract

To evaluate the effects of two different semi-dwarfing genes on yield, two cultivars of durum wheat bred at ENEA were used. "Creso" has the Rht1 gene and is insensitive to gibberellin at seedling stage. "Tito" has reduced stature due to a semi-dominant semi-dwarf (SD) mutation induced by gamma ray and is gibberellin responsive.

Sixty-six F_4 randomly selected lines coming from the cross of the above mentioned varieties were compared in agronomic trials. Half of them were gibberellin insensitive, like Creso and half gibberellin responsive, like Tito.

Grain yield of the SD lines was higher than that of the Rht1 lines, even though the difference was relatively small and not significant. In the subsequent generations F_5 and F_6 the best performing lines of these two semi-dwarf types gave on the average the same yield results; thus showing that the reduced height mutation (SD) appears as good as the Rht1 gene for breeding purposes. However, it has to be taken into account that the difference in plant height in favour of the taller SD lines (about 15 cm) may compensate for the Rht1 effect based on the positive yield-plant height correlation.

Introduction

The wheat production areas covered by semi-dwarf varieties are increasing year by year worldwide. This is due to the spread of a few height reducing genes, among which those deriving from Norin 10 appear to be the most relevant. Whether the great success of such genes is simply due to a fortunate case or to their pleiotropic effects on yield in addition to the benefit deriving from lodging resistance is still an open issue (1). Yet the induction of reduced height mutants

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in wheat through the use of both physical and chemical mutagenic agents is relatively easy (2). Such circumstances offer the possibility to evaluate genetic as well as environmental interactions of plant height reducing genes coming from different genetic sources (3).

Suitable material for this kind of investigations resulted from the durum wheat breeding program set up in late 1950's at Casaccia Nuclear Center under the aegis of the Italian Atomic Energy Commission (CNEN).

Materials and Methods

Creso and Tito are two semi-dwarf wheat cultivars released in 1974 and 1976, respectively (4). The former has the **Rht1 gene** which markedly reduces plant height, whereas the latter has reduced stature because of a semi-dominant SD (Semi-Dwarf) mutation induced by gamma rays (Table I).

TABLE I - Plant height, grain yield and test weight of two durum wheat cultivars. (Averages of 80 agronomic trials carried out in Italy for 8 years)

CULTIVARS	PLANT HEIGHT cm	GRAIN YIELD q/ha	TEST WEIGHT kg/hl
CRESO	75	58.83	81.95
TITO	87	58.43	77.37

Tito is 10 - 15 cm taller and 3 - 4 days later than Creso in ear emergence time. Furthermore, Tito shows good cold resistance, elastic culms and high yield potential. In some areas of Central and Northern Italy Tito can easily outyield Creso. However, it presents also some weak points, being susceptible to both leaf rust and powdery mildew. These diseases are rather endemic in Italy, but represent a high risk factor which accounts for the disappointing performance of Tito in terms of yield and test weight in some circumstances.

To evaluate the effects of the two semi-dwarfing genes on other characters, particularly yield, the cultivars Creso and Tito were crossed in 1980 and the progenies were analyzed in the following years according to the procedures described previously (3).

Field trials initiated with F_3 lines selected only for gibberellic acid response. By using the gibberellin test, consisting in growing germinated seeds in a solution with 10 p.p.m. of GA_3 for 6 - 8 days, three groups of F_2 plants were identified

1 - Homozygous GA_3 - Insensitive semi-dwarf plants (<u>Rht1/sd</u>).

2 - Homozygous GA_3 - Responsive semi-dwarf plants (<u>rht1</u>/SD).

3 - Homozygous GA_3 - Responsive tall plants (<u>rht1/sd</u>).

Assuming that the frequency of both useful and detrimental genes are homogeneously distributed in the three groups of plants as classified above, any difference in the mean values among the groups can be reasonably attributed to the action of the specific genes affecting plant height.

Starting with F_3 seeds a spaced-planted field experiment based on a total of 80 F_3 families replicated 5 times was carried out. However, for a better appraisal of the two major semi-dwarfing genes on yield and yield components, 33 Creso-like and 33 Tito-like F_4 lines were compared in a field trial. All lines and the controls (Creso and Tito) were sown in plots of 3 m^2 (1.5 x 2) according to a randomized complete block design with 3 replications.

Climatic conditions were nearly normal for wheat production in Central Italy. Shortage of rain occurred in April and May, but the grain yield was only slightly affected. Diseases and pests were negligible.

In the subsequent years the number of lines tested was restricted to the better yielding ones. In the F_5 generation the total number was 14, whereas in F_6 it decreased to 11. However, a balance between the semi-dwarf types was maintained. For the last two generations the size of the plots was 10^2 with the same randomized complete block design with 3 replications.

Results and Discussion

The results obtained from the F_3 families showed a small advantage in yield per plant for the gibberellic acid insensitive (GAI) progenies over the responsive semi-dwarf types. In the F_4 generation the grain yield of the SD lines (GA_3- responsive) was significantly higher than the **Rht1** lines (GA_3- insensitive), even though the difference was only 200 kg/ha (Table II). The SD lines showed also a higher test weight as compared with the **Rht1** lines, while the opposite situation occurred for Tito vs. Creso.

TABLE II - Performance of random selected F_4 lines coming from the cross CRESO x TITO

PARAMETERS GENOTYPES	N° OF F_4 LINES	PLANT HEIGHT cm	EAR EMERGENCE DATE	GRAIN YIELD q/ha	TEST WEIGHT kg/hl
CRESO **(Rht1/sd)**	-	84	MAY 9.3	64.1	82.4
TITO **(rht1/SD)**	-	103 **	MAY 10	64.7	81.4
F_4 LINES **(Rht1/sd)**	33	84.3	MAY 8.6	63.0	80.6
F_4 LINES **(rht1/SD)**	33	102.6**	MAY 9.1 *	65.0*	82.2*

*** Significantly different at $P < 0.05$**

**** Significantly different at $P < 0.01$**

The plant height of Creso and that of the corresponding Rht1 lines were on the average 84 cm and 84.3 cm, respectively. Tito and SD lines were taller (103 cm and 102.6 cm, respectively). All the genotypes tested showed an increase of the plant height in respect to the values obtained the previous year. The difference between Creso and Tito was also greater: 19 cm against 10-12 cm as normally occurs.

The results obtained from the comparison of the best 7 lines from each group showed that the yield potential of the F_5 selected lines was higher than that of the best control, irrespective of the presence of the either semi-dwarfing gene (Table III). In particular the F_5 Creso-like lines yielded 64.9 q/ha, whereas the Tito-like plants reached 63.1 q/ha. However, the difference of 180 kg/ha was not statistically significant. Test weights were similar.

TABLE III - Performance of 14 selected F_5 lines coming from the cross CRESO x TITO

PARAMETERS GENOTYPES	N° OF F_5 LINES	PLANT HEIGHT cm	EAR EMERGENCE DATE	GRAIN YIELD q/ha	TEST WEIGHT kg/hl
CRESO **(Rht1/sd)**	-	83	MAY 17.5	56.3	82.6
TITO **(rht1/SD)**	-	101 **	MAY 20.5	59.0	80.1
F_5 LINES **(Rht1/sd)**	7	82.9	MAY 16.4	64.9	81.3
F_5 LINES **(rht1/SD)**	7	95.4**	MAY 18.4	63.1	81.6

**** Significantly different at $P < 0.01$**

Following the same testing procedure, 6 F_6 lines (<u>Rht1</u>/<u>sd</u>) were compared with 5 F_6 lines (<u>rht1</u>/<u>SD</u>). The chosen lines were only those which in the previous year yielded at least 60 q/ha. Here again the two groups of lines behaved quite similarly (Table IV and V). The yield response of the control varieties Creso and Tito in the last field trial deserves a special comment. A dramatic collapse of the cv. Tito in terms of yield and test weight took place. It gave only 43.2 q/ha as compared with the

TABLE IV - Performance of 11 selected F_6 lines coming from the cross CRESO x TITO

PARAMETERS GENOTYPES	N° OF F_6 LINES	MILDEW Scale (0-9)	LEAF RUST Scale (0-9)	GRAIN YIELD q/ha	TEST WEIGHT kg/hl
CRESO **(Rht1/sd)**	-	4	0	63.5**	84.4**
TITO **(rht1/SD)**	-	9	7.5	43.2	79.4
F_6 LINES **(Rht1/sd)**	6	6.4	3.7	54.1	81.5
F_6 LINES **(rht1/SD)**	5	7.4	3.9	54.3	82.1

** Significantly different at $P < 0.01$

TABLE V - Summary of the yield data from short straw lines from the cross Creso x Tito, differing for two semi-dwarfing genes.

GENERATION	CRESO q/ha	TITO q/ha	LINES **Rht1/sd**		LINES **rht1/SD**	
			No.	Yield q/ha	No.	Yield q/ha
F_4	64.1	64.7	33	63.0	33	65.0
F_5	56.3	59.0	7	64.9	7	63.1
F_6	63.5	43.2	6	54.1	5	54.3
MEANS	61.3	55.6		60.9		60.8

63.5 q/ha produced by Creso. As to the test weight a difference of 5 kg/hl was observed (Table IV). Such a behaviour is no doubt related to both disease attacks (mildew and leaf rust) and drought stress throughout the maturity stage. Even the selected lines were partially affected at comparable levels and gave yield and test weights intermediate as compared with the control parents.

Therefore the results obtained after 3 years of field testing at normal sowing rate and conventional agronomic practices would indicate that the induced dwarfing gene is from the breeding point of view as good as the spontaneous Rht1 gene coming from Norin 10. However, it has to be taken into account that the difference in plant height in favour of the rht1/SD lines (about 15 cm) may compensate for the Rht1 effect if the positive yield-plant height correlation holds true.

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GENETIC ANALYSIS, GENETIC IMPROVEMENT AND EVALUATION OF INDUCED SEMI-DWARF MUTANTS IN WHEAT*

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Abstract

Recent results from breeding studies in T. aestivum wheats indicate that improved high yielding recombinants that carry the reduced height gene Rht13 from the semi-dwarf mutant Magnif 41 M1 in combination with Rht2 have been isolated. These improved lines should be useful in further breeding. In genetic analyses, additional data have confirmed that the reduced height gene Rht12 from the mutant Karcag 522M7K is strongly dominant, while typical epistatic, partially additive interactions may occur with other Rht genes and recombinations with different Rht or reduced height alleles can produce taller or shorter derivatives. Thus, the degree of dominance or recessiveness of Rht genes appears to be a continuum, with their expression in crosses further modified by epistatic interactions with other Rht alleles.

Mutant Burt M860 was found to carry a new mutant gene Rht20 that is partially dominant for reduced height. The reduced height gene rht11 of Bezostaja dwarf mutant Karlik-1 was largely recessive in the four combinations studied.

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In *T. turgidum durum*, the partially dominant *Rht14* gene of 'Castelporziano' showed independent inheritance from *Rht1*. The inheritance of two other partially dominant induced mutant genes, respectively *Rht16* of Edmore SD1 and *Rht18*, of 'Icaro' (from E.N.E.A., Italy) differed from *Rht1* and *Rht14*. The *Rht15* locus of 'Durox' showed less dominance than *Rht14*, and the two genes were independently inherited.

Significant new useful genetic variation for breeding improved semi-dwarf bread and durum wheat cultivars has been induced. These mutants offer breeders greater freedom in choosing *Rht* genes and combinations for cross-breeding to control straw height and lodging and to improve harvest index.

1. INTRODUCTION

Genetic sources for plant height reduction in cereal crops are now widely recognized as the most effective means for reducing lodging susceptibility. Plant height reduction usually is a major contributor to lodging resistance and to higher harvest index, permitting the greater exploitation of genotypic yield potential under more optimal production conditions. Moreover, some reduced height cultivars also are well adapted to lower production, drought stress conditions.

New genetic sources for plant height reduction are needed in wheat, either to complement the widely used 'Daruma' (*Rht1* and *Rht2*) gene and 'Akakomughi' (Akakomugi) sources (*Rht8* and *Rht9*) or to replace them whenever associated weaknesses, e.g., reduced coleoptile length, have yield limiting effects on stand establishment or on other properties [1]. There is also the concern for genetic vulnerability due to possible associations of disease susceptibilty with the reduced height genes currently in extensive use. However, as of now, only Karlik 1 (PI504549), of the new mutant semi-dwarfing sources in bread wheats, has been successfully exploited in improved reduced height cultivars. 'Odesskaya 75' and 'Odesskaya Polukarlikovaya' were developed and used in the USSR [2].

Several other height reduced mutant cultivars have been developed, but no other inducted mutant *Rht* gene is so widely distributed in cultivation or used in breeding hexaploid wheats as *Rht11* from Karlik 1. In the durum wheats, only *Rht1* of the Daruma genes is in wide use (*Rht2* is located in the D genome). Consequently, most of the semi-dwarf durum wheat cultivars in production today carry *Rht1*. However, six derived cultivars, 'Tito' and 'Augusto' (Italy), 'Miradur', 'Attila', 'Grandur' and Signadur (Austria) carry the induced mutant gene *Rht14* from 'Castelporziano' [3-12].

This report describes progress in on-going research with reduced height mutants of cultivated hexaploid and tetraploid wheats.

2. MATERIALS AND METHODS

2.1 Genetic and breeding stocks

T. aestivum wheat - Many studies still in progress involve the hard red winter wheat mutant Karcag 522M7K, described earlier [12, 14]. Karcag 522M7K is GA_3 sensitive, about 60% as tall as 'Karcag 522', and has normal lax spikes, but is later maturing. Its coleoptile length is about the same as the parent. Plants of the mutant have wide leaves, large diameter culms that tend to spread at the base. Burt M860 (PI503551), a reduced height mutant of the hard white winter wheat 'Burt', has normal lax spikes but may carry other mutant traits, since the plant is densely waxy, and a low frequency of defective (weak, semi-sterile) plants occurs in the progeny of crosses. 'Marzotto' (CI15267) is a very short Italian cultivar which probably carries *Rht8* and *Rht9* like 'Mara' (PI244854) [14]. Other 'tester' genotypes will be described where used.

T. turgidum L. *durum* wheats - *Rht1* sources used in crosses include Lloyd 'S' = Cando/Edmore Sel. from North Dakota, and two locally developed lines WA6518 (PI499364) and WA6754 (PI499366). The mutant sources in the studies included 'Castelporziano' (PIPI347731) which carries an incompletely dominant single gene Rht14 [15, 16]. Unlike the Rht1 stocks, Castelporziano is GA_3 sensitive, shorter in height than Lloyd 'S' and has compact (club-like), awned spikes. Edmore SD1 (PI499362) is a reduced height mutant induced recently in the tall, high quality cultivar Edmore (CI17748). It is GA sensitive, and appears to carry some secondary genetic changes. Durox (PI478306) is a reduced height mutant carrying Rht15, induced by EMS in the Washington State University research program. It is about 10-12 cm taller than the Rht1 genotypes. Durox was registered in France (1980) as 'Cargi Durox' by Gavadour Cargill and later (1984) released as Durox in Idaho, U.S.A [10]. 'Icaro' (PI503555), an Rht18 mutant from 'Anhinga' was released in 1986 in Italy (Rossi, E.N.E.A., personal communication, 1985). Studies at E.N.E.A. showed that Rht18 differs genetically from Rht1. The newer Rht19 mutant Vic SD1 (PI503553) has been subjected only to preliminary investigations. The only other reduced height durum mutant cultivar known to date, GO 367, released in Greece [9] was not available for these studies.

2.2 Breeding studies

Magnif 41 M1 (CI17689) derivatives - Reselections were made mainly from two crosses with this mutant and the Rht2 carriers 'Wared' (CI15926) or 'Borah' (CI17267) [11]. Several of these selections were evaluated in yield trials from 1983-85. Derivatives of a single backcross to Magnif 41 are in the F_5 generation. Generation advances were made with spring and winter crosses involving Karcag 522M7K, and selections with improved phenotype are under increase.

2.3 Gene interaction studies

Additivity, dominance, and epistastic interactions among reduced height genes in both bread and durum wheats have been investigated in the F_2 and later generation progeny of selected crosses, especially with sources of Rht1, Rht2 and the Rht mutants previously mentioned. Culm height segregation results from several crosses will be described.

2.4 Height measurements, coleoptile length measurements, GA_3 responses

Plant heights generally were recorded as the length of the longest culm of each plant. Coleoptile length measurements were made after growing seedlings in the dark for seven days [16]. For GA_3 response information, the seedlings used for coleoptile measurements were grown in 1/4 Hoagland nutrient solution plus 10 ppm GA_3 (commercial), then continued in the light for an additional seven days or until the first leaf sheath was fully extended and the second leaf was visible [17].

3. RESULTS AND DISCUSSION

3.1 Genetic analyses

T. aestivum wheats - Crosses to estimate the epistatic interactions, dominance and independence of several Rht gene sources were made and studied through F_2. Because of their number, only a few of the segregations obtained are illustrated (Figs. 1-12). Preliminary conclusions regarding the Rht traits and their designations are presented in Table I. In most instances, reciprocal crosses were analyzed, with essentially similar results, indicating that all genetic variation was due to nuclear factors. Parental height data were lost due to a field sampling error.

Based on the F_2 data from several crosses, Burt M860 appears to carry a single incompletely recessive gene Rht20, differing from others against which it was tested, including rht4. A few defective, weak progeny, appeared as segregates in the crosses indicating that Burt M860 carries some secondary genetic alteration(s). Karlik 1 carries a recessive semi-dwarfing gene, designated rht11, that is independent of Rht1, Rht2 and rht6. Burt background materials also evidently carry a 'grass clump' dwarfing factor (D_1, D_2, or D_3) complementary to that present in Karlik 1 (data not shown).

Text continued on p. 87.

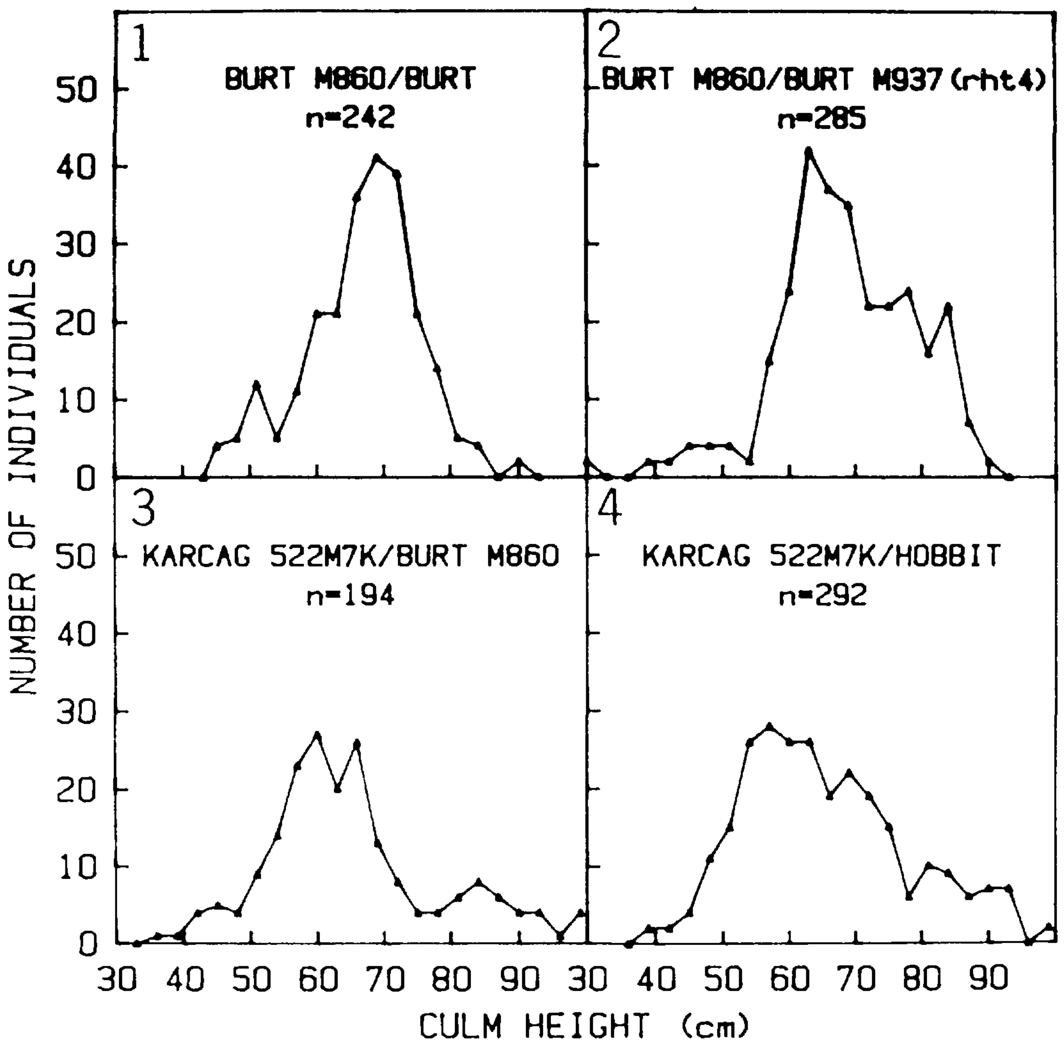

Fig. 1. F_2 culm height distribution for the cross Burt M860/Burt, indicating partially dominant inheritance of the mutant gene Rht20.

Fig. 2. F_2 culm height distribution for the cross Burt M860/Burt M937 (rht4), indicating independence, additivity and combination of one partially dominant and one recessive reduced height gene.

Fig. 3. F_2 culm height distribution for the cross Karcag 522M7K/Burt M860, indicating dominance of the Rht12 gene, probable independence of Rht12 and Rht20.

Fig. 4. F_2 culm height distribution for the cross Karcag 522M7K/Hobbit (Rht2+), indicating probable independence and near additivity of semidwarfing genes Rht12 and Rht2.

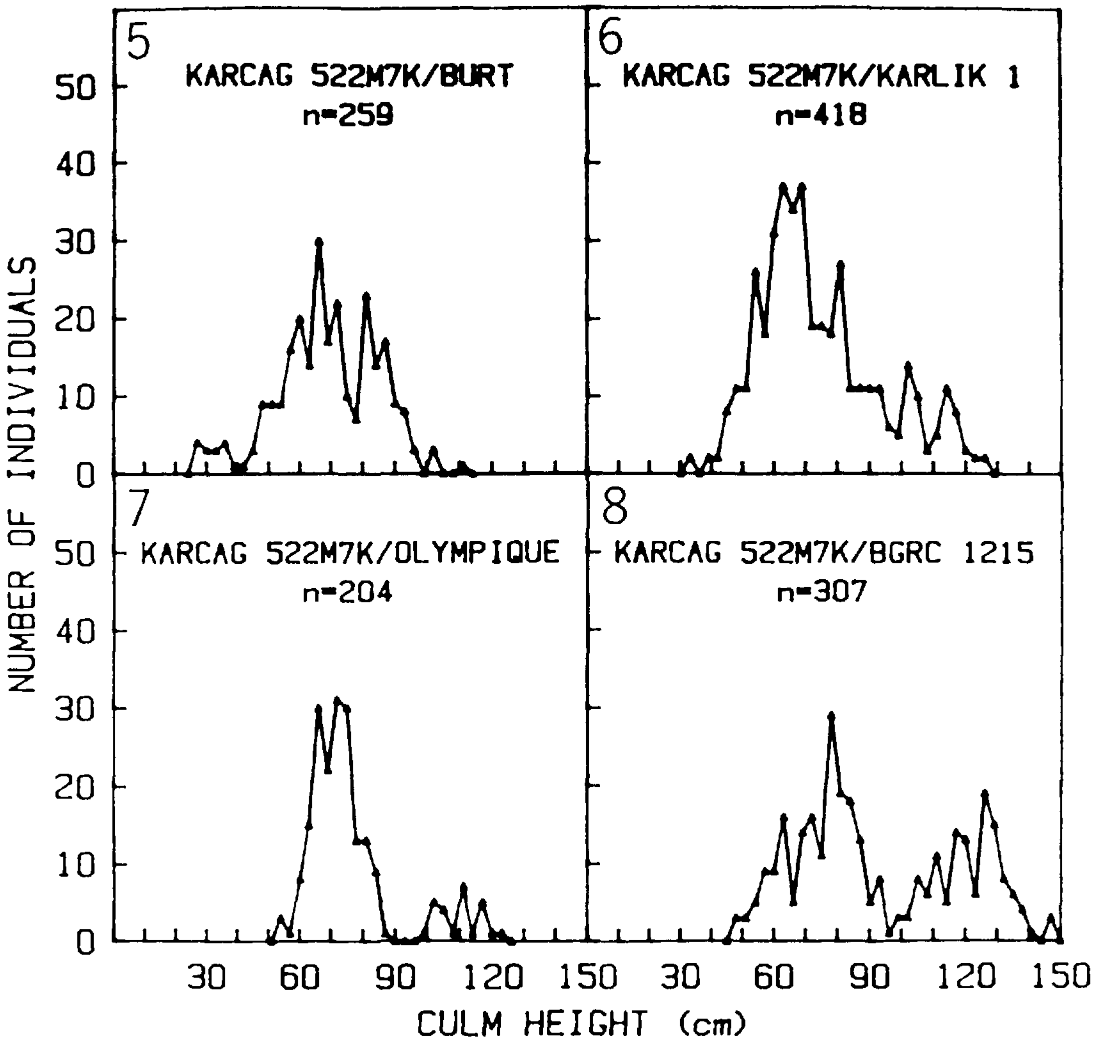

Fig. 5. F_2 culm height distribution for the cross Karcag 522M7K/Burt, showing possible additivity of rht6 and Rht12 genes, plus epistatic interaction among genes in parents.

Fig. 6. F_2 culm height distribution fot the cross Karcag 522M7K/Karlik 1, indicating a wide difference between the two stocks, additivity of semi-dwarfing genes, but details are somewhat blurred.

Fig. 7. F_2 culm height distribution for the cross Karcag 522M7K/ Olympique, indicating partial epistasis of gene Rht12 as a basis for the intermediate height F_1 (not shown here).

Fig. 8. F_2 culm height distribution for the cross Karcag 522M7K/ BGRC001215, indicating a much stronger epistatic interaction between the tallness factor of BGRC1215 with the gene Rht12 (and its recessive allele in Karcag 522) and the independence of alleles affecting height.

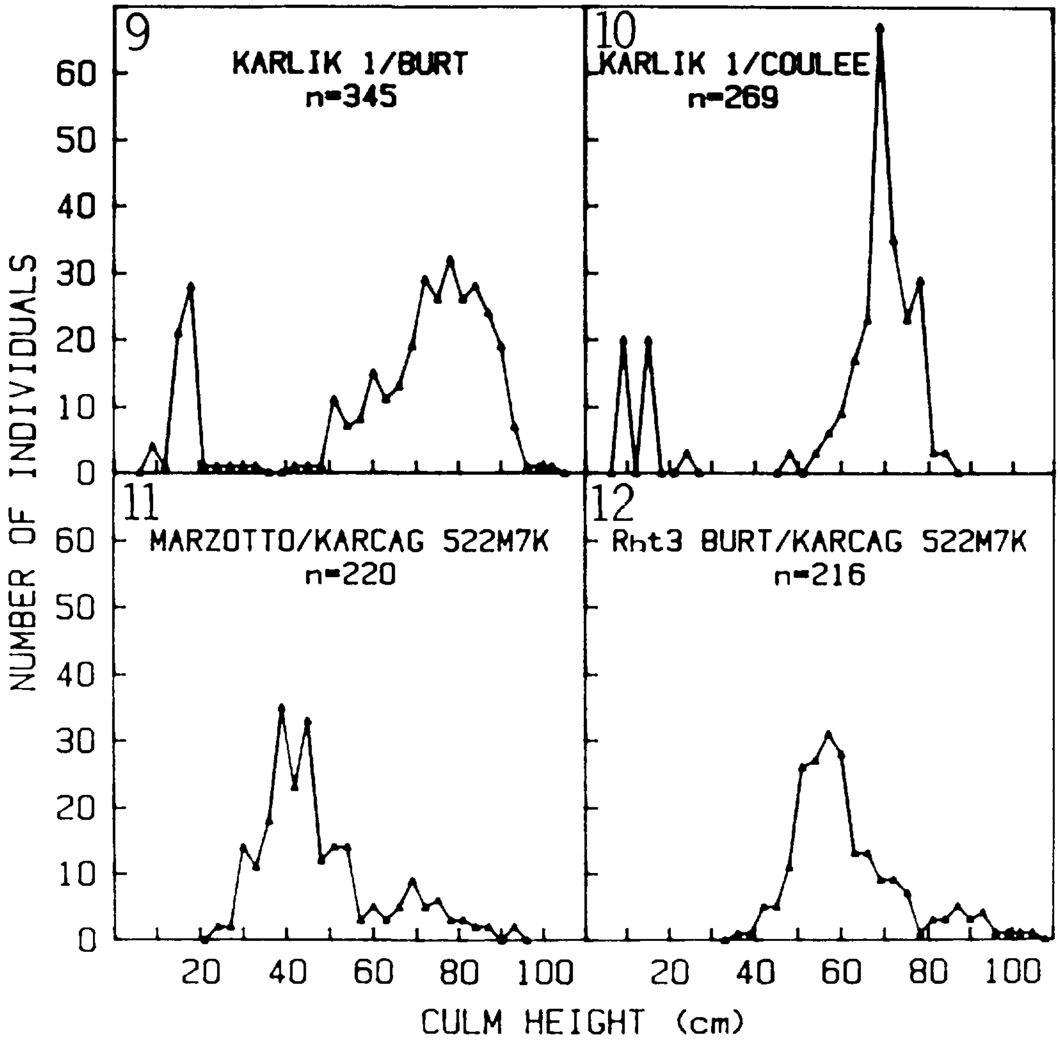

Fig. 9. F_2 culm height distribution for the cross Karlik 1/Burt (rht6), indicating possible segregation of rht6 and Karlik 1 rht11 recombinants.

Fig. 10. F_2 culm height distribution for the cross Karlik 1/Coulee, indicating independence and recessive inheritance of rht11 in Karlik 1 trait from Rht2 of Coulee (a Burt Rht2 derivative); in the effect or rht6 may be evident.

Fig. 11. F_2 culm height distribution for the cross Marzotto/Karcag 522M7K, indicating additivity and independence of the Rht genes in the parents, but the three genes segregating (Rht8, Rht9 and Rht12) make analysis difficult.

Fig. 12. F_2 culm height distribution for the cross Rht3 Burt/Karcag 522M7K. The major Rht genes interact in a partially additive fashion.

TABLE I. EVALUATION OF CROSS COMBINATIONS FOR NUMBER OF RHT GENES, DOMINANCE, AND EPSITATIC ADDITIVITY

Cross		Rht genes	Epistasis Additivity; Dominance Relations	Other
1.	Karcag 522M7K/Karlik 1	Rht12/rht11	Reduced additivity, E; ND/R	Fig. 6
2.	Burt M860/Burt	Rht20/0*	PD/-	+
3.	Burt M860/Coulee	Rht20/Rht2	Reduced additivity, E; PD/PD	-
4.	Burt M860/CI13438	Rht20/Rht1	Slight reduction in additivity, E; PD/PD	+
5.	Burt M860/Burt M937	Rht20/rht4	Reduced additivity, E; PD/R	+
6.	Karlik 1/CI13438	rht4/Rht1 rht6	Reduced additivity, E; R/PD,R	Seg. Dw
7.	Karlik 1/Coulee	rht11/Rht2 rht6	Reduced additivity, E; R/PD,R	Seg. Dw Fig. 10
8.	Karlik 1/Burt	Rht11/rht6	Limited additivity, E; R/R	Seg. Dw Fig. 9
9.	Karcag 522M7K/CI13438	Rht12/Rht1 rht6	Reduced additivity, E; D/PD,R	Seg. Dw
10.	Karcag 522M7K/Coulee	Rht12/Rht2 rht6	Reduced additivity, E; D/PD,R	Seg. Dw
11.	Karcag 522M7K/Burt M937	Rht12/rht4 rht6	Reduced additivity, E; D/R,R	Seg. Dw
12.	Karcag 522M7K/Burt M860	Rht12/Rht20 rht6	Sl. Reduced additivity, E; D/PD,R	Fig. 3

TABLE I. (continued)

Cross	Rht genes	Epistasis Additivity; Dominance Relations	Other
13. Karcag 522M7K/Marzotto	*Rht12*/*rht8* *rht9*	Distinct reduction in additivity, E; D/PD	Fig. 11
14. Karcag 522M7K/CI17329	*Rht12*/*rht1*	Reduced additivity, E; D/PD,R	
15. Karcag 522M7K/CI17324	*Rht12*/*rht2*	Reduced additivity, E; D/PD,R	
16. Karcag 522M7K/Rht$_3$ Burt	*Rht12*/*Rht3*	Reduced additivity, E; D/PD,R	Fig. 12
17. Karcag 522M7K/Burt	*Rht12*/*rht6*	Limited additivity strong dominance; D/R	Fig. 5
18. Karcag 522M7K/BCRC1215	*Rht12*/rht	Partial epistasis; ND	Fig. 8
19. Karcag 522M7K/Olympique	*Rht12*/0	Partial epistatsis ND	Fig. 7
20. Karcag 522M7K/Hobbit	*Rht12*/*Rht2*	Reduced additivity, E; D/PD	Fig. 4

D = Dominant, ND = near dominant, PD= partial dominant, R = recessive, Burt and Burt derivatives carry *rht6* in common.

E = Epistatic effects

Dw_{+} = Grass Clump Dwarfs

Reduced height gene rht6, present in Burt and related materials, was difficult to detect in some crosses because of its small effect. However, rht6 is readily detectable in Burt/taller wheat crosses.

Previous results had indicated that the mutant gene Rht12 of Karcag 522M7K was unique among height reducing factors in wheat [11]. New evidence presented here (Figs. 3-8, 11, 12) demonstrates that gene Rht12 expresses its own range of epistatic and additive genetic relations with other height gene sources. In crosses, gene Rht12 shows partial additivity also with other reduced height factors, including Rht3. Sutka (personal communication, 1986) has recently located Rht12 to chromosome 5A, revealing that a third chromosome group carries a major gene causing plant height reduction.

The tall cultivar 'Olympique' (BGRC20156) a soft red winter wheat from the German Collection at Braunschweig carries no reduced height genes. However, the cross with BGRC1215 indicated that Karcag 522M7K may carry a second reduced height gene which may be a common difference between the original Karcag 522 and the very tall BGRC1215 (see Fig. 8).

In T. turgidum durum, we analyzed the F_2 of several crosses involving new semi-dwarf mutants. Our results support those of Georgi [17] that the Rht14 gene of Castelporziano is inherited independently from Rht1, and transgressive recombinants have been recovered (Fig. 13). The F_2 segregation from crosses between Rht1 carriers WA6518 and WA6754/Edmore SD1 indicates the probable independence of their Rht genes (Fig. 14 a,b). F_2 data from the backcross to Edmore indicate that the Rht16 gene of Edmore SD1 is partially dominant (Fig. 15). Laboratory data showed that Edmore SD1 is GA_3 sensitive like Edmore, and it has a long coleoptile. Edmore SD1 maintains its semi-dwarf height under the rainfed conditions tested, but responds more in height under irrigated conditions than Rht1 carriers. In that respect, its response to environment is much like that of the Rht14 gene carrier Grandur. Edmore SD1 was induced in a high-quality cultivar, and thus should prove useful in further breeding. Backcrosses have been made to free it of extraneous genetic changes.

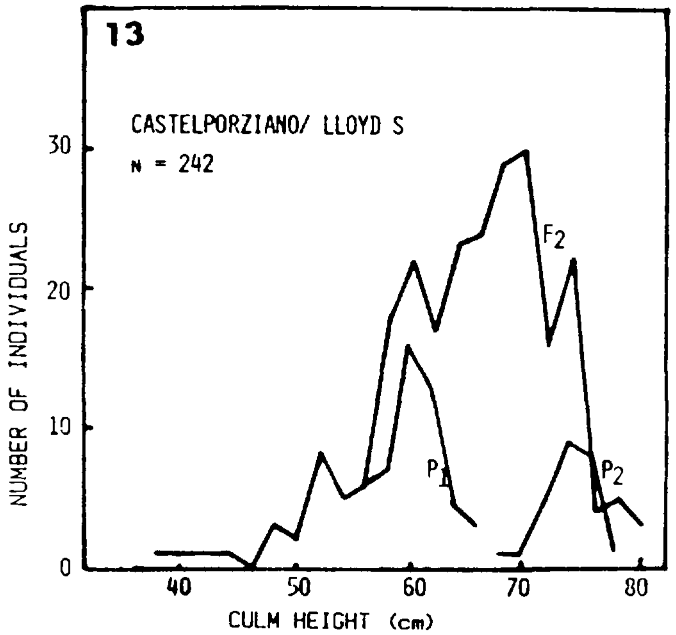

Fig. 13. F_2 culm height distribution for the cross Castelporziano/Lloyd 'S', indicating independent inheritance of the partially dominant Castelporziano gene Rht14 from gene Rht1 of Lloyd 'S'. Both additive and epistatic effects occur.

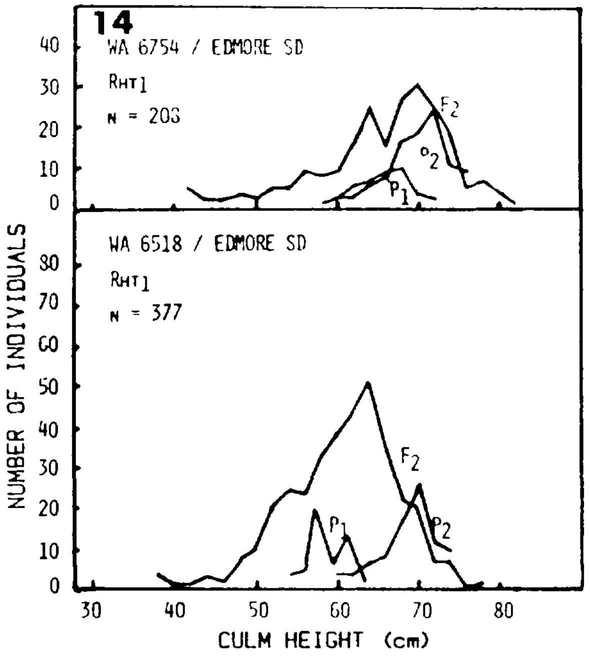

Fig. 14. F_2 culm height distribution for the crosses WA6518 and WA6754/Edmore SD1, indicating possible independence of new mutant gene Rht16 from Rht1 as well as epistatic interaction and partial additivity of the Rht genes.

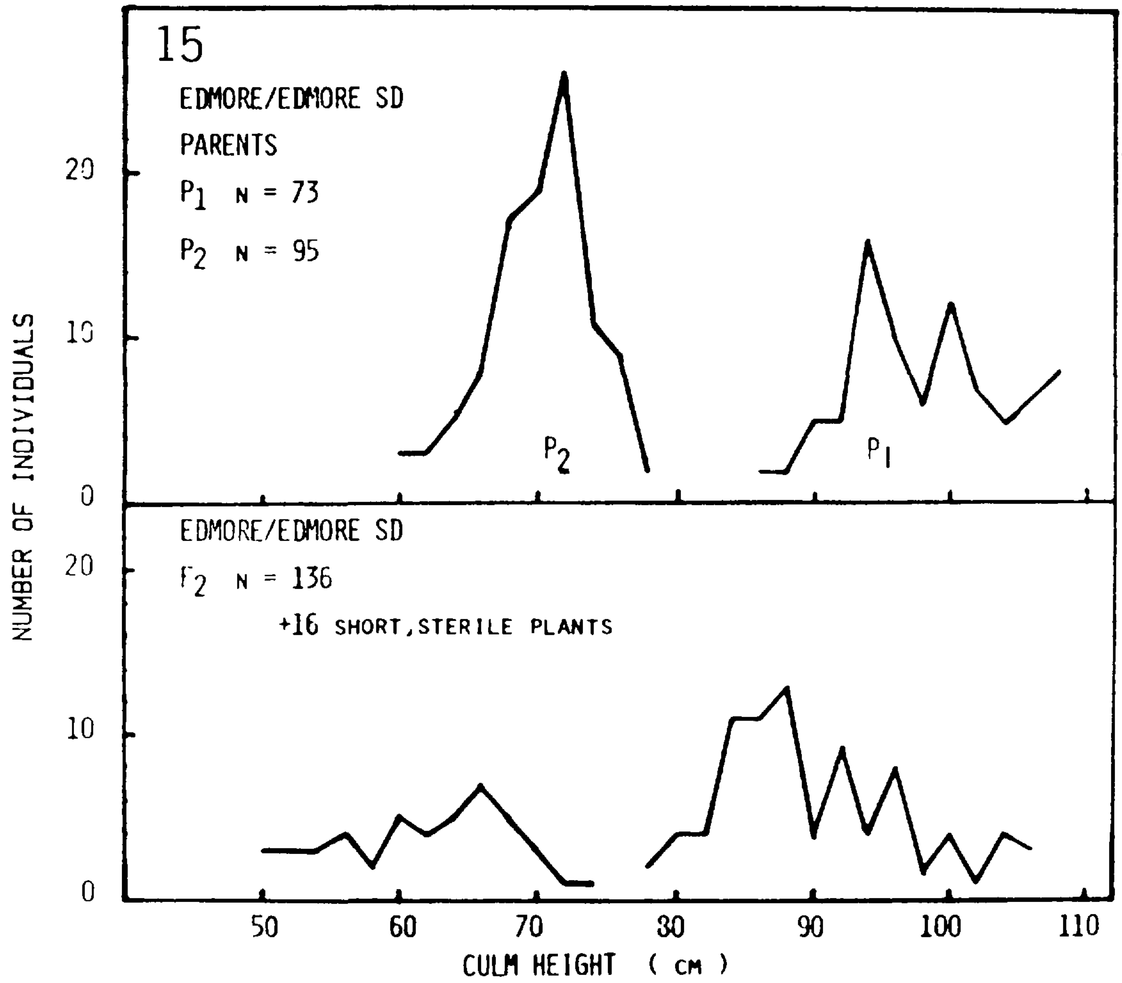

Fig. 15. F_2 culm height distribution for the cross Edmore/Edmore SD1, indicating partial dominant behavior of gene Rht16 of the mutant. Different parent heights are related to field location and plant density.

Crosses of Edmore SD1 the mutant gene Rht15 carrier with Durox or Grandur (Rht14), and with the newly acquired Rht18 mutant, Icaro, showed codominance and apparently independent segregation. The cross Grandur/Durox segregated a ratio of 9 (61) medium tall:4 (34) medium short:3 (17) short F_2 progeny, demonstrating the partial dominance of the Rht14 gene, and the partial recessiveness of the Rht15 gene and their epistatic interactions. Transgressive recombinants also were recovered from the cross Icaro/Durox indicating that Rht15 and Rht18 are independent. In fact, a list of the 20 reduced height genes and sources shows that most Rht genes show partial dominance and few are completely recessive (Table II).

TABLE 11. REDUCED HEIGHT (SEMI-DWARFING) GENES IN WHEAT[1].

Rht Gene	Chromosome Location	Source	Origin[2]	Initial Material	Associated GA3 Response[3]	Dominance[4]	Use in Breeding
Rht1	4A	Norin 10	S	Akadaruma?	I	P. dom.	Widest
Rht2	4Ds	Norin 10	S	Akadaruma?	I	P. dom.	Widest
Rht3	4A	Tom Thumb	S	?	I	Semidom.	Doubtful[6]
Rht4	?	Burt M937 (CI15076)	M	Burt	S	Rec.	Uncertain
Rht5	?	Marfed M1 (CI13988)	M	Marfed	S	Semidom.	Doubtful
Rht6	?	Burt (CI12696)	S	(Brevor)	S	Rec.	Probably Wide[7]
Rht7	2A	Bersee Mut	M	Bersee	S	Rec.	Doubtful
Rht8	2D	Mara, Sava	S	Akakomugi	S	Rec.	Moderate
Rht9	7B	Mara*	S	Akakomugi	S	Rec.	Moderate
Rht10	4D	Ai-bian 1 (PI504466)	S	Aiganzhao[8]	I	Semidom.	Uncertain
Rht11	?	Karlik 1 (PI504549)	M	Bezostaja 1	S	Rec.	Some
Rht12	5A	Karcag 522M7K (PI503552)	M	Karcag 522	S	Str. dom.	Uncertain
Rht13	?	Magnif 41M1 (CI17689)	M	Magnif 41	S	P. dom.	Uncertain
Rht14	?	Castelporziano (PI347331)	M	Cappelli[5]	S	Semidom.	Some
Rht15	?	Durox (PI478306)	M	K6800707[5]	S	P. rec.	Promise
Rht16	?	Edmore M1 (PI499362)	M	Edmore[5]	S	Semidom.	Promise

TABLE II. (continued)

Rht Gene	Chromosome Location	Source	Origin[2]	Initial Material	Associated GA3 Response[3]	Dominance[4]	Use in Breeding
Rht17	?	Chris M1 (CI17241)	M	Chris	S	Rec.	Uncertain
Rht18	?	Icaro (PI503555)	M	Anhinga[5]	S	Semidom.	Promise
Rht19	?	Vic M1 (PI503553)	M	Vic[5]	S	Semidom.	Promise
Rht20	?	Burt M860 (PI503551)	M	Burt	S	P. dom.	Promise

[1] Adapted from Gale and Youssefian (1985), Konzak (1982), Konzak (unpublished), Konzak (1986), McIntosh (1973), Heiner and Elsayed (1974) and Pepe and Heiner (1975).

[2] M = induced mutants, S = spontaneous, ? = unknown.

[3] I = Gibberellin A3 insensitive, S = gibberellin A3 sensitive.

[4] P. dom. = partial dominant, Semidom. = semidominant, Rec. = recessive, Str. dom. = strong dominant. Partial dominant = partial recessive, Semidominant = greater dominance, Strong dominant = almost complete dominant.

[5] Durums. [6] Doubtful = unless modified by additional genes; Some = recent use for several cultivars; Promise = good potential, Uncertain = promise, but still under investigation--modification seems possible.

[7] Likely responsible for "triple dwarf" effect in breeding; has comparably smaller effect than Rht1 or Rht2.

[8] Personal communication (Gao, Mingwei, P. R. China, 1985).

* By inference also in Italian *T. aestivum* cv 'Forlani' and 'Acciao', and *durum* derivatives 'Jucci', 'Montanari' and 'Ringo', based on geneology (Vallega, 1974).

3.2 Breeding studies

T. aestivum wheats - Many selections were made from crosses involving Magrif 41 M1, but the only possible carriers of the mutant gene *Rht13* which have been advanced to yield performance trials are lines which also carry *Rht2*. These lines are K7900085, K7900100, HP830007, and HP830015. All four lines are very short in height (>60 cm) when grown under irrigated conditions, and all are GA_3 insensitive due to the dominance of their *Rht2 Gai2* gene component. Their coleoptiles appear to be short like *Rht2* carriers, but are not as short as those of *Rht1 Rht2* semi-dwarfs. No direct descendents carrying only *Rht13* from CI17689 have yet proved worthy of yield tests. However, it is hoped that crosses involving these very short semi-dwarf lines will produce better progeny. Germplasm improvement studies are continuing in an effort to develop *Rht13* carriers with better agronomic properties.

4. ADDITIONAL COMMENTS AND CONCLUSIONS

Detailed investigations to establish the chromosome locations of mutant semi-dwarfing genes have not been possible in our more practically-oriented program. Thus, our analyses have been limited to "tester" type crosses and backcrosses, the progeny of which may have value in variety development or in germplasm improvement. Many of these combinations involve segregation of reduced height loci other than those under investigation, often making segregations difficult to interpret. Even so, such combinations often provide reasonable estimates of relationships which can later be tested in combinations that allow for greater analytical precision. Until better (isogenic) tester lines are produced for each mutant *Rht* locus, such crosses are a reasonable alternative since they also provide additional information on breeding value, whereas many tester or analytical crosses do not. The numerous crosses investigated suggest that the degrees of dominance vs. recessiveness of *Rht* or *rht* genes form a continuum of expressions which are further modified by epistatic interactions between and among *Rht* gene loci. Some of the newer *Rht* loci may yet prove to be different alleles of a single locus or pseudoalleles of closely linked genes.

Results, particularly with durum wheats, suggest that the mutation breeding methods have excellent potential both for direct variety development and for producing new reduced height gene resources, since productive mutants are readily isolated. Even so, it appears that the breeding value of semi-dwarf mutants may exceed their potential for direct use.

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BARLEY MUTANTS IN RELATION TO CULM LENGTH

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Abstract

The semi-dwarf type in barley is very heterogeneous, reduced culm length being more or less characteristical of a number of different groups of mutants in barley at the side of the short culm mutants proper. Thus, short culm may occur associated with the mutant characters *erectoides* (short spikes), *praematurum* (early), *globosum* (rounded seeds), *upright*, and *strawstiff*.

Covariation studies of 111 mutants in the five *ert*-loci *ert-a*, -*b*, -*c*, -*d*, and -*e* showed a positive correlation between culm length and yielding ability. The patterns of distribution differ among the loci, but favourable deviants of semidwarf type are indicated for all five loci. For the three main *mat*-loci *mat-a*, -*b*, and -*c*, no correlation between culm length and yielding ability was shown, mutants in *mat-a* generally had shorter culm length. Thus, mutants of the praematurum type compared with mutants of the erectoides type may offer more favourable materials for selecting high-yielding semi-dwarf types.

Among the 52 short culm mutants, those with extreme reduction of culm length showed, without exception, strong reduction of yield. Among the 23 strawstiff mutants, on the other hand, the majority, even among the shortest ones, exceeded the average yield of the mother variety.

In an M_2 material of cv Sv Frida, sodium azide compared with neutrons turned out to be clearly more efficient to produce short culm M_3 lines.

In studies of mutations affecting the development of lateral florets in the three two-row cv Sv Bonus, Sv Foma, and Sv Kristina single mutants of intermedium type showed no effect on culm length and maintained the varietal differences. Double *int* mutants, irrespective of their being derived from cross combinations within a variety or between varieties showed a marked reduction of culm length. Triple mutants, with the additional contribution of a six-row gene from locus *hex-v*, showed an apparent increase in culm length. A special interaction between the six-row locus on one side and *int*-loci on the other side is made probable.

INTRODUCTION

The Scandinavian mutation work in crop improvement has been carried on since about 50 years, with the plant-breeding institute at Svalöv as its centre, and with barley as its foremost research material. Being a diploid and self-pollinating, barley is easy to handle in repeated experiments and has become a model crop all over the world where barley is cultivated. At Svalov, a broad spectrum of easily recognized mutation types in barley has been screened for, and a collection of more than 9,000 isolated mutants is now available. Several of the mutant groups have been studied genetically and biochemically in deep detail and with regard to plant-breeding value and mutagen specificity. Since 19 , the collection of mutants is in the process of being stored in the Nordic gene bank.

The semi-dwarf type is of interest for plant breeding in barley. Genetically this type is very heterogeneous, reduced culm length being more or less chracteristic of a number of different groups of mutants in barley at the side of the short culm mutants proper. Thus, short culm may occur associated with the mutant characters *erectoides* (short spikes), *praematurum* (early), *globosum* (rounded seeds), *upright*, and *strawstiff*.

For the present "semi-dwarf project" some of the mutant groups have attracted our particular interest, namely *erectoides* mutants (with about 1,200 cases), *praematurum* mutants (with about 900 cases), *globosum* mutants (with about 200 cases), "*upright*" mutants (with about 150 cases), "*strawstiff*" mutants (with about 25 cases), and mutants with "*short culm*" (with about 50 cases). Concerning their yielding ability the globosum and upright groups have been fairly disappointing in earlier as well as in the present yield trials; they will not be included in this report.

COMPARISON OF THE ERECTOIDES (*ert*) AND PRAEMATURUM (*mat*) MUTANT GROUPS

The mutants discussed in the following are mainly derived from the Svalöf varieties cv Sv Bonus, Sv Foma, and Sv Kristina. The mutagens used are radiations (sparsely and densely ionizing), organic chemicals, and sodium azide.

In earlier investigations, by Arne Hagberg and Goran Persson, 26 erectoides (*ert*) loci have been identified, with altogether 205 localized mutations (1). More than 50% of these mutations are localized to the four loci *ert-a*, -*c*, -*d*, and -*m*. Locus *ert-m* is, however, not associated with reduction of culm length and is, therefore, of no interest in the present context.

There is a pronounced tendency to mutagen-specific reaction of the *ert*-loci (1). Especially the two loci *ert-a* and *ert-c* behave differently: among 33 *ert-a* mutations only 1 has been induced by densely ionizing radiations, in contrast to about 50% of the 36 mutations in the *ert-c* locus.

Earlier investigations have also shown the erectoides mutants induced by irradiation to be generally superior in yielding ability to mutants induced by chemical mutagens (2). These investigations have also shown that the different *ert*-loci affect yielding ability differently (2). The genic background of the varieties from which the mutants are derived also has turned out to be of importance for the practical value of the mutants (2).

Concerning the praematurum (*mat*) mutants, 9 *mat* loci, with altogether 85 localized mutations, have been identified. More than 80 of these mutations are localized to the three loci *mat-a*, -*b*, and -*c*. Only these three loci have been included in the semi-dwarf studies. It has long been known that mutants with extreme earliness have a shorter culm, usually lacking one of the internodes.

Covariation diagrams for culm length and yielding ability based on two-row plots from one year (1982) are available. Altogether 111 mutants in five *ert*-loci (*ert-a*, -*b*, -*c*, -*d*, and -*e*) showed a positive correlation between culm length and yielding ability. The patterns of distribution differ among the loci; but favourable deviants of semi-dwarf type are indicated for all five loci. In total, 16 of these mutants are indicated to be of potential practical value in a semi-dwarf project. About 200 mutants not localized but suspected, on morphological grounds, to belong to the ert-a or the ert-c locus again showed positive correlation between culm length and yielding ability, but now with only 6 favourable deviants recognized.

Covariation diagrams for the three main mat-loci (mat-a, -b, and -c) with altogether 68 mutants showed, surprisingly, no correlation between culm length and yielding ability. It is concluded that mutants of the praematurum type compared with mutants of the erectoides type may offer more favourable materials for the selection of high-yielding semi-dwarf types. The diagrams indicate differences among the mat-loci, mutants in mat-a generally having a shorter culm length. It is interesting in this connection that our recent observations indicate that sodium azide, compared with other mutagens, is less efficient in producing mutations in locus mat-a. Even in the absence of an adequate standard for the praematurum plots, several of these mutants are indicated to be of practical value in a semi-dwarf project.

"SHORT CULM" AND "STRAWSTIFF" MUTANTS

Previous data had not been particularly favourable, but the data being meagre a more extensive study of these mutants was done in the summer 1984.

A number of mutants of cv Sv Bonus, 52 of the short culm type, and 23 of the strawstiff type were studied in a field trial, with the mother variety as a standard. The tendency to reduced culm length showed a strong variation among the short culm mutants, and the mutants with extreme reduction of culm length showed, without exception, strong reduction of yield. Mild reduction of culm length was, as a rule, associated with mild reduction of yield; and a small number among these mutants were equal to or even exceeded their mother variety. There seem to be no bright prospects of finding short culm mutants combining some reduction of culm length with improved yielding ability; but the possibility certainly exists.

The strawstiff mutants, on the other hand, showed an interesting picture. On average, they showed a milder reduction of culm length. Of greater interest is the fact that they showed a much weaker association between yield and culm length. Remarkably, the majority among the strawstiff mutants, even among the shortest ones, exceeded the average yield of the mother variety. It will certainly be an important task to look for a verification of these tendencies in further trials, because neither of the two mutant groups have given particularly encouraging results in earlier trials.

MUTAGEN SPECIFIC REACTIONS FOR SHORT CULM PLANTS

In 1984, in connection with the harvest of plants that had been tagged as "early" in an M_2 material of cv Sv Frida, a number of short culm plants were also selected, 195 among them after treatment with sodium azide, and 66 after treatment with neutrons. These plants were distinctly more short-culm than the early plants selected in the same plots. The frequency of short-culm

M_2 plants was clearly higher after sodium azide treatment. Interestingly enough, sodium azide also turned out to be clearly more efficient to produce short-culm M_3 lines (67% among the lines, against 35% among the lines after neutron treatment).

In this material, culm length and spike length were, as expected, positively correlated in the M_3 lines, but deviants with shorter culm and longer spikes appeared after both mutagenic treatments, at about 10% frequency. Unfortunately, yield data for the M_3 lines (grown in 1985) are not yet available. Nevertheless, there are suggestive indications that culm shortening without reduction in yield may be achieved by mutation breeding.

CULM LENGTH IN RELATION TO MUTATIONS AFFECTING SPIKELET DEVELOPMENT

Some data will now be presented to show that mutations affecting spikelet development in barley may also interact to affect culm length. In normal two-row barley the two rows of main florets are each surrounded by two rows of small, sterile and awnless lateral florets. In six-row barley these lateral florets are well developed, fully fertile and carrying well developed awns. The two-row barley can mutate in a single step to six-row barley; these mutations have so far been localized to one and the same locus, named hex-v. Besides, two-row barley can give rise to mutants with enlarged lateral florets, where awn development, fertility, and kernel development vary in characteristic ways. These mutants have a spike development that is intermediate between two-row barley proper and six-row barley proper, and the mutations are therefore named intermedium mutations. A considerably greater number of gene loci - so far 11 int-loci have been detected - are able to influence the development of lateral florets in this way.

These genes generally act recessively. Two of the loci - hex-v and int-d - are semidominant. Increased development of lateral florets that is due to co-operation between such loci has been observed ([illegible], 4). Thus, different int-loci combined in double homozygotes frequently give rise to typical six-row type; and the combinations between such double mutants and the six-row gene in triple mutants have turned out to be able to produce extremely beautiful six-row types. The ensuing discussion will centre upon changes in culm length that have been found to be associated with these kinds of mutations in different combinations.

The int-mutants involved in this study are derived from three mother varieties, cv Sv Bonus, Sv Foma, and Sv Kristina. Mutants, mainly in the four int-loci -a, -c, -d, and -e, totally 53, plus the six-row gene hex 3 and the six-row variety cv Sv Agneta form the basic material for double mutant combinations between intermedium genes, and for triple mutant combinations between intermedium genes and six-row gene. Results are available from three conditions of cultivation, in 1984 and 1985. The year 1985 was characterized by droughts, which affected plant development harmfully. Consequently, culm lengths are generally higher in 1984.

The mother varieties differed in average culm length. Single mutants of intermedium type show no effect on culm length, and maintain the varietal differences. Double mutants, irrespective of their being derived from cross combinations within a variety or between varieties, show a marked re-

TABLE I. Varietal differences in culm length (means in cm) for intermedium genes in barley. Number of cases behind the means, in parentheses.

Year	Material studied	Mother variety			Remarks
		Bonus	Fama	Kristina	
1985a	Single mutants	54.5 (10)	56.7 (23)	48.7 (18)	
	Double mutants	48.7 (24)	49.7 (106)	47.8 (78)	
	King-size, *hex* 3	53.3 (7)	56.2 (51)	53.9 (34)	
	King-size, Agneta	54.4 (23)	54.6 (82)	53.4 (55)	
	Mother varieties	54.4	56.4	54.0	*hex* 3 = 59.6 *Agneta* = 50.9
1985b	Double mutants	57.1 (28)	58.9 (111)	56.9 (81)	
	King-size, *hex* 3	57.9 (15)	63.2 (50)	61.1 (39)	
	King-size, Agneta	61.8 (23)	64.4 (90)	62.0 (59)	
	Mother varieties	64.1	64.5	60.5	
1984	Double mutants	75.6 (28)	79.3 (106)	77.0 (72)	
	King-size, *hex* 3	80.1 (13)	83.6 (58)	83.8 (45)	
	King-size, Agneta	86.1 (26)	86.8 (93)	87.2 (51)	

TABLE II. Influence of varietal combinations on culm length (means in cm) for intermedium genes in barley. Number of cases behind the means, in parentheses.

Year	Material studied	Combinations of mother varieties				
		F + F	K + K	B + F	B + K	F + K
1985a	Double mutants	50.0 (25)	48.6 (11)	53.1 (12)	44.3 (12)	48.4 (44)
	King-size, *hex* 3	57.2 (15)	54.5 (7)	56.9 (4)	48.6 (3)	54.3 (17)
	King-size, Agneta	55.0 (21)	53.4 (7)	55.9 (11)	53.0 (12)	53.6 (29)
1985b	Double mutants	59.1 (28)	54.8 (11)	59.5 (12)	55.3 (16)	58.8 (43)
	King-size, *hex* 3	64.5 (13)	60.1 (7)	57.9 (7)	58.0 (8)	63.3 (17)
	King-size, Agneta	64.5 (25)	59.8 (8)	64.5 (10)	59.7 (13)	64.1 (30)
1984	Double mutants	80.5 (27)	77.7 (8)	78.0 (12)	73.8 (16)	78.0 (40)
	King-size, *hex* 3	85.0 (15)	88.8 (8)	82.9 (6)	77.8 (7)	82.0 (22)
	King-size, Agneta	86.9 (26)	91.4 (5)	87.0 (13)	85.3 (13)	86.6 (28)

duction of culm length. Triple mutants, with the additional contribution of the six-row gene from *hex* 3 or from the extremely short-culm six-row variety Agneta, show an apparent increase in culm length (and in spike length as well); accordingly, we have named them "King-size". Interestingly, King-size types involving Agneta are generally the strongest ones. To summarize.

Varietal differences in culm length (TABLE I) are, on the whole, maintained among the single mutants. Double mutants are clearly lower than single mutants. King-size plants are clearly higher than double mutants under all conditions of cultivation.

No heterosis effect can be demonstrated when *int*-genes from different varieties are combined (TABLE II). Nor can a heterosis effect be seen at the level of King-size types (*hex* 3 being derived from cv Sv Bonus). An effect of interaction between *int*-genes on one side, and six-row genes on the other side, is made probable.

Different culm lengths are associated with the four more closely investigated *int*-loci -*a*, -*c*, -*d*, and -*e* (the remaining 7 loci being excluded because of too few representatives (TABLE III). Interestingly, these differences for single mutants are maintained at the level of double mutants, even at different conditions of cultivation. King-size plants behave in a more irregular way.

TABLE III. Influence of gene loci on culm length (means in cm) for intermedium genes in barley. Number of cases behind the means, in parentheses.

		Intermedium loci			
Year	Material studied	a	c	d	e
1985a	Single mutants	52.5 (20)	52.1 (15)	53.1 (9)	62.7 (4)
	Double mutants	48.0 (54)	48.3 (73)	49.4 (63)	55.8 (10)
	King-size, *hex* 3	55.7 (24)	54.7 (34)	55.1 (28)	53.6 (5)
	King-size, Agneta	54.0 (44)	53.1 (55)	55.0 (46)	59.5 (8)
1985b	Double mutants	56.4 (60)	56.9 (77)	59.2 (66)	64.3 (9)
	King-size, *hex* 3	61.5 (28)	61.9 (37)	61.6 (30)	61.0 (6)
	King-size, Agneta	62.7 (48)	61.4 (58)	65.1 (51)	63.7 (7)
1984	Double mutants	76.6 (58)	76.8 (71)	79.0 (60)	87.4 (10)
	King-size, *hex* 3	81.6 (30)	82.2 (45)	85.7 (32)	86.0 (7)
	King-size, Agneta	85.5 (51)	84.9 (57)	89.2 (47)	91.0 (8)

There is no particular effect of interaction to be seen when different *int*-loci are combined in the double mutants (TABLE IV). The variation seems to be due mainly to the differences between individual *int*-loci (longer culm on average characterizing *int-d* and *-e*). This supports the conclusion that the King-size effect depends mainly on a special interaction between the six-row locus on one side and *int*-loci on the other side.

TABLE IV. Influence of gene loci combinations on culm length (means in cm) for intermedium genes in barley. Number of cases behind the means, in parentheses.

		Combinations of intermedium loci				
Year	Material studied	a + c	a + d	c + d	c + e	d + e
1985a	Double mutants	47.8 (32)	48.3 (22)	48.7 (32)	55.2 (6)	56.7 (4)
	King-size, *hex* 3	54.6 (14)	57.3 (10)	54.6 (16)	57.1 (3)	48.3 (2)
	King-size, Agneta	52.3 (26)	56.5 (18)	53.2 (21)	59.5 (6)	59.5 (2)
1985b	Double mutants	55.3 (36)	58.1 (24)	57.8 (33)	61.7 (5)	67.6 (4)
	King-size, *hex* 3	62.2 (17)	60.6 (11)	62.3 (15)	59.1 (3)	62.9 (3)
	King-size, Agneta	59.6 (27)	66.7 (21)	62.7 (23)	62.6 (5)	66.4 (2)
1984	Double mutants	76.2 (37)	77.4 (21)	77.2 (28)	83.9 (4)	89.8 (6)
	King-size, *hex* 3	79.7 (21)	86.1 (9)	84.8 (19)	82.4 (4)	90.7 (3)
	King-size, Agneta	83.3 (32)	89.1 (19)	87.0 (19)	85.3 (4)	96.7 (4)

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AGRONOMIC AND GENETIC EVALUATION OF INDUCED MUTANTS OF WHITE LUCHAI-112*

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Abstract

Early mutants induced in the photo-sensitive, tall and fine-grained variety White Luchai - 112 (WL.112) continued to show promise when tested in the region where the parent variety is popularly grown. Earliness in one such mutant is found to be governed by a gene system different from that of another early, semi-dwarf, long-slender grained and high yielding lines , TR-17. Several hundred selections combining desirable traits have been obtained in the progeny of the cross involving these two early parents. Differences in culm height, number of internodes and pattern of internode elongation and thickness were observed in the promising reduced height non-lodging mutants of WL.112. Studies upto F_2 in 21 crosses involving 13 mutants of W.L. 112 and 9 genetic stocks have revealed the following. Among the mutants, two carry the same dwarfing gene $\underline{sd_1}$ as that of Dee-geo-woo-gen while three others have its dominant allele. The remaining eight mutants are governed by seven single genes tentatively designatedas $\underline{sd_{x1}}$ to $\underline{sd_{x7}}$, $\underline{sd_{x1}}$ being common in two mutants and non-allelic to $\underline{sd_{x2}}$; one gene $\underline{sd_{x6}}$ is non-allelic to $\underline{sd_1}$; four genes $\underline{sd_{x3}}$, $\underline{sd_{x4}}$, $\underline{sd_{x5}}$ and $\underline{sd_{x7}}$ are non-allelic to the dwarfing gene $\underline{d_t}$ of TR-5, one gene $\underline{sd_{x4}}$ is non-allelic to $\underline{sd_{x'}}$ of Cheng-chu-ai-11. Among the remaining genetic stocks the $\underline{sd_2}$ gene of D66 is non-allelic to $\underline{sd_{x'}}$, similarly $\underline{sd_{x''}}$ of Tainan-5 is non-allelic to $\underline{d_t}$ while both $\underline{d_{x'}}$ and $\underline{d_t}$ are present in IR-28 dwarf.

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INTRODUCTION

Induction of desirable mutants like reduced height and/or early types in W.L.112 and their promising nature in preliminary evaluations were reported earlier (1,2). A comparatively rapid and convenient seedling height test for Gibberellin (GA) response in rice was described by Narahari and Bhagwat (3) who also used the method to distinguish different induced reduced height mutants of W.L.112 (1). Unlike wheat where induced dwarf mutants show a clear-cut presence or absence of GA-response, in rice the GA-response was found to vary in a continuous fashion (1,2), thus making it rather fifficult to distinguish between one or the other dwarf type, based only on this criterion Genetic experiments were, therefore, considered essential to determine the allelic nature or otherwise of the mutant genes with the known dwarfing genes. The present report deals with the agronomic evaluation and genetic analyses of some of the promising early and/or reduced height mutants of W.L.112.

MATERIALS AND METHODS

The photo-sensitive tall growing *indica* variety, W.L.112, its gamma ray- induced early and/or reduced height mutants and certain dwarf genetic stocks, all as detailed hereafter, were used in the experiments. Evaluation of 85 early flowering and 48 non-lodging dwarf mutants of WL.112 had shown that 59 of the former with grain yields over 4000 kg/ha and 32 of the latter with grain yields over 3500 kg/ha were of some promise (1). The promising early mutants have since been passed on for local testing at two locations - Sindewahi and Sakoli research stations - in the region where WL.112 is popular. Based on duration to ear-emergence and plant height these mutants were put into four different groups and tested in Initial Evaluation

Trials with randomised block design, three replications net plot size of 5.70 m x 1.20 m, spacing of 20 cm x 15 cm and fertilizer applications of 20 kg N + 50 kg P_2O_5 + 50 kg K_2O/ha at transplanting and 40 kg N/ha each at tillering and panicle initiation stages. Yield data obtained from these trials was subjected to statistical analysis. One of the early mutants, γA-42-32, having short fine grains and lodging habit like its parent was crossed with another early, semi-dwarf, non-lodging and high yielding culture TR-17 (4). The object of this experiment was to study the genetics of earliness and to isolate desirable high yielding recombinants. Thirteen reduced height mutants of WL.112 and 9 genetic stocks, of different height, as detailed in table-1, were crossed in 21 combinations. Crosses involving mutants of WL.112 numbered fourteen. They were :(a) two crosses among mutants - (1)γA-64-23 xγE-60-26 and (2)γA-69-7 x γE-60-26; (b) six crosses with sd_1 stocks - (3) TR-17 x γA-42-32; (4) γD-81-9 x I-geo-tze; (5) D_2IV-L_7-70-44 x Dee-geo-woo-gen; (6) E_2IV-L_8-75-56 x I-geo-tze; (7) γB-Ed-184-3-11 x I-geo-tze; (8) γB-LT-728-10-7 x I-geo-tze; and (c) six crosses with non-sd_1 stocks - (9) γD-81-9 x Cheng-Chu-ai-11; (10) D_2-IV-L_7-46-20-7 x Cheng-Chu-ai-11; (11) γD-37-9 x TR-5, (12) D_2-IV-L_7-46-20-7 x TR-5; (13) E_2-IV-L_8-74-56-10 x TR-5; and (14) γC-MD-2630-11-9 x TR-5. Crosses involving genetic stocks numbered seven as follows : (15) Dee-gee-woo-gen x I-geo-tze; (16) K-8 x I-geo-tze; (17) D-66 x Cheng-Chu-ai-11; (18) IR-28 dwarf x Cheng-Chu-ai-11; (19) IR-28 dwarf x TR-5; (20) TR-5 x IR-28 dwarf; and (21) Tainan-5 x TR-5. All the cross progenies in F_1 and F_2 were studied for plant height character. Care was taken to sow seeds thinly in the nursery bed and to separate the short from the tall seedlings before transplanting so as to avoid possible competition among them in the field. The mean, range and/or frequency distribution for height in parents, F_1 and F_2 were calculated and plotted to interpret the results.

RESULTS AND DISCUSSION

Yield evaluation of early mutants :

In 1984 wet season 21 of the 59 tested early mutants of WL.112 gave yields equal to or better than the parent and other check varieties (table-2). These mutants were promoted to Preliminary Variety Trials and results of 1985 wet season are yet awaited. From the results available so far, it can be reasonably inferred that induction of early mutants without the loss of parental yield potential is quite successful.

Breeding for earliness combined with other desirable characters :

Progeny of the cross TR-17 x γA-42-32 was studied for ear emergence, plant height and grain type characters in F_1 and F_2 in 1984 and 1985 wet seasons respectively. Ear emergence in parents was found to be within 90 days while in F_1 it was later by about 3 weeks. However, the F_2 showed 64 to 132 days transgressive pattern of segregation for this character (Fig.1). The results indicate that two different gene systems are involved in governing earliness in the parents. A total of 960 plants with desirable character combinations have been selected for further studies. These selections also include plants with duration to ear emergence being earlier or later than both the parents. It seems possible to isolate high yielding early lines combining the semi-dwarf non-lodging nature of TR-17 and fine grain character of γA-42-32.

Non-lodging mutants :

In 1984 and 1985 wet seasons 25 short non-lodging and 10 lodging resistant reduced height mutants of WL.112 were evaluated for their yield performance and internodal patterns. In 1984 all

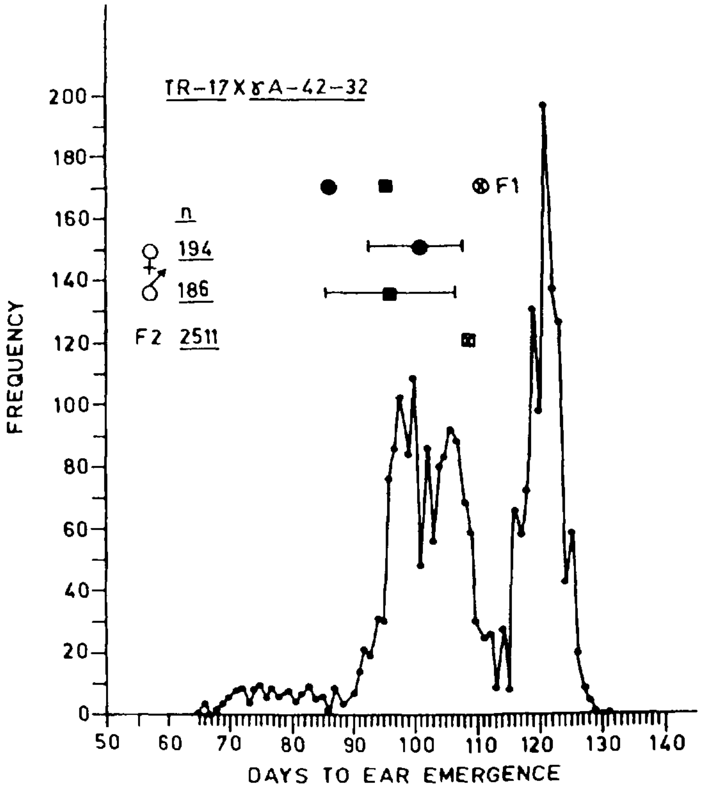

Fig. 1. Mean, range and/or frequency distribution for number of days to ear emergence in parents, F_1 and F_2 of the cross TR-17 x γA-42-32.

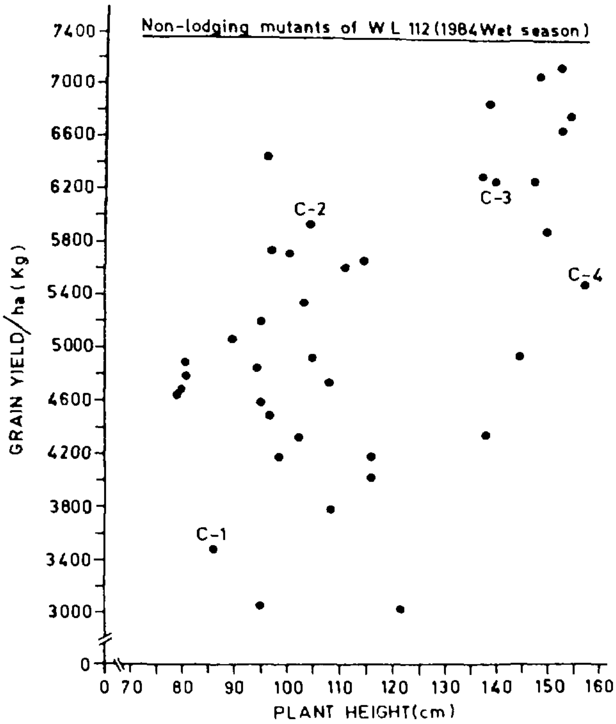

Fig. 2. Relationship between plant height and grain yield in 35 non-lodging mutants of W.L.-112 and four control varieties.

but three of the mutants yielded more than 4000kg/ha and in general plant height and grain yields were correlated (Fig.2). Eighteen of these mutants again gave similar high yields in 1985 wet season. All these mutants are now proposed to be sent for testing in the region where the parent variety is popular. The data on culm and panicle lengths, internode number and their elongation obtained for the short non-lodging mutants is summarised and depicted in Fig.3 . The average number of developed internodes in the short non-lodging mutants ranged between 5.7 and 8.7 (the maximum observed in any plant being 10), as against 7.8 in W.L. 112 and 8.0 in the check variety SKL-1. In the tall checks all the internodes, including the lower ones, were well developed; the short non-lodging mutants showed in general reduced length of internodes, moreso in those below the third internode. Some of the mutants, however, showed relatively uniform reductions in all the internodes. The relative

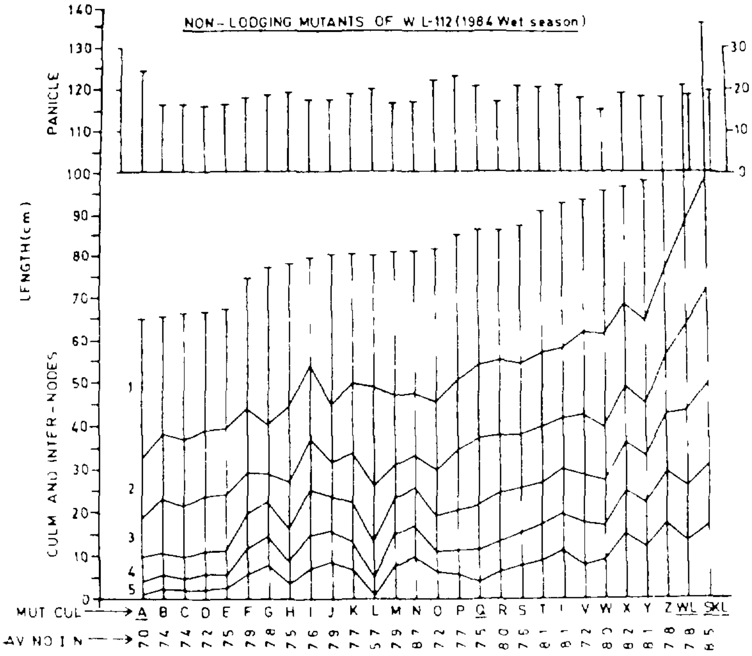

Fig. 3. Relative pattern of internode elongation, culm height, panicle length and mean number of internodes in 24 reduced height non-lodging mutants of W.L.112 and four check varieties (A, Q, WL & SKL).

contributions of the top two internodes to culm lengthwere generally far greater than 50 per cent in the mutants as against just about 50 per cent in the tall checks. The girth of the top four internodes also revealed similar variations. The first to fourth internodes showed a respective range between 0.90 and 1.30, 1.34 and 2.14, 1.50 and 2.06 and 1.65 and 2.34 cm in the mutants as against 1.12 and 1.17, 1.58 and 1.66, 1.86 and 1.91 and 2.00 and 2.13 cm in the tall check variaties.

Genetics of induced dwarfs :

Based on the normal height of the suspected natural crosses, 3 dominant : 1 recessive phenotypic segregation ratio in F_2 and 2 segregating : 1 non-segregating families in F_3, mono-genic receissive inheritance of several W.L.112 mutant phenotypes was inferred earlier (1,2), Similarly preliminary information on TR-5 dwarfing complex accompanied with characteristic cigar-shaped panicle was determined to be governed by $\underline{d_t}$ gene which is independent of $\underline{sd_1}$ gene of Dee-geo-woo-gen (1,2,5). Twenty-one crosses involving 13 reduced height mutants of W.L.112 and 9 genetic stocks, as described earlier, have since been studied in F_1 and F_2 generations. The range of height in the mutants was between 47 and 145 cm and in the genetic stocks between 51 and 101 cm while one variety K-8 was 139 cm tall (Table-1). Plant height differences in the parents, F_1 hybrids and patterns of segregation in their respective F_2 progenies of the 21 crosses are serially depicted in Figs. 4 to 24. In four crosses (Nos. 1,7, 8 and 15 vide Figs. 4 to 7 respectively) height differences among parents were almost absent, F_1 resembled both the parents and the F_2 showed unimodal normal segregation curves, thus indicating presence of the common gene pair in the respective parents of each of these

TABLE 1. PARTICULARS OF CROSS-PARENTS

(a) Induced mutants of W.L. 112

Sl. No.	Name	Plant Height (Cm)
1.	γD-81-9	145
2.	D_2IV-L7-70-44	138
3.	γA-42-32	124
4.	γE-60-26	108
5.	γA-64-23	105
6.	γB-Ed-184-3-11	102
7.	γD-37-9	101
8.	γC-MD-2630-11-9	95
9.	γB-LT-728-10-7	89
10.	D_2IV-L7-46-20-7	86
11.	E_2IV-L8-74-56-10	86
12.	E_2IV-L8-75-56	84
13.	γA-69-7	77

(b) Genetic Stocks

Sl. No.	Name	Gene Symbol	Plant Height (Cm)
1.	Dee-geo-woo-gen	$\underline{sd_1}$	101
2.	I-geo-tze	$\underline{sd_1}$	100
3.	TR-17	$\underline{sd_1}$	95
4.	D-66	$\underline{sd_2}$	80
5.	TR-5	$\underline{d_t}$	82
6.	Tainan-5	(non-$\underline{sd_1}$)	71
7.	Cheng-chu-ai-11	(non-$\underline{sd_1}$)	93
8.	IR-28 dwarf	(double dwarf)	51
9.	K-8	(Tall variety)	139

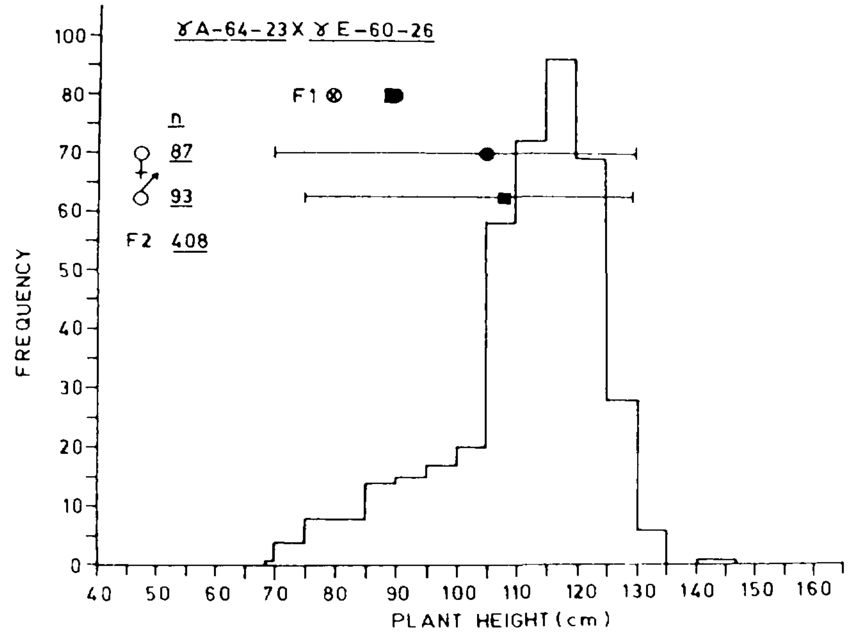

Fig. 4. Mean, range and/or frequency distribution for plant height in parents, F_1 and F_2 of the cross γA-64-23 x E-60-26.

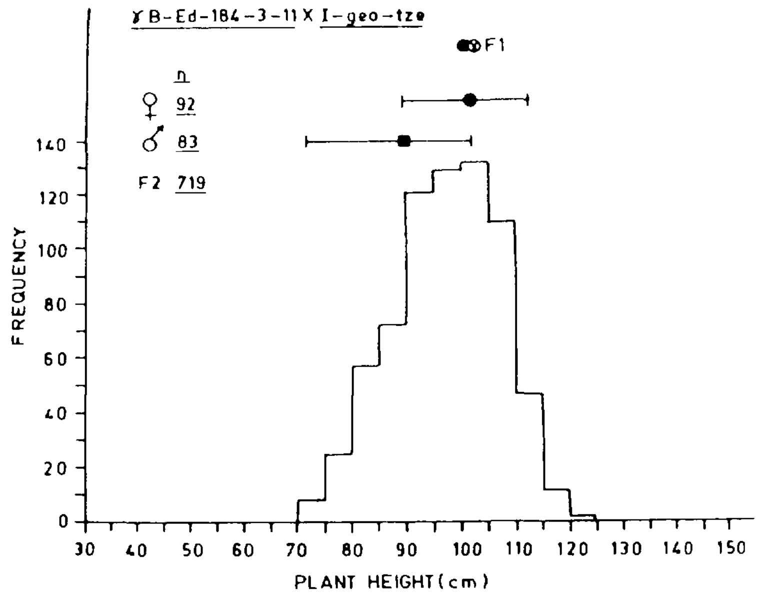

Fig. 5. Mean, range and/or frequency distribution for plant height in parents, F_1 and F_2 of the cross γB-Ed-184-3-11 x I-geo-tze.

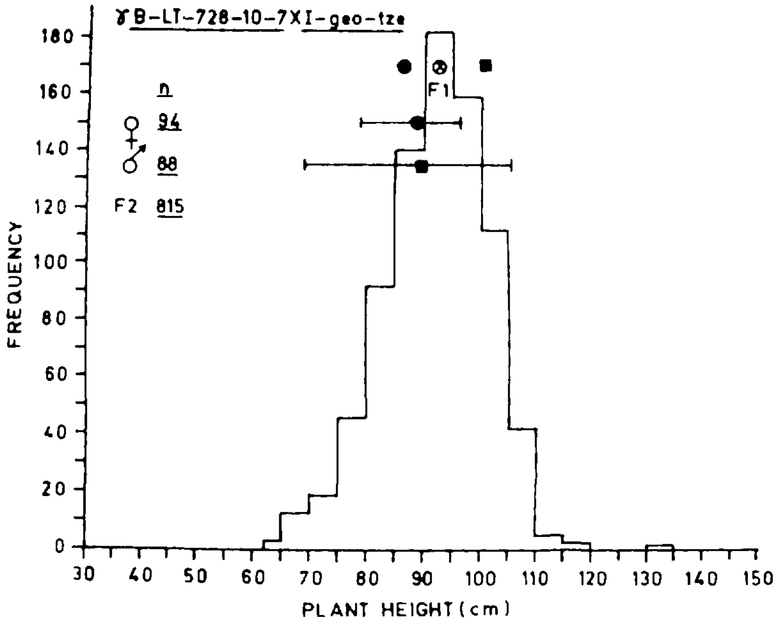

Fig. 6. Mean, range and/or frequency distribution for plant height in parents, F_1 and F_2 of the cross γB-LT-728-10-7 x I-geo-tze.

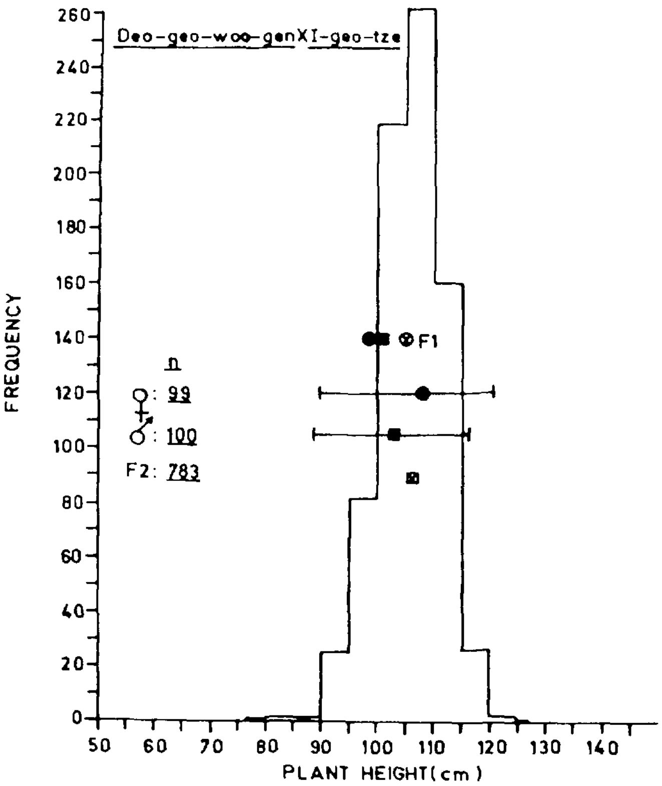

Fig. 7. Mean, range and/or frequency distribution for plant height in parents, F_1 and F_2 of the cross Dee-geo-woo-gen x I-geo-tze.

crosses. In three crosses (Nos. 5, 19 and 20 vide Figs. 8 to 10 respectively) parental height differences were present, F_1 resembled one of the cross parents and the F_2 segregation patterns were unimodal but skewed towards short height, thus indicating single gene pair differences among the parents of the particular cross. In five other crosses (Nos.3,4,9,16 and 18 vide Figs. 11 to 15 respectively) height differences were present among parents, F_1 resembled one of the cross parents and F_2 showed bimodal patterns of segregation, thus indicating again a single gene pair difference among the

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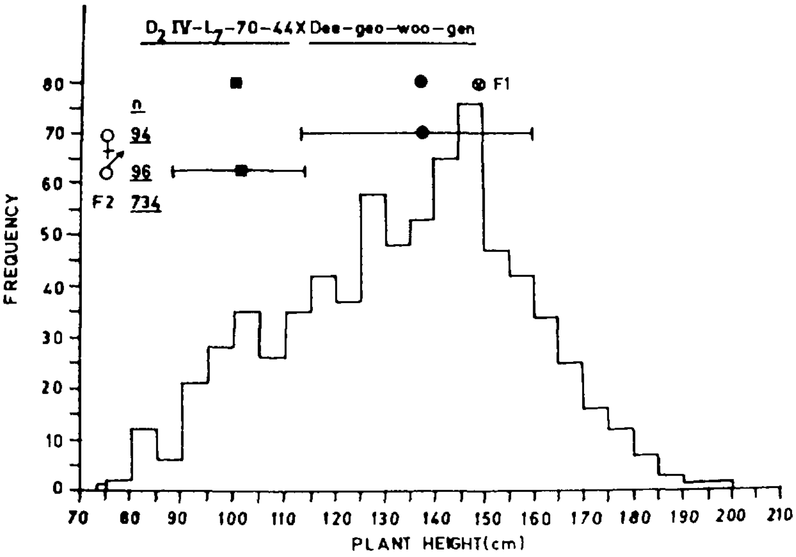

Fig. 8. Mean, range and/or frequency distribution for plant height in parents, F_1 and F_2 of the cross D_2IV-L7-70-44 x Dee-geo-woo-gen.

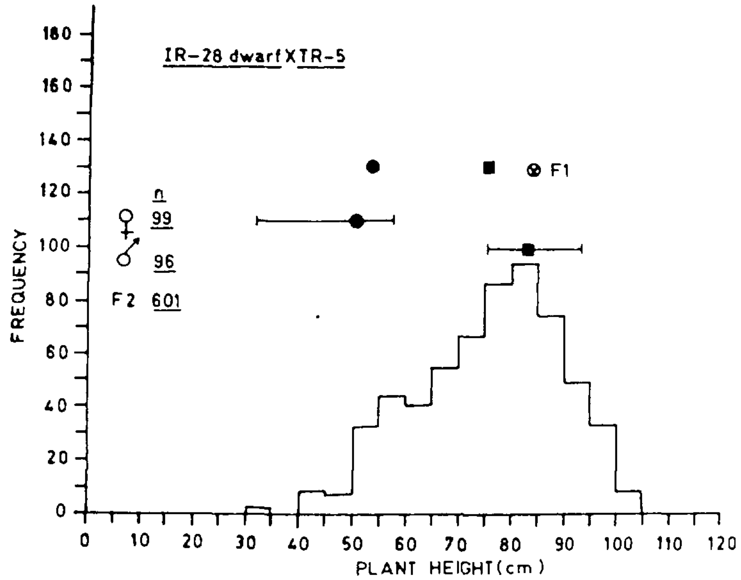

Fig. 9. Mean, range and/or frequency distribution for plant height in parents, F_1 and F_2 of the cross IR-28 dwarf x TR-5.

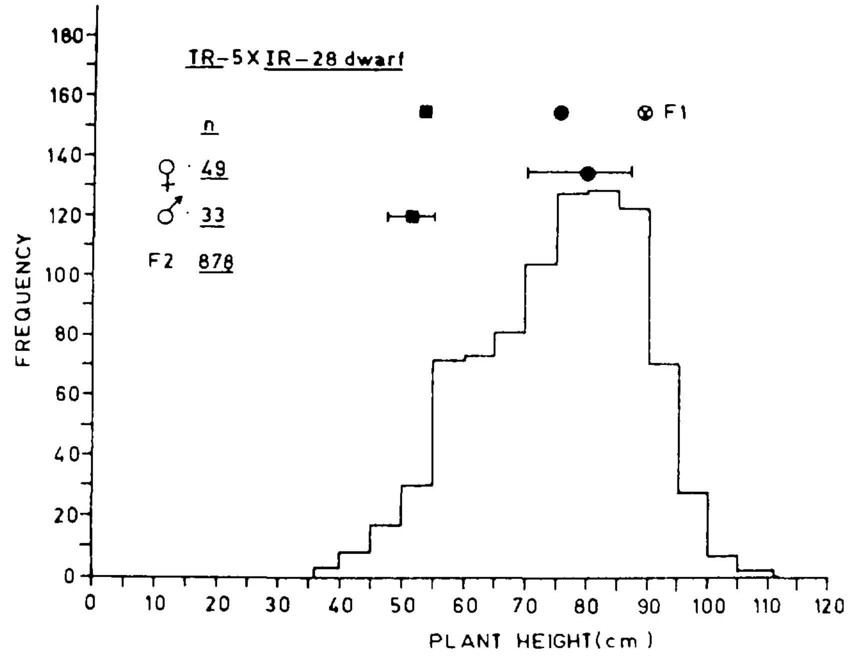

Fig.10. Mean, range and/or frequency distribution for plant height in parents, F_1 and F_2 of the cross TR-5 x IR-28 dwarf.

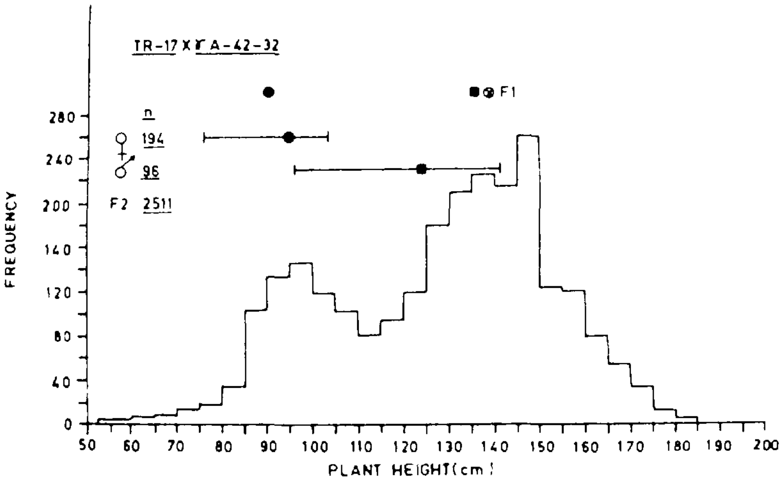

Fig.11. Mean, range and/or frequency distribution for plant height in parents, F_1 and F_2 of the cross TR-17 x γ A-42-32.

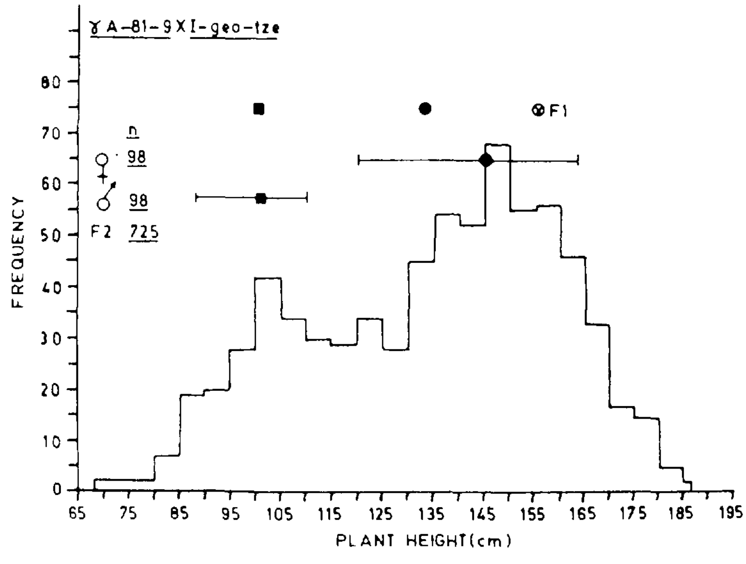

Fig.12. Mean, range and/or frequency distribution for plant height in parents, F_1 and F_2 of the cross γA-81-9 x I-geo-tze.

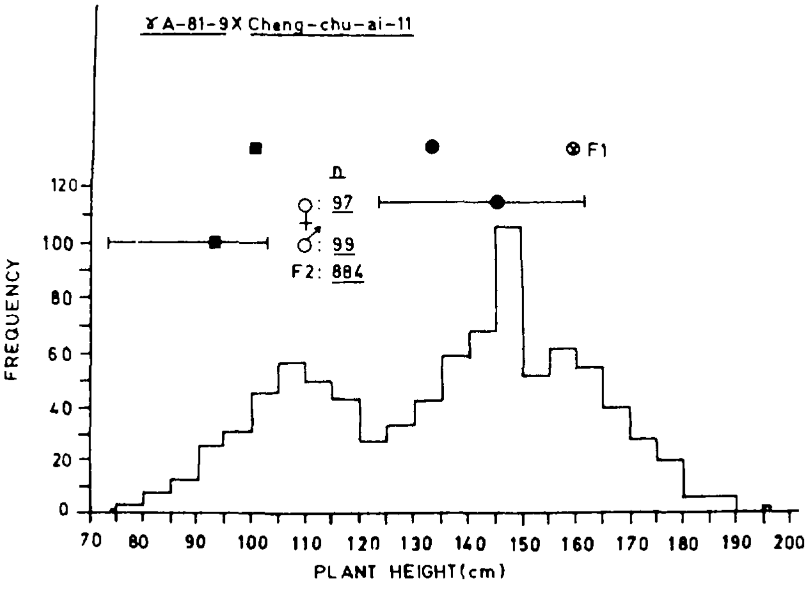

Fig. 13. Mean, range and/or frequency distribution for plant height in parents, F_1 and F_2 of the cross γA-81-9 x Cheng-chu-ai-11.

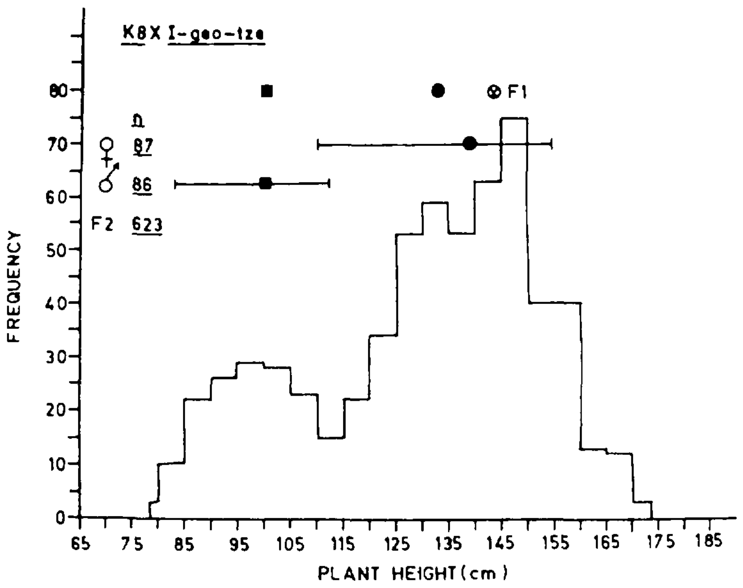

Fig.14. Mean, range and/or frequency distribution for plant height in parents, F_1 and F_2 of the cross K-8 x I-geo-tze.

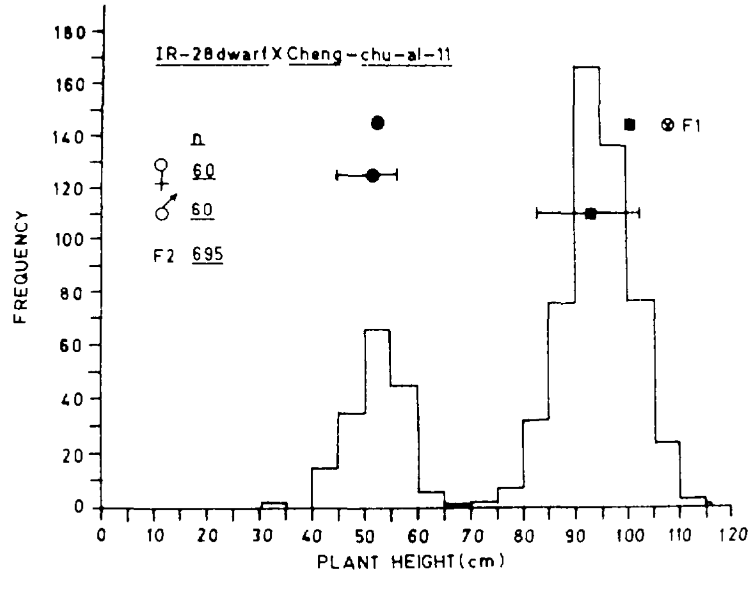

Fig.15. Mean, range and/or frequency distribution for plant height in parents, F_1 and F_2 of the cross IR-28 dwarf x Cheng-chu-ai-11.

parents of the respective crosses. In the remaining nine crosses (Nos. 2,6,10,11,12,13,14, 17 and 21 vide Figs. 16 to 24 respectively) parental height differred, F_{1s} were distinctly tall and F_2 showed multimodal and/or transgressive segregation patterns, thus indicating involvement of two different gene pairs in the respective cross parents. Genotypic segregations in F_2 families in all the cross progenies are still to be studied for confirmation of the F_2 phenotypic segregation patterns. Even so the above results do permit the following tentative deductions regarding the genetic constitution for dwarf or tall height of the mutants and genetic stocks. Those having genes allelic to sd_1 of Dee-geo-woo-gen and I-geo-tze are (a) γA-42-32, γD-81-9, D_2IV-L_7-70-44, E_2IV-L8-75-56 and K-8, all of them carry its dominant allelic pair (SD_1 SD_1); and (b) γB-Ed-184-3-11 and γB-LT-728-10-7, both of them carry the same recessive allelic pair (sd_1 sd_1). Non-sd_1 stocks are D-66 (sd_2 sd_2), TR-5 (d_t d_t and $sd_{x'}$, $sd_{x'}$), Cheng-Chu-ai-11 ($sd_{x'}$, $sd_{x'}$ and D_t D_t), Tainan-5 ($sd_{x''}$ $sd_{x''}$) and IR-28 dwarf ($sd_{x'}$, $sd_{x'}$, and d_t d_t). The following mutants are governed by single pair of semi-dwarfing genes in each case : γA-60-26 and γA-64-23 (both having the common gene pair (sd_{x1} sd_{x1}), γA-69-7 (sd_{x2} sd_{x2}), γD-37-9 (sd_{x3} sd_{x3}), D_2IV-L7-46-20-7 (sd_{x4} sd_{x4}), E_2IV-L8-74-56-10 (sd_{x5} sd_{x5}), E_2IV-L8-75-56 (sd_{x6} sd_{x6}) and γC-MD-2630-11-9 (sd_{x7} sd_{x7}). The sd_{x4} gene of the mutant D_2IV-L7-46-20-7 is found to be non-allelic to $sd_{x'}$ gene of Cheng-Chu-ai-11. While the dwarfing gene sd_{x6} of the mutant E_2IV-L8-75-56 is found to be non-allelic to the dwarfing gene sd_1 of Dee-geo-woo-gen, the relationships of the other dwarfing genes with sd_1 remain to be investigated.

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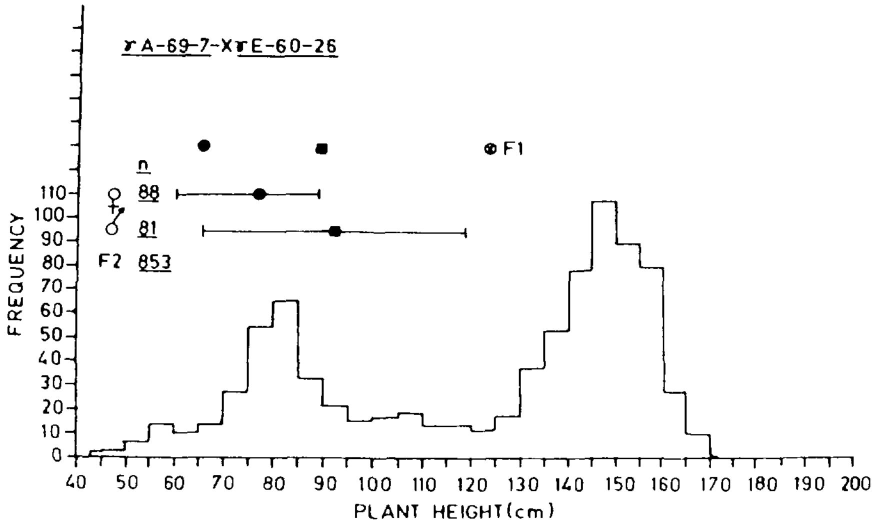

Fig.16. Mean, range, and/or frequency distribution for plant height in parents, F_1 and F_2 of the cross γA-69-7 x γE-60-26.

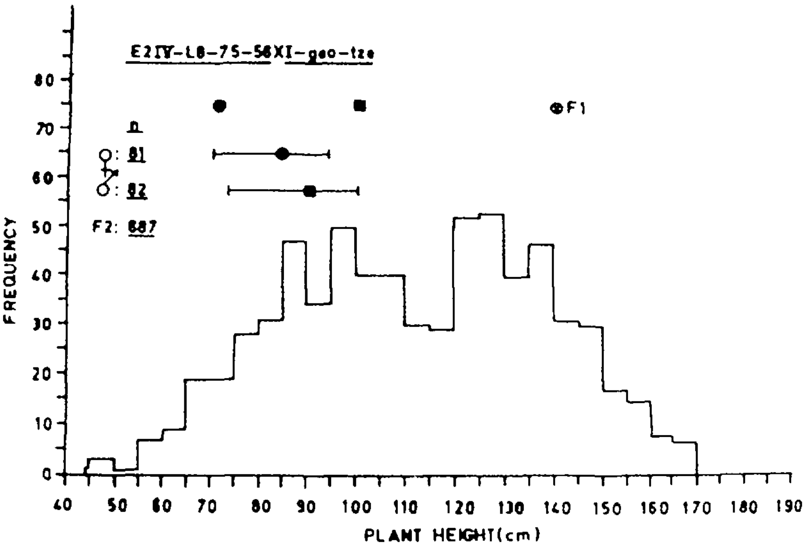

Fig.17. Mean, range and/or frequency distribution for plant height in parents, F_1 and F_2 of the cross E_2IV-L8-75-56 x I-geo-tze.

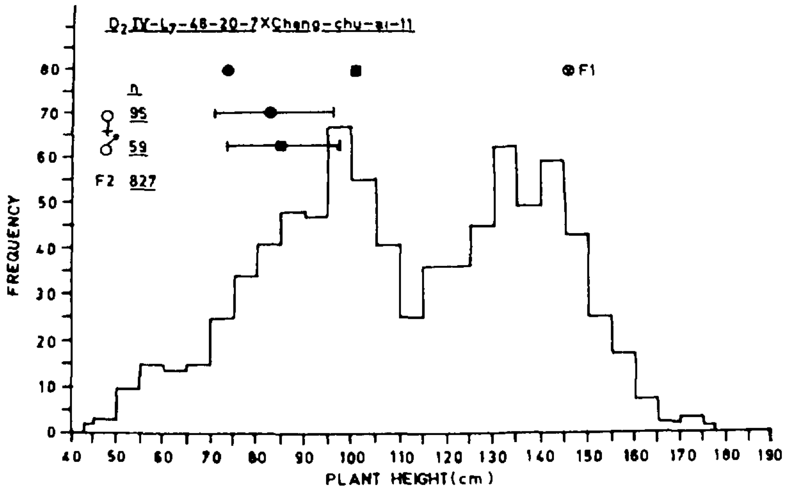

Fig.18. Mean, range and/or frequency distribution for plant height in parents, F_1 and F_2 of the cross D_2IV-L7-46-20 x Cheng-Chu-ai-11.

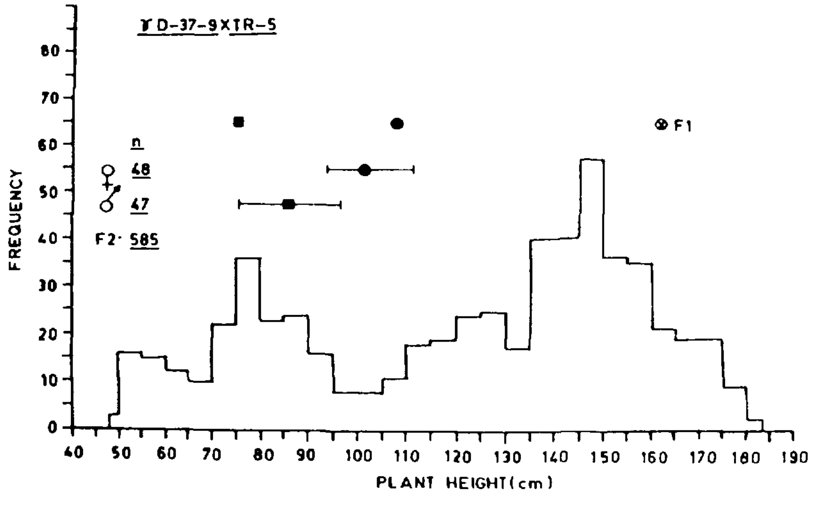

Fig.19. Mean, range and/or frequency distribution for plant height in parents F_1 and F_2 of the cross γD-37-9 x TR-5.

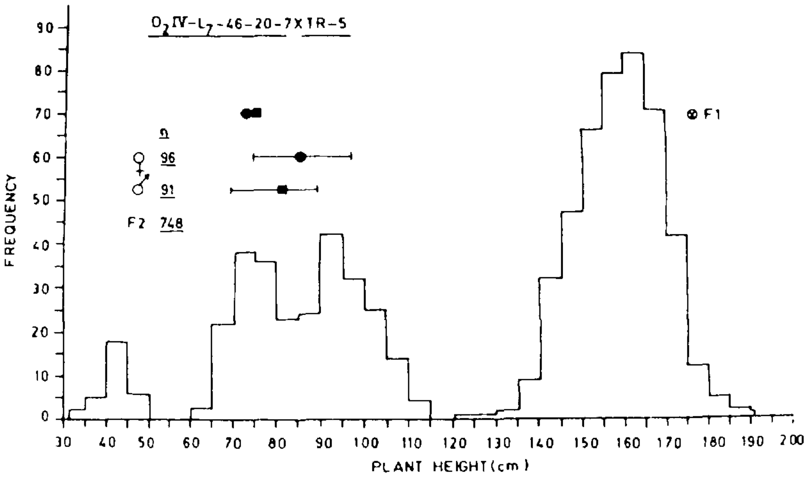

Fig.20. Mean, range and/or frequency distribution for plant height in parents, F_1 and F_2 of the cross D2IV-L7-46-20-7 x TR-5.

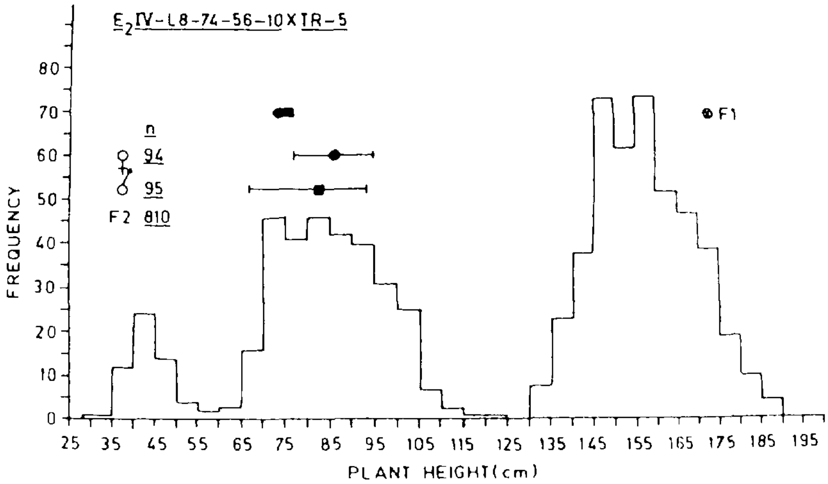

Fig.21. Mean, range and/or frequency distribution for plant height in parents, F_1 and F_2 of the cross E2IV-L8-74-56-10 x TR-5.

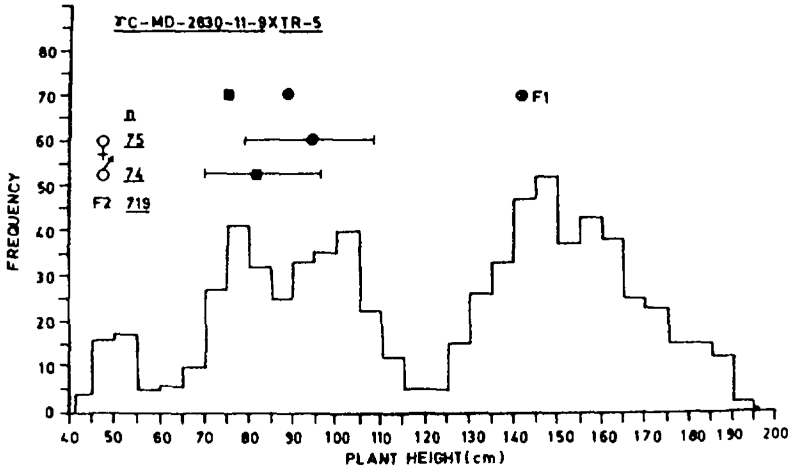

Fig.22. Mean, range and/or frequency distribution for plant height in parents, F_1 and F_2 of the cross γC-MD-2630-11-9 x TR-5.

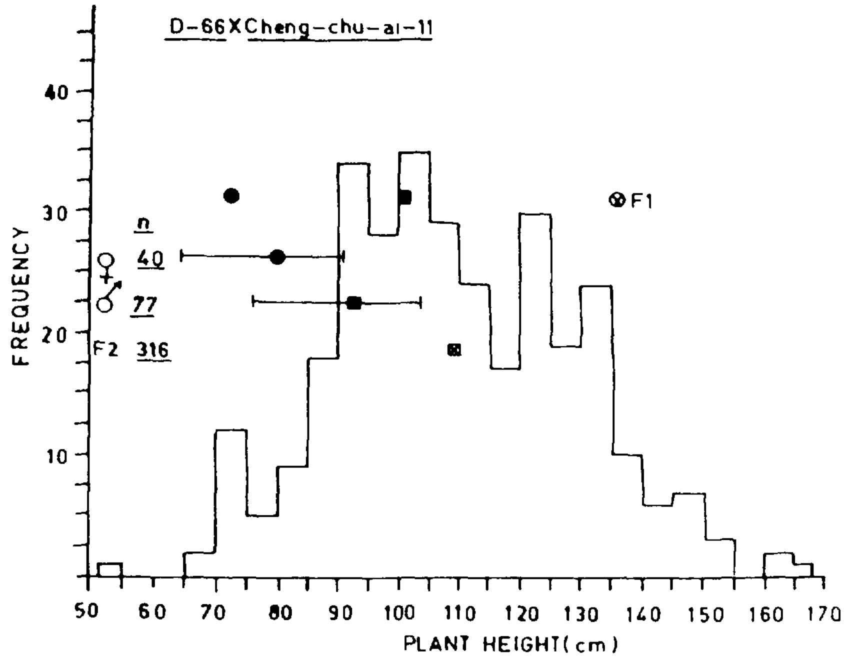

Fig.23. Mean, range and/or frequency distribution for plant height in parents, F_1 and F_2 of the cross D-66 x Cheng-chu-ai-11.

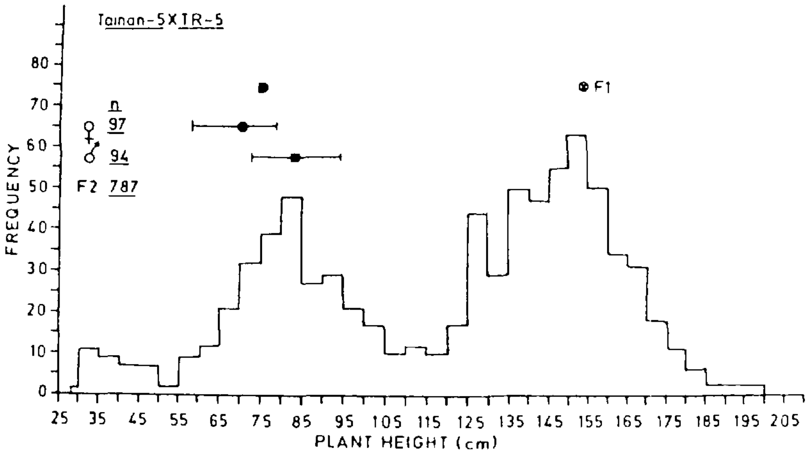

Fig.24. Mean, range and/or frequency distribution for plant height in parents, F_1 and F_2 of the cross Tainan -5 x TR-5.

TABLE 2.

PROMISING EARLY MUTANTS OF WHITE LUCHAI-112

1984 Wet Season

Sl. No.	Mutant Particulars		Days to E.E.	Plant Height (Cm)	Panicle Length (Cm)	Grain Yield/ha(Kg)		
	Code No.	Selection NO.				Sindewahi	Sakoli	Average
1	2	3	4	5	6	7	8	9
		Group I (16 Mutants + 3 Checks)						
1	TSSR-12	γE-23(b)-7-3	98	82.7	19.3	4239	4799	4519
2	TSSR-15	γC-ET-4145-12	100	104.7	20.0	3606	4434	4020
3	TSSR-13	γA-15-8	96	100.7	19.7	3241	4507	3874
4	TSSR-9	γE-23(C)-2	96	128.0	20.7	3289	4264	3776
5	TSSR-2	SL-Bulk-Ed-14-15	96	129.3	24.3	2364	5092	3728
6	TSSR-6	γC-ET-23-17-5	94	116.0	22.7	2485	4922	3703
7	TSSR-7	γE-23(C)-10-3	93	123.3	20.0	2070	4849	3459
8	-	Ratna (Ch)	90	89.3	24.0	3922	3241	3581
9	-	Hiramoti (Ch)	94	117.7	20.0	2144	2899	2521
10	-	Sye-44-3 (Ch)	87	90.7	20.3	3362	3118	3240
		CD/ha	-	-	-	NS	NS	-
		Group II (17 Mutants + 2 Checks)						
1	TSSR-27	A2-I-Ed-3	98	111.3	20.0	2606	4385*	3495
2	TSSR-19	D2-III-Ed-6	96	117.0	21.1	1948	4630*	3289
3	TSSR-26	A2-III-Ed-7	98	121.7	22.8	2631	3899	3265
4	TSSR-31	D2-III-Ed-13	99	120.7	20.1	1998	4264*	3131
5	TSSR-17	CA2-Ed-1	95	120.7	21.9	2364	3874	3119
6	TSSR-30	A2-I-Ed-2	100	122.7	21.6	1925	4166*	3045
7	TSSR-33	γG-3-3	100	123.0	21.1	2387	3630	3008
8	TSSR-20	γG-3-1	96	119.0	22.8	1802	4141*	2971
9	-	Hiramoti(Ch)	97	127.7	19.9	2588	3289	2923
10	-	SKL-1 (Ch)	111	115.0	19.7	2168	3606	2887
		CD/ha	-	-	-	NS	501 .	-

TABLE 2 (cont.)

1	2	3	4	5	6	7	8	9
		Group III (21 Mutants + 3 Checks)						
1	TSSR-38	γA-51-7	109	124.1	20.1	3703*	2533	3118
2	TSSR-35	γD-101-17	102	129.1	22.4	3660*	2358	2909
3	TSSR-53	A2-II-SL-ET-3	114	125.5	19.2	3095	2690	2892
4	TSSR-44	γE-49-12	107	109.2	18.6	2875	2519	2697
5	TSSR-50	γA-11-2	109	120.7	19.3	2758	2606	2682
6	-	SKL-1(Ch)	112	131.6	19.5	3606*	1925*	2765
7	-	Sye-75(Ch)	111	94.0	20.3	3362*	1973*	2667
8	-	WL-112(Ch)	112	126.5	18.3	2538	2704*	2631
		CD/ha	-	-	-	765	407	-
		Group IV (5 Mutants + 3 Checks)						
1	TSSR-55	γE-58-18	103	124.6	23.0	3776	1729	2752
2	-	R.P.4-14(Ch)	101	81.5	22.0	5656*	1633	3594
3	-	Sye-75(Ch)	111	95.9	19.4	4265	1267*	2766
4	-	W.L.-112(Ch)	111	116.0	16.4	3436	1754	2595
		CD/ha	-	-	-	860	-	-

Source: Punjabrao Agricultural University, Akola, Annual Report : Rice - 1984-85.

* Significantly higher or lower than W.L. - 112.

CONCLUSIONS

Early and/or reduced height non-lodging mutants have been successfully induced in the photosensitive, tall and fine-grained variety W.L.112. Several of these mutants retain the grain character and yield potential of the parent variety. It has been possible to select in F_2, early plants combining also the semi-dwarf non-lodging character of one parent TR-17 with the fine grain character of the other parent γA-42-32 which is an early and reduced height mutant of W.L.112. Promising reduced height non-lodging mutants exhibited different culm lengths, internode numbers and internode elongation and thickness patterns. Genetic analysis of reduced height character has been attempted by studying upto F_2 the progenies of 21 crosses involving 13 mutants of W.L. 112 and 9 genetic stocks, 8 of them semi-dwarfs . While two of the mutants carry the same $\underline{sd_1}$ gene as that of Dee-geo-woo-gen, eight others are governed by seven seperate single genes tentatively designated as $\underline{sd_{x1}}$ to $\underline{sd_{x7}}$. One of the genes $\underline{sd_{x1}}$ is common in two mutants and is non-allelic to $\underline{sd_{x2}}$. Another gene $\underline{sd_{x6}}$ is non-allelic to $\underline{sd_1}$. Genetic composition of the other parents and certain other inter-relationship among dwarfing genes are also inferred from the available results.

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EVALUATION, INHERITANCE, AND GIBBERELLIN RESPONSE OF INDUCED SEMI-DWARF MUTANTS OF RICE

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Abstract

The induced semidwarf mutant gene, sd_1, has been used to develop nine rice cultivars in California. This induced mutant gene is allelic to the semidwarfing gene in DGWG, T(N)1, IR8, and other Green Revolution cultivars. Six other mutants are non-allelic to sd_1. To date, none of these six has been as agronomically useful as sd_1, but three warrant further investigation if genetic vulnerability should arise from widespread usage of sd_1. Seedling response of genotypes to exogenous gibberellin (GA) fell into three broad categories: High response (second leaf sheath length at 10 ppm of GA more than 450% of control), intermediate response (300-450%), and low response (less than 300%). The high response category consisted of Tanginbozu and the semidwarf mutant Short Labelle. The low response category consisted of the tall indica cultivar Peta and the tall southern US cultivar Labelle. The remaining genotypes, including the semidwarf indica cultivar DGWG, the two tall japonica cultivars Calrose and M5, three japonica semidwarfs with the induced mutant gene sd_1, the indica-japonica derived semidwarf cultivar M9, and the other 8 induced semidwarfs, showed intermediate response. The major semidwarfing gene sd_1, whether derived from induced mutation or from the DGWG source, does not seem to affect GA-response in japonica backgrounds. In contrast, in indica backgrounds the sd_1 gene is associated with increases in GA-responsiveness. The implications of GA responsiveness of semidwarfs in rice breeding remain unclear.

1. INTRODUCTION

The three agencies cooperating in rice research in California (the University of California, the U.S. Department of Agriculture, and the California Co-operative Rice Research Foundation, Inc.) have made extensive use of induced semidwarf mutants in rice breeding [1]. In this cooperative effort the first two agencies investigate genetics and breeding methods, including induced mutation, while the last agency takes principal responsibility for cultivar development. Since 1976, two induced semidwarf mutants have been directly released as cultivars, while seven more semidwarf cultivars have been developed by cross-breeding with induced mutants or their derivatives. All nine cultivars carry the sd_1 source of semidwarfism, which is allelic to the major semidwarfing gene in DGWG, T(N)1, IR8, and other Green Revolution cultivars of the tropics.

Results achieved since 1980 within the Co-ordinated Research Programme fall into two categories: inheritance of additional semidwarf mutants which are nonallelic to the primary sd_1 source, and determination of the response of semidwarf mutants to exogenous gibberellin (GA). The first category was on-going when the Programme began and has been extended during this period, while the second category was initiated as a result of discussions at the first RCM.

2. INHERITANCE OF SEMIDWARFISM

The inheritance and allelic relationships of the several semidwarf mutants produced in California was recently reported [2] and hence will be summarized only briefly. Three independent, recessively inherited semidwarf genes, sd_1, sd_2, and sd_4, were induced in the original mutagenic treatments of the tall cultivar Calrose. Following the elucidation of the allelic relationships among these three genes [3, 4], subsequent studies with new mutants were concentrated only on determining the allelic relationship to the sd_1 reference source. Thus four additional induced semidwarfs are now known to be nonallelic to sd_1 (Figure 1). These semidwarf mutants

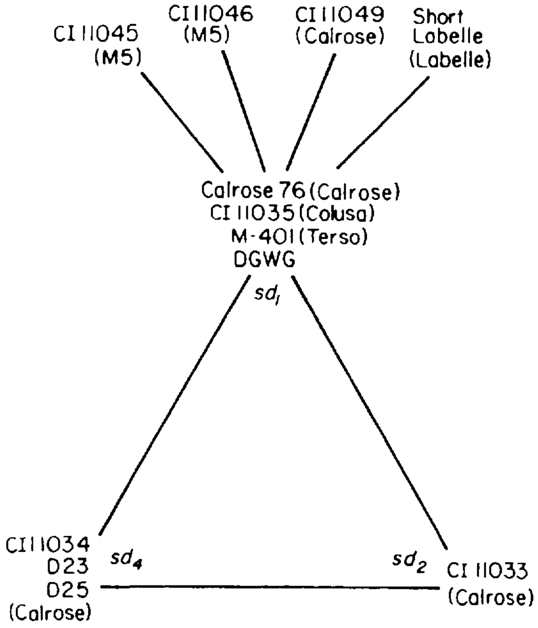

Figure 1. Allelic relationships of induced semidwarf mutants and DGWG. Genotypes at the same corner of the triangle are allelic; those at different corners are nonallelic. Genotypes in the "fan" at top are nonallelic to the sd_1 locus but their relationships to other loci are unknown. Parent cultivars are shown in brackets. From Rutger et al. [2].

and their tentative gene symbols are: Short Labelle, $\underline{sd}_{5-t}$; CI 11049, $\underline{sd}_{6-t}$; CI 11045, $\underline{sd}_{7-t}$; and CI 11046, $\underline{sd}_{8-t}$. The $\underline{sd}_1$ gene, which reduces height about 25 cm and leads to 15-20% yield increases, has been the most agronomically useful semidwarf source. Thus the $\underline{sd}_2$ source reduces height only 15 cm instead of the desired 25 cm; the $\underline{sd}_4$ source likewise reduces height only 15 cm and has an additional pleiotropic affect for a 20% reduction in seed size; and the $\underline{sd}_{6-t}$ gene has a pleiotropic effect for short narrow leaves and reduced seed size. The $\underline{sd}_{5-t}$, $\underline{sd}_{7-t}$, and $\underline{sd}_{8-t}$ genes produce the appropriate height reduction and seem to have little or no harmful pleiotropic effects; however ,none has yielded more than their respective tall parent [4]. A peculiarity of the Short Labelle mutant is that it shows considerable sterility when backcrossed to its tall parent, although Short Labelle itself is fully fertile [5]. Whether these latter three semidwarfs would benefit from a "cleaning up" process of crossing into other backgrounds is not known, but should the $\underline{sd}_1$ source ever prove genetically vulnerable, these three will certainly receive greater attention.

3. GIBBERELLIN RESPONSE

3.1 Introduction

Dwarf gene sources in cereals have been known to exhibit differential culm elongation responses to gibberellin (GA) at least since 1956, when Phinney [6] reported such differences in maize. Following discussions at the first RCM about the non-responsiveness of the widely used Norin-10 semidwarfing genes in wheat, we decided to investigate the GA response of our induced semidwarf mutants in rice. We were particularly interested in determining if the most useful induced mutant gene, $\underline{sd}_1$, showed any unique GA response pattern. Previous work by other researchers indicated that the $\underline{sd}_1$ source derived from DGWG was GA-responsive in indica backgrounds [7, 8] but not in japonica backgrounds [9].

3.2 Materials and Methods

Four experiments were conducted to determine response to exogenous GA of 12 induced semidwarf rice mutants plus appropriate dwarf, semidwarf, and tall controls, using the procedure outlined by Harada and Vergara [7]. GA response was expressed by the length of the secondary leaf sheath as a percentage of its control (treatment/control x 100 = GA response). Tanginbozu and DGWG were included in all four experiments as standard GA responsive dwarf and semidwarf genotypes, respectively. The induced semidwarf mutant Short Labelle also was included in all four experiments.

Experiment 1 included five genotypes. The ancestry, plant type, and semidwarf allele of these and subsequent genotypes are shown in Table 1. Rice seeds were surface sterilized in 2.62% sodium hypochlorite for approximately 15 minutes, rinsed several times with distilled water, placed in

Table 1. Ancestry, plant type, semidwarf allele, and length of second leaf sheath of 12 induced semidwarf mutants, 2 semidwarf cultivars, 1 dwarf cultivar, and 4 tall cultivars of rice used in GA-response studies.

Genotype	Ancestry[a]	Plant type[b]	Semidwarf allele	GA response category[c] Experiment 1	2	3	4
DGWG	I	S	$\underline{sd}_1$	I	I	I	I
Peta	I	T		L			
Tanginbozu	J	D		H	H	H	H
Short Labelle	S	IS	$\underline{sd}_{5-t}$	H	H	H	H
Labelle	S	T		L			L
Calrose 76	J	IS	$\underline{sd}_1$		I		I
Calrose	J	T			I	I	I
M9	I/J[4]	S	$\underline{sd}_1$		I		
M5	J	T					I
M-401	J	IS	$\underline{sd}_1$		I		I
CI 11033	J	IS	$\underline{sd}_2$			I	
CI 11034	J	IS	$\underline{sd}_4$			I	
CI 11035	J	IS	$\underline{sd}_1$			I	
CI 11045	J	IS	$\underline{sd}_{7-t}$			I	
CI 11046	J	IS	$\underline{sd}_{8-t}$			I	
CI 11047	J	IS	Unknown			I	
CI 11048	J	IS	Unknown			I	
CI 11049	J	IS	$\underline{sd}_{6-t}$			I	
CI 11050	J	IS	Unknown			I	

[a] I = indica, J = japonica, S = southern US type, I/J[4] = indica-japonica, backcrossed to japonica.

[b] S = semidwarf, T = tall, D = dwarf, IS = induced semidwarf.

[c] H = high response (second leaf sheath at 10 ppm of GA more than 450% of control), I = intermediate response (300-450% of control), L = low response (less than 300% of control).

glass petri dishes containing #2 Whatman filter paper with 5 ml of distilled water and set in the dark for 48 hours at 30 ± 2 C. The uniformly germinated seed were transferred to 20 x 125 mm glass test tubes, 8 seeds per tube, containing 0.5 ml solutions of GA_3[1] at concentrations of 0 and 10 ppm, and covered with transluscent plastic caps. Six replications were used, in randomized complete blocks. The test tubes were placed in continuous light, 180 microeinsteins $m^{-2}sec^{-1}$ at plant level, at 30 ± 2 C. On the third and sixth day 0.5 ml of distilled water was added to each test tube. Measurements of the second leaf sheath were taken on the eleventh day after initiation of the experiment.

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[1] Grade 111, 90% minimum GA_3 provided by Sigma Chemical Co.

Experiments 2, 3, and 4 were conducted over subsequent two-week intervals, in similar fashion to Experiment 1, with changes in test genotypes as noted in Table 1.

3.3 Results and Discussion

In Experiment 1, the cultivar Tanginbozu showed the expected high response to GA (Figure 2). The only indica cultivars in the experiment showed response patterns in line with previous reports [7, 8], in that the semidwarf cultivar DGWG showed more response than the tall cultivar Peta. The most interesting responses were shown by Short Labelle, which was the most responsive genotype in the experiment, and by its tall parent, Labelle, which was relatively non-responsive. In this and subsequent discussions, genotypes will be categorized as having high response to GA (second leaf sheath length at 10 ppm of GA more than 450% of control), intermediate response (300-450% of control), and low response (less than 300% of control).

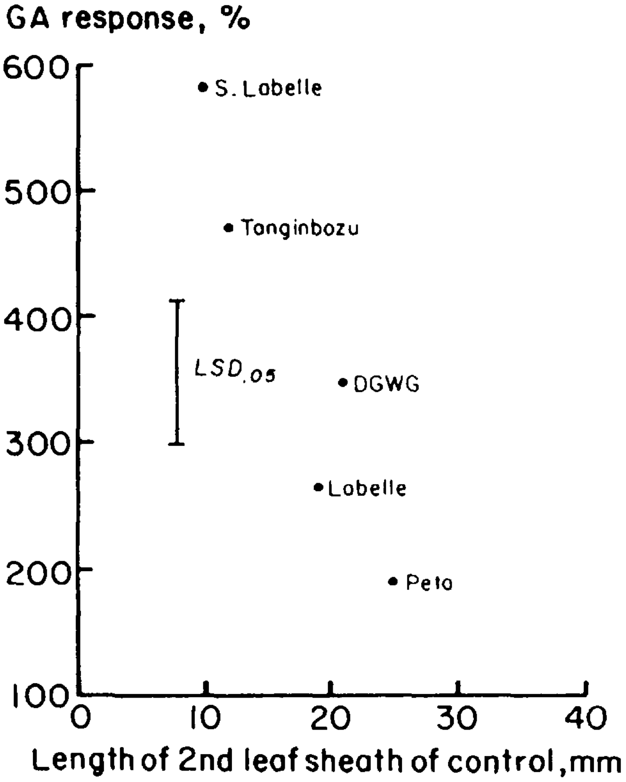

Figure 2. Relative elongation responses of second leaf sheath to 10 ppm of gibberellin of genotypes in Experiment 1.

In Experiment 2, Short Labelle and Tanginbozu again were the most responsive entries (Figure 3). DGWG showed about the same intermediate level of response as in Experiment 1. Three other semidwarfs, M-401, M9, and Calrose 76, all of which possess the same sd_1 locus as DGWG, showed responses similar to DGWG. The tall cultivar Calrose also showed response similar to DGWG. Calrose and Calrose 76 provide an interest-

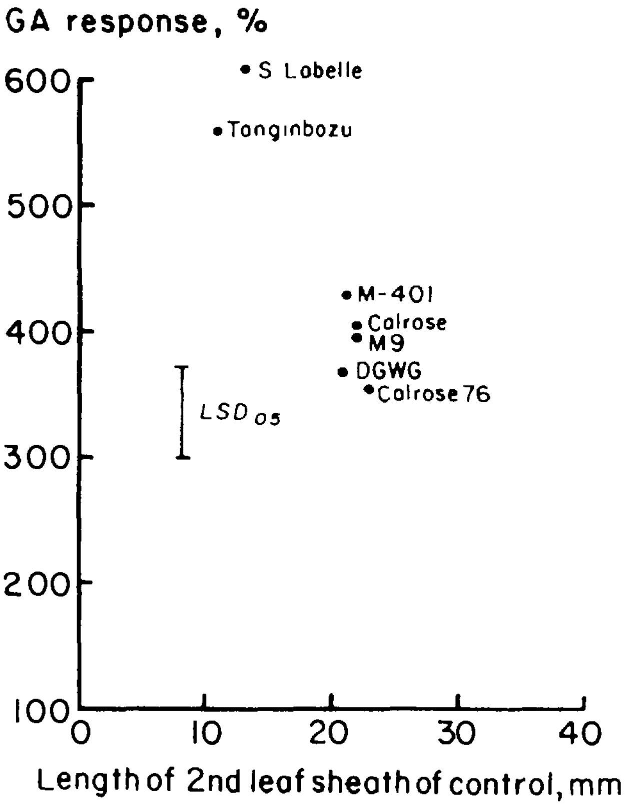

Figure 3. Relative elongation responses of second leaf sheath to 10 ppm of gibberellin of genotypes in Experiment 2.

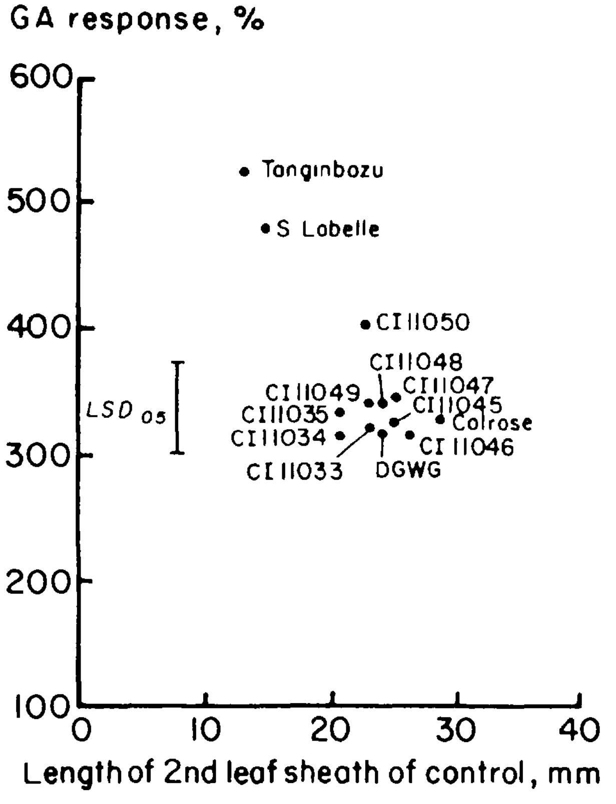

Figure 4. Relative elongation responses of second leaf sheath to 10 ppm of gibberellin of genotypes in Experiment 3.

ing comparison, in that the latter is a semidwarf mutant of the former, and that their GA responses are nearly identical. Thus, the $\underline{sd}_1$ gene in this japonica background has no distinctive effects on GA response.

In Experiment 3, Short Labelle and Tanginbozu again were the most responsive entries, although the response of Short Labelle was not as pronounced as in the two previous experiments (Figure 4). DGWG again showed an intermediate response level. The remaining semidwarf genotypes, and the tall cultivar Calrose, all gave intermediate level responses which were nearly indistinguishable from each other and from DGWG. Included in this intermediate group were the induced semidwarf mutants CI 11033 and CI 11034, which carry the $\underline{sd}_2$ and $\underline{sd}_4$ genes, respectively. Another member of the intermediate group, CI 11049, also is a non-allelic (to $\underline{sd}_1$) semidwarf mutant from Calrose. Yet another member, CI 11035, which is an induced semidwarf mutant from the tall California cultivar Colusa, has the $\underline{sd}_1$ gene.

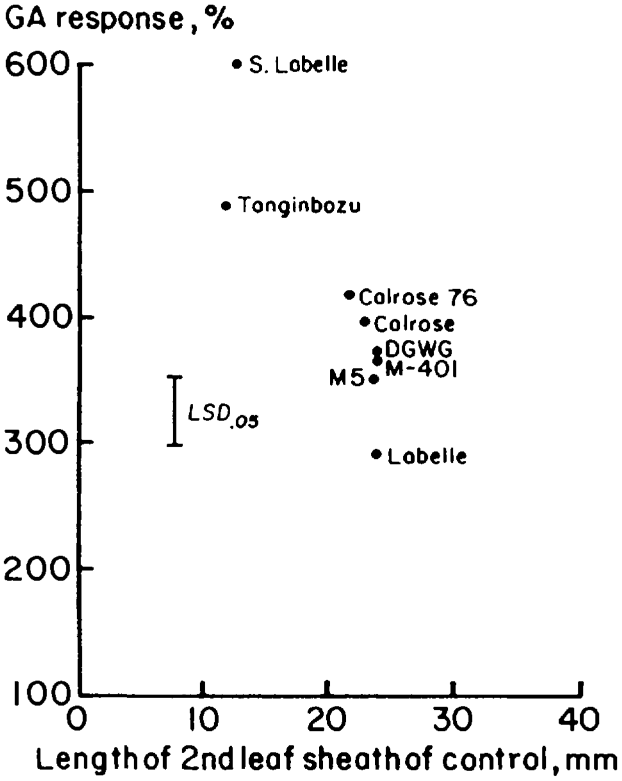

Figure 5. Relative elongation responses of second leaf sheath to 10 ppm of gibberellin of genotypes in Experiment 4.

In Experiment 4, Short Labelle and Tanginbozu again were the most responsive entries, and DGWG again showed an intermediate response level (Figure 5). Calrose 76 and its tall parent Calrose also were again in the intermediate group and showed nearly identical responses to each other. M-401, an induced semidwarf mutant from the tall cultivar Terso, also carries the $\underline{sd}_1$ gene and falls in the intermediate group, as does the tall cultivar M5. The tall cultivar Labelle showed a lower response level, as it did in Experiment 1.

An interesting finding in the present experiments was that the induced semidwarf mutant Short Labelle was highly GA-responsive, exceeding or at least being equal to Tanginbozu, which is the standard high-GA-response cultivar for GA tests. Short Labelle has been yield-tested in the southern U.S., but does not yield more than its tall parent [5]. As noted earlier, Short Labelle has the semidwarfing gene $\underline{sd}_{5-t}$, which is non-allelic to $\underline{sd}_1$.

A principal conclusion from these four experiments is that the induced semidwarf mutant genes in japonica backgrounds, $\underline{sd}_1$, $\underline{sd}_2$, $\underline{sd}_4$, $\underline{sd}_{6-t}$, $\underline{sd}_{7-t}$, $\underline{sd}_{8-t}$, and three unknown gene sources (CI 11047, CI 11048, and CI 11049) show the same intermediate GA response levels as do tall japonica cultivars. This is particularly noticeable for $\underline{sd}_1$, which has been widely used in California breeding programs, and which is at the same locus as the semidwarfing gene from DGWG, T(N) 1, IR8, and other Green Revolution cultivars. By contrast, in indica backgrounds it has been reported [7, 8] that cultivars with the $\underline{sd}_1$ gene are more GA-responsive than tall cultivars. The semidwarf DGWG versus tall Peta comparison, both of which are indica cultivars, in Experiment 1 of the present studies, confirmed those earlier findings. Thus, it appears, that the GA response of the $\underline{sd}_1$ locus is dependent upon background genotypes. Even when the $\underline{sd}_1$ gene was backcrossed into the japonica background from IR8 to produce the semidwarf cultivar M9, the semidwarf maintained its intermediate GA response level, which was not noticeably different from the intermediate GA-response level of the tall japonica cultivar Calrose (Experiment 2). Similar results were noted by Matsunaga et al. [9], who found little difference in GA-response between semidwarfs, developed by backcrossing the semidwarfing gene from T(N)1 into the tall japonica cultivar Norin 29, and the tall cultivar itself. The implications of GA-response of semidwarfs in rice breeding remain unclear.

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MUTATION BREEDING FOR SEMI-DWARFISM IN BARLEY*

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Abstract

Mutation breeding has been an important part of the barley breeding effort for reduced height and lodging resistance at Washington State University. Between 1980 and 1985, 264 sodium azide induced putative semi-dwarf mutants have been selected in the M_2 from 19 spring genotypes. Most have been discarded due to serions defects. Fifteen mutants were chosen for genetic analysis and agronomic evaluation to determine their usefulness for cross-breeding or even as potential cultivars. In addition to reduced culm length (65-88% vs normal), most mutants had similar or reduced culm diameters, while one mutant had larger culm diameters compared to their normal isotypes. Coleoptile lengths varied similarly. Six of these mutants are presented here as representative of the range of mutants studied. All mutants appear to carry a single recessive gene for semi-dwarfism. Most non-allelic mutant crosses gave 9:7 F_2 ratios, while one combination produced a double dwarf indicating additive gene interaction. Two of the mutants are erectoides (ert) types, and the rest appear to be sdw, semi-dwarf types. An incomplete diallele cross series revealed some allelic, but mostly non-allelic gene relationships. Mutant agronomic performance was variable, but most mutants were lower in yield than their respective normal isotypes. The mutant, Mo1, in 'Morex' was 19% higher in yield than Morex. Kernel quality (density, plumpness) tended to be lower in most of the mutants. Lodging was reduced in most mutants but not in all. Whereas some of the mutants appeared to be improved for several agronomic traits such as lodging resistance, yield and earliness none combined all the traits necessary for a new improved cultivar. The mutants, Mo1 and Mo4 in Morex and L2 in 'Larker' should be good parents for cross-breeding especially for lodging resistance or earliness. Cross-breeding should eliminate deleterious mutation effects and put the semi-dwarf genes into more suitable genetic backgrounds.

INTRODUCTION

Breeding for reduced height in barley (Hordeum vulgare L.) to primarily reduce the risk of lodging in commercial production remains an important objective in barley improvement programs around the world. This is especially true in areas with high rainfall or irrigated production. Most of the sources of semi-

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dwarfism used in barley breeding programs originated from induced mutations. Although some semi-dwarf barley mutants have been released directly, many have been modified by cross-breeding before release as commercial cultivars. Subsequent to their release most semi-dwarf mutant cultivars have given rise to other cultivars through cross-breeding. See Konzak et al. and Micke et al. (1,2) for recent reviews.

In addition to using a number of previously described spring and winter semi-dwarf barley mutant and cultivar sources (eg. 3, 4, 5, 6, 7,) in pedigree and recurrent selection breeding, the Washington State University (WSU) barley breeding program has been involved in induced mutagenesis with several locally adapted cultivars and breeding lines. The WSU mutation breeding program in general has involved the induction of new mutants, agronomic evalution, genetic analysis and use of selected mutants in breeding. The emphasis has been on proanthocyanidin-free (a quality factor) (8) and semi-dwarf (9, 10, 11) mutants.

Knowledge of the agronomic performance combined with knowledge of the number, action and interaction of the dwarfing genes of a pool of induced mutants is very important for the efficient selection of parents for cross-breeding. With this in mind, the objective herein, is to review the progress of the WSU semi-dwarf barley mutation breeding program by considering the various aspects of the program (listed above) with an emphasis on the agronomic and genetic evalution of a selected group of induced mutants. This report updates three previous reports (9, 10, 11).

MATERIALS AND METHODS

Mutant Induction. All mutant selection occurred in the M_2 generation, space planted from bulk-harvested M_1's in the field at Pullman, WA. Sodium azide was the mutagen applied at a concentration of 10^{-3} M at pH 3 for 2 h to presoaked seeds. This paper gives results from 6 years of selection beginning in 1980. The progenitor barleys have been malting 2-row and 6-row types from primarily western, but also midwestern North American adaptation (see TABLE I).

Genetic Analysis. Eighteen putative mutants in 9 genotypes selected in 1980 and 1982 were chosen for genetic analysis and agronomic evaluation in 1984 and 1985 in Pullman, WA after preliminary evaluations. Crosses were made in 1984 among semi-dwarf mutants and with their normal isotypes or other normal height genotypes to determine allelism, gene number and gene action and interaction. Parents, F_1's and F_2's (from greenhouse propagation) were planted in the field in 1985. F_1 and F_2 populations were space-planted in rows 2 and 3 m long, respectively, with parent rows flanking each population.

Culm length was determined by averaging the length of the 3 tallest tillers, measured from the base of the culm to the base of the spike, for each individual plant in the parent, F_1 and F_2 populations.

Allelism was determined by comparing the culm lengths of F_1's, F_2's, mutant parents as well as that of the normal isotypes for each cross by pooled t-test. Gene number, action and interaction were determined by appropriate chi-square tests for all segregating populations. Height classes were determined by considering mutant and normal isotype means and ranges for culm length.

Agronomic Evaluation. The 18 putative semi-dwarf mutants mentioned above along with their respective normal isotypes were planted in replicated yield trials in 1984 and 1985 in Pullman, WA using conventional farming practices. The design was randomized complete block with four replications. Each plot consisted of 4 rows 3 m long with 30 cm row spacing.

Data were collected on culm and spike length, days to heading, physiological maturity, lodging, tillering, grain yield, harvest index, volume weight, kernel plumpness, internode number and length, culm diameter, sterility and grain protein. Only culm length, culm diameter, days to heading, grain yield, harvest index, volume weight, kernel plumpness and lodging data are reported here. A full account of the genetic and agronomic experiments can be found in Aydin (12).

Culm length was measured from main culms of 5 randomly selected plants/plot from the ground to the base of the spike and culm diameter from these 5 main culms at the basal internode with a vernier calipers near harvest time. Days to heading were determined from the date of planting to when 50% of the plot was headed. Grain and shoot yields were measured from the two center rows (hand cut) of each plot. Harvest index was calculated as the ratio of grain to total shoot weight. Wolume weight and kernel plumpness (on 2.8×1.9 mm sieve) data were obtained from the kernels on a volume weight machine and timed sieve-shaker device, respectively. Lodging was measured as the percent of lodged culms in a plot at harvest maturity. Data were analyzed by analysis of variance and $LSD_{.05}$.

An experiment was conducted in the laboratory with seed from the 1984 field experiment to determine gibberellic acid (GA_3) response and coleoptile length using the Myhill-Konzak (13) seedling culture technique. Twenty-two seedlings (11 GA_3 treated, 11 untreated) of each mutant and normal isotype were grown 7 days in complete darkness and 7 days in light with 1/4 Hoagland solution and 10 ppm GA_3 in treated cultures. After the 14 days, seedling heights were measured. Coleoptile lengths of the untreated seedlings were also measured. Comparisons between mutant and normal isotypes were made by paired t-test.

Breeding. Selected mutants and/or their cross-bred progeny have been used as parents in a basically pedigree system for malting and feed types of barley and in male sterile facilitated recurrent selection (14).

RESULTS AND DISCUSSION

Mutant Induction. Over the 6 years since 1980, 264 putative semi-dwarf mutants induced in 19 cultivars or breeding lines were selected (TABLE I). A single row was planted of each putative mutant in the year after selection to increase the seed and make an initial evaluation in comparison with the respective normal isotypes (progenitors). Selected mutant lines were entered into preliminary yield trials the following year (TABLE I).

In most cases a large attrition occurred. Some putative mutants turned out not to be reduced in height, and in general, the majority of induced mutants showed severe defects in fertility, yield and other characters of agronomic importance. Undoubtedly a number of deleterious mutations usually occur simultaneously with each desired height reducing mutation. Neverthless, several mutants have emerged as parental material for cross-breeding which is described below.

TABLE I. Putative sodium azide-induced semi-dwarf spring barley mutants selected between 1980 and 1985 and their disposition.

Progenitor cultivar/line	Type	Mutants selected	Yield trial entries
		No.	No.
		1980	1982
Advance	6-row	32	12
Morex	6-row	10	2
WA 9037-75	2-row	28	4
WA 9044-75	2-row	30	5
		1981	1983
Cree	6-row	3	0
Dickson	6-row	7	0
Manker	6-row	29	3
Andre	2-row	2	0
		1982	1984
Morex	6-row	5	5
Manker	6-row	1	1
Larker	6-row	4	4
Norbert	2-row	2	1
Harrington	2-row	1	1
WA 10698-76	2-row	3	1
Klages	2-row	2	0
Bonanza	6-row	1	0
		1983	1985
Advance	6-row	10	4
Morex	6-row	15	1
Robust	6-row	20	1
Andre	2-row	15	2
Lamont	2-row	18	2
Klages	2-row	2	1
		1984	1986
Hazen	6-row	9	5
Clark	2-row	4	2
WA 8822-78	2-row	4	2
Harrington	2-row	1	1
		1985	1987
Hazen	6-row	4	?
Andre	2-row	2	?

Eighteen of the putative mutants after preliminary observation were selected for genetic analysis and agronomic evaluation. Three of these appeared not to carry a gene for reduced height. The remaining 15 are described in TABLE II. A mutant in 'Morex' and one in 'Norbert' are erectoides (*ert*) types, while the others are probably *sdw* types.

Mutant culm length reductions ranged from 12 to 35% compared to their respective normal isotypes. Culm diameter can be an important character for lodging resistance, and was unfortunately less for most of the mutants compared with the normal, but 5 mutants were unchanged, and one Morex mutant (Mo4) was larger in diameter than Morex. Coleoptile length is commonly less in semi-

TABLE II. Description+ of induced semi-dwarf spring barley mutants selected for genetic and agronomic evaluations.

Isotype		Probable gene type	Culm length		Culm diameter		Coleoptile length		Heading date vs normal
Mutant	Normal		M	vsN	M	vsN	M	vsN	
			-cm-	-%-	-mm-	-%-	-mm-	-%-	-d-
6-rows									
A1	Advance	*sdw*	53	78*	3.0	95	38	81*	-1
A2	Advance	*sdw*	52	76*	2.8	89*	37	79*	+1
Mo1	Morex	*ert*	58	72*	3.7	96	41	89*	+8
Mo2	Morex	*sdw*	67	84*	3.8	98	42	91*	0
Mo3	Morex	*sdw*	67	84*	3.8	98	42	91*	0
Mo4	Morex	*sdw*	52	65*	4.1	106*	36	78*	+1
Ma1	Manker	*sdw*	65	82*	4.1	100	35	85*	-1
L1	Larker	*sdw*	71	83*	3.5	92*	44	110*	-1
L2	Larker	*sdw*	62	72*	3.0	79*	45	112*	-5
L3	Larker	*sdw*	63	73*	3.1	82*	46	115*	-5
L4	Larker	*sdw*	65	76*	3.2	84*	44	110*	-5
2-rows									
WA371	WA9037-75	*sdw*	66	85*	2.7	92*	31	82*	0
WA441	WA9044-75	*sdw*	56	76*	2.8	93*	43	105	-1
N1	Norbert	*ert*	62	85*	3.2	100	29	103	+1
H1	Harrington	*sdw*	56	88*	2.8	85*	23	70*	+1

* Significant mutant-normal differences determined by LSD$_{.05}$, except coleoptile length by paired $t_{.05}$-test. Heading date data were not analyzed.

\+ Based on 2 year averages from field, except coleoptile length.

dwarf cereals compared to normal. Mutant coleoptile lengths in this group of barley mutants ranged from 70 to 115% compared with the respective normal isotypes; 9 mutants had shorter, 2 equal, and 4 mutants had longer coleoptiles than their normals. The coleoptile lengths of these mutants may or may not be a factor in field emergence (3, 9, 10). Response to gibberellic acid has been of interest in semi-dwarf cereal studies. All mutant and normal isotypes responded to GA$_3$ in this study. Heading date was little affected in most of the mutants, but Mo1 in Morex was 8 days later than Morex and three Larker mutants (L2, L3, L4) were 5 days earlier than Larker. The variability among these mutants for the traits in TABLE II in some cases is a factor in the agronomic performance of the mutants as described below. Six of these 15 mutants were selected for the genetics and agronomic discussions to simplify the presentation, and because these 6 represent the range of reponses well and probably contain the best candidates for further breeding work. Further details and all data can be found in Aydin (12).

Genetic Analysis. All mutants examined appear to carry a single recessive gene for semi-dwarfism. All backcross and other mutant/normal height genotype cross progeny were normal height in the F$_1$ (12) and segregated 3:1 (normal:semi-dwarf) in the F$_2$ (TABLE III). The mean culm lengths of the F$_1$ populations from one pair of reciprocal crosses was significantly greater than the

TABLE III. Culm length distribution and chi-square analysis of F_2 populations from induced semi-dwarf barley mutants crossed with normal height genotypes.

Cross	Normal	Semi-dwarf	Total	χ^2 for 3:1	P
	--- No.	of plants	---		
Advance/A1	60	28	88	2.1819	.25-.10
A1/Advance	57	24	81	0.9259	.50-.25
Ant531+/Mo1	44	15	59	0.0056	.95-.90
Morex/Mo4	35	13	48	0.1111	.75-.50
Larker/L2	30	12	42	0.2857	.75-.50
WA441/WA9044-75	28	14	42	1.5556	.25-.10
WA441/Morex M‡	32	9	41	0.2032	.75-.50
N1/Morex M‡	34	11	45	0.0075	.95-.90
Morex M‡/N1	43	18	61	0.6612	.50-.25

+ Morex proanthocyanidin-free mutant of normal plant height.

‡ A Morex "mutant" line derived from a single M_2 plant of normal plant height.

normal parents mean culm lengths (Morex M/N1, F_1: 61, 52, 64 cm, respectively and N1/Morex M, F_1: 55, 64, 75 cm, respectively) presumably due to heterosis as the 6-row Morex and 2-row Norbert *ert* mutant genotypes vary considerably.

Most mutant/mutant intercross F_2 progeny segregated in a typical two gene fashion giving acceptable 9:7 ratios (12). The three recessive genotypes aaBB, AAbb, aabb were not usually distinguishable. Double recessives particularly were not discernable. However, an apparent double recessive plant was recovered in the F_2 population from a cross between the two *ert* mutants, N1/Mo1 (24:22:1 for 9:6:1 χ^2=2.5886, p=.50-.25) indicating additivity between the two genes. The double dwarf culm length was only 25 cm, while the rest of the population ranged between 36 and 81 cm.

Allelic relationships are presented in TABLE IV. The only known allelism among the six mutants emphasized herein is between A1 and L2. Several mutants have been shown to be non-allelic to each other and several combinations are yet to be tested.

Agronomic Evaluation. Agronomic performance was variable among the mutants (TABLE V). Mutant grain yield was generally lower than that of respective normal isotypes. However, the mutants A1 and L2 were statistically equal and Mo1 was higher in yield than Advance, Larker and Morex, respectively. The 19% increase in yield of Mo1 compared with Morex is quite high. However, the comparison involved only two trials. The difference in yield between mutant and normal can not be attributed to a difference in harvest index in this case as might be expected. Harvest index varied considerably among the mutants; from .33 to .52 or 82 to 113% of the normal isotype.

TABLE IV. Allelism relationships among induced semi-dwarf mutants of barley.

Semi-dwarf mutants	A1	A2	Mo1	Mo2	Mo3	Mo4	Ma1	L1	L2	L3	L4	WA 371	WA 441	N1	H1
A1		×		/	/	/	×	/	+	×	×				/
A2				/	/	/	×	/	+	+	+				/
Mo1														−	
Mo2					−	−	−	−	/	/	/	−	/?	−	
Mo3				**			−	−	/	/	/	−	/?		
Mo4				**			−	−	−	/	/				
Ma1				**	**	**		−	+	×	+				−
L1				**	**	**	**		/	/	/	−			
L2						**				+	×				/
L3											+				/
L4															/
WA371				**	**			**					+?	/?	−
WA441														−	/?
N1			**	**									**		
H1							**					**			

\+ Allelic.
− Non-allelic.
× Allelic by inference based on other allelic relationships.
/ Non-allelic by inference based on other allelic relationships.
** Culm height differences (p=.01) between F_1/F_2 vs Parents/Normal Isotypes in non-allelic situations determined by pooled t-test.

TABLE V. Agronomic characterization of selected induced semi-dwarf barley mutants, Pullman, Washington.

Isotype		yield/ plot^{+}		Harvest index^{+}		Volume weight±		Plump kernels±		Lodging±	
Mutant	Normal	M	vsN	M	vsN	M	N	M	N	M	N
		-g-	-%-		-%-	-kg/hl-		----- % -----			
A1	Advance	496	89	.50	102	61	61	37	50*	40	58
Mo1	Morex	574	119*	.49	102	58	63*	52	71*	25	86*
Mo4	Morex	318	66*	.41	85*	61	63*	73	71	23	86*
L2	Larker	544	101	.52	113*	59	65*	46	80*	68	50
WA441	WA9044-75	439	77*	.33	82*	64	69*	30	88*	0	33*
N1	Norbert	467	85*	.37	97	59	64*	96	94	0	10

* Significant mutant-normal differences determined by $LSD_{.05}$.
\+ 2 year averages, 1984 and 1985.
± 1985 averages only, except lodging in 1984 only.

Volume weight and kernel plumpness were generally lower in the mutants compared to their normals. Kernel density and size assortment did not seem to correlate positively with yield. The highest yielders tended to have the poorest kernel characteristics.

Lodging did not occur in the trial in 1985, but did so relatively severely in 1984. The mutants were generally more lodging resistant than their normals. In the case of Mo4 it could be at least partially explained by an increase in culm diameter as well as decrease in culm length (TABLE II, V); lodging 25%*, culm length 65%*, and culm diameter 106%* of Morex, the normal isotype. On the other hand L2 had lodging 136%, culm length 72%* and culm diameter 79%* of Larker, the normal isotype. The trend was similar but opposite. Combining the data from TABLES II and V, one can see that straw strength is related to more than just culm length, and not only culm diameter, but internode length and probably chemical composition and configuration as well.

The number of internodes (six) did not vary among the mutants or in the mutants vs normals. Height reduction in the mutants occurred because of shorter internodes. The greatest shortening percentagewise occurred in the two internodes nearest the base of the culm. The greatest shortening in absolute numbers occurred in the uppermost internode or peduncle (12).

CONCLUSIONS AND BREEDING PROGRESS

The selection of reduced height mutants in barley induced with sodium azide was relatively easy. However, the vast majority of these putative mutants were rejected. About 20% of the mutants selected were entered into yield trials in the 6 years of this study, and most of these were culled after one year.

The experiments specifically reported herein showed that the response was variable for the various agronomic traits among the mutants compared to their normal progenitors or isotypes. Whereas some of the mutants appeared to be improved for several agronomic traits such as lodging resistance, yield, harvest index and earliness, none combine all the desirable traits necessary for a new improved cultivar. Since sodium azide is a potent mutagen in barley, and most mutations are deleterious, it is not surprising that some deleterious mutations simultaneously occur in most mutants selected for a desirable improvement. Also the background genotype of a given induced mutant gene may or may not be suitable to the optimum expression of the mutant trait.

Several of the mutants in this study should be good germplasm sources in cross-breeding. Mo1 in Morex has relatively good yield potential and good lodging resistance, but poor kernel characteristics and extreme lateness. If the desirable traits can be transferred without the undesirable traits improved progeny will result. Mo4 has good straw and kernel characteristics, but poor yield potential. The Larker mutant L2 has good yield potential, long coleoptile length and earliness, but poor straw. The allelic Advance mutant A1 has better lodging resistance, but shorter coleoptile length and poorer kernel characteristics. Differences in background genotype can be seen in these two allelic semi-dwarfs.

The above mentioned semi-dwarf mutants could be good germplasm sources for improving barley, especially in regard to lodging resistance or earliness. These mutants could be used in a pedigree program, as they are all malting types, or they could be used in new or existing male sterile facilitated recurrent selection (MSFRS) populations. The Advance, Morex and WA9044-75 mutants have already been used in crosses in our pedigree program and in MSFRS populations particularly modifications of CCXXXII (6). Also, selections have been made from this study's genetic analysis backcross and intercross progeny for channelling into our pedigree program. In any event the goal should be to find the optimum genetic background for each potentially valuable semi-dwarf gene and to overcome any deleterious mutations in the various induced mutants.

Little detail has been provided here concerning the genetic analysis of the various semi-dwarf mutants. There have been some special problems encountered in interpreting culm length results when different genetic backgrounds are combined. This is to be expected especially with a quantitative trait such as culm length. Ideally isogenic series would be developed for a set of mutants to be analyzed. We have tried to overcome the problem of differing genetic backgrounds by comparing progeny with parents, and where different, normal progenitor genotypes all planted in the same way and in close proximity to each other.

Additional genetic work remains to be done with these mutants, such as isogenic line development, as well as continued allele testing among these mutants and other older and newer known sources of dwarfing genes (1, 15, 16). Chromosome location of new genes could also be done.

To conclude on a positive note, the WSU barley breeding program has released a new semi-dwarf winter feed barley called 'Showin' (2905-75) for short winter barley. Showin has resulted from mutation and pedigree breeding efforts. The pedigree of Shown includes the semi-dwarf 'Jotun' mutant and WSU's 'Luther' (an X-ray induced semi-dwarf mutant from 'Alpine'). Agronomic data from Showin has previously been reported (10, 11). In addition to short straw and lodging resistance, averaged over 68 location years at the time of release (1985), it had yielded 118 and 110% of 'Kamiak' and 'Boyer', respectively, the leading commercial winter barley cultivars in Washington. It is anticipated that success in breeding semi-dwarf, lodging resistant barley cultivars will continue with the use of newer induced semi-dwarf mutants.

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SELECTION OF COLD TOLERANT MUTANT LINES IN NAKED BARLEY *(Hordeum vulgare L.)*

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Abstract

In order to screen the cold tolerant mutant lines in naked barley, seeds of recommended variety Baekdong were treated with x-rays of 25 kR. Two cold tolerant mutant lines were selected under field conditions with the lowest winter temperature being from -13°C to -20° C, during M_2 to M_5 generations. The number of vascular bundles of cold tolerant mutant line BM-5 was decreased and culm diameter was thicker than that of the parent line. Important characters of cold tolerant mutant line BM-5 were similar to those of the parent line.

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1. Introduction

Induced mutations can be used to generate useful variation in inherited characters and such mutants can provide a new source of germplasm for improvement of different traits. The main advantage of mutation for plant breeding is the increase of the genetic variability within a relatively short period, including genetically controlled traits not existing in the non-mutated material. Mutagenesis has become a unique method for breeding in cases where the range of genetic variability in existing population is limited. Until now most of the mutant lines from various crops were selected for visible characters such as short plant height, early flowering and maturing, flower shape and size or seed and fruit size. Since mutants have expressed various adaptability to environmental conditions, as compared to their parent lines, the possibility of selection for certain physiological characters important for crop plants has increased (Gregory, 1965; Konzak, 1959; Lapins, 1965; Mastenbroek, 1956; Micke et al., 1985; Toriyama and Futsuhara, 1960).

Cold tolerance in barley is one such important character for productivity and stable cultivation. Particularly, though naked barley in Korea has high yield, its cultivation area is limited to the southern part of the country because of susceptibility to cold (Fig. 1). There are no available germlines for improvement of naked barley in this character. In such case, induced mutations may be an actual and a unique breeding method for naked barley. We have selected, under field conditions, useful cold tolerant mutant lines in naked barley after treatment with radiation. This paper reports on selection procedure and important characters of cold tolerant lines.

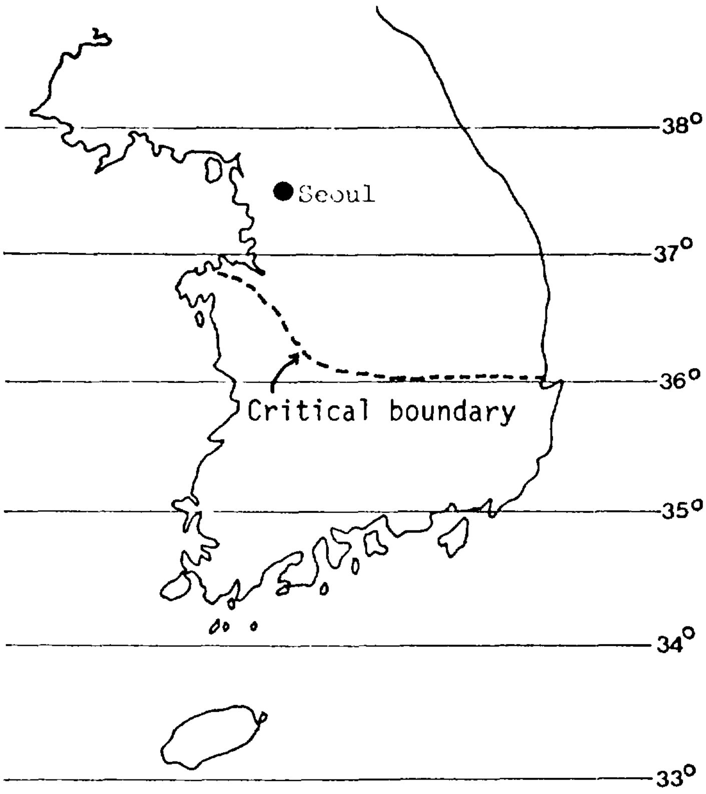

Fig. 1. Critical northern boundary for naked barley growing in Korean peninsula.

2. Material and Method

Recommended variety Baekdong of naked winter barley was used as material for treatment of matured seeds with 25 kR of x-ray in the Korean Atomic Energy Research Institute. Harvest of M_1 generation followed the mutation breeding method recommended for cereals. Cold tolerant lines were screened under field conditions using routine cultivation methods (Table 1). Investigation of the number of vascular bundles and culm thickness were carried out and compared to the parent variety. The evaluation of such important characters as length of culm, nodes, spikes, number of nodes, number of grains per spike and 1,000 seeds weight was conducted on 20 plants.

3. Results and Discussions

About 5,000 grains were irradiated with a dose of 25 kR, which is recommended for mutation breeding in cereals such as barley and rice. Four spikes per M_1 plant and 10 grains per spike were harvested in bulk for M_2 planting. About 100,000 plants in M_2 population were grown for screening of cold tolerant mutant lines. The experimental farm where this screening was done for M_2 - M_5 generation is located in a region excluded for cultivation of existing naked barley varieties due to low winter temperature. Surviving plants in M_2 population may include epigenetic as well as genetic variants because of environmental factors.

Table 1. Selection process of cold tolerant mutant line from naked winter barley variety Baekdong

Generation	Year	Winter temp. (at lowest °C)	No. of seeds harvested or selected lines	Remarks
	1976			Radiation treatment
M_1*	1976/77		ca. 100,000 seeds	
M_2	1977/78	-15.0	ca. 500 plants	Selection **
M_3	1978/79	-13.0	ca. 30 lines	Selection **
M_4	1979/80	-18.5	5 lines	Selection **
M_5	1980/81	-20.0	2 lines	(Mutant line BM-5 and BM-11)
M_6	1981/82			Seed multiplication, field evaluation and basic study
M_7 to present				

* Planted in Gyeongsang National University

** Screened in experimental farm of Korean Atomic Energy Research Institute, Seoul

The grains from screened M_2 plants were single row planted in M_3 and it was possible to confirm whether surviving M_2 plants were genetically changed. Among the cold tolerant mutants selected in M_3 there existed mutant lines with negative characters. Such lines were eliminated from the experiments in succeeding generations. Two mutant lines BM-5 and BM-11 were selected in M_5 generation (Table 1). Mutant BM-5 was better than BM-11 from the point of agronomic characters. Mutant line BM-5 has similar characters to its parent variety, contrary to cold tolerant mutant BM-11 with short and stiff straw, smaller grain size, less number of grains per compact spike and higher susceptibility to fungi.

Histological observation has been made using culms of mutant and control line (Table 2). Culm diameter of mutant lines was thicker than the parent variety but the number of vascular bundles decreased in mutant BM-5. It is not known whether there is any relation between cold tolerance and culm thickness or number of vascular bundles. BM-5 mutant line has a similarity to the mother line in all important agronomic traits and differs only, besides cold tolerance, in its slightly dark green leaves (Table 3). Yield trials for official release are in progress in different regions. Until now its grain productivity has been successfully evaluated. Furthermore, it would be interesting to investigate the genetic background and physiological characteristics of the resistance to cold temperature. Crossing programme between the mutant BM-5 and the parent line or with other mutants will be initiated in the future.

Table 2. Number of vascular bundles and culm thickness of parent variety and mutant BM-5

	No. of vascular bundles	Culm diameter * (mm)
Mother variety	32.4	4.20
Mutant BM-5	24.6	4.70

* 2nd internode from spike

Table 3. Important characters of naked barley parent variety Baekdong and its cold tolerant mutant BM-5

	Culm length (cm)	No. of nodes	I*	II	III	IV	V	VI	Spike length (cm)	Grains per Spike	1000 seed weight(g)	Response to fungi
Baekdong mother variety	69.7	5	24.3	16.3	12.2	8.4	6.3	2.2	6.4	67.3	31.8	susceptible
Mutant BM-5	70.0	5	24.3	16.5	11.7	8.5	6.5	2.6	6.1	65.6	31.9	tolerant

* Spike internode

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GENETIC ANALYSIS OF INDUCED SEMI-DWARF MUTANTS IN RICE

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Abstract

A number of semi-dwarf mutants of rice were evaluated for 15 agronomic characters laying stress on plant type and grain yield, and some of them were genetically analyzed for the genes controlling the semi-dwarfness.

1) Principal component analysis for 14 characters revealed that various plant types of semi-dwarf mutants could easily be induced by mutagenic treatment, and the pattern of induced plant type was largely affected by the genotype of the original variety. Grain yield of the induced mutants tended to be higher in the mutants with larger "general size" irrespective of the original variety, but did not show particular relationship to the culm trait as represented by both internode elongation and culm thickness. 2) Five of the nine induced semi-dwarf mutants analyzed were each proved to be controlled by single semi-dwarfing gene, and these mutated genes were considered to be non-allelic to each other. Among the remaining four, one carried two semi-dwarfing genes and three were brought by the mutations in minor gene system. 3) Based on the above results, the high possibility was indicated for creating new semi-dwarfing gene resources for breeding, and the necessity was pointed out for appropriate choice of materials to be adopted and analysis of both induced and existing semi-dwarfing genes.

1. INTRODUCTION

It is widely known that the appearance of semi-dwarf varieties has brought a remarkably elevated and stabilized productivity of rice crop in Japan as well as in many other countries. According to some recent reports, however, the existing semi-dwarfing gene resource base is quite smaller than expected [1,2]. Such a situation is far from the idea that the genetic diversity should be maintained, and suggests that exploiting of new useful semi-dwarfing genes is of urgent necessity.

Breeding by induced mutations has already succeeded in producing a number of excellent varieties and strains over various crops, and much has been expected from mutation breeding for further creating of new gene resources [3]. However, there are as yet few reports where induced semi-dwarfness was genetically analyzed together with the agronomic characteristics related to the semi-dwarfness.

In this paper, (1) various semi-dwarf mutants of rice were evaluated for 15 agronomic characters and their mutual relationships, laying stress on plant type and grain yield, and (2) the genes responsible for semi-dwarfness were analyzed with 9 induced semi-dwarf mutants.

2. MATERIALS AND METHODS

2. 1. Agronomic characteristics of semi-dwarf mutants

Out of a large number of mutant stocks which were induced with γ-rays, EI or NMUA from 3 varieties, Gimbōzu, Nōrin 8 and Nihonmasari, a total of 69 semi-dwarf mutants, *viz.*, 23 from Gimbōzu (Group G), 23 from Norin 8 (Group M) and 23 from Nihonmasari (Group N), were selected on the basis of the panicle weight and seed fertility not inferior to original variety. These groups G, M and N are here each referred to as mutant group. The 3 original varieties were included in respective groups.

In addition, a total of 39 diverse types of varieties and induced mutants were also used as Group V for comparison with the mutant groups ; 6 semi-dwarf mutants (Hokuriku 100, Kantō 79, Reimei, Fukei 71, W 12 and W 24), 3 their original varieties (Koshihikari, Fujiminori and Wakaba) and 30 check varieties comprising 20 semi-dwarf and 10 tall ones.

Seeding and transplanting were made on 6th of May and 30th of June in 1982, respectively. Fertilizers applied were 6, 9 and 9 kg/10a for N, P_2O_5 and K_2O, respectively, and plant spacing was 10 x 30 cm.

Ten plants per variety or mutant were measured for 15 characters ; culm length (CL), panicle length (PL), panicle number (PN), number of elongated internodes (EIN), first to fifth internode lengths (IN1-IN5), first to fifth internode diameters (IT1-IT5) and grain yield per plant (GY). Principal component analysis was applied to all the 14 characters other than grain yield to obtain an integrated figure of plant type and to discuss the relationship of plant type to grain yield.

2. 2. Gene analysis of semi-dwarf mutants

Out of the above described Groups G, M and V, 3, 4 and 2 semi-dwarf mutants were respectively selected (TABLE II) without particular choice other than semi-dwarfness. They were crossed with their respective original varieties and analyzed for semi-dwarfing gene in the F_2 and F_3 generations. In each cross combination, about 300 F_2 plants and about 50 F_3 lines which were raised from randomly selected F_2 plants, were subjected to the analysis. These materials were grown in paddy field together with the cross parents and measured for culm length and heading date.

3. RESULTS AND DISCUSSION

3.1. Agronomic characteristics of semi-dwarf mutants

TABLE I indicates the first and second component vectors (z_1 and z_2, respectively) extracted from the principal component analysis. As shown in this table, the cumulative contribution of z_1 and z_2 exceeded 70 percent in each group, suggesting that the total multivariate variance of plant type could safely be explained by these two components. In all the groups, z_1 gave positive loading to all organs. This means that z_1 indicates the isometric phase of variation in "general size", where all organs vary in size almost proportionally. On the other hand, z_2 seemingly indicates the allometric phase of variation between "upper-internode elongation with thick culm" and "lower-internode elongation with slender culm" for 3 groups other than Group N, while only between "upper-internode elongation" and "lower-internode elongation" for Group N.

Figure 1 gives the scatter diagram of plant type for all the varieties and mutants used in this experiment, according to the scores

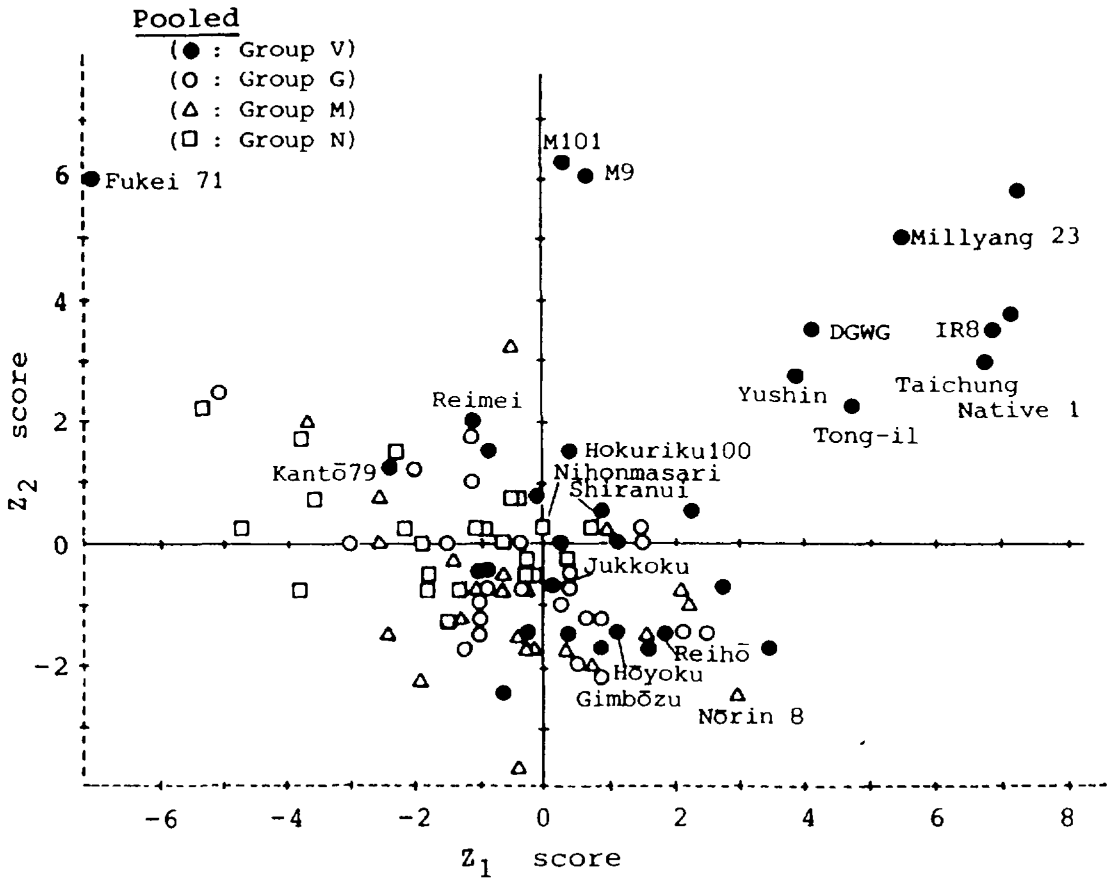

FIG. 1. Scatter diagram of 108 varieties and mutants according to the scores given by the first and second component vectors extracted from PCA for 14 characters.

obtained from pooled z_1 and z_2 (TABLE I). As clearly seen from this figure, the variation of plant type was far larger in Group V than in the others, where the distribution of mutants was confined to a relatively small extent. Among the 3 mutant groups, Group G and Group M each showed larger variation than Group N, and the majority of the mutants were characterized by "lower-internode elongation with slender culm". The variation of Group N was almost limited to the small area confined by z_1 below zero and z_2 near zero, and did not show such a trend of culm trait as observed in Groups G and M.

Figure 2 shows the scatter diagram of plant type for the varieties and mutants in each group according to the scores of z_1 and z_2.

In Group V, 4 varieties possessing Jukkoku semi-dwarfing gene (Jukkoku, Shiranui, Hōyoku and Reihō ; Jukkoku-type varieties), 6 varieties possessing Dee-geo-woo-gen (DGWG) semi-dwarfing gene (DGWG, Taichung Native 1, IR 8, Tong-il, Yushin and Millyang 23 ; DGWG-type varieties) and 2 varieties possessing sd_1 gene (M-9 and M 101 ; SD-type varieties) bore close resemblance in plant type, respectively : Jukkoku-type varieties, characterized by "lower-internode elongation with slender culm" were situated far from the other two, which were conversely characterized by "upper-internode elongation with thick culm". From this and the fact that the above 3 semi-dwarfing genes have recently proved to be identical with or isoallelic to one another, it is suggested that the semi-dwarfing gene acts not only on culm length but also on culm trait as evaluated by length and thickness of internodes, but its action is strikingly influenced by the genetic background which the gene belongs to.

TABLE I. COMPONENT VECTORS EXTRACTED FROM PRINCIPAL COMPONENT ANALYSIS

Character*	Group V		Group G		Group M		Group N		Pooled	
	Z_1	Z_2	Z_1	Z_2	Z_1	Z_2	Z_1	Z_2	Z_1	Z_2
CL	.851	.297	.808	-.309	.829	.081	.863	-.004	.872	-.200
PN	.823	.222	.769	-.105	.827	.026	.792	.356	.846	-.171
PL	.851	-.212	.802	-.155	.761	.146	.835	.392	.883	.090
EIN	.755	.401	.814	-.321	.766	-.321	.867	-.203	.781	-.350
IN1	.742	-.324	.803	.249	.775	-.213	.821	.378	.849	.298
IN2	.818	.109	.728	.383	.745	-.373	.768	.174	.824	-.178
IN3	.837	.173	.819	-.016	.833	.003	.824	-.261	.818	-.304
IN4	.772	.484	.830	-.309	.794	.448	.837	-.178	.838	-.374
IN5	.803	.347	.823	-.272	.805	.371	.796	-.372	.841	-.208
IT1	.784	-.447	.799	.113	.844	-.247	.804	-.241	.845	.414
IT2	.863	-.408	.846	.299	.840	-.232	.856	-.122	.880	.379
IT3	.861	-.422	.835	.343	.865	-.373	.849	-.135	.864	.430
IT4	.862	-.413	.846	.307	.861	-.370	.849	.120	.867	.416
IT5	.789	.277	.755	-.211	.659	.531	.761	.129	.802	-.338
Contribution (%)	66.6	11.7	65.0	6.9	64.3	9.7	67.9	6.1	71.2	9.9

* CL:Culm length, PN:Panicle number, PL:Panicle length, EIN:Number of elongated internodes, IN1~IN5:First to fifth internode lengths, IT1~IT5:First to fifth internode diameters.

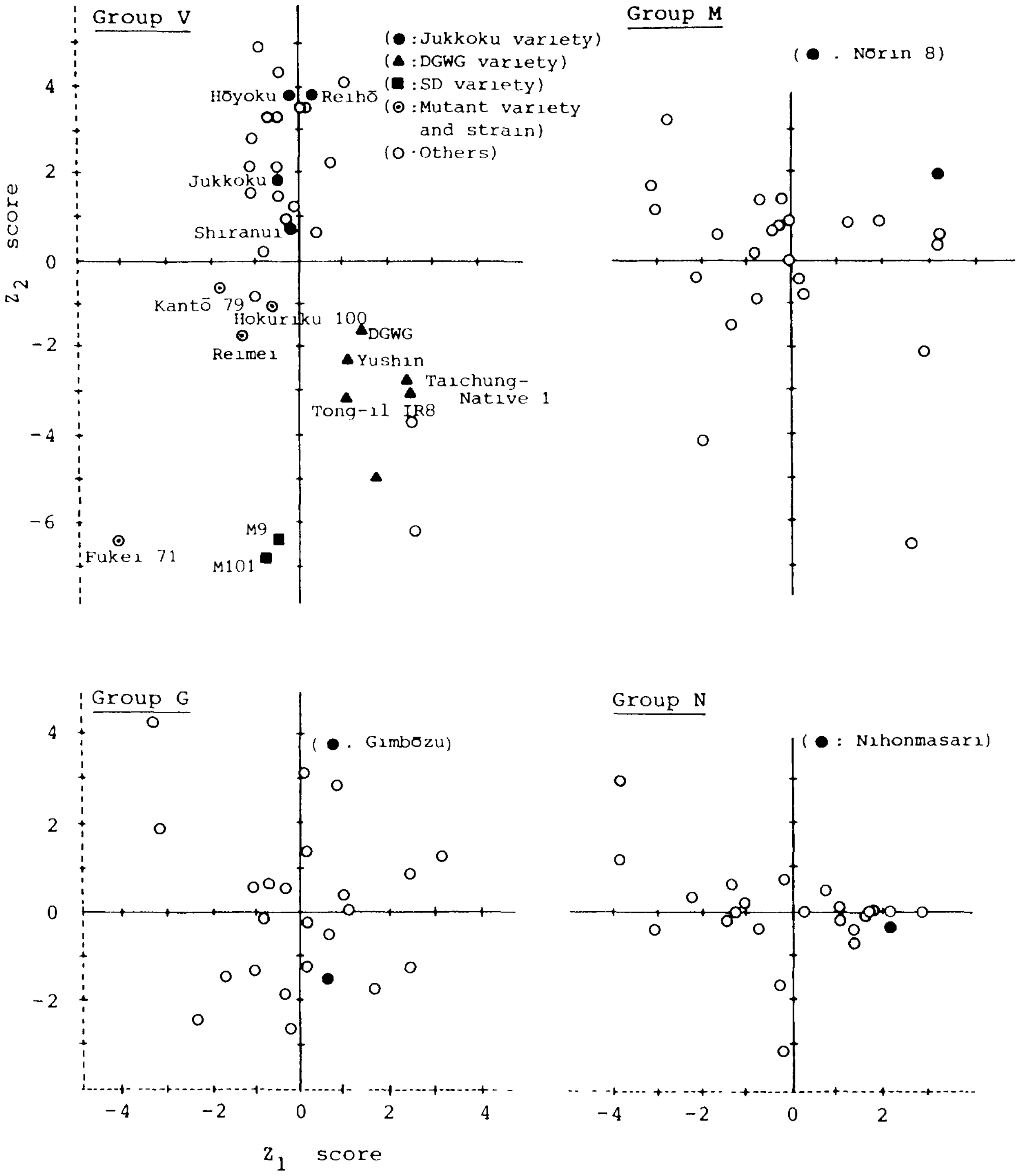

FIG. 2. Scatter diagram of varieties and/or mutants in each group according to the scores given by the first and second component vectors extracted from PCA for 14 characters.

Four variety or promising mutant strains induced with γ-rays (Reimei, Fukei 71, Hokuriku 100 and Kantō 79) exhibited the plant types conspicuously different from those of the above three types of varieties. The 3 mutant strains as well as the variety, Reimei, have intensively been used as excellent cross parents in breeding for semi-dwarfness. However, taking account of the fact that Reimei, an induced mutant, and Jukkoku possess an identical semi-dwarfing gene [1, 4, 5] in spite of a great difference of plant type between the two, it is considered that promising mutants appearing henceforth as well as these mutamt strains should be analyzed for the genes controlling the semi-dwarfness prior to the entry as new gene sources.

In Group G, the distribution of z_2 scores of the mutants had a strong bias in positive direction against the original variety Gimbōzu. This indicates that the semi-dwarf mutants induced from Gimbōzu mostly manifest the plant type characterized by "upper-internode elongation with thick culm" compared with the original variety (TABLE I).

In Group M, there was observed a strong bias of z_2 scores in negative direction but substantially similar to that in Group G, indicating that the same trend of plant type as observed in Group G is true also of the mutants induced from Norin 8 (TABLE I). An observable difference of Group M from Group G was that mutants exceeding the original variety in "general size" could not be found in the latter.

In Group N, it was recognized as a distinguishing feature that most of the mutants came together near the z_2 of the original variety, and thus did not show such a trend of "upper-internode elongation with thick culm" compared with the original variety as observed in Groups G and M. This may be due to that the original variety, Nihonmasari, itself is a semi-dwarf variety of "upper-internode elongation with thick culm" (Fig. 1).

The relationships of grain yield to z_1 and z_2 scores are depicted in Figs. 3 and 4, respectively. In Group V, higher yields were observed in the varieties with the scores of z_1 above zero (Fig. 3) and z_2 below zero (Fig. 4), which were all indica or indica-type varieties (Fig. 2), indicating that the varieties of "large general size" and "upper-internode elongation with thick culm" tended to give higher grain yield. In Groups G, M and N, the relationship between grain yield and z_1 score was substantially similar to that in Group V, namely, higher yielding mutants tended to show "larger general size". Between yield and z_2 score, however, no particular relationship was observed, except that some mutants with larger z_2 score in Group G and a few mutants having z_2 score distant from that of the original variety in Group N gave remarkably reduced yield. In any case, it deserves attention that in Group N, some mutants showed better yield than the original variety despite of equivalent z_2 scores to the latter.

Some reports [2, 6, 7] have suggested that "upper-internode elongation" type of rice is more favorable for productivity than "lower-internode elongation" type. In this sense, it appears somewhat curious that in each of Groups G and M, some mutants of "lower-internode elongation with slender culm" type showed rather high yield, notwithstanding these two groups were derived from "lower-internode elongation with slender culm" type varieties (Fig. 1). This may be due mainly to the lower level of fertilizers applied in this study.

Thus, the results derived from the analysis of principal components suggest that the agronomic characteristics of induced semi-dwarf mutants are strikingly affected by the genotype of the original variety used, and that an appropriate choice of materials to be adopted is highly significant for the successful induction of useful semi-dwarf mutants.

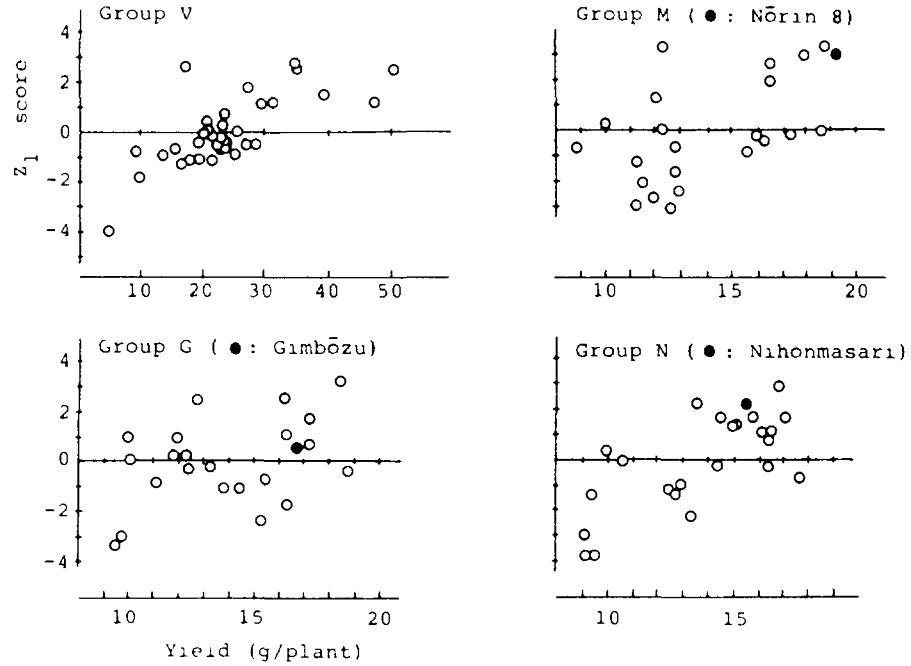

FIG. 3. Relationship between yield and Z_1 score extracted from PCA for 14 characters in each group.

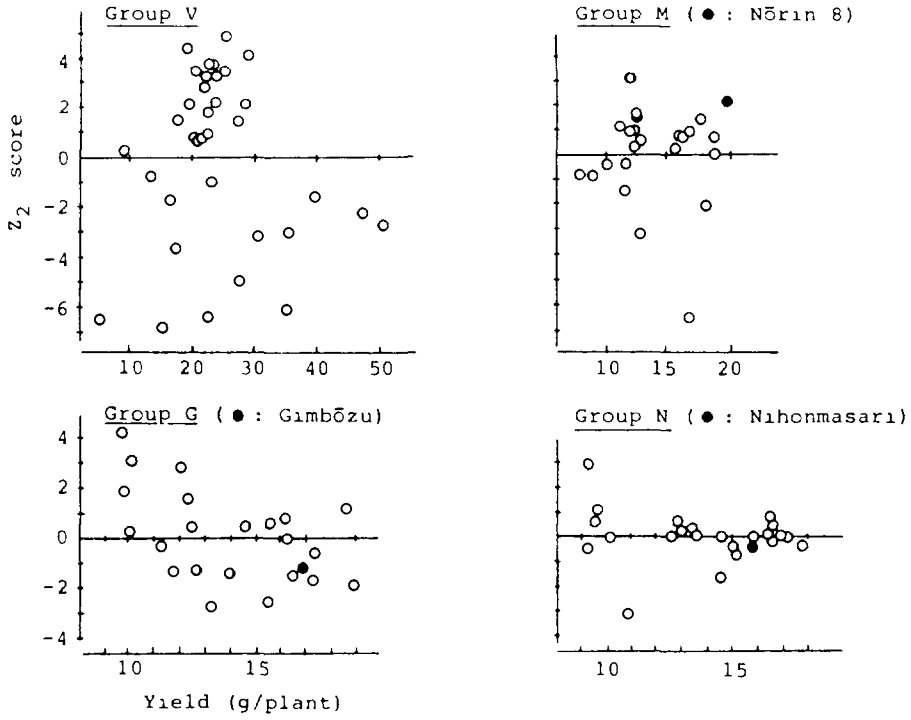

FIG. 4. Relationship between yield and Z_2 score extracted from PCA for 14 characters in each group.

3. 2. Gene analysis

The mutants submitted to the analysis of semi-dwarfing gene and their original varieties are presented in TABLE II along with culm length and heading date. The analysis of the gene was made on the basis of segregation in culm length, referring to the influence of heading time on this trait. The frequency distributions of culm length in F_2 and the results of progeny tests in F_3 are summarized in Fig.5 and TABLE III, respectively.

TABLE II. SEMI-DWARF MUTANTS ANALYSED AND THEIR ORIGINAL VARIETIES

Mutant or original variety	Mutagen	Culm length [cm]	Heading date
HS90	γ-rays	50(64)	9.03
X82	γ-rays	66(85)	9.06
IM222	γ-rays	72(92)	8.30
Gimbōzu		78	9.01
M686	γ-rays	61(74)	9.03
M700	γ-rays	69(84)	9.03
M803	γ-rays	59(72)	9.03
M1007	γ-rays	56(68)	9.02
Nōrin 8		82	9.01
W12	EI	61(81)	8.20
W12	EI	63(84)	8.22
Wakaba		75	8.26

( ) . Percent of the original variety.

(1) HS 90

As seen from Fig. 5, the F_2 following the cross between HS 90 and the original variety showed trimodal distribution of culm length, and allowed to infer that HS 90 has one semi-dwarfing gene which causes a remarkable reduction of culm length and its genotypic value in heterozygous state is almost equivalent to that of midparent. TABLE IV illustrates 3 different types of frequency distribution within line observed in F_3 generation, corresponding them to the types of parental F_2 plants. As clearly indicated by this table, all the 48 F_3 lines could easily be classified into 3 groups corresponding to the 3 types of F_2 plants, and, as shown in TABLE III, the segregation of F_3 lines, *viz.*, 10 [homozygous, tall] : 23 [heterozygous, intermediate] : 15 [homozygous, semi-dwarf], was quite well consistent with the expected ratio of 1 : 2 : 1. This result confirms the adequacy of the above consideration about the semi-dwarfing gene of HS 90.

TABLE III. CLASSIFICATION OF F_3 LINES BASED ON THE TYPE OF CULM-LENGTH SEGREGATION

Cross	Genotype and phenotype of F_2 parent	Segregation ratio for F_3 lines	
		Observed	Expected
Gimbōzu x HS90	Dominant homo : tall	10	1
	Hetero : intermediate	23	2
	Recessive homo : semi-dwarf	15	1
	(Test for one-gene segregation : χ^2=1.13, 0.500 $<$ P $<$ 0.750)		
Gimbōzu x X82	Dominant homo : tall	12	1
	Hetero : tall	26	2
	Recessive homo : semi-dwarf	10	1
	(Test for one-gene segregation : χ^2=0.50, 0.750 $<$ P $<$ 0.900)		
Gimbōzu x IM222	Dominant homo : tall	13	1
	Hetero : tall	24	2
	Recessive homo : semi-dwarf	13	1
	(Test for one-gene segregation : χ^2=0.08, 0.950 $<$ P $<$ 0.975)		
Nōrin 8 x M686	Dominant homo : tall	10	1
	Hetero : tall	21	2
	Recessive homo : semi-dwarf	19	1
	(Test for one-gene segregation : χ^2=2.76, 0.250 $<$ P $<$ 0.500)		
Nōrin 8 x M700	2 gene-dominant homo : tall	2	1
	1 gene-dominant homo, 1 gene hetero : tall	10	4
	2 gene-hetero : tall	12	4
	1 gene-hetero, 1 gene-recessive homo : intermediate	15	4
	1 gene-dominant 1 gene-recessive homo : intermediate	6	2
	2 gene-recessive homo : semi-dwarf	5	1
	(Test for two-gene segregation : χ^2=2.56, 0.750 $<$ P $<$ 0.900)		
Nōrin 8 x M803	Minor genes : semi-dwarf to tall	(Continuous distribution)	
Nōrin 8 x M1007	Dominant homo : tall	11	1
	Hetero : tall	27	2
	Recessive homo : semi-dwarf	11	1
	(Test for one-gene segregation : χ^2=0.52, 0.750 $<$ P $<$ 0.900)		
Wakaba x W12	Minor genes : semi-dwarf to tall	(Continuous distribution*)	
Wakaba x W24	Minor genes : semi-dwarf to tall	(Continuous distribution)	

Homo : homozygous, Hetero : Heterozygous,

* : High correlation was observed between culm length and heading date.

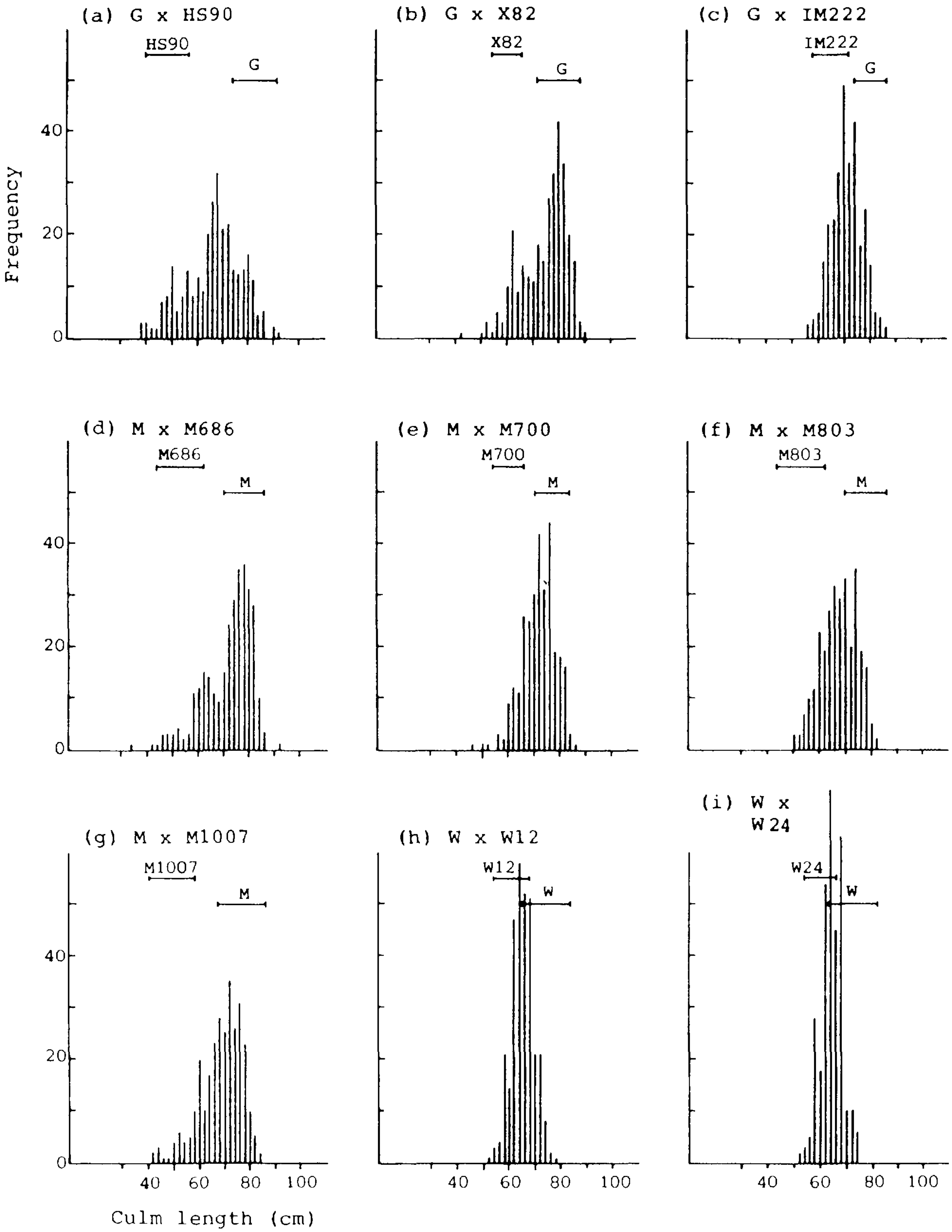

FIG. 5. Frequency distribution of culm length in 9 F_2 populations.

G : Variety Gimbōzu
M : Variety Nōrin 8
W : Variety Wakaba

TABLE IV. FREQUENCY DISTRIBUTIONS OF CULM LENGTH IN SOME REPRESENTATIVE F$_3$ LINES WHICH WERE DERIVED FROM THREE DIFFERENT TYPES OF F$_2$ PLANTS (Gimbōzu x HS90)

Genotype and phenotype of F$_2$ parent	Culm length (cm)																											
	40	.	.	.	.	50	.	.	.	.	60	.	.	.	.	70	.	.	.	.	80	.	.	.	.	90	.	.
Dominant homozygous : tall																			3	1	4	4	1	4	4	1		
																			1	2	5	4	5	3	1	1		
Heterozygous : intermediate						2	1	2	0	0	0	0	1	1	1	2	1	4	2	2	0	0	1	3	1			
				2	0	0	2	1	0	1	1	0	0	3	1	3	4	0	0	0	2	1	0	1				
Recessive homozygous : semi-dwarf			1	2	1	7	7	4	1																			
			1	5	2	4	5	3	2																			

(2) X 82, IM 222, M 686 and M 1007

As to the frequency distributions of culm length in 4 F_2 populations with X 82, IM 222, M 686 and M 1007, it was somewhat difficult to find out any break points of the distribution and, in its turn, to estimate the segregation ratio (Fig. 5). In each of the 4 cross combinations, however, all the F_3 lines could easily be classified into 3 groups as in the case of HS 90, and the observed segregation ratios of [homozygous, tall] : [heterozygous, tall] : [homozygous, semi-dwarf] were well fitted with the expected ratio in one-gene segregation, 1 : 2 : 1 (TABLE III). From this ratio and the culm length of each mutant (TABLE II), it can be said that the 4 mutants analyzed each has one semi-dwarfing gene, but the degree of gene action to reduce culm length varies with mutants, being in the order of M 686 = M 1007 > X 82 > IM 222.

With X 82 and M 686, however, there was observed a close relationship between culm length and heading time as illustrated in Fig. 6, which depicts the case of Gimbōzu x X 82. As seen from this figure, recessive-homozygous genotype for culm length apparently delayed heading time compared with dominant-homozygous and heterozygous genotypes. A similar tendency was observed also in the case of Nōrin 8 x M 686. This suggests that each of the semi-dwarfing genes of these 2 mutants is closely linked with some mutated late-heading gene or has a pleiotropic effect on delaying heading time.

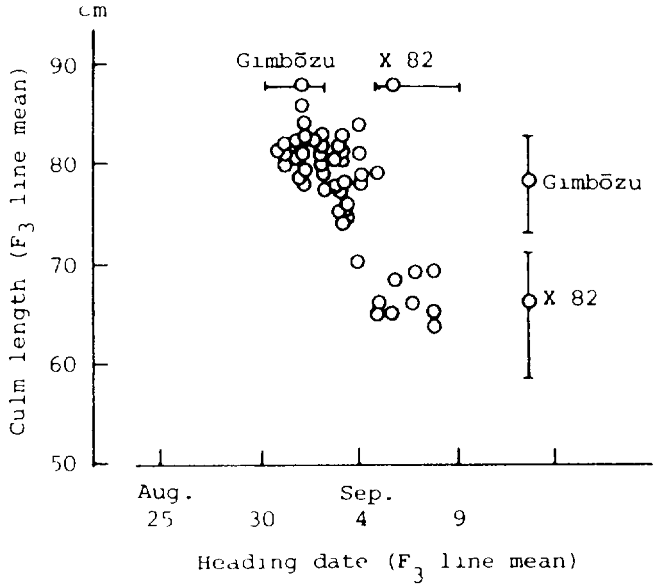

FIG. 6. Relationship between culm length and heading date in the cross of Gimbōzu x X82.

(3) M 700

Regarding M 700, there was observed a continuous frequency distribution of culm length in F_2, which did not allow the discrete grouping (Fig. 5). However, the 50 F_3 lines could comparatively with ease be classified into 6 groups. The observed segregation ratio of 2 [2 gene homozygous, tall] : 10 [1 gene homozygous, 1 gene heterozygous, tall] : 12 [2 gene heterozygous, tall] : 15 [1 gene heterozygous, 1 gene homozygous, intermediate] : 6 [2 gene homozygous, intermediate] : 5 [2 gene homozygous, semi-dwarf] was quite well consistent with the ratio of 1 : 4 : 4 : 4 : 2 : 1, which is expected for two-gene segregation. From this segregation ratio and the culm length of M 700, it can be presumed that this mutant has 2 semi-dwarfing genes with rather weak action on reducing culm length.

(4) M 803 and W 24

In regard also to M 803 and W 24, the discrete grouping of F_2 plants was impossible, because of their continuous frequency distribution of culm length (Fig. 5). Furthermore, it was also impossible to classify the F_3 lines, because their respective phenotypic variances took similar value to each other and the line means formed nearly a normal distribution curve, which had a comparatively wide variation and a peak around the midparent value. Such frequency distributions of culm length as observed in F_2 and F_3 suggest that the semi-dwarfnesses of M 803 and W 24 were due to the mutations induced in respective minor gene systems. The fact that no transgressive segregants were observed may indicate that the mutations occurred unidirectionally.

(5) W 12

With respect to W 12, the segregation behaviors in F_2 and F_3 were almost the same as observed in those with M 803 and W 24, indicating that minor gene mutations are responsible for the culm length of this mutant. As shown in Fig. 7, however, the culm length and heading time of F_3 line were positively correlated with each other so highly that most of the F_3 lines were distributed on and closely to the line of linear regression. These facts suggest that the semi-dwarfness of W 12 was brought about by the mutations of minor genes which are responsible also for heading time. Both W 12 and W 24 are the mutants rated high in that they possess also early maturity and blast resistance which were induced coincidentally with the semi-dwarfness. Therefore, the results from the above gene analysis may suggest that a semi-dwarfness induced by minor gene mutations are worthy to be examined for its utility in certain cases.

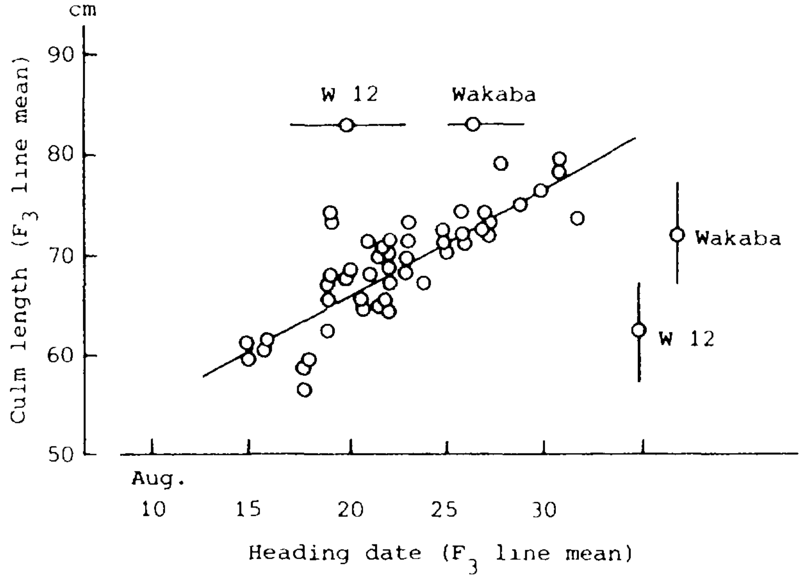

FIG. 7. Relationship between culm length and heading date in the cross of Wakaba x W12.

As mentioned above, as many as 5 of the 9 semi-dwarf mutants analyzed here were yielded through the single gene mutations for culm length, and the semi-dwarfing genes detected were considered to be non-allelic to each other. These facts indicate that various semi-dwarf mutants with different genotypes for semi-dwarfness can comparatively with ease be induced by simple genetic alteration, suggesting the high possibility for creating new semi-dwarfing gene resources and the necessity for gene analysis with induced semi-dwarf mutants.

On the other hand, the astonishing fact that most of the semi-dwarf varieties being widely cultivated in Japan, south-east Asia and California have same semi-dwarfing gene, sd_1 [4], urges to find out and exploit new substitutive semi-dwarfing genes. In this connection, Yamagata *et al.* [6] and Tanisaka *et al.* [8] detected a useful semi-dwarfing gene non-allelic to sd_1 in an experimental stock. Moreover, Tanisaka *et al.* (unpublished) have recently found that a Japanese super-variety Kinmaze has a useful semi-dwarfing gene non-allelic to sd_1, sd_2 and sd_4. Even only from these two findings, it may be suggested that within close range of us, there are many as yet unexplored useful semi-dwarfing genes other than sd_1. Thus, it is considered that the analysis of semi-dwarfing genes should be advanced also for existing varieties.

4. CONCLUSION

Through the analysis of principal component, it became clear that various plant types of semi-dwarf mutants could easily be induced by mutagenic treatment, and the pattern of the induced plant type was largely affected by the genotype of the original variety, so that an appropriate choice of the material to be used was highly significant for the successful induction of useful semi-dwarf mutants.

From the analysis of semi-dwarfing genes, it was suggested that the semi-dwarfness of induced mutants was in many cases brought by single gene mutation, and that the mutated semi-dwarfing genes were non-allelic to each other in not a few cases.

These facts suggest that there exist a lot of loci concerning semi-dwarfness and an appropriate induction of mutations gives a high possibility to create new semi-dwarfing gene resources. In any case, it seems to be an immediate subject of importance to examine whether the induced semi-dwarfing genes are useful for practical breeding or not. For this purpose, the effect of those genes on agronomic characters, especially on yield, should be analyzed. At the same time, the analysis of semi-dwarfing genes should be advanced also for existing varieties and mutants as well as induced ones.

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INVITED PAPERS

TRANSFER OF ALIEN GENES INTO CULTIVATED WHEAT AND TRITICALE GENOTYPES BY THE USE OF HOMOEOLOGOUS PAIRING MUTANTS*

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Abstract

Alien species, both wild and cultivated, can greatly help in broadening the genetic base of presently cultivated wheats, thanks to the large reservoir of useful genes they contain. However, introduction of substantial portions of alien genomes into cultivated genotypes in most cases makes them unfit for commercial use, due to the contemporary presence of positive and negative traits carried by the alien material. Therefore, what appears to be generally needed is to obtain transfers that include the shortest possible alien segment(s). Exchanges induced between functionally related (=homoeologous) chromosomes are the most likely to produce balanced and, thus, agronomically well performing transfer lines. Since more or less close homoeology has been proved to exist between each alien chromosome and certain wheat chromosomes, but, in spite of this, they are prevented from pairing and recombine by the wheat pairing control system and mainly by the *Ph1* gene, the use of *ph1* mutants, available in common and *durum* wheat and, recently, also in a primary 6X triticale background, allows to efficiently overcome such an obstacle.

Preliminary results are reported here of a work aimed at transferring to common wheat a gene for resistance to powdery mildew derived from *Triticum longissimum*, a diploid species with a very closely related genome to the B genome of polyploid wheats. As donor line a *T.longissimum* ditelosomic addition to Chinese Spring was used. The alien telo, bearing the resistance gene(s), corresponded to the short arm of chromosome G, whose homoeology with the group-3 chromosomes of common wheat had been established. A transfer scheme has been adopted that, within two generations of crosses, of which one with the *ph1* mutant of Chinese Spring, provided plants having in single dose the alien telocentric, its wheat homoeologue (alternatively 3A, 3B or 3D) and a 5B/*ph1* chromosome. Such plants were then pollinated by the standard euploid for the recovery of recombinant types. The frequency of recombinant resistant plants detected so far suggests that the pairing frequency between the alien telo and its wheat homoeologues was quite high, most probably ranging between 25 and 30%, on an average.

(*) Contribution n°104 of the Divisione Tecnologie Biologiche ed Agrarie - E.N.E.A.

With the major objective of improving triticale bread making quality, extensive use is also being made of the phl 6X triticale as one the parents in various wide crosses with different D genome sources, including common wheat and synthetic amphiploids. The remarkable increase in homoeologous pairing observed in all the F1 plants, together with the fairly successful obtainment of BC derivatives to cultivated triticales, allow good confidence in the phl mediated recombination as a promising method for triticale improvement.

Ir nature the regularity of meiosis and thus the success, in evolutionary terms, of allo- and also autopolyploid plants has been largely achieved through a diploid-like behaviour of chromosomes, resulting in almost exclusive bivalent type of pairing at first metaphase. Polyploid wheats are the most typical and best studied examples to this respect. In contrast to what happens, for istance, in allopolyploid Festuca species (1), where the genes restricting pairing to homologous pairs appear to be ineffective in the hemizygous state, thus creating virtually no barrier to recombination of characters between related species, the type of pairing control existing in polyploid wheats, mostly due to the homoeologous pairing suppressor gene Ph1, is fully effective in the hemizygous state and, therefore, it represents a major obstacle to interspecific and intergeneric transfers between wheat and related Triticinae.

The approach to resort to alien species as a method for the improvement of cultivated wheats is probably, at present, in its transition phase from being a cytogenetic curiosity to being an increasingly urgent need for further achievements in wheat breeding. Indeed, the genetic material present in the world assortment of cultivated wheat varieties is being exploited to the fullest. Besides this, its range of variation is rapidly decreasing as a result of the enormous selection pressure applied on this crop during the last century. No doubt that the trends dictated by the "Green Revolutions" resulted in outstanding successes in several cases. However, nowadays breeders are certainly facing a number of newly arisen problems which appear to have limited and, sometimes, not available solutions if only the cultivated gene pool and strickly conventional breeding methodologies are used. As several analyses of population growth, economics of farming and energy needs in fact underline, there is an urgency of further raising the ceiling to yield, both in quantity and quality, coupled with enhanced stability of output. This is true for several main crops, including wheat. More than this, poor pest menagement and poor tolerance of our crop varieties to many different environmental constrains have to be solved. In this connection the genetic approach is certainly the most economical and the least damaging to the environment (2,3) Wild species can help a lot in enlarging the genetic variability of crop varieties; they not only contain resistance genes to several wheat diseases, but also represent a large reservoir of genes for getting wheats with high protein content, higher amount of essential aminoacids, improved drought resistance and salt tolerance; more than this, the cytoplasms as well as nuclear genes of different wild relatives of wheat induce male sterility, a trait which may be profitably used for a "clean" production of hybrid wheat.

Complete homology may exist between the genomes of the alien and the cultivated species and, in this case, gene transfer can be accomplished by conventional breeding techniques. However, with progressively decreasing cytogenetic affinity, alternative and more sophisticated approaches have to be followed, most if not all demanding a constant reference to the chromosome status of the breeding material.

Introduction of substantial portions of alien genomes into cultivated genotypes, such as in synthetic amphidiploids and also in addition and substitution lines, in most cases makes them unfit for commercial use, due to the contemporary presence of positive and negative traits carried by the alien material. Therefore, what appears to be generally needed is to obtain transfers that include the shortest possible alien segment(s).

One way for transferring less than an entire chromosome is by the use of ionizing radiations. The first experiment of this type involved the transfer of an Aegilops umbellulata chromosome segment, bearing resistance to wheat leaf rust, to chromosome 6B of wheat (4). One of the resulting lines was then used at Purdue to develop the variety Riley 67, which has been recently utilized in Italy by Dekalb to breed the variety Chiarano, showing an outstanding performance in several fiels trials this last year. Another successful radiation-induced transfer involved the translocation of an Agropyron elongatum chromosomal segment, carrying resistance to stem rust, to wheat chromosome 6A (5). This resistance has been incorporated into four Australian cultivars (Eagle, Kite, Jabiru and Avocet), which were reported by Driscoll (6) to represent a 7% of the Australian wheat crop at the beginning of the 80ties. However, in spite of these and a few other notable exceptions, generally radiation-induced translocations suffer from a number of limitations, inherent to the method in itself: breakages occur essentially at random; interchanges are mainly terminal; the majority of them are genetically unbalanced, as they involve non related chromosomes; their male transmission is consequently quite low.

Fortunately, it turns out that, with few exceptions, each alien chromosome has retained considerable relatedness with certain wheat chromosomes and is only prevented from pairing with them by the wheat pairing control system. Therefore, by altering the genetic environment, that is operating so to "relax" the restriction to homologous pairing only, transfers can be obtained between functionally related (=homoeologous) chromosomes. This, of course, highly reduces the probability that a deletion, though a small one, of a wheat segment will result in negative effects, since homoeologues are known to be able to largely compensate for each other from the functional point of view. Wheat, especially common wheat, is a particularly amenable species for works of this kind. In fact, not only almost all the possible aneuploid lines for entire or telocentric chromosomes are available, thanks to the exceptional work of E. R. Sears, but also several addition and substitution lines of single alien chromosomes or telosomes into the wheat background have been produced, which give fundamental informations for the establishment of the feasibility of a transfer work. They in fact first of all allow to ascertain whether or not the alien gene expresses itself once introduced into the recipient genome, but also permit to quickly determine the relationships of homoeology existing between the alien chromosomes and their wheat counterparts. Once this is made, what one needs to do is to create a "pairing permissive" condition for the critical homoeologous partners. To this respect, we are at present more lucky than cytogeneticists of some years ago. We do not have, in fact, to use a nullisomic condition for the entire 5B chromosome (with its consequences in terms of aneuploidy etc.) to induce homoeologous recombination. Mutants at the *Ph1* locus have been obtained in recent years in hexaploid wheat (7), in *durum* (8) and also in a primary 6X triticale (9). In a *ph1* background, added or substituted alien chromosomes can pair and exchange genetic material with their wheat homoeologues, especially if proper aneuploid lines are used as recipient and donor parents which allow to create a chromosomal situation, in particular a monosomic condition for the crutial chromosomes, due to which they are already prone to pair. Experiments based on the use of *ph1* mutations as pairing induction system have not been very numerous so far. In all the reported cases, always an *Agropyron* chromosome (in 3 cases *A. elongatum* and in 1 *A. intermedium*) was used as donor of resistance to various diseases (10, 11, 12, 13).

Preliminary results are reported here of a work recently undertaken (14) with the aim of transferring to common wheat resistance to wheat powdery mildew derived from *T. longissimum* (= *Ae.*

longissima), a diploid species with a very closely related genome to the B genome of polyploid wheats. As donor line, a *T. longissimum* ditelosomic addition to Chinese Spring, previously produced by the author(15), was used. The alien telosome, bearing the resistance gene(s), corresponded to the short arm of chromosome G. The available markers for this arm allowed to establish functional homoeology of chromosome G with the group-3 chromosomes of common wheat (15, 16). Based on these observations, a transfer scheme has been adopted (Fig. 1) that, within two generations of crosses, one of which with the *ph1b* mutant of Chinese Spring, provided plants having in single dose the alien telocentric, its wheat homoeologue (alternatively 3A, 3B or 3D) and a 5B*ph1* chromosome. Plants with such a genetic constitution were then pollinated by the standard euploid for the recovery of recombinant types. Meiotic analyses, made rather difficult by chromosome clumping, indicated a 10% pairing frequency between the alien telo and its wheat homoeologues, on an average (Tab. 1). According to the observed types and relative frequencies of the paired configurations involving the alien telo (heteromorphic bivalent and telotrivalent, see tab. 1), a preferential pairing seems to exist between the telo and its closer wheat homoeologue, i.e. 3B. The frequency of recombinant resistant plants recovered so far suggests that the meiotic data represented an underestimate and that the actual pairing frequency most probably ranged between 25 and 30%. Since in *Ph1* condition no such pairing occurs, the efficacy of the inductive system adopted appears remarkable. Meanwhile the size of the experiment is being enlarged, in order to widen the number and the spectrum of the resistant transfers, those already recovered are being characterized as for male transmission of the recombinant chromosome, wheat chromosome involved in the transfer, fertility etc., in order to select the best ones to be introduced as mildew resistant parents in conventional breeding programs.

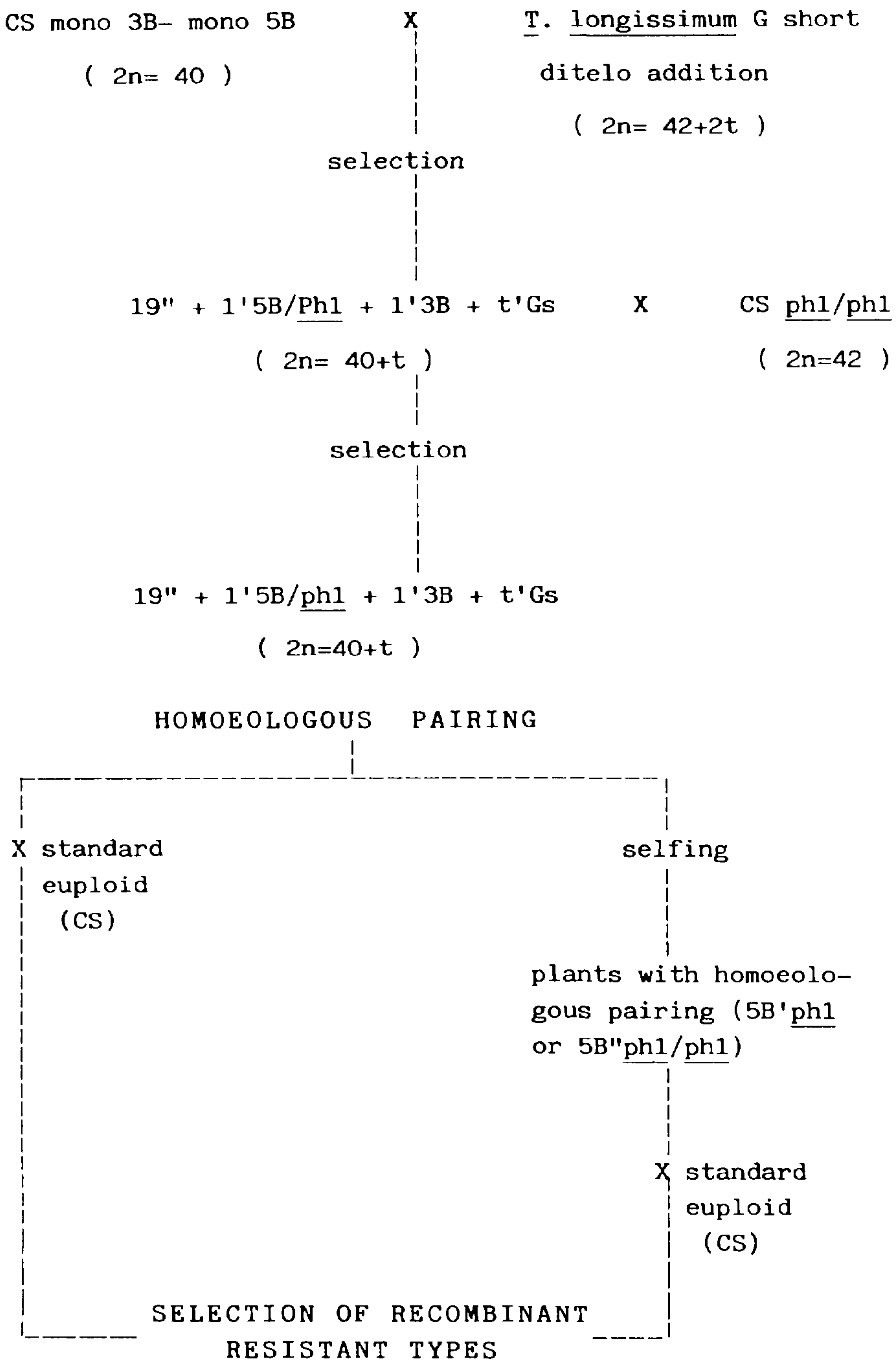

Fig. 1 . Procedure followed to induce pairing between *T. longissimum* chromosome G short arm telosome and its group-3 wheat homoeologues (CS = cv. Chinese Spring).

Table 1. Metaphase I pairing of the *T. longissimum* short arm telosome in 2n=40+t plants having in monosomic condition chromosome 5B/*ph1*, the alien telo and, in turn, each of its wheat homoeologues.

Group-3 wheat monosome	N° of plants	N° of cells	% cells with telo paired		
			Hetero-morphic bivalent	Telo-trival.	Total
3A	6	142	5.6	2.8	8.4
3B	3	173	10.4	1.2	11.6
3D	2	60	5.0	3.3	8.3

Another set of experiments is presently under way in which extensive use is being made of a recently obtained *ph1* primary hexaploid triticale (9). This triticale mutant, which was produced by colchicine treatment of the F1 between the *ph1 durum* wheat of the Italian cv. Cappelli and a rye inbred line, allows to approach the problem of reducing certain defects of the presently cultivated triticales through chromosome manipulation techniques. With the major objective of improving bread making quality, the effectiveness of the *ph1* mutation in inducing recombination between the chromosomes of different D genome sources and those of triticale is being exploited. To this aim, various wide hybridization crosses have been performed, in which one of the parents was always represented by the *ph1* 6X triticale and the other, besides carrying a D genome, either carried a *ph1* mutated 5B chromosome or lacked the entire B genome. A *ph1* homozygous or hemizygous condition could thus be established for the occurrence of homoeologous recombination, most probably involving the D genome chromosomes of one parent and those of the A and B genomes of the *ph1* triticale line. At present, work is particularly concentrated on the following cross combinations.

a) 6X *ph1* triticale x *ph1* common wheat cv. Chinese Spring.

This represents the most direct way to introduce D genome chromosomal segments from the wheat parent into triticale. Obtainment of F1 seeds was relatively easy, but sterility problems are being encountered in selfed and BC generations to normal triticale.

b) *Ae. ventricosa*/*S. cereale* amphidiploid x 6X *ph1* triticale;

Ae. ventricosa/*T. boeoticum* amphidiploid x 6X *ph1* triticale.

Crossability of both these amphidiploids with triticale and the seed set of their F1s was fairly good. BC's to cultivated triticales are yielding seeds in considerable amount.

Meiotic analyses of the triticale x wheat F1s (9), as well as of the hybrids involving the D genome carrying synthetic amphidiploids (17, see also tab. 2) showed the appearance of multivalents, both trivalents and higher rank configurations, in considerable amount

Table 2. Metaphase I pairing behaviour of *Ae. ventricosa*/*S. cereale* amphidiploids crossed by normal and *ph1* hexaploid triticale. Mean values and ranges (in brackets) are reported. 100 PMC's were scored per hybrid combination (data partly from Ceoloni *et al.*, 1986).

Hybrid genomic formula	Unival.	Bival.	Trival.	Quadriv.	Quinq.	% paired chrom.
$M^{v}DABR_{1}R_{3}$(*)	32.42 (26-42)	4.74 (0-8)	0.02 (0-1)	0.01 (0-1)	-	22.8
$M^{v}DAB(\underline{ph1})R_{1}R_{2}$	19.75 (10-31)	8.39 (3-15)	1.44 (0-4)	0.20 (0-1)	0.05 (0-1)	52.74

(*) R_1 = a French rye inbred line; R_2 = an inbred rye obtained from P.B.I., Cambridge; R_3 = a Hungarian rye variety.

when the Phl suppressor activity was absent. C-banding analysis of the meiotic configurations of the phl triticale x phl common wheat hybrids above mentioned revealed that, as expected, homoeologous pairing mostly involved A-, B- and D- genome chromosomes and only rarely wheat and rye chromosomes (Jouve, pers. comm.). Although this, of course, does not prevent from reaching the purpose of transferring D genome chromosomal segments to hexaploid triticale, it perhaps indicates that with very distantly related species from wheat, such as rye, the potency of the phl mutation might not be sufficient to induce practically usable levels of recombinations.

The possibility has still to be definitely ascertained whether the phl ceiling for homoeologous pairing may be further raised by combining it with mutations for other suppressors or with increased doses of pairing promoters. Alternatively, greater advantage than in the case of transfer induction between closely related species can be taken from the use of radiations, including the pollen irradiation technique. "Genome restructuring" genes, such as the one recently discovered in an Aegilops longissima accession (18), may also prove useful to induce exchanges of genetic material between distant homoeologues or between non-homologous chromosomes in interspecific and intergeneric hybrids. However, knowledge in this field has certainly to be increased before the best use can be made of their potential for practical purposes.

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EFFECT OF GAMMA RADIATION ON IMMATURE WHEAT EMBRYO CULTURE

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Abstract

The possibility of using in-vitro cultures of common wheat in connection with mutagenic treatment is discussed. Seeds, pro and young embryos and callus were irradiated with various doses of gamma rays. The observed variation in the awn types is suggested as an indicator of the mutagenic effect in this combined treatment. The variants frequency for the pooled M_1 donors in S_1 generation was about 14% in comparison to 6% of the in-vitro control and over the usual frequency for awning mutation in M_2 and M_3 generations in wheat.

Introduction

It has often been reported that a great diversity of desired variants could be frequently detected in somaclonal progenies originating from somatic tissue culture, particularly from immature wheat embryo culture (Larkin et al, 1981 and 1984). This has aroused the extensive interest of wheat breeders. Some workers have used either chemical mutagens (Hibberd, 1982) or ionizing radiation in in-vitro culture systems in an attempt to enhance the variation frequency and spectrum. Results were obtained in tobacco, potato, rice and many other species (Van Harten et al., 1981); (Shanghai Institute of Plant Physiology, 1975) but rarely in wheat.

Two major problems should be considered prior to adding mutagenic treatments to the in-vitro culture system. First, the effect of the added treatments on callus formation and shoot differentiation. Treatments would be of little practical value if a severe reduction occurs in callus induction and plant regeneration. Secondly, the magnitude of contribution made by additive mutagenesis to somaclonal variation. One of the studies we have made in the past two years was aimed at finding the answers to the above-mentioned questions, with emphasis on evaluation of the feasibility of the combined technique in plant breeding. Several sets of experiments were involved in this study. Some of the results are given as follows:

Seeds irradiated with gamma rays

Dry dormant seeds (13% moisture content) of seven wheat genotypes were exposed to 30 kR gamma rays at 30 R/min, then grown as M_1 plants, from which the immature embryos (embryos of M_1 plants constitute already M_2 progeny), 15 days after anthesis (April of each year), were excised, surface sterilized and placed on modified MS culture medium. The constituents of medium and the changes of 2,4-D content at various stages were the same as described in previous works (Sears et al., 1982). Calli were produced within two weeks, shoot regeneration was not promoted until late October, the season for wheat sowing in our region. The regenerated plantlets were transplanted to the field or to the pots in greenhouse.

In 1984, more than 230 plantlets regenerated from seed-irradiated parents were obtained, and 168 seed bearing plants were harvested for investigation. The response of explants from 7 genotypes to in-vitro conditions is demonstrated in Table 1.

Table 1. Comparison of callus induction frequency (CIF) and shoot differentiation frequency (SDF) between explants from M_1 and from the control

Explant donors	No. of embryos	CIF		SDF	
		%	difference (M1 - CK)	%	difference (M1 - CK)
(1) Young 4, CK	64	43.8		32.1	
Young 4, M1	160	95.0	51.2**	17.0	-15.1
(2) R. R. 2, CK	98	63.3		45.1	
R. R. 2, M1	40	92.5	29.2**	51.4	6.3
(3) 79 p-17, CK	56	25.0		7.7	
79 p-17, M1	196	88.5	63.5**	22.3	15.3
(4) Nu. A. 1, CK	161	37.5		25.0	
Nu. A. 1, M1	98	86.7	49.2**	31.7	6.7
(5) 908, CK	416	34.9		14.5	
908, M1	143	42.7	7.8	34.5	20.0*
(6) Zhe. 2, CK	248	64.1		16.7	
Zhe. 2, M1	48	91.7	27.6**	18.2	1.5
(7) 2. E., CK	16	6.3		0	
2. E., M1	55	83.6	77.3**	22.2	22.2**
Total and mean of CK group	1059	44.2		21.2	
Total and mean of M1 group	740	83.6	39.4**	25.0	3.8

* and **: significant at 0.05 and 0.01 level.

The results revealed that both callus induction frequency (CIF) and shoot differentiation frequency (SDF) are genotype-dependent. The CIF value of some of the genotypes in the M_1 group reached to above 90%, whereas the lowest one was merely 43%. A similar case happened to SDF value in the same M_1 group.

The comparisons made in each pair between M_1 donor and parent donor favoured the M_1 explants which on the average, had a higher CIF value than the parental explants, the mean difference (39.4%) being statistically significant. As regards SDF value, the difference between the two groups was so small (3.8%) that it could be regarded as a consequence of experimental error. Thus, the seed irradiation, or more precisely, the mature embryo irradiation, seemed to play an active role in promoting callus induction, while keeping the same regeneration capacity as the untreated one. Furthermore, seed irradiation also helped to extend the duration of plant regeneration by about 10 days, leading to a 10% increase in plantlet production.

Frequency of variants in S_1 somaclones

A diversity of morphological variations were found in S_1 population (here S_1 designates the first generation of regenerated plants). Among somaclonal variation the change of awn type was probably the most common one, like the leaf colour variations appearing in M_2 generation in mutation breeding of diploid species. Awn type in hexaploid wheat has been considered a stable inherited character with high heritability. Extensive studies on awning inheritance have shown that awn development was controlled by five or six major genes (Ausemus et al., 1973). Alteration of awn type in wheat, therefore, would be a result of gene mutation or chromosomal aberration, both of which might have taken place in the course of in-vitro culture.

It might be appropriate to take the awn type variation as an indicator to reflect and measure the mutagenic effect of in-vitro culture. Based on this, variants frequency with regard to awn type in S_1 population was determined, and comparisons of frequencies between two groups and in each pair were also made. The results are shown in Table 2.

The explant-donors tested here were the same as listed in Table 1, but classified into three groups according to awn types. Group I included three pairs of fully awned donors, in each pair, one being the parent donor, another M_1 the counterpart. No variation was seen in the parent donors. Among 84 regenerated plants from M_1 donors, 7 plants were found to be awnless, one awnleted, the variants frequency amounted to 9.5%. Abundant evidence has indicated so far that awnless and awnleted are dominant over awned (Ausemus et al., 1973). Hence, variants of such kinds could be assumed to have resulted from gene mutation in the dominant direction if the variants had normal fertile spikes, or from chromosome aberrations if there were problems in variants' seed setting.

Most of the variants were normally fertile in our study, thus the awnless and awnleted character would most probably be the outcome of gene mutations with dominant genes in homozygous or in heterozygous stage.

Group III consisted of another three pairs of awnless donors. There appeared two awnleted variants from parent donors, two awnleted plus ten awned variants from M_1 donors, the variants frequency being 3.7% for parent and 20.3% for M_1 donors respectively. The value of the latter was three times that of the former, showing an additive mutagenic effect of the mature embryo irradiation on the enhancement of awning variation.

It is obvious that the awned variation appearing in Group III (awnless donors) had a higher frequency than the awnless and awnleted variants in Group I (awned donors). This phenomenon seems to coincide with the empirical fact that recessive mutation occurs more often than dominant mutation.

From the bottom line of Table 2 we can see that the variants frequency for the pooled M_1 donors in S_1 generation was 14.3%, predominating that of the control (5.9%) and over the actual frequency obtained for awning mutation in M_2 or M_3 generation in wheat mutation breeding practice.

Table 2. Comparison of awn variation in S_1 populations

Varieties and	No. of	No. of variants				Freq. of
M1 counterparts	plants	awnless	awnleted	awned	total	
I. Awned:						
(1) Young 4, CK	14	0	0		0	0.000
Young 4, M1	33	6	1		7	0.212
(2) R. R. 2, CK	21	0	0		0	0.000
R. R. 2, M1	18	0	0		0	0.000
(3) 79 p-17, CK	1	0	0		0	0.000
79 p-17, M1	33	1	0		1	0.030
Sum: CK	36	0	0		0	0.000
M1	84	7	1		8	0.095
II. Awnleted:						
(4) Nu. A. 1, CK	11	3		1	4	0.364
Nu. A. 1, M1	25	4		0	4	0.160
Sum: CK	11	3		1	4	0.364
M1	25	4		0	4	0.160
III. Awnless:						
(5) 908, CK	35		1	0	1	0.029
908, M1	37		0	7	7	0.189
(6) Zhe. 2, CK	19		1	0	1	0.052
Zhe. 2, M1	13		0	1	1	0.077
(7) 2.E., CK	0	No plant regenerated				0.000
2.E., M1	9		2	2	4	0.444
Sum: CK	54		2	0	2	0.037
M1	59		2	10	12	0.203
Total CK	101	3	3	0	6	0.059
M1	168	11	3	10	24	0.143

Inheritance of the various awn characters

Samples were taken from S_2 lines derived from the S_1 variants for studying the inheritance of varied awn characters. Of the four S_2 progeny lines derived from S_1 awnless variants in Group 1 (awned donor, M_1 Young 4), one segregated for awnleted and awned with a ratio of 1:1, another one completely restored to awned, the other two breed true for awnless. The S_2 lines from S_1 awned variants in group 3 (awnless donors, M_1 908) breed true for awned.

Results of analysis on the inheritance of varied characters including awn type from S_1 to later generations will be given in other papers.

According to our observation, a large number of the varied characters ocurring in S_1 generation were inheritable. It is suggested that field selection should be carried out in S_1 generation if tissue culture really becomes an effective tool for plant breeding.

Pro-embryos and young embryos irradiated with gamma rays

Adult plants of four wheat genotypes were exposed to 1 kR gamma rays at either the pro-embryonic or young embryonic stage (1 or 8 days after anthesis respectively). Immature embryos (15 days) were excised from the seeds of treated plants, followed by the same steps as described above.

Pooled results showed that explants treated at the pro-embryonic stage had a greater average of CIF value (88.3%) which was twice as much as the control, but a lower SDF value (7.6%), only half as much as the control, and finally causing a marked decrease in plantlet production.

Explants treated at the young embryonic stage produced a better result, the SDF value going up to 30.3%, while that of the control was only 12.1%. As regards CIF value, both the treated and the control reached a high level, 84.7% for the treated group and 86.3% for the control; no significant difference was found between them. Consequently, the treatment brought in more regenerated plants than the control.

It is also evident that treatment at the young embryonic stage was preferable to that at the pro-embryonic stage, because the former helped to produce more plantlets.

Based on the above facts and using the untreated ones as a standard, the different types of response of treated embryos could be summarized at three stages: callus initiation, shoot regeneration and shoot production (Table 3).

Table 3. Different types of response of treated embryos

Developmental stage	Dose	% Callus induction	% shoot differentiation	% shoot production
Proembryo	1 KR	ascending	descending	descending
Young embryo	1 KR	equal	ascending	ascending
Mature embryo	30 KR	ascending	equal	ascending

As far as the shoot production rate is concerned, the second and third treatments in Table 3 seemed to be more effective than the first one. That the second treatment had a higher rate depended mainly on the increase of SDF value, while the third relied on CIF value increase. Unfortunately, no alterations in awn type were found in any of the 266 S_1 regenerated plants from explants treated at either pro-embryonic or young embryonic stages. On the basis of awn type variations, it is therefore impossible to make a comparison of frequency of variants and mutagenic effects between pro-embryo and young embryo treatment, or between these two treatments and the treatment of mature embryo. At the present time, no conclusion can be drawn from the limited facts that treatment at pro-embryonic or young embryonic stages is less effective as compared to treatment of mature embryos.

Calli irradiated with 1 kR or higher dose of gamma rays

Calli from twelve genotypes were irradiated with 1 kR gamma rays 66 days after inoculation. The results were very discouraging. On the whole, a drastic reduction in shoot differentiation was observed in the treated cultures as compared to the control, the frequency falling to 4.7%, significantly lower than the control's 21.0%. In addition, nearly half of the genotypes completely lost the capacity to regenerate after treatment with radiation. The facts implied that 1 kR might be, in general, too high a dose for wheat callus.

Morphological observations and investigations of the treated callus were conducted 3 months after treatment. Most of the irradiated culture was characterized by a peculiar dark yellow color, hard texture, lower proliferation rate, fewer fresh weight grain, larger mean size of cell, more giant cells, and poor cell content, etc. When callus was treated with TTC solution, only a few spots that scattered sparsely on the callus' periphery turned light red in colour. Above all, the cultures were in a senility stage, having entered the senescence phase much earlier than the control.

Calli from 29 other genotypes were alloted to be exposed to 5, 8, 12, 25, 35, 45, 55 and 100 kR gamma rays respectively. All irradiated calli failed the regeneration of plantlets. According to our observation, the dose of 5 kR seemed to be the starting dose which caused the calli to cease shoot differentiation, and 25 kR was a critical dose that completely inhibited the callus growth. In conclusion, it must be pointed out that treatment of callus with a dose higher than 1 kR is of less practical value in wheat mutation breeding due to its detrimental effects on callus regeneration potential.

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IN VITRO CULTURE IN BARLEY BREEDING*

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Abstract

One of the most useful biotechnics for plant breeders is in vitro culture of anthers or miscropores to induce haploids and homozygous diploids. High frequency of microspore-derived diploid plants could be produced by culturing anthers on Ficoll medium. The segregation ratios of certain morphological characters were not random and could be shifted by culture conditions. It was reported by a number of authors that true breeding and highly productive genotypes were obtained from microspore - derived diploid plants and doubled haploids derived from bulbosom techniques. There is a great possibility that a selective system for desirable characters can be built in an in-vitro culture system.

Where haploids can be induced in crop plants, they provide the most rapid technique for producing homozygous lines. Since the genetically controlled factors in homozygous lines are fixed and will be identical in the future generations, it becomes possible for a plant breeder to evaluate quantitative characters such as yield and quality very early in the breeding program [1].

There are two methods which have been used extensively for production of homozygous diploid barley plants. They are bulbosum techniques and anther culture methods.

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* NRCC No. 25107.

A. The bulbosum techniques [2,3].

When diploid cultivated barley were pollinated by pollen from Hordeum bulbosum, fertilization did occur. However, the H. bulbosum chromosomes were selectively eliminated resulting in seeds that contained immature embryos of haploid cultivated barley. Some of the embryos could develop into plants by embryo culture technique. Homozygous diploid plants could be obtained by treating these haploids with colchicine.

B. Anther culture methods.

Since Clapham [4] reported in 1973 that haploid barley plants could be produced by culturing anthers on agar medium, very little progress was made in the next 10 years. Huang et al [5] in 1984 reported that the maximum yield of plants from the bulbosum technique was 15.4 per 100 florets pollinated, while the highest yield of green plants was 0.5 per 100 anthers cultured from the same hybrid material. They concluded that until the problems of poor regeneration and high numbers of albinos from microspore calli can be overcome the bulbosum technique will remain the method for haploid production in barley breeding programs.

We, at the Plant Biotechnology Institute, (former Prairie Regional Laboratory), National Research Council of Canada, have spent sometime to study techniques for induction of haploid or homozygous diploid barley plant by the anther culture method. We are now able to produce up to 100 green plants per hundred anthers cultured. This was made possible by overcoming two obstacles in barley anther culture techniques. One obstacle was that the anthers or calli tended to sink and die within a few days in a liquid medium, and the other that the pH of our culture medium usually gradually acidified to such an extent that the calli were no longer able to grow. These problems were resolved by increasing the initial pH of our culture media, the buffering ability of the culture media with $KHCO_3$, and by increasing the density of the culture media with Ficoll [6,7,8].

Our results indicated that the frequency of pollen callus formation in anthers could be substantially increased when barley anthers were made to float on a liquid medium containing Ficoll 400 (Table I).

Table I. Effect of Ficoll 400 on pollen callus formation

	No. of calli per 100 anthers cultured					
Variety	M/BT	K42M/BT	59/Jet	TR/SF	S/S	T275/S
With 10% Ficoll	309	220	37	82	117	38
Without Ficoll	10	4	33	2	19	2

Furthermore, we were able to increase the frequency of pollen plant formation by increasing the Ficoll content and reducing the sucrose content (Table II).

Table II. Effect of Ficoll on pollen plant formation.

Osmotica g/l			Experiment 1		Experiment 2	
Ficoll	Sucrose	Glucose	Calli per 100 anther	plants per 100 anther	Calli per 100 anther	Plants per 100 anther
100	90	17.5	356	11	-	-
200	60	17.5	436	40	716	15
300	25	5.0	-	-	200	40

We are now routinely adding 300 g/l (w/v) Ficoll 400, 42.5 g/l sucrose and 2.5 g/l (w/v) glucose in our barley anther culture media. The composition of one of our media is listed in Table III.

Table III. A medium for culturing barley anther.

a)	Mineral Salt (mg)		Sequestrene 330Fe	38
	NH_4NO_3	600	KI	0.75
	$(NH_4)_2SO_4$	67	H_3BO_4	3.00
	KNO_3	2200	$MnSO_4.H_2O$	10.00
	$CaCl_2$ $2H_2O$	445	$ZnSO_4.7H_2O$	2.00
	$MgSO_4$ $7H_2O$	310	$Na_2MoO_2.2H_2O$	0.25
	KH_2PO_4	170	$CuSO_4.5H_2O$	0.025
	NaH_2PO_4 H_2O	75	$CoCl_2.6H_2O$	0.025
	KCl	150		
b)	Sugars (g)			
	Glucose	2.5	Ficoll	300
	Sucrose	42.5	Xylose	0.15
c)	Organic acids (mg) (adjusted to pH 5.5 with (NH_4OH)			
	Sodium pyruvate	5	Malic acid	10
	Citric acid	10	Fumaric acid	10
d)	Vitamins (mg)			
	Inositol	100	Folic acid	0.2
	Nicotinic acid	1	p-Aminobenzoic acid	0.01
	Pyridoxine.HCl	1	Biotin	0.005
	Thiamine.HCl	2	Ascorbic acid	1
	D-Calcium pantothenate	0.5		
e)	Hormones (mg) 2 row barley			6 row barley
	2.4-D	0.5	2.4-D	0.5 - 1.0
	Zeatin riboside	1.0	Zeatin riboside	0.25
f)	Vitamin-free casamino acids (mg)	250		
g)	Coconut Water (ml) From mature fruits: heated to 60°C for 30 min and filtered	10		
h)	Glass distilled water (ml)	1,000		
i)	pH 6 (NaOH). Filter-sterilized			

The procedures of our anther culture method are as follows: Anthers (20-30 in number) containing microspores (pollen) at early to late uninuclear stages were removed aseptically from barley plants and allowed to float on 2-3 ml of Ficoll medium (Table III) in a 60 mm Falcon petri dish and incubated in dim light (less than 50 lux for 9 hrs per day) at 20-22°C. When the pollen calli grew to a diameter of 0.1 to 1 mm in size (ca 20-25 days), they were exposed to stronger light (ca 1000 lux) and were provided with additional culture medium with lower level of 2.4-D. After another 10-20 days, the calli (or embryoids) were placed on a floater in liquid medium with the same components as the anther culture medium (Table III) except that it contained no Ficoll, the amount of sucrose was increased to 60 g/l, 2.4-D was reduced to 0.1 mg/l and zeatin-riboside to 0.5 mg/l for 2 row barley. The amount of 2.4-D and zeatin-riboside for 6 row barley were 0.25 and 0.1 mg/l respectively. The floater was made by coating the edges of a piece of polyester fabric with paraffin. Some of the calli (or embryoids) developed into seedlings in one to two weeks time. The seedlings were transferred onto agar medium devoid of plant growth substances and then into pots. Over 95% of the transferred seedlings survived.

Some morphological characters and isozyme patterns in two small populations of green pollen plants from F_1 hybrid barley have been studied. The results indicated that the segregation of certain characters were not random (Table IV) [9].

The segregation ratio of 2 row to 6 row types could be shifted by changing the amount of zeatin-riboside in anther culture media. Generally, in the pollen plants derived from 2 row x 6 row (or 6 x 2) F_1 hybrids more 6 row than 2 rows were found when the F_1 hybrid anthers had been cultured in low zeatin-riboside and high 2.4-D medium (Table V). The result indicated that selection would be achieved during androgenesis [8].

Table IV. Segregation of morphological characters in pollen plants from F_1 hybrids.

Characters	Segregation ratio	
	Observed	Expected
2 row : 6 row[1]	3:14	1:1
2 row : 6 row[2]	15:45	1:1
Rough : Smooth Awn	11:6	1:1
Rough : Smooth Awn	27:33	1:1
Black : Yellow Lemma	12:3	1:1
Green : Orange Lemma	3:14	1:1
Covered : Naked Seeds	6:5	1:1
Black 6 row Orange : Others	8:7	1:7
Tetraploid : diploid : haploid	2:40:18	?

Table V. Effect of zeatin-robiside on segregation ratio of pollen plants from F_1 hybrid barley.

Source of anthers	Zeatin-riboside mg/l	No. of plants	6 row vs 2 row Segregation ratio	
			Observed	Expected
50 x Jet F_1	0.5-1	17	1:1.43	1:1
(6 x 2)	0.25	21	1:0.21	1:1
K149 x K169 F_1	0.5-1	38	1:0.52	1:1
(2 x 6)	0.25	74	1:0.3	1:1

When one barley pure line which had produced 27% green pollen plants (a total of 15 plants) was crossed with another pure line which had produced 30% green pollen plants (a total of 57 plants), the F_1 produced 54% green pollen plants (a total of 271 plants). A pure line which consistently produced a high frequency (82%) of green plants was selected out from them. These results indicated that genetic recombination is involved.

When a variety which had produced essentially albino pollen plant (a total of 8, all albino) as female parent was crossed with the pure line which had produced 82% green pollen plants (a total of 105 plants), the F_1 produced 61% green pollen plants (a total of 31 plants) indicating that the tendency to form green pollen plants is controlled by nuclear gene(s) [8].

Agronomic performance of homozygous diploid barley

Song et al [10] reported that there were no differences in yield when the best 10 lines from bulk plot derived lines were compared with best 10 lines from haploid derived lines by the Bulbosum technique. Reinbergs et al [11] reported that under Ontario conditions, homogeneous and homozygous lines derived by Bulbosum techniques were as good agronomically and have yields as stable as the licensed cultivars developed by conventional breeding methods.

Friedt and Foroughi-Wehr [12, 13] reported that true breeding and highly productive genotypes were obtained from microspore (pollen)-derived diploid plants. The mean grain yield of doubled haploids was usually lower than the mid-parent mean. However, the yield of the best doubled haploid lines was slightly poor or better than the best parents. Dr. Rossnagel of the University of Saskatchewan (Personal communication) indicated that the best doubled haploid lines from F_1 of Abee x Norbert was slightly lower in yield than the best pedigree lines from the same cross combination. Most of the doubled haploid lines ripened slightly earlier than the pedigree lines.

Variation in agronomic characters of microspore-derived plants of Hordeum cv. Sabarlis has been reported [14]. However, Rossnagel (Personal communication) indicated that microspore-derived diploid lines of cv. Elrose by Ficoll method were uniform within the lines and among the lines in agronomic characters studied. All the lines from F_1 hybrids of Abee x Norbert were uniform within the lines.

Perspective

Since large numbers of microspore-derived calli can be induced by anther culture methods, we should be able to increase further the frequency of microspore-derived plants. There is a great possibility that a selective system can be established in the in-vitro system for isolating salt tolerant and disease resistant plant at the microspore stage; perhaps even yield, if we could find the correlation between the growth vigor in vitro and in vivo.

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SEMI-DWARF MUTANTS AND HETEROSIS IN BARLEY
I. The use of barley sd-mutants for hybrid breeding

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Abstract

Short stature, spring barley mutants obtained after chemical or physical mutagenic treatment were evaluated as a possible source of dwarfism in barley. Genetic analysis of 35 mutants indicated non-allelic relationships. Two dwarfing genes dw-1 and sdw-b were localized on chromosome 3. It was observed that out of 190 various morphological, physiological or isoenzymatic mutants, 122 were mutated simultaneously also for semi-dwarf or dwarf stature. In many crosses between mutants or between mutants and parents, a very high heterosis was observed. The heterosis manifestation in F_1 was observed for four characters; height, tillering or number and weight of grain per plant. The use of induced semi-dwarfs as an alternative for hybrid barley production is discussed.

Evaluation of semi-dwarf forms for spring barley improvement was initiated at the Department of Genetics, Katowice, about 12 years ago. The investigations followed earlier studies on the susceptibility of different varieties to chemical and physical mutagens. 35 varieties cultivated at the time in Poland were treated with the mutagens. The mutagenic treatments as well as the growing of M_1 plants was usually performed in our Department. The M_2 to M_4 generations were grown in the experimental fields of the Plant Breeding Station Lagiewniki. The chemical mutagen MNH (N-nitroso-N-methylurea) was used in most cases. The method of treatment was described in detail before by Maluszynska (1978); Maluszynska and Maluszynski (1983). Fast neutrons (from U-120 cyclotrone of the Institute of Nuclear Physics in Cracow) and sodium azide (NaN_3) were used in a few cases (Skawinska-Zydron and Fatyga, 1978; Szarejko and Maluszynski, 1980).

1. GERMPLASM COLLECTION OF SHORT STATURE SPRING BARLEY MUTANTS

1.1. Evaluation of dwarf and semi-dwarf mutants

Collections of mutants with semi-dwarf or other desirable characters were assembled and grown in the two locations for the following purposes:

Department of Genetics, Katowice	- genetic analysis and agronomic evaluation of mutants
Plant Breeding Station, Lagiewniki	- evaluation of yield and disease resistance and cross breeding

Mutants from 13 varieties were maintained in both collections. Semi-dwarf forms were collected from 10 varieties. Out of 515 mutants collected in 1984, 333 were semi-dwarf and dwarf. Genetic investigations using barley marker stocks and allelism tests between mutants are still in progress.

Table 1 lists the number of mutants in our collections and their relative height. In the selection of mutants from M_2 to M_4 progenies, other than semi-dwarfness agrobotanical traits were mainly considered. Many dwarf forms for this reason, mainly from the varieties Julia, Delisa and Plena were not included in the collection. It means the number of short stature forms now existing in the collection does not reflect the total frequency of induced sd-mutations. For example, the highest frequency of mutants with this character was observed in the M_2 progeny of Julia variety but there are now only 39 mutants in the collection from this variety. Over a period of a few years (1980 to 1984) Aramir and Karat became the most popular varieties in our experiments, enabling us to collect numerous mutants from them.

TABLE 1: Number of dwarf and semi-dwarf mutants in our collection in height classes related to the height of their parent cultivars (1984).

Cultivar		Mutants' height classes (% of parent variety)					
Name	Height (cm)	40-49	50-59	60-69	70-79	80-89	Total
Julia	77.4 ± 3.2	1	11	23	3	1	39
Delisa	80.5 ± 2.0	28	-	7	6	1	42
Plena	95.2 ± 1.6	--	2	5	--	--	7
Aramir	79.3 ± 4.9	1	6	18	13	12	50
Diva	86.9 ± 4.6	2	4	8	6	5	25
Georgia	71.2 ± 4.6	--	1	--	3	2	6
Mg 4170	76.9 ± 7.2	1	8	7	8	2	26
Trumpf	74.0 ± 1.7	--	2	--	4	27	33
HDM	77.0 ± 2.4	--	--	1	6	5	12
Karat	71.8 ± 3.4	2	7	11	14	59	93
TOTAL		35	41	80	63	114	333

It should be noted that all parent varieties used by us are already short statured in comparison with the varieties grown in Europe at the turn of the century (Riggs et al., 1981). Still, the high frequency of sd-mutants induced in our experiments demonstrated that shorter stature mutants can be very easily induced by mutagenic treatment in them too. The cultivar Trumpf, which was released in the DDR in 1973, originated from the cross of several sources of disease resistance with the mutant variety Diamant, itself a semi-dwarf form obtained after x-ray treatment of the "tall" variety Valticky. We collected, after mutagenic treatment, 33 mutants of Trumpf with shorter straw - of which 2 were dwarf.

It appears that stem length and spike length are under separate genetic control. An almost normal or even longer spike was observed in 96 short stature mutants of different varieties (Table 2). Five mutants, 3 semi-dwarf and 2 dwarf (080 AR, 081 AR, 218 DV, 033 AR, 282 AR) have a very long (lax) spike. An independent mutation, induced simultaneously with the sd-mutations is supposed to be responsible for this type of spike. This suggestion will be the subject of a separate genetic investigation.

TABLE 2: Number of short stature mutants in different classes of straw or spike length in relation to their parent varieties.

	Length classes (% of parent variety)								
	30-39	40-49	50-59	60-69	70-79	80-89	90-99	100-110	111+	Total
Straw	6	34	54	65	67	104	3	--	-	333
Spike	-	5	16	30	69	117	71	20	5	333

Generally it is possible to say that straw length was reduced more often and more drastically than the length of the spikes. Examples of mutants with very short straw and normal spike length are presented in Table 3.

TABLE 3: Examples of short stature mutants with almost normal length of spike

Mutant	Straw length, % of parent cultivar	Length of spike, % of parent cultivar
571 DK	38.9	97.1
639 DK	60.8	98.1
845 PK	57.8	99.0
040 AR	67.7	90.8
215 DV	59.3	93.3
438 MG	68.2	94.0

It is encouraging that grain yield components of short stature mutants were often not affected. Since 1983, 305 mutants have been evaluated for the number of grains per plant. 33 of them have a similar or higher number of kernels than the parent cultivars, even such successful ones as Aramir, Trumpf and Diva (Table 4). Examples of semi-dwarf mutants with high yielding capacity are given in Table 5. The shortest-straw mutant in this high yielding group had still 73.2% of the parent variety's height. Shorter, dwarf type mutants usually displayed poor yielding capacity.

TABLE 4: Number of semi-dwarf mutants in grain number classes related to grain number per plant of their parent varieties (1984).

Variety	Grain number per plant	Yield classes (% of parent variety)										
		< 21	21-30	31-40	41-50	51-60	61-70	71-80	81-90	91-100	101+	Total
Julia	140 5 ± 29.7	-	--	4	11	9	6	4	3	1	--	38
Delisa	161.1 ± 62.2	--	6	5	7	12	5	3	3	1	--	42
Plena	126.6 ± 21.3	--	--	2	1	--	--	--	1	1	2	7
Aramir	145.3 ± 24.9	2	3	9	5	7	5	3	1	2	--	37
Diva	165.2 ± 28.9	--	3	5	6	2	4	3	-	-	2	25
Georgia	153.8 ± 24.3		--	2	3	--	1	--		--	-	6
HDM	137.4 ± 22.4	-	--	--	3	2	4	1	-	-	1	11
Mg4170	116.3 ± 28.3	2	4	3	3	2	3	2	3	--	3	25
Trumpf	164.9 ± 38.6	--	2	4	5	6	4	3	3	1	5	33
Karat	171.1 ± 10.8	4	4	12	8	12	9	10	8	5	9	81
	Total	8	22	46	52	52	41	29	22	11	22	305

Table 5: Semi-dwarf mutants with the highest number of grain per plant.

Mutant	Height, % of parent cultivar	Grain No./plant, % of parent cultivar
223 DV	74.4	152.3
428 MG	88.0	153.2
445 MG	73.2	143.2
562 TR	88.8	138.3
715 Q	85.5	155.2
823 Q	75.1	179.1
862 Q	88.6	176.0
888 Q	89.2	126.0
914 Q	86.3	130.6
364 HD	76.1	129.4

1.2. Genetic analysis of short stature mutants

Allelism test has been carried out on 55 cross-combinations of 35 mutants in order to clarify their genetic relationships. Non-allelic relations were found in 44 cross combinations. Crosses of 7 non-allelic semi-dwarf or dwarf mutants with their respective parent varieties gave in the F_2 monogenic segregations (Table 6). This means that 7 independent loci, responsible for shortness of plant height were already found among investigated mutants. The detailed genetic analysis of interaction among these genes is presented in the second part of this paper (Szarejko et al., 1987). Continuous variability in plant height was observed in F_2 progenies from crosses between 3 other non-allelic mutants and their parent varieties. Significant morphological differences among these 35 mutants together with preliminary results of complementation tests suggest that the number of independent semi-dwarf or dwarf loci is much higher.

TABLE 6: F_2 segregation in crosses of 7 short stature mutants with their parent varieties.

Cross combination	No. of plants			X^2	P
	Total	Tall	Dwarf or semi-dwarf	(3:1)	
392 JK x Julia	497	373	124	0.00*	0.98-0.95
409 JK x Julia	423	314	109	0.13	0.80-0.70
555 DK x Delisa	555	424	131	0.58	0.70 0.50
599 DK x Delisa	109	85	24	0.52	0.70-0.50
648 AK x Aramir	325	243	82	0.01	0.95-0.90
862 PK x Plena	417	303	114	1.21	0.30-0.20
267 MK x Mg4170	379	276	103	0.96	0.50 0.30

*/ X^2 = 0.00067

Results of genetic analysis using translocation lines led to the following conclusions (Szarejko and Maluszynski, 1984a and b):

- dwarf mutant 648 AK (new number in our collection 035 AR) is determined by a new, recessive allele of brachytic (br) locus on chromsome 1, first described by Powers (1936).
- the locus dw-1 located on chromosome 3 is responsible for the dwarf character of the mutant 862 PK
- the semi-dwarf character of the mutant 267 MK (new number in our collection 437 MG) is determined by the locus sdw-b on chromosome 3

With the exception of mutant 648 AK, other analysed mutants were non-allelic to the br gene and none was allelic to uz gene. Both uzu and brachytic mutants are usually considered as the most important sources of dwarfness - even though they have undesirable pleiotropic effects in many climatic conditions.

2. HETEROSIS

2.1. High frequency of mutagenic events in cell nucleus after mutagenic treatment with "supermutagenes"

NMH and also sodium azide, which was used in a few cases, are very effective mutagens for mutation induction in barley (Konzak et al., 1975; Maluszynski, 1982; Szarejko and Maluszynski, 1980). Our experiments furnished sufficient evidence to confirm the conclusions of Loesch (1964) and Hänsel (1966) that by using highly effective mutagens in the methodology we developed usually more than one mutation event per cell nucleus is induced in the generative line (mutation rates per surviving M_1-nucleus). The commonly accepted opinion, that only one gene's difference exists between the various mutant lines and their parent varieties, is based on studies with a limited number of characters. Listed below are examples of many cases of semi-dwarf or dwarf mutants which have an identical phenotype and similar agrobotanical performance but are differing in the isoenzymatic spectrum (Table 7):

Table 7: Mutations in isoenzymatic loci of short stature mutants.

Parent varieties and their mutants with identical sd- or dwarf-phenotype	Isoenzymatic loci**				
	Est 1	Est 3	Est 4	6-Pgdh1	AcPh 1
Plena	fast	slow	slow	slow	-
841 PK*	fast	fast	fast	fast	-
862 PK*	slow	slow	fast	slow	-
Julia	slow	slow	-	-	-
392 JK	slow	slow	-	-	-
399 JK	fast	fast	-	-	-
Delisa	slow	-	fast	-	-
628 DK	slow	-	fast	-	-
638 DK	fast	-	slow	-	-
Diva	slow	-	-	-	slow
203 DV*	fast	-	-	-	fast
256 DV*	slow	-	-	-	fast

*/ Genetic analysis indicated allelism of these forms for sd-character.

**/ Nomenclature after Kahler et al. (1981). Loci Est 1 and Est 3 are closely linked (Kahler and Allard, 1970).

Similarly, out of 190 various morphological, physiological or isoenzymatic mutants described in our collection, 122 were mutated simultaneously also for semi-dwarf or dwarf stature. It should be noted that in this case all lines in the collection were screened for only 15 traits (Table 8). The genetic analysis of 10 independently induced mutants with the 6 row character indicated that they were allelic to locus _v_ on barley chromosome 2.

Similarly, the elongated glume mutation was allelic to gene _e_ located on chromosome 2. Out of 24 investigated mutants with dense spike, one (321 GR) already was found as allelic to locus _ert-c_ on chromosome 3 (Gaj and Maluszynski, 1985). Two short hair rachilla mutants were allelic to gene _s_ located on chromosome 7.

TABLE 8: Examples of morphological, physiological and isoenzymatic mutants in collection of 515 mutant stocks (data from the 1984 season).

Mutation	Number of mutants	
	Total	With simultaneous mutation(s) in dwarf or sd-loci
Chlorophyll	27	11
Improved powdery mildew resistance	21	16
Isoenzymatic loci	69(108*)	42(66*)
Increased internode number	1	1
Grain and spike morphology		
Dense	27	23
Lax	5	5
6-row	11	-
Intermedium	1	-
Collar mutation	14	14
Elongated glume	1	-
Short awn	5	4
Lateral flower reduction	1	1
Short hair rachilla	5	4
Hooded (calcaroides)	1	1
Black lemma and pericarp	1	-
Total	190	122

*/ Number of recorded mutations, some mutants contained 2 mutations or more.

The concept of high frequency of mutations per cell nucleus was confirmed in our experiments on heterosis effect in F_1 progeny of crosses between mutants from the same parent variety.

2.2. Heterosis manifestations in mutant crosses

To avoid a heterosis effect in such a character as plant height, mainly semi-dwarf or dwarf mutants and their parent varieties were used in these experiments. To describe the potential heterosis effect in crosses between mutants the F_1 plants were grown space planted (24x25 cm.) in most field experiments. Because of the lack of gametocides all crosses were done by hand. For this reason the number of plants for each replication and the number of replications had to be limited to 5. The general scheme of experiments is presented on Fig. 1.

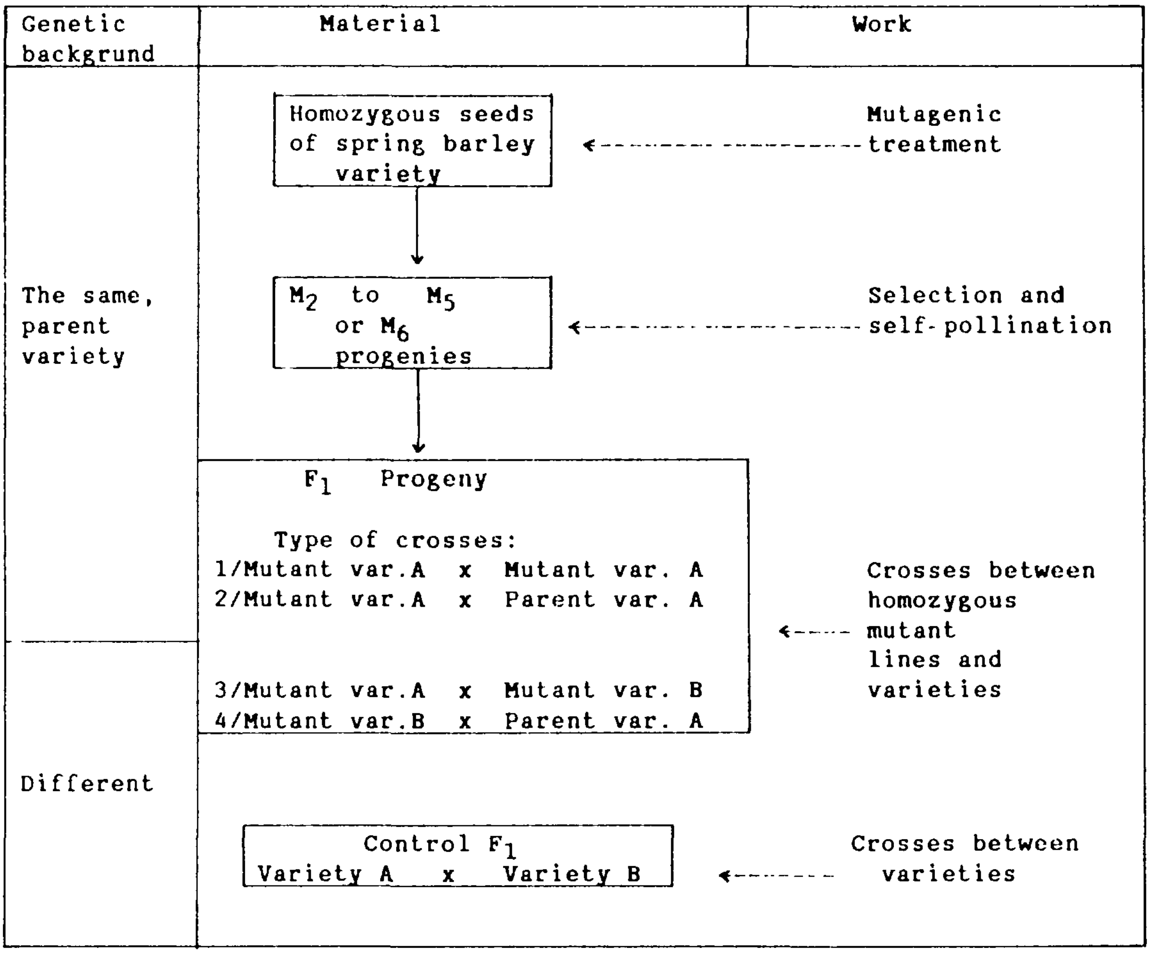

Figure 1: Design for asessment of heterosis effect in crosses of spring barley mutants with each other or with parent cultivars

The first results of these experiments were published by Maluszynski (1982). In many cases a very high heterosis effect was observed in F_1 crosses between mutants as well as between mutant and parent or other variety. At the same time, heterosis was noted in only a few control combinations, especially between distantly related varieties. Results focusing on heterosis in mutant crosses are presented in this paper.

Four lower-yielding mutants of the variety Aramir were used in experiments carried out in 1985. Agronomical characteristics of these lines are presented in Table 9. Two semi-dwarf lines, 282 AR and 239 AR, had very low yield components in comparison with the parent variety Aramir which is one of the leading varieties in Poland. Low number of grains per plant (54.2% and 28.3%, respectively) in comparison to Aramir, as well as very low grain weight (only 37.0% and 17.9%) is due in part to very poor tillering even in widely spaced growing conditions.

TABLE 9: Agronomic characteristics of four mutants from the variety Aramir (data from 1985).

Mutant or parent var.	Height (cm)	Tillering	Grain per plant	
			Number	Weight (g)
Aramir	89.4 ± 7.6	16.4 ± 6.5	273.9 ± 98.4	8.9 ± 3.7
233 AR	91.2 ± 7.4	12.7 ± 4.4	213.1 ± 82.8	6.1 ± 2.7
239 AR	64.9 ± 8.5	4.2 ± 1.7	77.6 ± 46.4	1.6 ± 1.0
280 AR	87.9 ± 4.7	15.8 ± 4.3	237.4 ± 80.1	6.9 ± 2.4
282 AR	70.1 ± 9.4	11.6 ± 2.5	148.5 ± 73.9	3.3 ± 1.5

The heterosis manifestation in F_1 282 AR x 239 AR was observed for four characters in comparison to both parents and their average. These two poor yielding mutants produced F_1 hybrids with an extremely high increase of both yield components (Table 10). Grain weight per plant increased in relation to better parent for 263.6% and grains number per plant for 171.5% Similar data in relation to poorer parent is of course much higher. It was only due in part by increase of tillering from 11.9 or 4.2 to 18.8 tillers per plant but other factors had to be responsible for yield improvement as well. Such extremely high results of heterosis can be used to demonstrate the potential value of various, often poorly performing mutants for heterosis programmes.

These results may be explained by a high level of heterozygosity in F_1 hybrids. This may indicate again that MNH as a "supermutagen" induced a great number of mutations per surviving M_1-nucleus. However, taking into consideration that the significant heterosis effect was observed only in 4 of about 20 crosses of various semi-dwarf Aramir's mutants, it is possible to conclude that specific genes, which directly or indirectly affect yield had to be involved in mutation events and that heterozygosity as such is no sufficient explanation of the phenomenon.

TABLE 10: Heterosis manifestations (% of increase) in F_1 progenies of Aramir's mutant cross 282 AR x 239 AR in relation to both parents (1985).

Character	Percent of increase over:		
	mutant 282 AR	mutant 239 AR	parent mean
Plant height	30.9	41.4	36.0
Tillering	62.1	347.6	138.0
Grain No/plant	171.5	419.6	256.6
Grain weight/plant	263.6	650.0	389.8

It was very clearly demonstrated on the basis of a cross between mutants 282 AR and 239 AR that agronomic performance of mutants can not be used as screening criterium for good combining ability. The mutant 239 AR was used as a cross component three times in the four crosses where the heterosis effect was observed (Table 11). Very low yield and poor tillering of this mutant drastically increased the indexes of all hybrid F_1 characters in relation to the same mutant characters. Its agrobotanical characteristic is not at all attractive for plant breeders: short stature (only 65 cm) with weak straw, large leaves, lax spike (about 14cm) inside a flag leaf sheath and late maturated.

Hybrid plants showed better tillering in all cases, especially in the F_1 of 233 AR x 282 AR. The increase of plant height was not significant in relation to parent variety due to the use of semi-dwarf mutants as components for these crosses. This result demonstrates that the use of mutants from the same parent variety as cross components for F_1 hybrids gives opportunity to avoid the complementation effect of great number of genes regarding such character as plant height (tall hybrid) because of similar genetic background of both parents except one or two sd-genes which differ both these mutants.

TABLE 11: Heterosis manifestations (% of increase in relation to Aramir) in F_1 of Aramir's mutant crosses (data from 1985).

Cross	Height		No. of spikes per plant		Grain per plant			
					Number		Weight	
	(cm)	(%)	(No.)	(%)	(No.)	(%)	g	(%)
Aramir x 239 AR	90.8	1.6	16.6	1.2	362.4	32.3 *	11.2	25.8 *
280 AR x 239 AR	94.4	5.6	18.4	12.2	374.4	36.7 *	12.9	44.9 *
282 AR x 239 AR	91.8	2.7	18.8	14.6	403.2	47.2 **	12.0	34.9 **
233 AR x 282 AR	95.6	7.0	20.6	25.6 *	424.8	55.1 **	13.2	48.3 ***

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

The highest heterosis manifestation was observed in the F_1 of 233 AR x 282 AR. Very high grain number (424.8), as well as weight of grains (13.2g) per plant were observed parallel to very rich tillering (20.6 spikes). It should be noted that the difference in height of plants between the tallest parent (233 AR - 91,2 cm) and F_1 plants was only 5,5 cm and was not significant in relation to the parent variety Aramir.

Statistics proved that a heterosis effect was observed also in the cross of the parent variety Aramir with its mutant 239 AR. It manifestated in both grain yield components but without any changes in such characters as height or tillering.

The heterosis manifestation was not limited to mutants of the variety Aramir. A similar effect was observed in crosses between semi-dwarf or normal tall mutants from variety Diva, for example. Heterosis was observed in crossing Diva with its mutants as well. Manifestation of heterosis effect varied, in this experiment, from year to year but still exceeded the better parent. The F_1 hybrids between parent variety Diva and its mutant 125 DV achieved the highest yield in this group of crosses in both years. This hybrid combination gave in 1985 a very high yield increase 87.6% and 115.3% for grain number and grain weight per plant, respectively. The same semi-dwarf mutant 125 DV was the component of two other mutant crosses in which the heterosis effect manifestation was very high (Table 12).

TABLE 12: Heterosis manifestations (% of increase) in crosses of Diva's mutants in comparison with the better parent and check cultivars in years 1984, 1985.

Cross		Height		Spikes No./plant		Grain per plant			
						Number		Weight	
		1984	1985	1984	1985	1984	1985	1984	1985
DV x 134 DV	a	-1.0	5.8	-0.8	34.6*	0.7	53.8*	-8.1	52.9*
	b		14.8		44.1		67.2		73.3
DV x 125 DV	a	-2.1	11.5*	31.6*	62.7*	45.5*	87.6*	61.5*	115.3*
	b		14.8		43.4		67.7		86.7
DV x 116 DV	a	-4.2	3.9	22.8*	59.5*	33.4*	82.6*	37.2*	80.0*
	b		7.1		40.6		63.5		56.0
125 DV x 134 DV	a	1.3	4.0	1.5	38.6*	14.4	68.9*	16.5	72.9*
	b		12.9		48.3		83.7		96.0
116 DV x 134 DV	a	-4.8	-2.1	0.8	8.5	6.4	19.9	2.2	24.7
	b		6.3		16.1		30.3		41.3
125 DV x 116 DV	a	18.2	19.4	17.5*	20.6	20.8	32.7*	19.2	38.5*
	b		4.5		9.6		18.8		20.0

*/ $p < 0.05$, calculated only to better parent (')
a/ increase in comparison to better parent
b/ increase in comparison to check cultivars

The expression of heterosis in relation to the original variety or better parent was evidently lower in our experiments in 1984, perhaps because of extremly good climatic conditions for spring barley cultivation: a very wet and cool May with a relatively dry summer. Hybrids of barley mutants seem to express a better adaptability in adverse climatic conditions, as in 1985. A dry May together with a delay in sowing due to a wet last decade of April was probably the reason for relatively lower yielding parental and check varieties (Table 13).

TABLE 13 Meteorological data of 1984 and 1985 for the experimental field Boguchowalowice (Katowice).

Month	Temperature (°C)		Rainfall (mm)	
	1984	1985	1984	1985
March	0.4	2.2	17.0	37.2
April	7.4	7.4	17.9	64.5
May	12.4	14.2	128.3	58.0
June	14.0	13.4	52.6	103.9
July	15.0	17.1	74.4	93.4
August	16.9	16.8	53.8	153.2

2.3. Induced semi-dwarfs, an alternative for hybrid barley production?

Hybrid vigour in barley mutant crosses has been described in many mutation breeding experiments. Gustafsson (1946) in a review paper on the effect of heterozygosity on variability and vigour presented examples of hybrid vigour in F_1 of crosses between spontaneous or induced chlorophyll mutants. Heterosis manifestation in vigour was found under short day conditions also in crosses of early mutants (Gustafsson et al. 1973). Hagberg (1953) obtained "superdominance" in total plant weight, yield of grain per plant and tillering in crosses of different erectoides mutants with their parent line.

Similar results were published for other crops. A suprisingly large amount of heterosis up to 104 percent in grain yield above the more productive parent was described by Jones (1945) in crosses of maize spontaneous mutants with parental homozygous lines. It has to be noted that all these mutants were classified as carrying "degenerative changes" such as: narrow leaf, dwarf type of growth, pale chlorophyll and crooked stalks, blotched leaf and late flowering. The author concluded, from results of these crosses that "heterosis could result from a single allelic difference if the change involved more than a single function". Unfortunately, data about segregation in F_2 progenies have not been presented nor other results which could provide additional support for this statement. Gottschalk (1968, 1976), on the basis of detailed genetic studies of Pisum mutants, clearified that seemingly pleiotropic action of mutated genes very often can be explained as simultaneous mutations of neighbouring, closely linked genes. The concept of so called "monohybrid heterosis" was revised first by Doll (1966) and than by other authors (Lönnig, 1982; Gottschalk and Wolff, 1983)

Craigmiles (1968) informed about heterotic effects of dwarf genes on forage production in Sorghum vulgare var. sudanense Hitch. Gorny (1976) described heterosis effect in crosses of Petunia axillaris (Lam.) Juss. mutants obtained after MNH treatment. Drastic increases of green matter production, considerably above the level of the parent variety, were observed in the F_1 of low glucoside induced mutant crosses of Melilotus albus DESR (Micke, 1976). Stoilov and Daskalov (1976) presented data on general and specific combining ability of induced maize mutants. Inter-mutant F_1 hybrids of Sesamum indicum L. gave a heterotic effect in seed yield and number of capsules (Murty, 1979). Qiu and Lu (1982) found that heterosis in the F_1 of Arachis hypogea L. mutant crosses was more significant than the F_1 heterosis of original varieties. It should be noted that these mutants can not be directly used as new, improved varieties.

Lehman (1981) in paper with title "Where is hybrid barley today?" presented a few ideas on the use of new techniques for hybrid barley seed production. As a basis of all of them, there is a need to have hybrid plants which are profitable. We demonstrated that semi-dwarf mutants used as components of hybrid crosses can express a very high level of heterosis in such important characters as number or weight of grain per plant and tillering. These results were obtained in space planted trials which gave an opportunity to describe the yielding potential of inter-mutant or mutant x parent variety crosses. Stiff, semi-dwarf mutants with proper differences in plant height between both parents for more effective pollination of a sterile, female component, will necessarily be needed for hybrid seed production with the use of gametocides.

Collections of semi-dwarf mutants can be an alternative for traditional sources of hybrid cross components if the mutant heterosis will be confirmed in farmers field conditions. This approach with the use of semi-dwarf, intra varietal mutants for hybrid seed production has important advantages in comparison to the traditional one. Mutants used for hybrid production differ only in limited number of genes. It means that other important characters, particularly of the grain, such as grain quality, cooking quality, taste, malting characteristics could remain in homozygous stage and therefore would not segregate in the harvested product of F_1 plants (as would be easily the case in distant hybridization) because of the same genetic background both cross components which originated from the same parent variety. This is extremly important for cereal hybrid production where semi dwarf stature of the plant, with its physiological implications, has to be maintained together with other important characters of the present day ideotype to win competition with high yielding comercial varieties.

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BIOCHEMICAL ASPECTS OF COLD AND HEAT SHOCK RESPONSE IN BARLEY*

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Abstract

Studies with barley plantlets (cv. Onice) showed the appearance of at least five major proteins(MW = 22, 47, 70, 85, 94 Kd) after a temperature shift up (heat shock) from 24° to 40°C.

The synthesis of these heat shock proteins has been related with acclimation or thermoprotection of barley seedlings to elevated temperatures. We have also analyzed the modifications in proteins synthesis following a temperature shift-down from 24° to 0°C (cold shock). The synthesis of three to five polypeptides (cold shock polypeptides, CSP) was obtained under this condition. Synthesis of these CSP was followed in different parts of the same plant and in two different cultivars: Onice and Georgie.

The identification of specific HSPs and CSPs could offer the opportunity to clone some of their structural genes by complementary DNA synthesis and to analyze in the molecular details their genetic regulation and their dependence on the specific genes.

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Temperature variation is one of the major factors controlling plant growth and reproduction. In crop plants, when the final yield or the reproductive capacity is influenced, the stress may result in an economic disaster. In fact the agronomical practice has evidenced that in cultivated plants rapid step-up and down above the normal temperature (heat shock and cold shock) can modify the expression of a particular genotype.

However the plant can react to the thermal insult in several manners. These mechanisms have the purpose to survive the plant to those environmental modifications which represent the temperature extremes of their habitat (1, 2).

Survival to these otherwise lethal temperatures had deserved particular interest also because there could be some relationship with the capacity to adapt at those temperature variations which can occurr in the field.

Therefore the search for crop plants which can go through the temperature variations of their habitat without significant modifications of their final yield can have a benefit for the comprehension of the mechanisms regulating the cellular response to temperature insult.

Among the adaptative response to temperature changes, two molecular mechanisms have been identified which are triggered respectively by a rapid temperature increase (heat shock) or by an equally rapid temperature decrease (cold shock). Both types of response involve a modification in gene expression since the activity of some genes is shut-off whereas other genes are shut-on. Consequently preexisting "normal" proteins are no longer translated during the stress whereas an increased synthesis of new proteins is induced (2, 3).

The response to heat shock in vascular plants has been described for several species including barley (4, 5, 6). The major features of the plant response as compared with animal response were the presence of a large number of low molecular weight heat shock proteins (LMW) in plants not detected in the animal systems (7). Genes coding for some of these LMW as well as genes coding for the high molecular weight heat shock proteins (HMW) have been cloned and sequenced (8). Investigations on the hydrophilic/hydrophobic properties of some of the heat shock polypeptides (HSPs) showed a conservation of structural features between HSPs from soybean, Caenorhabditis, Drosophila and the mammalian lens α-crystallin(9).The structural analysis carried out in one of those cloned HS genes (a gene coding for a 153 amino acids protein) showed the presence of an open reading frame and several potential promoters homologous to the Drosophila heat shock consensus sequence CT-GAA-TTC-AG with 15 bp upstream from the TATA box (for a review, see Ref. 10).

The possible function of these HSPs and of the putative HS genes have been investigated. In barley, as well as in soybean (11), a short exposure (two-four hours) at high temperatures (50°C) determines a block in the growth process of the young seedlings, which can not resume several days after the seedlings are returned to normal temperature.

Restoration of the elongation process following a HS as well as any other thermoprotecting effect can be easily detected by using this system. In fact, when the seedlings, before the shock at 50°C, are exposed for a short period to temperatures which induce synthesis of HSPs (37°-40°C) but which do not affect significantly the elongation process, inhibition of the growth by the second exposure was only partial or ceased (6).

Therefore the first mild temperature shock provides the plant with an acclimation or a protection toward the exposure to otherwise lethal temperatures. A positive correlation has been established between acclimation or protection against high temperatures and accumulation of HSPs (2).

Other agents different from temperature which can induce HSPs (e.g. arsenite) are able to furnish a similar thermoprotection to the plant, confirming the role of the HSPs in this phenomenon (11, 12).

Since the proteins induced by the HS could be different (at least in barley) in the different parts of the plant, this raises new questions about the mechanism of acclimation and thermoprotection in the various parts of the plant.

Firstly, is the synthesis of these different proteins related with a tissue specific requirement in the process of acclimation and thermoprotection? Secondly, are different HSPs really synthesized by different organs in response to HS also in adult plants, or are these differences a peculiarity of the seedling tissue?

A modification in the synthesis of individual proteins has also been evidenced when barley seedlings were exposed few hours to temperatures of 5°C or 0°C (3). Attempts were made to study protein synthesis below the freezing temperatures, but the results were not significantly different at -5°C. The effect of temperature step-down (cold shock) involved the synthesis of new proteins and the disappearance of some of the normal proteins. At 0°C only the cold shock proteins (CSPs) were present, whereas most of the normal proteins disappeared. Different organs of the seedling synthesized some CSPs which were common whereas other CSPs were detected only in a specific organ.

The synthesis of CSPs as the synthesis of HSPs could also be directed in vitro by purified messenger RNA isolated from shocked tissues or plants and this is currently utilized to produce cDNA clones of the major HSPs. The comparison by hybridization of these cDNAs with the cloned HS genes will elucidate the differences and the similarities existing in the genetic determinants for cold and heat shock proteins.

The function of the CSPs is under investigation, but the preliminary experiments indicate that they could have a role in thermoprotecting the plant against the very low temperatures.

Within the important issue of both cold and heat tolerance in plants a particular interest deserves the study of mutants in the genes encoding for the cold and heat shock proteins and of the effect of mutations in these genes upon the acclimation and thermotolerance of the whole plant.

Mutants of these genes can be identified in the progenies derived from mutagenized parents as well as constructed in vitro by using the cloned shock genes. Forthis purpose the availability of reliable techniques for mutagenizing both plants and isolated genes will be of major interest in the development of plants with modified responses to temperature stress.

The selection among these mutants, from those with particularly favorable adaptability to extreme environments, should produce proper genetic material from which seed productivity and other economic traits can be evaluated.

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PHYSIOLOGICAL TESTS FOR DROUGHT TOLERANCE IN BARLEY AND DURUM WHEAT*

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Abstract

Physiological tests for characterizing drought tolerance in barley (H. vulgare L.) and durum wheat (T. durum L.) were evaluated. These tests involved: 1) germination in osmotic solution (-13 atm by d-mannitol); 2) thermal stress on seedlings (42°C for 5 hours); 3) stability of the cellular membrane under osmotic stress (PEG 6000 at 43%). For both species genotypic variability which was associated with drought tolerance in the field was identified by the laboratory evaluation techniques.

Based on these procedures, the two-row barley types were more drought tolerant than six-row types. In durum wheat, only some local populations and some varieties, bred in drought environments, showed high laboratory test values. Weak and not significant correlations were found between the physiological tests indicating that the genetic mechanisms which control these traits may be independent and process-specific. Yield trials, in barley, have been carried out in environments with drought conditions and the correlation between grain and physiological tests were significant.

Of the three procedures evaluated in this study, the dry matter increase after a period of thermal stress and electrolyte leakage seemed to be most reliable and potentially useful for screening for drought tolerance in barley and durum wheat.

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INTRODUCTION

High temperature and drought are notorious both for their unpredictable variability from location to location and their ability to limit the yield of crop plants in many agricultural regions of the world (Lewis and Christiansen 1981; Blum 1983; Martiniello 1984). In cereals most of the progress in breeding for drought tolerance has been achieved by using empirical methods. In practice a drought tolerant cultivar is defined as the one making the greatest yield with limited soil moisture. However better progress could be made if the physiological responses of plant to drought were known and could be used in selection programs.

Breeders and physiologists are looking for ways to measure and analyse the underlying physiological mechanisms of tolerance. The chain of physiological events between gene and phenotype is not known but if a key step in the chain were known to be correlated with stress tolerance, the breeder can select directly for it rather than select for the final phenotype (Sullivan and Eastin 1974; Blum and Ebercon 1981; Larsson 1982; Martiniello and Lorenzoni 1985). The success of the physiological approach will require evidence that the characters tested in the laboratory reflect drought tolerance, under field conditions.

Among cereals, barley and durum wheat are two of the most important crops in the areas were high temperature and erratic rainfall occur during the life cycle. The following three laboratory tests have been applied in these two species. They include: a) seed germination in osmotic solution (GOS); b) dry matter increase in seedling (DN_1-DW_0) after a period of thermal stress carried out in the greenhouse; and c) evaluation of the stability of the cellular membrane in presence of osmotic stress applied at flowering time (LEOS).

PHYSIOLOGICAL LABORATORY TESTS - DESCRIPTION

Seed germination under osmotic solution.

Seeds of all progenies were immersed for 5 minutes in a sodium chloride solution (5% v/v), washed three times and dried to prevent mould infestation, then transferred to filter paper in Petri dishes. Fifty seeds for each genotype and for each replication were distributed on the filter paper and 10 ml of d-mannitol solution at ψ = -13 atm were added. Three series of dishes were arranged in a germinator at 25°C, following a randomized block design. A fourth replication using distilled water served as control. After 8 days, the percentage of germinated seeds (GOS) was determined.

Seedling evaluation in heat temperature chamber.

The genotypes were grown in pots of 15 cm diameter filled with a compost consisting of an algae-rich soil and arranged on a greenhouse bench without supplementary light. A randomized block design was used with four replications for evaluating dry matter increase after the period of thermal stress plus two replications for the control. Fifty seedlings were considered for each genotype in each replication. Plants at four-leaf stage (15-20 days old) were well watered and the following day placed in an unlighted forced air dryer at 42°C for 5 hours. After the exposure period, the plants were returned to the greenhouse and then rewatered. On control replications, a sample of thirty plants from each genotype was collected for determining the initial plant dry weight (DW_0). Seven days later the seedlings were picked up and final plant dry weight (DW_1) was evaluated for each genotype.

Leaf tissue discs under desiccation stress.

Progenies were arranged in the field in a randomized block design with four replications. The plot consisted of 10 m^2 with a seed rate of 350 seeds/m^2. Two sets of flag leaf-discs were cut from plants with equivalent growth stage (determined by the flag-leaf) in each plot. Two sets of fifty discs, 5 mm in diameter, were collected in vials containing 20 ml of deionized water. One set of discs was used for the stress treatments and the other set served as the control. The loss of electrolytes under osmotic stress (LEOS) was measured by electrical conductivity following the procedures described by Blum and Ebercon (1981).

EXPERIMENTAL RESULTS

Barley

A preliminary screening test based on seedling dry matter increase after a period of heat stress, has been carried out on fifty genotypes (cultivars and breeding materials).

Sixteen of them have been selected and evaluated in three environments where conditions of drought are frequent (Catania, Cagliari and Foggia).

In all physiological tests we were able to identify variability among genotypes (Table 1). The lack of correlation (Table 2), among physiological tests, at different stages of growth, means that the genetic mechanisms of drought tolerance are independent, process-specific for each stage of development and governed by specific mechanisms with some interference in their expression.

Correlations between grain yield and physiological tests were found (Table 3). The highest value was between electrolyte leakage (LEOS) and yield, which means this test may be a good indicator of drought tolerant lines. The grain yield average (Table 4) of the genotypes (45.9 q/ha), with high values of the physiological tests, was 15% higher than the mean of the discarded ones (39.4 q/ha). From these varieties we found that only Georgie shows, in all 3 stages of development, a good drought tolerance. Other genotypes have different response at different stages.

Table 1. Ear type (D= two-row; P= six-row), grain yield and physiological tests averages (GOS = germination under osmotic solution; DW_1-DW_0 = seedling dry matter increases after thermal stress; LEOS = loss of electrolytes under osmotic stress) of the barley genotypes in the experiments.

Genotype	Ear type	Grain yield (q/ha)	GOS (%)	$DW_1 - DW_0$ $\frac{g\times10^{-2}}{plant}$	LEOS (%)
Georgie	D	51.7	30.7	20.7	54.3
Gitane	"	46.3	54.7	14.0	69.4
Igri	"	46.8	6.4	24.5	63.4
Panda	"	43.7	1.8	12.9	67.2
Pepite	"	34.3	8.3	15.0	78.8
Arda	"	45.8	2.7	18.3	49.0
Alpha	"	41.8	29.3	13.2	67.7
Porthos	"	47.7	36.1	15.0	55.1
Tipper	"	44.6	14.7	18.6	73.0
Arma	P	37.4	1.3	9.5	75.6
Fior 26	"	41.2	2.1	8.5	69.9
Robur	"	40.4	0.7	6.7	75.5
Pirate	"	43.3	0.1	25.4	68.5
Vetulio	"	36.9	3.0	8.6	69.6
Barberousse	"	46.0	2.4	19.9	75.5
Jaidor	"	40.5	2.4	17.6	77.8
$LDS_{(0.05)}$		3.3	6.9	3.1	5.7

Table 2. Correlations among physiological tests in barley.

	GOS	$DW_1 - DW_0$
GOS	-	
$DW_1 - DW_0$	0.05 ns	-
LEOS	-0.32 ns	-0.38*

Table 3. Correlations among grain yield and physiological tests in barley.

	GOS	$DW_1 - DW_0$	LEOS
Yield	0.48*	0.56**	-0.69**

Tipper seems to be highly resistant at the early growth stages, while Arda and Igri are tolerant from tillering to maturity (Table 4). In all of these materials only Barberousse is a six-row type. Within the two-row type we found tolerance in both spring and winter types. This means that the two-row materials could be the basis for developing winter or spring cultivars for environments with drought conditions.

Table 4. Barley genotypes with high test values.

GOS	%	$DW_1 - DW_0$	$\frac{g \times 10^{-2}}{\text{plant}}$	LEOS	% injury
Gitane	54.7	Igri	24.5	Arda	49.0
Porthos	36.1	Georgie	20.4	Georgie	54.3
Georgie	30.7	Tipper	18.6	Porthos	55.1
Alpha	29.3	Arda	18.3	Igri	63.4
Tipper	14.7	Barberousse	19.9	Panda	67.2
$\bar{X}$	33.1		20.3		57.8
$\bar{X}$ Discarded Genotypes	2.8		12.4		72.8

Genotypes without underlining appear only in one test.
" with dashed underlining appear in two tests.
" " solid " " " three tests.

Durum wheat

Considering durum wheat the most important cereal crop in the areas where drought and high temperature are frequent, we applied two physiological tests on the basis of the results obtained in barley.

We chose 46 genotypes: 21 local populations have been tested for germination and dry matter increase in seedling (Table 5) and 25 common varieties were evaluated for germination in the osmotic solution test (Table 6).

Table 5. Physiological test values in durum wheat cultivated varieties (GOS) and local populations (GOS and DW_1-DW_0). For the code used in the test see Table 1.

Varieties	GOS (%)	Local populations	GOS (%)	$DW_1 - DW_0$ $\frac{g \times 10^{-2}}{\text{plant}}$
Adamello	53.2	Cannizzara	60.0	8.3
Aldura	48.6	Capeiti 8	*	10.0
Appio	96.0	Cappelli		13.1
Appulo	65.2	Castiglione G.	42.5	14.2
Arcangelo	57.0	Cotrone	66.7	15.6
Brunda	52.9	Creso	*	5.6
Capeiti 8	95.0	Farro Lungo	64.2	8.1
Castello	75.6	Gioia	92.6	8.0
Creso	62.4	Karel	*	12.0
Duilio	54.2	Lina	58.6	13.3
Gabbiano	61.1	Martinella	53.7	17.2
Japigia	42.6	Produra	*	9.1
Karel	68.5	Realforte	69.5	8.3
Lira	20.0	Ruscia	71.9	13.6
Messapia	61.1	Russello	64.9	0.4
Norba	43.7	Scorsonera	69.3	11.3
Nora	81.8	Semenzella	45.5	4.7
Piceno	67.4	Sicilia Lutri	47.0	10.1
Procace	63.8	Sicilia R.N.	47.4	10.3
Produra	73.0	Timilia	70.8	13.4
Solitario	78.0	Trinakria	62.6	8.2
Trinakria	62.6	Tripolino	51.1	11.2
Valforte	42.0	Tunisina	51.7	18.0
Valnova	51.4	Urria	42.9	13.5
Vespro	50.0	Valle L.G.	56.4	10.3
$LSD_{(0.05)}$	11.6		24.4	2.7

*) See column number 1.

For germination in osmotic solution (GOS) a large variability within in two groups of genotypes was found. Within the commonly cultivated varieties (Table 6) were found eight promising genotypes: Appio, Capeiti 8, Nora, Solitario, Castello, Produra, Karel and Piceno which are largely cultivated in drought environments. All these varieties have been bred for specific adaptation in stressed environments. On the contrary, the new high yielding varieties, bred in high fertile environments, have greater yield instability (Mariani et al. 1983), when grown in stressed environments, and

Table 6. Durum wheat varieties and local populations with high physiological test values.

Varieties		Local Populations			
GOS	%	GOS	%	$DW_1 - DW_0$	$\frac{g \times 10^{-2}}{plant}$
Appio	96.0	Gioia	92.6	Tunisina	18.0
Capeiti 8	95.0	Ruscia	71.9	Martinella	17.2
Nora	81.0	Timilia	70.8	Cotrone	15.6
Solitario	78.0	Realforte	69.5	Castiglione G.	14.2
Castello	75.6	Scorsonera	69.3	Ruscia	13.6
Produra	73.0	Cotrone	66.7	Urria	13.5
Karel	68.5	Farro Lungo	64.2	Tinilia	13.4
Piceno	67.4	Trinakria	62.6	Lina	13.3
$\bar{X}$	79.3		70.9		14.8
$\bar{X}$ Discarded Genotypes	56.0		51.8		8.8

showed low germination values of the applied test. Within local populations we found eight genotypes with high scores when germinated in the osmotic solution test (GOS): Gioia, Ruscia, Timilia, Realforte, Scorsonera, Cetrone, Farro Lungo, Trinakria (Table 6).

For thermal stress a large variability was also found. Six genotypes showed a good ability to recover after a period of stress (DW_1-DW_0) (Table 6). The two local populations (Timilia and Ruscia) seem to have physiological attributes of tolerance rather than avoidance (early maturity), because they are facultative and late maturity genotypes.

As in barley, no correlation was observed between these two tests. This could suggest that the genetic mechanisms which control the traits are independent.

CONCLUSIONS AND REMARKS

On the basis of these results, wide genetic variability for drought tolerance can be identified by physiological laboratory tests applied at different growth stages (seed, seedling and adult plant). Obviously, the data do not explain the physiological mechanisms that are the basis for the correlations between stress tolerance in field conditions, and the results of the laboratory tests. However, it is likely that the genotypes showing positive reactions to tests possess the genetic mechanisms for recovering their normal conditions of growth after a period of environmental stress. The absence or weak correlation between traits evaluated by physiological tests indicate that those traits are independent and may be regulated by different genetic mechanisms (Sullivan and Ross 1979; Levitt 1980; Trapani and Motto 1984; Martiniello and Lorenzoni 1985).

In this regard, the durum wheat varieties, released in stressed environments, and some local populations contain more favourable gene combinations for stress than the new varieties which have unstable yields in stressed environments (Fischer and Maurer 1978; Mariani et al. 1983).

The genotypes selected using these tests have been introduced in breeding programs for drought tolerance for developing synthetic populations through the recurrent selection method. With this cyclical method, it is possible to increase the frequency of the favourable genes and to maintain a large amount of genetic variability. From these populations new advanced breeding materials can be extracted: a) parents for new cycle of recurrent selection; b) new varieties and c) lines as parents for developing F_1 commercial hybrids. This last program can be facilitated, in the near future by using gametocides to induce male sterility.

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COMPARATIVE ANALYSIS AT THE MORPHOLOGICAL, BIOCHEMICAL AND GENETIC LEVEL TO DEVELOP A STRATEGY FOR STUDYING BIOLOGICAL SIMILARITIES IN *Triticinae*

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Abstract

The current affinities ascertained among Triticinae species are reviewed.

The search of a strategy to ascertain biological similarity among Triticinae species is still a debated issue. The best approach to be used must exclude analogies as deceptive and affinities be constructed only on homologies.

Previous morphological studies ascertained that the genera Secale and Dasypyrum were different each other, and both were different from Triticum and Aegilops. However more recent cytological studies showed that certain rye chromosomes are homoeologous to that of wheat; cytoplasm studies indicated that Dasypyrum villosum cytoplasm is affine to that of the donor of the S or B cytoplasm to wheat; isoenzyme proteins and seed storage proteins pointed out to the presence in D. villosum of genes orthologous to those in the A or B genome of wheat.

Definitive answers on species affinities have been obtained in few cases when in vitro DNA-DNA hybridization technique have been used.

Therefore for future work it will be interesting to use pairwise DNA-DNA hybridization of representative species of all Triticinae genera in order to have a broad and significant answer on the biological similarities of the species belonging to this subtribe.

INTRODUCTION

The subtribe Triticinae includes many important crop plants and many grazing grasses. The use of the crop plants or cultivated species of Triticinae has been related to different plant structures but the kernels are the most important plant organ to be used. The kernels are used for making basic foods for humans (two-layered flat breads, single-layered flat breads, pasta, cous cous, burghul, frekeh, etc.) and animals (straw and foodstuff). The wild species belonging to the subtribe Triticinae are grasses covering pasture land of many European, Mediterranean, and Near East areas.

To the taxonomists working with economic plant, the Triticinae species group is of particular interest because a large number of its species has been used. To the plant evolutionist interested in phylogeny , the Triticinae are an important group of species for testing hypothesis on evolutionary mechanisms and speciation events.

The Triticinae species have given rise to perplexing problems of evolution, phylogenesis and sistematics, which cannot be resolved by separate and partial studies on plant structure, plant proteins, cytogenetics, genetics and molecular biology. As matter of fact, currently the studies around the ascertainment of affinities among species of Triticinae is still cause of thorny debates .

The criteria that have been used to search affinities, among species ranged from geographical distribution to DNA - DNA hybridization. In no instance have all the species of the Triticinae been contemporarily compared for geographical distribution, morphology, biochemical properties (isozymes, seed storage proteins, aminoacid sequence of proteins, antigen-antibody reaction, flavonoids, etc.), gene and chromosomal homoeology, cytoplasm type, organelle (chloroplast and mitochondria) DNA restriction mapping, ribosomal gene sequence, in-situ hybridization of labeled DNA or RNA with metaphase chromosomes, DNA-DNA hybridization of not-repeated sequence, and nucleotide sequence of a certain gene. This is because some Triticinae genera (Triticum , Aegilops and Secale) have been studied more profusely than others (Agropyron and Dasypyrum), and even in the genera more analyzed not all the above criteria have been used.

In reality to search biological similarity, that is the fundamental procedure for constructing phylogenetic trees and follow the evolution of a taxa, not all the above cited criteria are necessary.

Gould (1) indicates that biological similarity includes two entirely different reasons for being. Two organisms may 'look alike' because both inherited a set of similar features from a common ancestor. Similarities based upon descent are called homologies. But features of two organisms may also look alike because both evolved independently in response to life in similar environments. Similarities based upon independent evolution are called analogies. (Wheat and broad bean are annual plants and cauchgrass and common waterlily have rhizomes, by analogy only. Wheat and cauchgrass remain Graminae by descent).

Therefore statements about similarities among Triticinae species must be based on genealogy and this can be ascertained only if similarities are excluded as deceptive and the affinities are constructed only on homologies.

The object of this paper is to review some of the work that has been done at the Agricultural Biology Institute, University of Tuscia, Viterbo, Italy on the search of affinities in Triticinae using the approach of sorting out homologies from analogies.

CYTOGENETICAL AFFINITIES

Various models of genetic and genome differentiation and affinities in the species of Triticinae have been developed on the basis of chromosome pairing studies in interspecific hybrids.

Many authors agree that the A genome is closely related to the genome of Einkorn wheats (2); the B genome appears to be related to the S genome that is present in the species Ae.speltoides, Ae.longissima, Ae.sharonensis, Ae.searsii, and Ae.bicornis of the section Sitopsis of the genus Aegilops (3). The D genome was contributed by Ae.tauschii (4).

The study of the homoeology between genomes can be done by crossing the species carrying different genomes and observing the amount of pairing at methaphase I. However pairing of homoeologous chromosomes is often prevented by the activity of suppressor genes, located on the long arm of chromosome 5B (gene Ph) (5), on chromosome 3A (6) and 3D (7).

The assumption that reduced methaphase I chromosome pairing in interspecific hybrids of Triticinae is caused by structural differentiation of chromosomes (inversions and translocations) does not agree with experimental observation (8).

More reasonable seems a chromosomal differentiation associated with speciation that occurs in numerous sites in the genome and by not altering gene sequence . Uneven rates of nucleotide sequence change has been postulated to occur at the genetic level in the chromosome of the A and B genomes. Therefore it has been proposed that differentiation among Triticinae species has both a structural and a molecular component (8).

This hypothesis indicates that many loci in the Triticinae although orthologous have evolved alleles that code for very different gene products. Therefore any affinity study in the Triticinae that is devoid from cytogenetics has to have a genetic background that involves many loci.

Recently genome affinities has been studied using the C-banding technique. Gill and Kimber (9) comparing the C-banding of the diploid species T.monococcum, Ae.speltoides, Ae.squarrosa with that of A, B, and D genomes, respectively, in hexaploid wheat, conclude that Ae.speltoides could not be the donor of the B genome and T.monococcum and Ae.squarrosa are the donors of the A and D genomes respectively.

Teoh et al. (10), however, gave evidence that on the basis of affinities in the C-banding patterns Ae.speltoides and Ae.sharonensis could be the possible donor of the B genome of wheat.

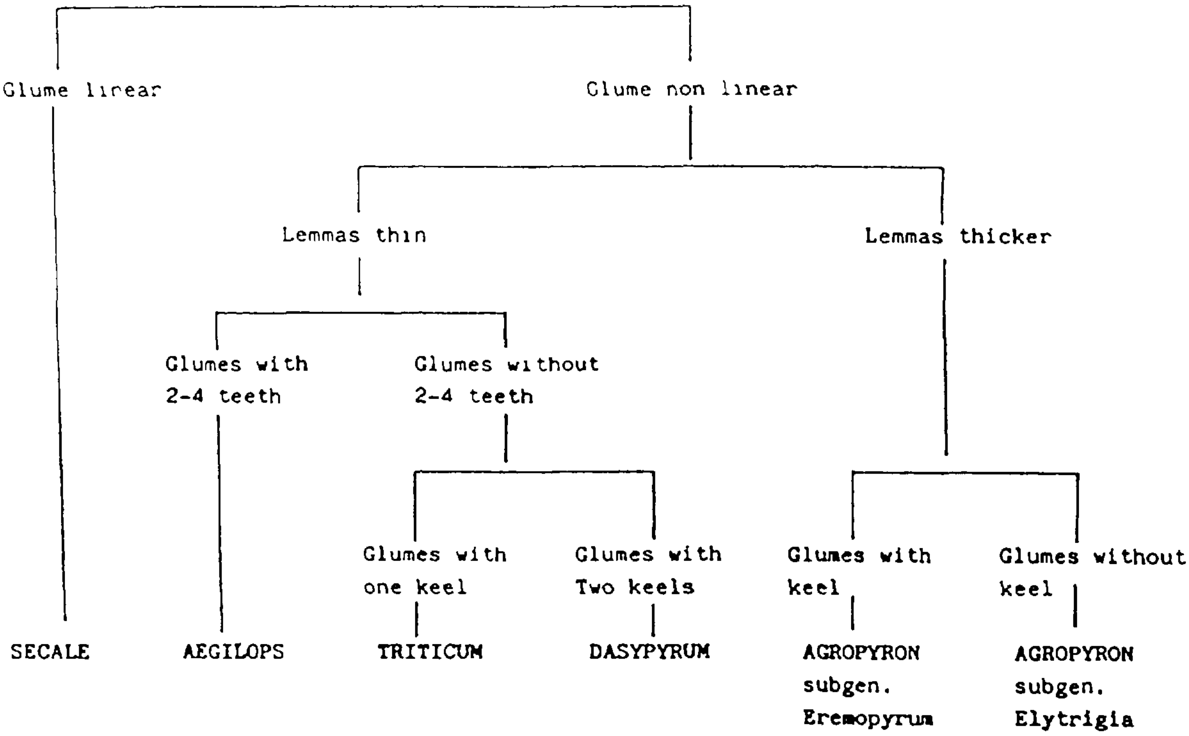
MORPHOLOGICAL AFFINITIES

The morphological approach has been used in botany at every taxa level to create grouping and subgrouping and to establish key to the taxa themselves. When key to the taxa are constructed the morphological system considered often do not consider phylogenetical, geographical, ecological and agronomical aspects. As matter of fact the key to the genera of Triticinae used for i.e. by Maire (11) is based on differentiation of few plant organs such as glume and lemma morphology (Tab. I) whose alternate traits of these characters are often controlled by alleles at one locus.

Therefore morphology, even in the situation when it is treated with sophisticated multivariate statistical tools, cannot separate analogy from homology unambiguosly.

TABLE I

KEY TO THE TRITICINAE GENERA ACCORDING TO MAIRE (9)



CYTOPLASM AFFINITIES

Sasakuma and Maan (12) found that cytoplasm of T. dicoccoides, Ae.kotschyi, Ae.variabilis, and Dasypyrum villosum did not have any adverse effect on the male fertility of the alloplasmic durum plants.

This indicates some affinity of D.villosum cytoplasm with that of the female parent in thehybridization process that lead to T.durum and represents the first affinity evidenced between D.villosum and wheat. The cytoplasmic affinity between D.villosum and tetraploid Triticinae is in line with the affinities that will be evidenced below for protein genes between D.villosum and the diploid wheats T.monococcum and T.urartu

PROTEIN AFFINITIES

In the 1950s and 1960s it was realized that comparison of protein sequences or electrophoretic patterns could have offered potential advantages in discovering homologies because phylogenetic conclusions were based on the analysis of primary gene products. If we assume that similar isozyme phenotypes are produced by genes with similar base composition, then the comparison of isozyme phenotypes of different species is an indirect comparison of the genes coding for those isozymes. In Tab.II a direct comparison of several diploid, tetraploid and hexaploid Triticinae is performed on the basis of the genetic information available for seven isozymes and two seed storage protein fractions (13) (14) (15) (16) (17) (18) (19, and paper in preparation). In wheat the genes for the isozyme phenotypes ADH-1 (20), GOT-2 , GOT-3 (21), EST-1, EST-2, EST-3, EST-4 (22) and for the seed storage proteins gliadin and glutenins (23) have been studied and their loci located on the A, B and D genomes Therefore if a Triticinae species shows an electrophoretic phenotype for a protein, that is similar to the wheat electrophoretic phenotype for the same protein, and the gene coding for that protein and its chromosomal location is known, then the species is indicated as carrying alleles similar to those in wheat (i.e. ADH-1 phenotype band 3 (Tab.III) is coded by genes on chromosomes 4B and 4D in wheat and the same band is present in Ae.speltoides. Therefore Ae.speltoides is considered to have an allele for ADH-1 similar to those on genome B and D of wheat). T.boeoticum and T. urartu for 60% of the patterns studied carry alleles similar to those in the A genome of wheat (Tab.IV). Ae.speltoides for 43% of the protein has alleles similar to those on the A genome of wheat and for 36% has alleles similar to those in the D genome of wheat. Ae.searsii and Ae.longissima have alleles similar to those of the D genome only. Ae tauschii has alleles similar to those of the genome D in 70% of the cases. D.villosum in 57% of the cases shows alleles similar to those in the A genome.For Secale information

TABLE II

SIMILARITY AMONG TRITICINAE SPECIES AS INDICATED BY ISOENZYMATIC AND SEED STORAGE PROTEIN, GROUPING THEM ON THE PRESENCE OF ALLELES LIKE THOSE CODIFIED BY CHROMOSOMES OF GENOMES A, B, D IN T.AESTIVUM.

Species	ADH-1			GOT-2			GOT-3			EST-1			Protein EST-2			EST-3			EST-4			Gliadins			Glutenins			Total(*)		
	A	B	D	A	B	D	A	B	D	A	B	D	A	B	D	A	B	D	A	B	D	A	B	D	A	B	D	A	B	D
Diploids (2n=2x=14).																														
T.monococcum		+	+	+	+		+			+			+								+	+			+		+	(6)	(2)	(3)
T.urartu		+	+	+	+		+						+						+			+			+		+	(6)	(2)	(2)
Ae.speltoides		+	+	+	+		+	+	+		+	+		+							+		+		+		+	(3)	(6)	(5)
Ae.searsii											+	+						+			+		+			+		(0)	(3)	(3)
Ae.longissima		+	+			+	+				+	+						+			+		+			+		(1)	(4)	(5)
Ae.tauschii		+	+			+	+	+	+		+	+			+			+			+			+			+	(1)	(3)	(9)
D.villosum				+	+		+			+												+		+		+		(4)	(2)	(1)
S.cereale							+																	+				(1)	(0)	(1)
Tetraploids (2n=4x=28):																														
T.turgidum	+	+	+	+	+		+	+	+	+	+	+	+	+			+		+	+		+	+		+	+		(8)	(9)	(3)
T.timopheevi		+	+	+	+		+	+	+	+	+	+	+	+														(4)	(5)	(3)
Esaploids (2n=6x=42):																														
T.aestivum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	(9)	(9)	(9)

(*) absolute frequency of alleles similar to those controlled by chromosomes of A, B, and D genomes in T.aestivum

TABLE III

ANALOGY AMONG <u>TRITICINAE</u> SPECIES FOR ELECTROPHORETIC PATTERN (1) OF ADH-1 ISOZYMES.

Species	Band number				
	5	4	3	2	1
Diploids (2n=2x=14)					
T.monococcum			│		
T.urartu			│		
Ae.speltoides	│	│	│		
Ae.longissima			│		
Ae.tauschii			│		
D.villosum	│				
S.cereale	│				
Tetraploids (2n=4x=28)					
T.turgidum			│	│	│
T.timopheevi			│		
Esaploids (2n=6x=42)					
T aestivum			│	│	│

\- ______________ +

TABLE IV

PERCENTAGE OF AFFINITY OF ALLELES OF <u>TRITICINAE</u> SPECIES WITH THOSE CODIFIED BY CHROMOSOME OF GENOMES A, B, D IN <u>T.AESTIVUM</u>

	Percentage (*)		
Species	A	B	D
Diploids (2n=2x=14)			
T.monococcum	55	18	27
T.urartu	60	20	20
Ae.speltoides	21	43	36
Ae.searsii		50	50
Ae.longissima	10	40	50
Ae.tauschii	7	23	70
D.villosum	57	29	14
S.cereale	50		50
Tetraploids (2n=4x=28):			
T.turgidum	40	45	15
T.timopheevi	33	42	25
Esaploids (2n=6x=42):			
T.aestivum	33	33	33

(*) percentage of alleles similar to those controlled by chromosomes of A, B, and D genomes in <u>T.aestivum</u>

was available only for two proteins and in one case this species showed alleles similar to those of the D genome of wheat

These data indicate that T. monococcum, T.urartu and D.villosum show the same type of allele for about 60% of the proteins and that these alleles are similar to those in the A genome of hexaploid wheats. Ae.tauschii shows proteins strongly similar to those determined by genes on the D genome. Ae.searsii and Ae.speltoides seems to show an equal proportion of protein phenotypes similar to those determined by genes on the B and D genomes.

The tetraploid wheats show 20% of the protein that are electrophoretically similar to those coded by genes on genome D of wheat. Because the cytogenetical evidence exclude the D genome in the tetraploid wheats, it is inferred that several genes of the A and B genomes share homology with those on the D genome. Although these data confirm the cytogenetical evidence that Ae.tauschii is the putative donor of the D genome, they do not support any cytological conclusion about the donor of the A and B genomes. In addition D.villosum has showed a great affinity with other diploid wheats and this is the second evidence that indicates genetic homology between D.villosum and the wheat species.

These conclusions correspond in part to the affinities deduced from cytology, morphology and geographical distribution of the Triticinae.

DNA AFFINITIES

Dvorak and Scheltgen (24)investigated the homology of nucleotide sequences among the DNAs of wheat, rye, and the diploid Agropyron elongatum by using the technique of DNA-DNA hybridization.It was shown that A. elongatum DNA is less different from wheat DNA than from rye DNA, while rye DNA appears equally different from wheat and Agropyron DNA.

Using a different DNA-DNA hibridization technique Nath et al.(25) were able to identify in Ae. speltoides the G-genome donor to T. timopheevii.

In the same study (25) there were indications that the repeated DNA fractions from the diploids Aegilops searsii, Ae. speltoides , Ae.sharonensis, Ae. longissima, Ae. bicornis and from the tetraploid T. timopheevii are more similar than the unique DNA fractions.

GENERAL DISCUSSION AND CONCLUSIONS

The evidence collected on the affinities among species indicate that no unique grouping with evolutionary significance can be traced on the basis of separate criteria of affinities. The reasons for this reside, in our opinion, is the fact that whenever affinities have been found the information stemmed from a narrow genetic background.

As a matter of fact the reviewed literature showed that, except for cytological affinities, evolutionary trends were postulated on the basis of few proteins coded by two or three genes, or on DNA segments (rDNA genes) that covered less than 1% of the total genomic DNA.

However cytogenetical data are often contradictory depending on the strain used for a certain species in the hybridization process to study chromosomal homoeology or in comparing C-banding patterns. This has caused different authors to reach contradictory conclusions on the affinities relationship among Triticinae.

In order to avoid inconsistency, all that is required is a method for recognizing homology and eliminating analogy.

Gould (1) indicated that the most promising tool to search for homology is the use of DNA-DNA hybridization. This is because the unique sequence of DNA differs from morphology in just those properties that capture homology. DNA is complex enough to preclude analogy as a cause of overall similarity. DNA-DNA hybridization works in finding similarity for two reasons of mathematical probability: its matches are too complex to evolve independently, and its sample size (all nucleotides of all single-copy DNA) is so large that any superficial analogy may be shaded by similarity of shared descent.

Using DNA-DNA hybridization phylogenies in Triticinae can be deduced completely independently from morphology and cytogenetics and the affinities of species such as D.villosum to the cultivated Triticinae can be evaluated on a secure base. Probably one result of such study will be that the apparent divergence of D.villosum from other Triticinae is less than the real genetic distance deduced at the overall DNA level.

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AGRONOMIC EVALUATION OF INDUCED SEMI-DWARF MUTANTS IN DURUM AND COMMON WHEAT*

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Abstract

The durum wheat cultivar "Anhinga" and two cultivars of common wheat "Anza" and"N. Strampelli" were irradiated with fast neutrons to induce short straw mutants with greater lodging resistance and yield potential.

From a total of about 4,000 irradiated seeds 1,702 M_1 plants were harvested. These gave rise to a population of about 25,000 M_2 plants among which 1,010 plants were isolated as potential mutants showing promising agronomic characters.

Following progeny tests and selection 280 true-breeding mutants entered preliminary yield trials.

After several years of testing only two lines one of durum wheat and one of common wheat showed a higher yield potential than the control varieties. In particular the durum wheat mutant (FM 027), after 7 years of field trials spread over 10 selected places, outyielded the cv. "Creso" (the leading variety in Central Italy) by about 13.6%.

The mutant fD 14 from Anza after 5 years of testing in 8 locations, performed on the average better than the mother variety and outyielded the standard cv. "Orso" by 12.1%.

These two lines have been submitted for registration in the National Register of Varieties. Other mutants are being used for cross-breeding.

Introduction

Mutation breeding has been relatively easy and quite effective in cereals, especially for the induction of short straw mutants capable of improving lodging resistance. The mutagenic approach in durum wheat breeding started at Casaccia Nuclear Center at the end of the 1950's, proved to be very successful (1, 2). Four

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short straw mutants were released as new cultivars 11 years after radiation treatment (3).

Other varieties such as Creso, Mida, Tito and Augusto were the result of indirect use of mutants through cross-breeding (4, 5).

In common wheat the search for lodging resistant mutants was discouraged due to the availability of semi-dwarf varieties carrying dwarfing genes from Japanese wheat germ-plasm.

The introduction of new genotypes from International Agriculture Research Centre, such as CIMMYT, offered the possibility of their direct use in Italian conditions because of their wide adaptability. However, very often minor phenotypic adjustments were necessary to meet the farmer's needs. This was the case of the wellknown cv. Anza widely cultivated in California (USA) and in many other countries. Such a variety was tested in Italy since the early 70's, proving to be high yielding but moderatly susceptible to lodging because of its plant height a few centimeters over the normal. A similar situation was found with the cv. N. Strampelli, obtained from the cross Libero x (S.Pastore x Jacometti 49)

In durum wheat, a variety with good agronomic traits named Anhinga proved to be too tall in field experiments and then highly susceptible to lodging . For such reasons it was envisaged to start a mutation breeding programme in the early 1970's with the main aim of inducing suitable short straw types.

Materials and Methods

Seeds of the cultivars Anhinga, N. Strampelli and Anza were subjected to a mutagenic experiment with fast neutrons by applying a total dose of 3 Krad. The radiation treatments were carried out at the Casaccia Nuclear Reactor "Triga" in 1971 for Anhinga and N. Strampelli and in 1974 for Anza. One thousand seeds were irradiated for each of the first two varieties and 2,000 for the third. The irradiated seeds were sown in the field along with a comparable number of unirradiated seeds taken as controls. M_1 plants were individually harvested and classified according to the number of spikes per plant and to the fertility per spike (when only one culm was present). From each M_1 plant a number of seeds varying from 1 to 25 were sown for scoring variants. The plants that appeared promising from the agronomic point of view were progeny tested and moved to the next generation for further analyses and observations.

Selection based on the pedigree method was practiced up to an acceptable level of uniformity and stability.

Starting from M_4 the mutant lines were tested in preliminary agronomic trials based on a single row 5 m and 30 cm apart, replicated two times. In some cases, particularly in common wheat, mutated plants isolated in M_2 were subjected to selection up to M_{13} to attain a sufficient extent of uniformity.

The most promising lines were subsequently tested in 10 m^2 plots following a randomized complete block design with 3 replications. Depending on the results obtained and the amount of seed available, multilocation field trials were carried out in 8 - 10 places disseminated in the central part of Italy and repeated for 5 - 7 years.

Special attention was focused on plant height, lodging resistance, disease resistance, test weight and yield.

Results and Discussion

Durum wheat

A total of 548 M_1 plants, corresponding to a survival of 55% of the seeds irradiated were harvested (Table 1). In M_2 513 plants out of 8,447 M_2 seeds sown were isolated as probable mutation carriers.

As expected, the number of mutants increased parallel to the higher level of damage estimated on the basis of tiller number and seeds per spike (Table 2).

The mutation frequency was 6.07 %, based on the number of M_2 seeds sown, which shows the high efficiency of the treatment especially if one considers that the visibly useless plants were omitted.

Table 1. Data related to the materials and methods used for mutagenic experiments with fast neutrons.

Cultivars	Year of irradiation	Dose applied (Krad)	No. of seeds irradiated	Percentage of germination %	No. of M1 plants harvested
Durum wheat					
ANHINGA	1971	3	1000	55	548
Common wheat					
STRAMPELLI	1971	3	1000	65	431
ANZA	1973	3	2000	45	723

Table 2. Frequencies of short straw mutated plants of cultivar Anhinga (T. durum) according to the number of spikes per plant and fertility.

M_1 plants sub-group	No. of spikes per plant	No. of M_1 plants	No. of M_2 seeds from each M_1 plant	No. of M_2 seeds sown	No. of putative mutants selected	Frequency of mutated plants in M_2 (%)
Sg 1	4	54	25	1,350	48	3.5
Sg 2	3	137	20	2,740	148	5.4
Sg 3	2	203	15	3,045	216	7.1
Sg 4	1^a	110	10	1,100	79	7.2
Sg 5	1^b	9	ALL	72	9	12.5
Sg 6	1^c	35	ALL	140	13	9.3
TOTAL		548		8,447	513	6.07

a. **With 10 or more seeds**

b. **With 8-9 seeds**

c. **With less than 8 seeds**

Starting from M_4 to M_{10}, 99 mutant lines were entered into preliminary field trials (Table 3). From fifteen M_6 lines a mutant line FM 027 was isolated.

Table 3. Number of mutant lines isolated in different generations and tested for the first time in agronomic trials for yield performance.

GENERATION	ANHINGA	ANZA	STRAMPELLI
M4	42		
M5	27		
M6	15*	60*	3
M7	7	49	12*
M8	1	33	1
M9	3		2
M10	4		
TOTAL	99	142	18

*** The best lines have been selected in these entries.**

After several field tests it was decided to submit it to the Variety Register Office for inclusion with the National Register of Varieties.

FM 027 is 20 cm shorter than the mother variety Anhinga and has erect leaves. After 7 years of field trials in 10 selected sites it outyielded the cv. Creso (the leading variety in Central Italy) by 13.6 %. It is 4 - 6 days earlier than Creso and 10 cm taller. (Table 4).

The quality of FM 027 though acceptable is lower than that of Creso based on test weight and percentage of yellow berry.

Table 4. Performance of a durum wheat mutant line FM027 (from cv. Anhinga) tested in 10 places of Central Italy for 7 years. (Means of 34 agronomic trials).

CULTIVARS	PLANT HEIGHT cm	LODGING INDEX (0-5)	EAR EMERGENCE DATE	YIELD q/ha	TEST WEIGHT kg/hl	YELLOWBERRY %
FM027*	85.9	0.7	MAY 5.9	60.9	80.0	12.2
CRESO	76.9	0.3	MAY 11.2	56.1	81.6	5.0
VALNOVA	89.1	1.1	MAY 7.0	53.6	78.1	7.8

* To be released as ICARO

Table 5. Frequencies of short straw mutated plants of common wheat cultivar Strampelli, according to the number of spikes per plant and fertility.

M_1 plants subgroup	No. of spikes per plant	No. of M_1 plants	No. of M_2 seeds from each M_1 plant	M_2 seeds sown	No. of putative mutants selected	Frequency of mutated plants in M_2 (%)
Sg 1	4	33	25	825	28	3.39
Sg 2	3	85	20	1,700	52	3.06
Sg 3	2	143	15	2,145	82	3.82
Sg 4	1[a]	170	10	1,700	82	4.82
Sg 5	1[b]	51	ALL	300	22	7.33
TOTAL		431		6,670	266	4.00

a. With 10 or more seeds

b. with less than 10 seeds

Common wheat

The frequency of survival was lower for common wheat than for durum wheat. For the cv. N. Strampelli, 266 M_2 variants were identified from 6,670 M_2 seeds sown. Here again a positive relationship between overall plant damage and frequency of plant variants seemed to occur (Table 5). Progeny tests carried out in M_3 confirmed that 86.8 % of the putative mutants identified in M_2 were short mutants (Table 6). In the following generations starting from M_6 to M_9 a total number of 39 mutants were entered in preliminary agronomic trials (Table 3). Two short straw mutants, St 116 and St 121 showed a slightly higher yield and improved lodging resistance than the mother variety (Table 7). However,after further trials it was decided to stop the efforts aimed at their direct utilization and try instead to use them for cross-breeding.

Table 6. Progeny test in M_3 of short straw mutants of common wheat cultivar Strampelli

M_1 plants subgroup	No. of spikes per plant	No. of M_2 mutated plants	No. of M_3 seeds from each M_2 plant	M_3 seeds sown	M_3 progenies confirming mutations	Percentage of mutations confirmed in M_3
Sg 1	4	28	11	308	22	78.6
Sg 2	3	52	11	572	46	88.5
Sg 3	2	82	11	902	78	95.1
Sg 4	1[a]	82	11	902	69	84.1
Sg 5	1[b]	22	11	242	16	72.7
TOTAL		266		2.929	231	86.8

a. With 10 or more seeds

b. with less than 10 seeds

Table 7 Performance of two common wheat mutant lines St 116 and St 121 (from cv. Strampelli) tested in 7 places of Central Italy for 1 year (Means of 7 agronomic trials)

CULTIVARS	PLANT HEIGHT cm	LODGING INDEX (0-5)	EAR EMERGENCE DATE	YIELD q/ha	TEST WEIGHT kg/hl
St 116	92.1	2.5	MAY 2.0	58.7	79.1
St 121	83.9	1.6	MAY 4.6	57.9	77.8
STRAMPELLI	100.1	3.5	MAY 2.0	56.6	80.3

These mutants are being used for cross breeding.

The mutagenic approach applied to the cv. Anza was more successful. From more than 10,000 M_2 plants 400 agronomically valuable variants were selected and taken to the next generation. Further selection was carried out on a single plant basis up to M_{13}. Starting from M_6 a total of 142 mutants were tested in preliminary agronomic trials to evaluate the yield potential (Table 3). Despite the severe selection pressure applied, the mutant fD 14, shorter than Anza by about 8 - 10 cm, after 5 years of testing in 8 locations in Central Italy proved to be a good yielding line, not only in comparison with the mother variety Anza but also with an outstanding variety named "Orso" (Table 8). In 21 agronomic trials the mutant fD 14 outyielded Orso by 12.1%, showed a better lodging resistance, a higher test weight and flowered 4 - 5 days earlier (Table 8). Since fD 14 was tested in a wide range of climatic conditions and in different agrotechnique regimes it appears this genotype has wide adaptation. Other noteworthy characters of fD 14 are moderate disease resistance, slender culms, short narrow and erect flag leaf, small but highly fertile spikes and average seed weight. These yield components are suitable for a dense stand by which it is possible to get a high number of spikes per m^2.

Table 8. Performance of a common wheat mutant line fD14 (from cv. ANZA) tested in 8 places of Central Italy for 5 years (Means of 21 agronomic trials).

CULTIVARS	PLANT HEIGHT cm	LODGING INDEX (0-5)	EAR EMERGENCE DATE	YIELD q/ha	TEST WEIGHT kg/hl
fD14*	81.4	0.1	MAY 2.8	71.1	80.0
ANZA	88.3	0.6	MAY 3.3	69.9	79.9
ORSO	87.6	1.2	MAY 7.6	63.4	78.5

*** To be released as SPINNAKER**

In conclusion, mutation breeding aimed at improving existing varieties by reducing plant height seems to be a realistic goal with a relatively short development time and moderate costs.

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SEMI-DWARF MUTANTS AND HETEROSIS IN BARLEY

II. Interaction between several mutant genes responsible for dwarfism in barley

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Abstract

Dwarf and semi-dwarf mutants obtained after mutagenic treatment were chosen as material for genetic investigation of this character. A non-allelic relationship was found among six analysed mutants. The interaction was observed between all analysed genes responsible for dwarfness, including uz and br genes. It was demonstrated that expression and interaction of dwarfing genes strongly depend on the genetic background of recombinants.

Morphological mutants of a few characters, obtained in various laboratories after mutagenic treatment of different barley varieties, were genetically investigated in detail. The following types of mutants have been analysed very thoroughly: eceriferum forms (Lundqvist and von Wettstein, 1962; Lundqvist et al., 1968), chlorophyll mutants (von Wettstein and Kristiansen, 1973), ear morphology mutants as erectoides (Persson and Hagberg, 1969; Persson, 1969a,b), breviaristatum (Kucera, 1975; Kucera et al., 1975), hexastichon and intermedium (Fukuyama et al., 1972; Fukuyama et al., 1975; Gustafsson and Lundqvist, 1980), laxatum (Larsson, 1985a,b). In organized mutant germ-plasm collections, from a few up to several dozen different loci were found and localized on barley chromosomes for each group of mutants.

Dwarf and semi-dwarf barley mutants were not so intensively investigated. None of the barley genetics centers have organized collections of dwarf or semi-dwarf mutants similar to the collections of ecerifrum, erectoides or chlorophyll stocks. Short stature barley mutants were not chosen as an objective for broad, basic genetic investigations. The very high frequency of dwarf and semi-dwarf mutants obtained in the Department of Genetics, Katowice after MNH treatment of different spring barley varieties (Maluszynski, 1982; Maluszynski et al., 1987), has provided an opportunity for genetic analysis of this character.

Material and Methods

Nine short stature spring barley (Hordeum vulgare L. convar. distichon /L./ Alef.,) mutants were chosen for investigation from more than 300 dwarf and semi-dwarf mutants existing in the collection of the Department of Genetics, Katowice. Mutants were obtained from five barley varieties cultivated in Poland at this time. The following true breeding stocks of M_8 progeny, after mutagenic treatment of MNH, were used for genetic analysis:

648 AK from variety Aramir
862 PK from variety Plena
267 MK and 270 MK from variety Mg 4170
392 JK and 409 from variety Julia
538 DK, 555 DK and 638 DK from variety Delisa

Three of these mutants (270 MK, 538 DK and 638 DK) indicated non-monogenic inheritance of the sd-character in F_2 progeny of crosses with parent varieties. A detailed description of these mutants and results of the genetic analysis of the remainder were presented by Szarejko (1982) and Szarejko and Maluszynski (1984a,b). Brachytic and uzu mutants with genes br from chromosome 1 and uz from chromosome 3 were used additionally for the study of interaction among different semi-dwarf or dwarf mutant genes.

The F_1 progenies were planted in a greenhouse during the winter or spring season, the F_2 and F_3 in an experimental field.

Results and Discussion

A non-allelic relationship was found among analysed mutants from our collection (Table 1). In contrast, the F_1 of marker stock brachytic with mutant 648 AK reached a height similar to the parent carrying the br locus. Both forms, mutant 648 AK and stock brachytic, expressed a similar phenotype in both greenhouse and field conditions. In these experiments the mutant 648 AK was always about 10 cm shorter. The genetic analysis confirmed the localization of both of them in the same locus (Szarejko and Maluszynski, 1984a). We did not find an allelic relationship between other mutants and marker stocks brachytic or uzu.

Dwarf double recombinants were found in F_2 progenies of all crosses between non-allelic mutants. The height of recombinant plants was dependent on the interaction of two genes responsible for dwarf or semi-dwarf character of parental mutants. The next (F_3) progeny of self-pollinated recombinants indicated their homozygosity.

The interaction was observed between all genes responsible for dwarfness, which effected the phenotype of double recessive recombinants (Fig. 1). The recombinants were always shorter (up to as much as 40%), than the dwarf parental mutant. The highest effect of interaction was observed between gene uz and genes of dwarf mutants 392 JK and 555 DK. In these cases, double recessive recombinants where extremely short and never exceeded 20-21 cm. The interaction of genes responsible for semi-dwarf height of mutants 862 PK and 267 MK was much smaller. The expression of both these genes gave plants only about 10 cm shorter than the mutants.

Table 1: Results of complementation test (F_1) between investigated dwarf or semi-dwarf mutants (greenhouse)

Cross combination	Height of F_1 plants ± SD (cm)	
	winter season	spring season
648 AK x 392 JK	66.6 ± 3.1	
648 AK x 409 JK	63.1 ± 7.0	
648 AK x 555 DK	66.4 ± 6.3	
862 PK x 267 MK	74.5 ± 8.1	
862 PK x 409 JK	66.7 ± 7.6	
862 PK x 555 DK	71.2 ± 7.2	
267 MK x 392 JK		79.8 ± 3.6
267 MK x 409 JK	70.7 ± 10.3	
267 MK x 555 DK	68.7 ± 4.1	
392 JK x 862 PK	79.0 ± 4.2	
392 JK x 409 JK		75.8 ± 2.5
409 JK x 555 DK	65.0 ± 4.0	
555 DK x 392 JK	63.7 ± 4.9	
648 AK x *br*		63.5 ± 5.2
862 PK x *br*	59.4 ± 10.1	
267 MK x *br*	79.0 ± 7.0	
392 JK x *br*		78.9 ± 7.1
409 JK x *br*	65.0 ± 5.9	
555 DK x *br*	61.7 ± 10.4	
648 AK x *uz*	57.9 ± 5.6	
862 PK x *uz*		76.7 ± 8.3
267 MK x *uz*		78.2 ± 3.8
392 JK x *uz*	61.5 ± 4.9	
409 JK x *uz*		77.6 ± 4.5
555 DK x *uz*	63.0 ± 5.7	
mutants: 648 AK	39.8 ± 4.9	48.0 ± 4.3
862 PK	52.8 ± 5.2	69.2 ± 7.0
267 MK	56.7 ± 6.8	73.2 ± 2.6
392 JK	41.2 ± 3.0	50.0 ± 4.1
409 JK	47.0 ± 6.8	60.7 ± 3.0
555 DK	41.8 ± 3.6	46.7 ± 2.4
brachytic	48.0 ± 4.0	59.2 ± 6.4
uzu	46.0 ± 10.1	63.5 ± 3.8

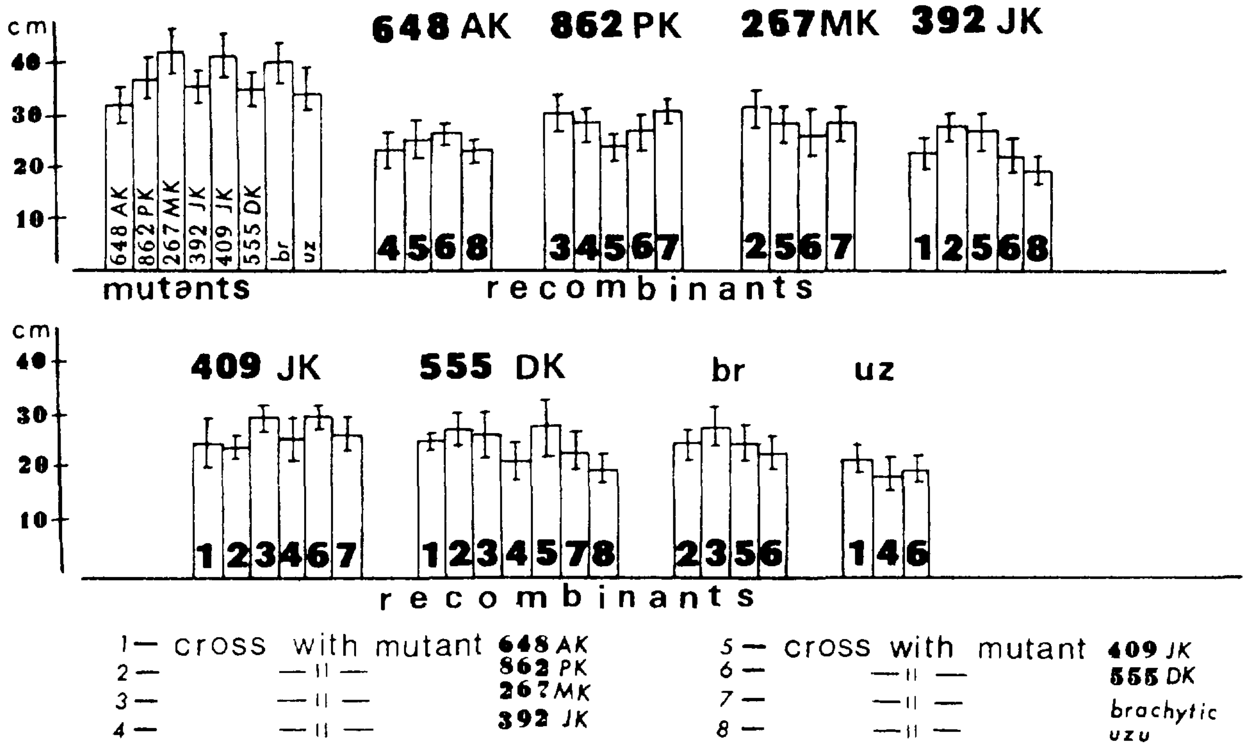

Fig. 1. The height of double recessive recombinants (F_3) from crosses between dwarfing mutants

In our experiments, similar to results published by Konishi (1977), the expression of dwarfing genes and their interaction depended considerably on the genetic background of recombinants. For example, the height of double recessive recombinants with the gene responsible for dwarfness of mutant 555 DK varied in different crosses from 59.5% to 81.6% in comparison to particular parent forms (Table 2).

Table 2: The height of double recessive recombinants as a result of the interaction of mutant 555 DK gene with other dwarfing genes

Cross combination	Height of recombinants in relation to second parent (%)
555 DK x 648 AK	81.6
" x 862 PK	74.5
" x 409 JK	70.3
" x 267 MK	64.0
" x uzu	62.1
" x 392 JK	61.1
" x brachytic	59.5

The number of internodes of analysed double recombinants was usually similar to the parent forms. Only recombinants of the mutant 267 MK inherited, in all crosses, the higher number of internodes (from 7-9), as well as the leafy bract in the collar region, which typical for the mutant phenotype. Similarly, the pleiotropic effect of dwarfing genes uz and br was expressed in all their recombinants. Double recessive recombinants with uz gene were always of semi-prostrate growth type: short, broad leaves; extremely short flag-leaf; very dense ear inside a flag-leaf sheath with drastically shortened awns. Recombinants with gene br grew erect, had dark-green leaves and rather long spike with short awns. The pleiotropic effect of mutant 862 PK (light green rosette) was expressed in all recombinants of this mutant.

The fertility and vitality of recombinants with two dwarfing genes was often decreased in comparison to the parents. A high level of sterility was always noted in all recombinants with gene uz.

A limited number of extremely short plants (from 12-18 cm.) was observed in the F_2 progenies of several crosses with mutants 862 PK, 409 JK and 555 DK. These segregants very often died in the rosette stage and were always sterile. The normal double recombinants were observed parallel to these forms.

The interaction of genes br and uz in double recesive forms was noted by Leonard et al. (1956) as well as Takahashi and Hayashi (1956). The effect of interaction between these genes, reported by the authors, was much bigger than observed in our experiments. Double recombinants br uz, as reported by Takahashi and Hayashi, reached only 20-25% of the mutants height while the same recombinants with "Aramir brachytic allel" reached about 70% of the height of both mutants. It should be noted that in our experiments the interaction effect between these genes was observed in relation to the length of culm and spike of recombinants but not to the length of awns. The various expression of different alleles from loci br and uz or extremely different genetic background of investigated mutants as well as climatic conditions could effect unlike interaction of these two genes, both in the past and in our experiments.

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EFFECT OF THE RYE MUTANT EM1 DWARFING GENE ON OCTO- AND HEXAPLOID TRITICALE

Preliminary report

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Abstract

Preliminary results presented show a strong influence of the EM1 rye dwarfing gene on plant height of octoploid triticale and on F_2 hexaploid cross progenies. The possible use of this source in triticale breeding is briefly discussed.

In the Poznanska Hodowla Roslin Station's winter wheat programme crosses with semi-dwarfs from different sources were used. Among them was the dwarf mutant Bezostaya, which contrary to material based on Norin 10 did not give rise to any promising advanced line.

In the rye programme the EM1 mutant of Kobylyanskii [1] semi-dominant for the dwarf character was used and this type has been much improved in plant type, yield and resistance to diseases. It proved, however, impossible to fix the semi-dwarf type as much taller plants always segregated as a result of distant open pollination and some back mutations. A characteristic of this material is presented in Table 1.

The idea of using short rye to produce short triticale was initiated by CIMMYT breeders [2]. One of the improved EM1 rye strains $L506_{79}$ was used by Tarkowski and co-workers* to produce octoploid triticales in combination with several wheat varieties and lines. Some other octoploids were obtained from crosses of short wheats with a tall rye strain M11. Selected octoploids were crossed with our own hexaploid triticale material. Some of the F_1 obtained seemed very promising, being short, lodging resistant, fertile, uniform and having plump kernels. The F_2 progenies were strongly selected for hexaploid plant type, fertility and plump grain.

Table 2 shows the plant height of four octoploids as compared to their parents of the F_2 selected hexaploid plants. As seen, the EM1 dwarfing gene exercises a strong shortening effect on hybrids, and transgressive forms are obtained. When the wheat parent is a semi-dwarf itself, extremely short ocotoploid material is obtained, so that not much success in crossing to hexaploids may be expected. When a short wheat was crossed to a tall rye an intermediate triticale was obtained.

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Table 1. Characteristic of the improved EMl semi-dwarf rye material as compared to the tall standard Dankowskie Zlote (Laski, 1983).

Variety-strain	Grain yield t/ha	1000 grain weight gr	Test weight kg/hl	Plant height cm	Lodging resistance 9 p. score 9- best
$L506_{79}$-s-dwarf	5.7	30	72	120	9.0
Dańkowskie Złote /tall standard/	6.2	30	75	160	7.8

Table 2. Plant height of 4 octoploid triticale strains as compared to their wheat and rye parents and the same character in respective F_2 octo-hexa hybrids (Laski, 1985).

Pedigree /wheat -rye/	Plant height in cm				Number of F_2 preselected plants
	wheat	rye	octopl	F_2 hexapl.	
$C1113_{78}$-$L506_{79}$	85	120	55	-	-
Lanca-$L506_{79}$	104	120	70	85-125	14
Aurora-$L506_{79}$	110	120	90	95-120	5
$C521_{77}$-M11	86	150	100	105-125	11

F_2 hexaploid plants are longer than the octoploid parent, but on the shortest among them the influence of the octoploid may be observed. The longer ones correspond in that respect to the hexaploid strains used as parents, the cv. Lasko among them.

Semi-dwarf EMl rye material was also directly crossed to hexaploid triticale. In this case, however, the progenies, although short, did not seem promising, with a certain similarity to the Bokolo type, based on Tom Thumb wheat [3].

The epistatic effect of the EMl rye dwarfing gene on the wheat genome of octoploid triticale was also found by Wandelt and Szigat [4].

Several problems arise in connection with the possible impact of the EMl rye on the breeding of short triticale. The first is connnected with the danger of unfavourable pleiotropic effects similar to those associated with the Rht$_3$ wheat gene. In this case, however, some of the octoploids and hexaploids derived from them seem to be more promising than the offspring of direct triticale-rye hybrids.

Another problem to consider is the possibility of obtaining short, homogeneous triticale varieties. In both cases, that is in octoploid-hexaploid and hexaploid-rye hybrids short, fairly uniform strains were obtained and no wide segregation was noted of the type occurring in rye material carrying the EM1 germplasm.

As mentioned before, some of the F$_1$ octo-hexa were perfect in plant type, although late. In the F$_2$ no such good plants could be selected and much grain shrivelling was observed, but the fertility was quite satisfactory. With a longer period of segregation occurring in octo-hexa crosses improvement of seed type may be expected in further generations. This has to be verified in the near future.

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WINTER TRITICALE BREEDING IN POLAND

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Abstract

Winter triticale is becoming an important cereal crop in Poland, as it's acreage is expected to reach 1 million ha in 1990. It is to occupy a large part of the rye growing area. Three cultivars are registered and 11 more are under official trials. The actual state of breeding in connection with the main goals is presented. Breeding methods leading to further progress are briefly discussed.

The present acreage under triticale is not large in Poland, despite the great interest of farmers in the new crop. It is estimated that around 20,000 ha were grown in 1984/85 and this should double by the Fall of 1985. An official seed supply of close to 5,000t is a good starting point for the planned 1 million ha in 1990.

The chief aim of triticale introduction is partial substitution of winter rye, grown in Poland in a proportion unique in the world (above 3 million ha). A major part of rye grain is used as fodder, although it's protein content is low and it contains some growth inhibitors. Possibilities of replacing rye with wheat and barley are limited owing to the predominance of acid soils. Frequent late or dry springs are less favourable to growing spring than winter cereals.

Three varieties of winter triticale - Lasko, Grado and Dagro - have been registered in Poland up to 1985, 4 others are in the third year of official trials and 7 more are being tested in the first or second year. Three varieties - Lasko, Salvo and Bolero - have obtained breeders rights protection and three others are in official trials in several countries. All the named varieties originate from the Poznan Plant Breeding Stations, Choryn and Laski. Another large programme is being carried out at the Institute of Plant Breeding and Acclimatization, mainly in Malyszyn Experimental Station. Several winter and spring triticales have been submitted to official trials.

The efforts of breeders are concentrated on improvement of several complex characters and on combining them in a favourable manner.

The grain yield potential may be regarded as quite satisfactory. In the official trials, 1982-84, the widest grown cultivar, Grado, had mean yields 3% higher than standard rye and 4% higher than standard wheat variety. The opinion of most growers is positive if not enthusiastic. Furthermore, in 2 years new strains will become varieties. These candidates gave, in the preliminary trials, more superior yields than Grado (Table 1). Further progress may be expected.

Table 1. Some characters of two new winter triticale varieties as compared to standards in preliminary trials at 10 locations in 1984 and 8 locations in 1985 (2 years means).

Variety	Grain yield in % of mean stand	Resist. to lodg in 9 p. score	Test weight kg/hl	Falling number sec.	Frost resist % surv. labor
LAD 285	111	6.4	68.9	156	74
Presto	109	5.2	70.5	70	44
Lasko stand.	101	4.6	69.2	126	14
Grado stand.	99	5.5	66.9	73	30
Mean stand. t/ha	5.71				

In official trials Grado has proven superior in lodging resistance, to the rye standard Dankowskie Zlote by 0.8 on a 9 point score. Dagro is still better by 0.5 and LAD 285 showed a superiority over Grado of 0.9 points in preliminary trials. A number of short and semi-dwarf strains were obtained mainly as transgressions, but were unsatisfactory in yield. Some strains carry different wheat and rye dwarfing genes. As triticale has to replace rye on sandy soils, it would be interesting to obtain relatively tall varieties with a good lodging resistance, analogous to Danko rye.

Winter hardiness in triticale is very important for Poland due to the frequent and heavy frost and also because of snow-mould. In this respect the first Polish triticale, Lasko, is unsatisfactory for local conditions. Later released Grado and Dagro represent an improvement in winter hardiness, but only some of the newer lines are good enough to assume safe cultivation in north-east Poland. Two of them advanced in tests, unfortunately have some serious drawbacks, but in some less advanced lines good combinations of winter hardiness with other agronomic characters are expected to be found.

Aluminum tolerance is decisive for replacing rye on acid soils. Triticales differ widely in that respect. Lasko expressed good tolerance [1] and Grado is even better [2].

Most triticales are rather late in development and ripening, and breeding for earlier types is desirable. A certain progress in earliness was obtained in Presto. Resistance to pre-harvest sprouting in triticale utilized as fodder is only important as far as it assures safe and stable seed production. Lasko is relatively good in that respect, similar to most rye varieties and to receptive wheats like Maris Huntsman. Most other triticales are inferior to Lasko. It was also found in the rainy season 1981 that Laski material is superior to that of the CIMMYT crossing-block [2]. Among recent varieties LAD 285 has a superior falling number. In the rainy harvest 1985, seed production, mentioned before and based chiefly on Grado, was possible, but with more losses.

The grain filling of many triticales is also unsatisfactory. Test weight, generally used as a measure of this character, is important, chiefly in view of it's correlation with yield [3], but also of it's impact on milling value and even a certain value in the feeding of poultry.

Disease has become quite a problem in the development of triticale breeding and growing. The resistance of most hexaploids to mildew and rusts did not decline drastically up to now - with some exceptions for rusts. Susceptibility to Fusarium and Septoria is an unhappy recombination of parental species traits and is one of the most difficult problems to be solved. Recently however, eye-spot has become the most serious disease of triticale. Among Choryn and Laski varieties Presto (CHD 775_{81}) seems to be quite resistant - followed by Largo (LAD 183).

Protein content is on the average 2% higher in triticale than in Polish rye cultivars. An improvement of this character is desirable, but it should not be obtained at the cost of agronomic value, as triticale is above all a source of energy. The feeding value is in most varieties satisfactory [4].

Triticale breeders dispose of good sources of most desired characters, which does not mean that optimal combinations of those characters have been obtained. Still, improvement of resistance to sprouting and some diseases seems extremely difficult with the available germplasm.

The main methods leading to improvement of triticale characters mentioned above is recombination breeding in the broad sense. This method offers a very wide range of possibilities. Different types of crosses, within hexaploid triticale as well as with direct or indirect introduction of wheat and rye germplasm, may be used. On the other hand, interaction between wheat and rye genomes makes results of crosses more unpredictable than within wheat or rye. Transgressions in many characters are often noted. They were observed in Choryn and Laski in resistance to lodging, winter hardiness, earliness, test weight and falling number. In most intergeneric crosses the influence of the wheat and rye parent is below expectations. So it is with sprouting resistance (Merker, personal communication). On the other hand, dominant dwarfing genes of wheat and rye exert a visible effect on plant height of octo and hexaploid offspring. Undesirable pleiotropic effects of the Tom Thumb Rht_3 gene make the utility of this type of dwarfness doubtful although the rye EM1 gene may prove less harmful.

Mutation breeding has not been of much use in triticale. The only success known to the author (personal communication) is that Nalepa has obtained a promising short Lasko mutant, using a chemical mutagen. This would be the case of improving one character of an otherwise good variety.

CONCLUSIONS

Winter triticale is rapidly growing in importance in Poland. The present state of breeding indicates a real possibility to replace rye by the new crop on 1 million ha in 1990. A further increase of triticale acreage may be foreseen.

The three registered varieties and eleven more in official trials represent desired characters in different extents and combinations.

Recombination breeding offers a broad range of possibilities owing to the wide application of inter-generic crosses. Still, the intergenome interactions in many cases make results unpredictable and are the cause of many observed transgressions. Some recent communications speak in favour of the possible effective use of mutation breeding in improvement of triticale.

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