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培育磷高效小麦品种的遗传学与生理学基础

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Genetic and Physiological basis for breeding Phosphorus Nutrient Efficient Wheat Varieties

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Deficiency of available phosphorus in alkaline soil is a serious problem in northern China. This contrasts with the finding that the total amount of phosphorus in the soil is, actually, very high (over 200 times of its available form). Starting from 1990, my colleagues and I initiated a new research project with a longer term aim to breed phosphorus efficient wheat varieties. From among 500 wheat lines, several genotypes that could tolerate low phosphorus level in the soil were identified. In one of the genotypes, the enhanced phosphorus utilization trait is now found to be controlled by a single dominant gene. Physiological analysis showed that the phosphorus efficient genotypes could secret a higher amount of organic acids (such as malic acid, citric acid, succinic acid, etc) into the soil under low supply of phosphorus. The organic acids may assist the solublization of the insoluble phosphorus in the soil. These results shed light on the genetic and physiological basis of phosphorus utilization by wheat plant and suggest that, with appropriate selection strategies, phosphorus efficient wheat varieties can be bred in the future.

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用于小麦染色体工程的蓝粒小麦单体系列材料的创制

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Establishment of a Set of blue Grained Wheat Monosomic Lines for Wheat Chromosome engineering studies

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The American geneticist, E. R. Sears, was the founder of wheat chromosome engineering. He established the monosomic series of common wheat, which greatly facilitated cytogenetic analysis of wheat. However, problems of univalent shift and labor involved in chromosome counting have limited the common usage of these materials. To circumvent these problems, I developed an alternative set of monosomic lines, in which the presence of the univalent chromosome was indicated by the production of blue pigmentation in the aleurone tissue of seeds. The gene(s) responsible for the blue pigmentation were carried on a short chromosomal fragment of *Agropyron elongatum*. This chromosomal fragment has been transferred to the different chromosomes of common wheat using radiation-induced translocation. On the spike derived from a blue-grained monosomic wheat ($2n = 41$, the univalent chromosome carries the gene for the blue pigmentation), four types of seeds are produced. The deep-blue seed has 42 chromosomes, the medium-blue and light-blue seed has 41 chromosomes, and the white seed has 40 chromosomes. The monosomic genotype is easily identified based on the color of the seed, without the use of microscope. So far, blue-grained monosomic lines have peoduced 11 of the 21 different wheat chromosomes. In the course of propagating the blue-grained monosomics, I found that the fertility of the nullisomic lines ($2n = 40$, represented by white seeds) could be improved by continued selfing and reselection. Using the resulted self-fertile nullisomic lines, I established an efficient procedure for producing alien substitution lines of wheat. The utilization of the blue-grained monosomic lines and the self-fertile nullisomic lines may facilitate chromosome engineering studies in wheat.

禾本科植物大小基因组间在基因密度上的共线性与保守性

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Micro-colinearity and Conservation of High Gene Density in Small and Large Grass Genomes

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Comparative genomic analysis at the genetic map level has shown extensive conservation of the gene order between the different grass genomes in many chromosomal regions. However, little is known about the gene organization in grass genomes at the microlevel. Comparison of gene coding regions between maize, rice and sorghum showed that the distance between the genes is correlated with the genome size. We have investigated the microcolinearity at *Lrk* gene loci in the genomes of four grass species: wheat, barley, maize and rice. The *Lrk* genes, which encode receptor-like kinases, were found to be consistently associated with another type of receptor-like kinase (*Tak*) on chromosome groups 1 and 3 in Triticeae and on chromosomes homoeologous to Triticeae group 3 in the genomes in rice and maize. On Triticeae chromosome group 1, Tak and *Lrk* together with genes putatively encoding NBS/LRR proteins form a cluster of genes possibly involved in signal transduction. Comparison of the gene composition at orthologous *Lrk* loci in wheat, barley and rice revealed a maximal gene density of one gene per 4 ~ 5 kb, very similar to the gene density in *Arabidopsis thaliana*. We conclude that small and large grass genomes contain regions which are highly enriched in genes with very little or no repetitive DNA. The comparison of the gene organization suggested various genome rearrangements during the evolution of the different grass species, including a duplication of the *Lrk* region specific for the Triticeae on group 1 chromosomes. We are now analyzing the gene organization in the *Lrk* regions using BAC clones of the A genome (from *T. monococcum*) and the D genome (from *Ae. tauschii*). In addition, we are investigating the A, B and D genome in hexaploid wheat using a cosmid library. The accumulation of sequence information around the *Lrk* loci in several species (orthologs) and in the same species (paralogous genes) has allowed comparisons of genome relationships in the investigated regions.

利用栽培一粒小麦的 BAC 文库精细定位小麦基因

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High Resolution Mapping of Wheat Genes Using a *Triticum monococcum* BAC Library

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The wheat genome is large (1.6×10^{10} bp) and complex (hexaploid with the A, B and D-genomes). Map-based cloning in such genomes requires at least one, but frequently several walking steps on a chromosome to reach the gene of interest, even if very closely linked markers are available for a "chromosome landing" approach. Chromosome walking in wheat has often been considered to be very difficult or impossible due to size and complexity of the wheat genome and the high content of repetitive sequences. We are interested to clone two genes on chromosome 1AS by map information only: the *Lr*10 leaf rust resistance gene and the *Pm*3 powdery mildew resistance gene. As no large insert library of wheat was available at that time, a collaborative effort of several research groups was started to create a BAC library of *T. monococcum*, a cultivated diploid with a close relative of the A genome in hexaploid wheat. The BAC li-

brary contains more than six genome equivalents and is double spotted on filters which are available from our lab. A mapping population of 3150 F2 plants segregating for the *Lr*10 gene has been established and a marker closely linked to the gene (0.1 cM) was found. This marker was the starting point for the assembly of a physical contig in *T. monococcum*. The use of subcloned BAC ends for mapping was only successful in a few cases but in general was problematic. To derive probes from BAC clones for genetic mapping we developed a rapid" low pass "sequencing protocol. Shotgun DNA libraries from BAC clones were generated and sequenced at 1.5 × genome equivalents. The obtained sequence data were sufficient to identify coding regions (usually good probes for mapping) as well as non-coding, non-repetitive sequences which sometimes can also be mapped and used as probes for further walking steps. Probes derived from sequencing have also to be physically mapped on the BAC clones to identify sequences close to the ends of the BACs. Four walking steps have been completed until now using these approaches. This resulted in a physical contig spanning around 440 kb on chromosome 1AS. Progress will also be reported on the mapping of the *Pm3b* gene.

图位克隆小麦抗叶锈基因 *Lr1*

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Towards map-based cloning of the leaf rust disease resistance gene *Lr1* in wheat

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Leaf rust, caused by *Puccinia recondita* Rocb. ex Desm. f. sp. *tritici* Eriks. & Henn, is one of the most important diseases in wheat worldwide. There are more than 40 resistance genes against wheat leaf rust and used in wheat breeding. The *Lr1* resistance gene is one of them, originates from hexaploid wheat and is present in a number of cultivars. It is a dominant gene located at the distal end of chromosome 5DL of wheat. We are working on isolation of the *Lr1* gene using a map-based gene cloning approach. Generation of a saturated map around the target gene is the first step of map-based gene cloning. Two segregating F_2 populations Thatcher *Lr1* × Thatcher (2814 individual plants) and Thatcher *Lr1* × Frisal (832 plants) are used for fine mapping of the *Lr1* gene. Three micro-satellite markers (GWM654, GWM269 and GWM272) and four RFLP markers (BCD1421, Psr567, pTAG621 and ABC718) are used to analyze the two mapping populations. The micro-satellite marker GWM272 and the RFLP marker ABC718 are tightly linked to *Lr1* gene. The two markers are located at 0.1 cM from the *Lr1* gene. For physical mapping of the *Lr1* gene, genomic BAC and YAC libraries of barley and *T. tauschii* (D-genome) have been screened with the RFLP marker ABC718. Five BAC clones from a genomic library of *T. tauschii*, six from a genomic library of barley and one YAC clone from a YAC library of barley were isolated. All ends of BAC and YAC clones have been isolated and analyzed. The ends of BAC and YAC clones from barley could not be used for mapping because they are repetitive or did not hybridize with wheat DNA. Most of BAC ends from *T. tauschii* BAC clones showed repetitive sequences. Two BAC ends isolated from BAC clone L-1 121K23 (100 kb) are polymorphic and were mapped at the same position as the RFLP marker ABC718. We did not find any recombinants in the 100 kb region around the RFLP marker ABC718.

认识和改良中国小麦蛋白质量的遗传基础:策略与现有的研究

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Understanding and Manipulating the Genetic Basis of Protein Quality in Chinese Wheat: Strategies and Current Experiments

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Seed protein content, nutritional balance and processing property of flour are the three major aspects of wheat protein quality. Most Chinese wheat cultivars are comparable to their Western counterparts in terms of seed protein content and nutritional balance. However, relatively few of them possess good processing property. The main reason underlying the poor processing property of hexaploid Chinese wheat varieties is the weakness in gluten strength. Considering that wheat gluten is mainly composed of a mixture of a finite number of storage protein species and that the storage protein species may determine gluten strength through combinatorial controls, we formed the following strategies in our studies on understanding and manipulating the genetic basis of protein quality in Chinese wheat. 1. Genetic analysis. By performing well-structured genetic analysis, we hope to identify two types of storage protein genes, those genes whose presence is associated with good processing property (the desirable genes, or the D-type genes) and those whose presence is always associated with undesirable processing property (the undesirable genes, or the U-type genes). Two sets of genetic analysis are being conducted currently. The aim of the first set of analysis is to obtain nonfunctional mutants for the majority of the genes whose products are present in the gluten. This analysis is expected to yield information on the function of individual members of storage proteins, some of which may be encoded by the D type genes, in gluten strength control. The aim of the second set of analysis is to identify potential genetic factors that may be responsible for causing weakness in gluten strength in Chinese wheat through the use of recombinant inbreed lines. This analysis may produce information on the function of the storage proteins specified by the U type genes. 2. Molecular analysis. On the basis of above genetic analysis, a molecular approach will be undertaken to clone the D- and U-type genes. The cloned genes will be characterized in terms of genetic diversity in cultivated wheat and wild species related to wheat and potential application in molecular breeding for processing property improvement. Because of the known association between the HMW glutenin subunit 1D×5 and good processing quality, we are now searching wheat related wild species for better versions of the 1D×5 subunit and testing their potential in wheat processing quality improvement. 3. Molecular breeding. The above genetic and molecular analysis should result in sufficient gene and marker resources suitable for wheat processing quality improvement through molecular breeding. The D-type genes will be transferred into high yielding, hexaploid wheat varieties using the transgenic technology. The molecular markers linked to the U-type of genes will be used to screen breeding materials for an early avoidance of this type of genes in breeding programs. In summary, the combination of theoretical and applied investigations described above should contribute to wheat protein quality improvement in both China and abroad. In the future, wheat quality breeding will be a more productive and efficient enterprise worldwide.

比较遗传学研究在认识禾本科植物基因组与基因功能中的应用价值

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Cereal Comparative Genetics-research Opportunities

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Map colinearity of gene order is now accepted as the rule rather than the exception over the Poaceae family which includes all the major cereals and represents 60 millions years of evolution. Some nine different cultivated crops rice, foxtail millet, sorghum, sugar cane, maize, wheat, barley, rye, oats, together with some of their wild relatives - are now included in the grass consensus map. Others including turf and the forage grasses, ryegrass and fescue will shortly be added. Synteny provides a means by which orphan crops can benefit from the research years spent on the genetics, physiology and biochemistry of the major cereals, particularly rice, wheat and maize. Crossable wild relatives are increasingly sought by breeders as sources of novel alleles for cultivated crops, particularly for wheat and rice. Comparisons of the organization of wild and cultivated genomes are necessary to predict which genes can be easily transferred. Such comparisons also give new insights into evolution, but few have, as yet, been made. Much rests on the degree of colinearity maintained between the larger maize and wheat genomes and the smaller rice genome. Extensive genetic and genomic facilities already exist in rice. If the relationship is precise enough, then genes may be isolated in rice from knowledge of their map position in the larger genomes. Work is underway at JIC to isolate the chromosome pairing inhibitor gene, *Ph*1, by this means. Although success has not yet been attained, all the indications are that colinearity between the critical homoeologous regions on chromosome 5B in wheat and chromosome 9 in rice is very precise indeed. The method being used involves inducing a number of small (~100s of kbs) deletions in the large genome target, in this case wheat. The target gene is then localized by identifying areas of minimum overlap, and the relevant region of the rice genome sequenced. Once the entire rice genomic sequence is available this could represent a very rapid way of isolating genes from wheat, barley or maize.

小麦与环境互作的遗传学基础及其在提高小麦产量中的作用

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The Genetics of Adaptation in Wheat and Its Role in Maximising Yield Potential

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To maximise yield potential in any environment, wheat cultivars must have an appropriate flowering time and life cycle duration, which fine-tunes the life cycle to the target environment. For plant breeders to produce such varieties by conventional plant breeding combined with marker-assisted selection, or by genetic engineering, a detailed knowledge of the genetic control of the key components is required. Genetic analysis in wheat using precise genetic stocks, particularly substitution lines and recombinant substitution lines, has revealed that there are three genetically independent systems controlling life-cycle duration in wheat, namely those controlling vernalization response (*Vrn* genes), photoperiod response (*Ppd* genes) and developmental rate ("earliness *per se*", *Eps* genes). This paper discusses our current knowledge of these systems and their role in modifying life cycle duration and yield potential. In addition, comparative mapping of these genes in other *Triticeae* species, particularly barley, is indicating new target genes for discovery in wheat, and comparative mapping with rice is indicating that rice may have orthologues of *Triticeae* flowering time genes, and, hence rice may provide a strategy for cloning *Vrn* and *Ppd* genes using rice molecular tools. The major genes controlling photoperiod response in wheat, the *Ppd-1* genes, have been shown to be located on the homoeologous group 2 chromosomes. These have been shown to have dramatic effects on yield potential in different environments. In temperate northern latitudes it is advantageous to have late spring flowering, and hence a long vegetative period, mediated by response to longer day-length, and hence varieties need to possess photoperiod sensitive alleles. In autumn sown spring wheats in sub-tropical regions, or southern European winter wheats, it is advantageous to flower early in the spring to complete the life cycle before desiccating summer temperatures, and, hence, varieties possess strong alleles for photoperiod insensitivity, such as *Ppd-D1a*. These genes on 2A, 2B and 2D are homoeologous to a gene on barley chromosome 2H, *Ppd-H1*. However, mapping in barley also indicates that there are photoperiod response loci on barley chromosomes 1H and 6H, indicating that homoeologous series should exist on wheat group 1 and 6 chromosomes. These have not yet been mapped.

The need for vernalization determines the difference between winter and spring wheats. The major genes controlling vernalization response have been located both genetically and physically on the long arms of the homoeologous group five chromosomes. These genes are homoeologous to each other and to the vernalization genes on chromosomes 5H of barley and 5R of rye. By using rice RFLP probes and a rice mapping population it was shown that a region homoeologous to the *Triticeae Vrn-1* region exists on rice chromosome 3. This finding was confirmed using deletion lines, where probes from rice chromosome 3 and probes co-segregating with Vrn-A1 all mapped in deletions associated with a flowering time effect. Comparative analysis also indicates that another series of vernalization response genes may exit on chromosomes of homoeologous group 4 (4B, 4D, 5A), and mapping studies in *Triticum monococcum* support this. Apart from the ability to protect plants from winter kill by delaying reproductive development, the Vrn genes do not appear to have major effects on yield potential once vernalization requirement is satisfied. Nevertheless, in some environments, lengthening of the life cycle by introducing vernalization sensitivity can increase the canopy size, and hence, yield potential.

In wheat, to date, very few "earliness per se" loci have been located. Only those on chromosomes of homoeologous groups 2 and 3 have been mapped in any detail, and then only as QTL effects and not precisely as major genes. Also, little is currently known on the pleiotropic effects of different alleles on yield potential in different environments. In barley, all chromosomes appear to carry such loci, indicating that series of loci that affect developmental rate independent of environment remain to be discovered on the other homoeologous groups of wheat. Overall, comparative studies indicate that there are probably twenty-five loci, controlling the duration of the life-cycle, *Vrn*, *Ppd* and *Eps* genes, that remain to be mapped in wheat.

Although our knowledge of the genetic control of flowering time genes has greatly improved there are major gaps in our knowledge of their detailed physiological effects on the timing of the life cycle from different sowing dates. This is being addressed by studying the phenology of isogenic and deletion lines in both field and controlled environmental conditions. This has indicated that the vernalization genes have major effects on the rate of primodia production, whilst the photoperiod genes affect the timing of terminal spikelet production and stem elongation, and these effects interact with sowing date.

John Innes 中心禾本科植物系所从事的研究：为认识小麦的生物学而努力

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Towards Understanding the Biology of wheat: Work in the Cereals Research Department at the John Innes Centre, Norwich, UK

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The John Innes Centre is a major centre for research into the genetics, cytogenetics, molecular biology, pathology and biotechnology of cereals. Most work focuses on wheat, but barley, rye, maize, rice and millet species are also studied. Work on cereal cytogenetics is concerned with studying chromosome pairing and transferring new variation, particularly for biotic and abiotic stress resistance, from related alien species into wheat. The transfer of genes for mildew resistance, aluminum tolerance and salt tolerance are recent successes. Particularly significant at the present time is work to clone the gene *Ph1*, responsible for controlling the diploid meiotic behavior of hexaploid wheat. Using a comparative mapping approach and rice molecular tools, possible rice homologues of *Ph1* have been isolated on rice BACs, and sequencing of these BACs has identified candidate genes.

Work on cereal genomics is concerned with developing new molecular markers, particularly SSR markers, and using these for mapping and fingerprinting European wheat germplasm. Work to develop Single Nucleotide Polymorphism (SNP) systems has been initiated. Additionally, new genomic tools are being developed such as a hexaploid wheat BAC library, and the Department is involved in the ITEC EST sequencing and databasing, and the development of wheat DNA microarray technology.

The Department has large projects concerned with identifying new major genes and QTL controlling important agronomic traits using molecular marker-mediated forms of genetic analysis and precise genetic stocks, particularly recombinant substitution lines and recombinant doubled haploid populations. Major targets are genes controlling adaptation, drought and salt tolerance, pre-harvest sprouting tolerance, bread-making and animal feed quality, and adult plant resistance to fungal pathogens.

The Department is a major centre for cereal transformation with programs on the genetic engineering of wheat, barley and rice, mainly, at present, using biolistics. A non-destructive marker system using the luciferase gene is used routinely, mediated by special JIC developed transformation cassettes. A major component of this work is technology development, where systems for *Agrobacterium* mediated transformation are being developed so that marker-free, clean-gene technology can be used. In rice, the major target traits being engineered are for pest and disease resistance into West African varieties, particularly the use of protease inhibitor constructs effective against nematodes, and a homology-dependant induced resistance mechanism against rice yellow mottle virus. In barley, quality traits are being modified, such as the introduction of a fungal enzyme to increase starch conversion during the malting process, and a gene for lysine biosynthesis to improve nutritional value. Alongside technology development, molecular analysis of transgene structure, expression, and the physical and genetic mapping of transgenes is being carried out. Work on cereal fungal pathology is concerned with studying pathogen variation and molecular biology, and discovering new host resistance genes against isolates of the major UK fungal pathogens; yellow and brown rust, powdery

mildew, *Septoria tritici*, eyespot and *Fusarium* species. A mixture of conventional pathology and molecular pathology approaches are used in this work, and a major target is the cloning of avirulence genes in the pathogen and resistance genes in the host, and understanding the mechanisms of virulence and resistance. New genes for resistance to *Septoria* species on chromosome 7D have recently been mapped. For resistance breeding against *Fusarium* species, new molecular diagnostic tools have been developed to quantify infection levels using quantitative PCR, so that the effects of specific species on infection levels in the stem base and in the head can be characterized. Details of the work can be viewed at the web site : www.jic.bbsrc.ac.uk.

杀配子染色体的作用机制及其应用

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Action and application of gametocidal chromosomes

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When monosomically introduced into wheat, the so-called gametocidal chromosomes of wild species belonging to genus *Aegilops*, which is related to the genus *Triticum*, are known to induce chromosomal mutations, either lethal or sublethal, in gametes lacking the *Aegilops* chromosome. The chromosome mutation involves breakage in chromosomes or chromatids and repair or fusion of the broken ends, resulting in the generation of deletions and translocations. Two such chromosomes, chromosome 2C derived from *Ae. cylindrica* and chromosome 3C from *Ae. triuncialis*, have been used to induce chromosomal structural changes in each of the barley and rye chromosomes added to common wheat. In the progeny of the wheat lines that are disomic for the barley or rye chromosome and monosomic for the *Aegilops* chromosome, chromosome mutations involving the barley or rye chromosome occurred in more than 10% of the plants examined. Sequential chromosome banding (N-banding or C-banding) and *in situ* hybridization (FISH and/or GISH) revealed that there were many wheat-alien translocations, as well as terminal deletions of the alien chromosomes. All these aberrant barley and rye chromosomes in common wheat would be useful for physical mapping of the respective alien chromosomes using DNA markers. Besides, alien-to-wheat translocations could be employed in wheat breeding programs. Terminal segments of the satellite of rye chromosome 1R have been transferred to the tips of different wheat chromosomes by the gametocidal action of chromosome 3C. By using this strategy, it is possible to obtain a 1R satellite segment carrying the disease resistance genes but not the Sec 1 locus, which is proximally located.

利用杀配子染色体 2C 诱导大麦染色体产生结构变异

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Generation of structural changes in barley chromosomes by the gametocidal chromosome 2C

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Introgression of agronomically important traits from barley to wheat is important for the improvement of wheat. Also, knowledge about precise location of genes in barley chromosomes is a prerequisite for map-based gene isolation. The isolated genes would be introduced into wheat by crop transformation and consequently improve wheat. Many attempts have been conducted to achieve chromosome-mediated introgressions from barley to wheat and to construct chromosome maps of barley showing physical locations of genes in the past. In the present study I have developed an alternative chromosome-mutation-inducing system capable of producing barley/wheat translocations and deletions in barley chromosome. The following is the summary.

1) In the attempt to induce breakage in barley chromosomes, I introduced a gametocidal chromosome 2C into six wheat-barley addition lines. Chromosome 2C, from *Aegilops cylindrica*, which is a related species of wheat, has a gametocidal action causing chromosome breakage in the progeny of the monosomic 2C addition line of Chinese Spring wheat. The critical plants (21″ + H″ + 2C′), disomic for each of barley chromosomes and monosomic for the 2C chromosome, were obtained.

2) The six critical lines were either selfed or backcrossed with the respective wheat-barley addition lines. The selfed and backcrossed progeny of these lines were cytologically investigated by N-banding and FISH using the barley probe HvT01 that is specific to the subterminal repeats of barley chromosomes. Various types of structural aberrations, most of which were deletions and translocations, were detected for all barley chromosomes with frequencies ranging from 10.8% to 27.9%.

3) Chromosome 7H was chosen to investigate the distribution of the breakpoints in the aberrations. Reciprocal crosses were made between the mutation-inducing common wheat line (or critical lines) (21″ + 7H″ + 2C′) and the 7H addition line of common wheat (21″ + 7H″) to obtain more 7H deletions and 7H/wheat translocations. There were various types of aberrations as observed in the previous study. The breakpoints of these deletions and translocations appeared to distribute along the entire length of chromosome 7H.

小偃麦附加系 Z1 和 Z2 中外源染色体 2Ai-2 的结构组成

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关键词:小偃麦;附加系;染色体

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Structural Organization of an Alien Group 2 Chromosome (2Ai-2) in Wheat-*Thinopyum intermedium* Addition Lines Z1 and Z2

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The barley yellow dwarf virus(BYDV) resistance lines of Z1 and Z2 were derived from Zhong 5, a partial amphiploid resulted from the cross between *Triticum aestivum*(wheat) and *Thinopyrum intermedium*. Genomic *in situ* hybridization (GISH) was used to analyze the chromosome constitution of Zhong 5 by using genomic DNA of *Pseudoregneria strigosa* (StSt, 2*n* = 14) as the probe. The GISH results showed that zhong 5 contains 42 wheat chromosomes and 14 *Th. intermedium* chromosomes composed of 4 St, 4 Js, 4 St-J translocation and 2 St-Js Robertsonian translocation chromosomes. The chromosome constitution of Z1 and Z2 was analyzed by GISH using genomic DNA probes from *Th. intermedium* and *Ps. Strigosa*. The GISH results indicated that both Z1 and Z2 possess 42 wheat chromosomes and 2 *Th. intermedium* chromosomes that were identical to a pair of St-J translocation chromosomes in Zhong 5. The *Th. intermedium* chromosomes, designated as 2Ai-2 chromosome derived from Zhong 5, mostly belong to the St genome except the middle region (about one third of the long arm) belonging to the E(J) genome. A detailed RFLP analysis was conducted for Z1, Z2 and their parents, St and E (J) genomes. The results of RFLP analyses demonstrated that the *Th. intermedium* chromosomes(2Ai-2, St-J) in Z1 and Z2 are extensively homologous to the Wheat group 2 chromosomes. The results of RFLP analyses on the genome composition of the 2Ai-2 chromosome were in agreement with the GISH results. Presence of psr 928 on 2AS and 2DS but absence on 2Ai-2S suggests some internal structural differences between 2Ai-2 and the wheat group 2 chromosomes. Some RFLP markers specific to the 2Ai-2 chromosome were identified and may be effectively used to select translocation lines with small segment of the 2Ai-2 chromosome and to localize the BYDV resistance gene in wheat background.

Key words: wheat; *Thinopyrum intermedium*; barley yellow dwarf virus (BYDV); genomic *in situ* hybridization (GISH); RFLP; homoeologous group 2

抗大麦黄矮病的小偃麦易位系的创制与鉴定

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关键词:大麦;小偃麦;易位系

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Development and Characterization of Common wheat-*Thinopyrum intermedium* Translocation Lines with Resistance to Barley Yellow Dwarf Virus

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Barley yellow dwarf virus (BYDV), vectored by several aphid species, is the most significant viral pathogen of wheat and other grain cereals. Significant economic losses resulting from BYDV in wheat, barley and oats have been reported in many countries. The most economic means of controlling BYDV is to develop wheat varieties with resistance to BYDV. So far no BYDV resistance has been described in wheat collections except one gene in some cultivars tolerant to BYDV. However, *Thinopyrum intermedium*, two octoploids Zhong 4 awnless and TAF46, and the disomic addition lines, L1, Z1, Z2 and Z6 all showed resistance to BYDV. We developed several wheat-*Th. Intermedium* translocation lines, Yw642, Yw443 and Yw243 etc., showing good BYDV resistance from L1 by inducing homologous pairing using CS *Ph1* mutant. It was found that their BYDV resistance was controlled by a single dominant gene. Characterization of these wheat lines was carried out by GISH and RFLP analysis. The results of GISH showed that the lines, Yw642, Yw443 and Yw243 etc., were homozygous wheat-*Th. intermedium* translocation lines containing 20 pairs of wheat chromosomes and 1 pair of wheat-*Th. intermedium* translocation chromosomes, in which the chromosome segments of *Th intermedium* were transferred to the dis-

tal end of a pair of wheat chromosomes. RFLP analysis indicated that the translocation chromosome of the wheat lines was T7DS·7DL-7XL translocation. The breakpoint of translocation is located on the distal end of 7DL, between Xpsr965 and Xpsr680, about 90-99 cM from the centromere. The BYDV gene is located on the distal end of 7XL around Xpsr680, Xpsr687 and Xwg380. The RFLP markers of psr680, psr687 and wg380 co-segregated with the BYDV resistance and could be used for marker-assisted selection (MAS) in wheat breeding program for BYDV resistance.

Key words: *Thinopyrum intermedium*, BYDV, disease resistance, translocation, GISH, RFLP, homoeologous group 7

多枝赖草 DNA 导入小麦引起重要农艺性状变化及相应的分子证据

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关键词:小麦;多枝赖草 DNA;农艺性状

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Important Agronomic trait Changes in Wheat Caused by Introduction of *Leymus racemoses* DNA and Some Molecular Proofs

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In 1989, *Leymus racemoses* DNA was introduced into a spring wheat cultivar "761" through pollen tube Pathway. In the resulted F3 progenies, apparent trait changes were observed. In the F4 progenies, many lines, possessing longer ears, more grains per ear, higher 1000 grain weight and higher protein content, were obtained. We reasoned that some genetic components of *Leymus racemoses* might have been transferred and integrated into the wheat genome. In order to get molecular proof for above reasoning, RFLP, RAPD and storage protein analysis were carried out. The following results were obtained. 1. RFLP analysis. Four repetitive DNA sequence (pHv7161, pHv7179, pHv7191 and pHv7293) clones from barley (*Hordeum vulgare*) genome were used for molecular hybridization using the genome DNA of *Leymus racemoses* (donor), spring wheat 761 (receptor) and the putative transformed wheat lines. Our results revealed some bands, common to *Leymus racemoses* and the putative transformed wheats, were absent in spring wheat 761. 2. RAPD analysis. 160 operon primers were used to amplify polymorphic bands using genomic DNA of *Leymus racemoses*, spring wheat 761 and the putative transformed wheats. 20% of the primers was polymorphic. The calculated genetic relationship based on the RAPD analysis were 35% between *Leymus racemoses* and spring wheat 761, 90% between the putative transformed wheats and spring wheat 761. 3. Storage protein analysis. After comparison of the

patterns of gliadin and glutenin by SDS-PAGE among *Leymus racemoses*, spring wheat 761 and the putative transformed wheats, we found that, in gliadin analysis, a new band appeared in the putative transformed wheats with an electrophoretic mobility similar to that of a gliadin polypeptide of *Leymus racemoses*. In glutenin analysis, a few high molecular weight glutenin subunits appeared in the putative transformed wheats, which might be derived from *Leymus racemoses*. These subunits may have direct impact on flour processing properties, and are worthy of further investigations.

利用异源双代换系杂交产生染色体易位系

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Production of Chromosome Translocation via Crossing Two Different Alien Substitution Lines in Wheat

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Translocation lines with useful genes derived from wheat related species are valuable in wheat variety improvement. But it is not easy to produce them. The frequency of translocation line production is usually low in wheat. So far, most of translocation lines have been obtained by spontaneous translocation method. It was once suggested that univalent chromosomes would mis-divide, reunion, and form chromosome translocation at meiosis. This theory has been supported by some experiments. We designed a series of experiments to increase univalent number at meiosis to test if univalent chromosome number was related to translocation frequency. Our results showed that crossing two different wheat-alien substitution lines could indeed increase the frequency of chromosome translocations.

过去 50 年中中国小麦品种在 *Glu-A1*, *Glu-B1* 和 *Glu-D1* 位点上等位基因的变化

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关键词 小麦 基因
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Allelic variation of *Glu-A1*, *Glu-B1* and *Glu-D1* in Chinese Commercial wheat Varieties in the Last *50* Years

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The high-molecular-weight (HMW) glutenin subunits from 85 varieties, including 22 corner stone breeding parents, 45 widely-cultivated varieties in the different period of Chinese wheat production and 18 recent varieties with good processing quality, were fractionated by SDS-PAGE. The 22 corner stone breeding lines were found to carry either null (c) or 1(a) subunits on *Glu-A1* locus. Five types of subunits were detected for *Glu-B1*, which were 7 + 8 (b), 7 + 9 (c), 14 + 15 (h), 17 + 18 (i) and 6 + 8 (d), with subunit pairs 7 + 8 and 7 + 9 being the two major types. Six types of subunits were detected for *Glu-D1*, which are 2 + 12 (a), 2 + 10 (e), 5 + 10 (d), 4 + 10 (j), 4 + 12 (c) and 2 + 11 (g), with subunit pairs 2 + 12 and 2 + 11 being the two major types. With respect to the *Glu-D1* locus, among the corner stone breeding lines, Early premium from American carried 5 + 10, St2422/464 from Italy carried the 14 + 15, whereas two related varieties, Mara and Alondra, carried 5 + 10. In the 45 commercialized varieties, only Yangmai 5 possessed the 5 + 10 subunit gene pairs at *Glu-D1*, Xiaoyan 6 and Yumai 7 possessed the 14 + 15 subunit gene pairs at *Glu-B1*. The *Glu-B1* locus of four varieties, Funo, Nongda 139, Zhengzhou 683 and Fan 6 all specified the 17 + 18 subunits. The 18 varieties recommended by the National Agricultural Ministry in 1992 as being the ones possessing good processing property could be classified into two groups, one carried the 5 + 10 subunit pair encoded by the *Glu-D1* locus, the other carried the 14 + 15 subunit pair encoded by the *Glu-B1* locus. Zhongzuo 8131 and its selections possessed the 5 + 10 subunit pair, the coding genes of which were derived from either Yecora F-70 or IRN68-181. Xiaoyan 6 and its derivative varieties carried the 14 + 15 subunit pair, the coding genes of which were derived from St2422/464, a variety that was bred in the early part of the 20th century in Italy by N. Strampelli. The 17 + 18 subunit encoding genes in Chinese varieties were derived from Funo or its selections. These results provided for the first time a basic pattern of allelic changes at the *Glu-1* loci in Chinese wheat varieties in the past 50 years, which may have important implications in the improvement of protein quality in Chinese wheat in the future.

化学杀雄剂导致小麦雄性不育分子机制的初步研究

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Preliminary Study of Molecular Basis of Male Sterility Induced by Chemical Hybridizing Agent in Wheat (*Tritium aestivum*)

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The exploitation of heterosis to increase crop yield and improve the end-using quality is an important achievement won by agricultural scientists in the 20th century. However, in wheat hybrid seed production,

though“ three lines ”and“ two lines ”systems have been studied and tested for many years, wheat hybrids have not been sown in a large area by farmers. So in 1980s, agriculturists and chemists began to test chemical hybridizing agents (CHA) and much progress has been achieved. Today, the sowing area of hybrid wheat produced by CHA has reached 1 million-hectare. The action of CHA has been studied intensively in wheat. But most of them deal with development of anther and pollen, and transport, metabolism of CHA in the wheat plant. Few reports dealt with molecular basis of the action of CHA. In this study, cDNA-RAPD technique has been employed to study the molecular basis of the action of CHA. We sprayed the wheat plant with the CHA Genesis manufactured by Monsanto company. Thirteen days later the anthers of the treated and control plants were collected for RNA extraction. Reverse-transcribed cDNAs from RNA isolated from the treated (sterile) and control (fertile) anthers were used as template for PCR. Amplification by PCR was performed using a single arbitrary RAPD primer. Products of PCR were separated by agarose gel electrophoresis. The results are described below.

In the 47 primers tested, 245 fragments were produced, the number of bands per primer ranged from two to ten. All primers except OPJ20 gave products specific to one or two types of anthers and the number of specific PCR fragments varied from one to eight per primer. Of the total fragments, 91 were specific to the control, their absence in the treated anthers indicated that the expression of their representative genes is inhibited by CHA. 74 fragments are specific to the treated anthers, which suggested that the genes represented by these fragments were activated by CHA. Of the 27 fragments (amplified by 23 primers) common to both samples, 9 exhibited a lower intensity in the control sample, 18 showed an increased intensity in the treated sample. The remaining 53 fragments were found in both samples in identical intensity. The results demonstrated that the normal gene expression program in the anther was disrupted by CHA treatment, which ultimately led to abnormality in anther development and male sterility.

利用小偃麦附加系对 *Agropyron elongatum* 生化标记进行染色体定位

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Chromosomal Location of *Agropyron elongatum* Markers in Wheat-*Agropyron elongatum* (2*n* = 14) Addition Lines

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Isoelectric focusing (IFF) technique was used to locate biochemical loci in *Agropyron elongatum* by using wheat-*Agropy-*

ron elongatum addition lines. There were six loci being located initially in all. The structural genes of Est-E5 and Est-E8 were located in 3EL, β-Amy-1 in 4EL, Per-E1 in 7E, and Per-E4 in 5E. The α-Amy-E1 was relocated in 6EL. Chromosome location of these genes provide evidence of homoeology between wheat groups 3, 4, 6 and *Agropyron elongatum* chromosome 3E, 4E, 6E, respectively. It also indicated that chromosome rearrangement probably took place between 1E and 7E chromosomes during the evolution of the E genome.

Key words: chromosome location, biochemical mark, isoelectric focusing, addition lines

小麦春化相关基因的分子克隆与功能分析

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关键词:小麦;基因;分子克隆
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Molecular Cloning and Functional Analysis of Vernalization Related Genes in Wheat

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*Ver*203, a gene related to vernalization in winter wheat was cloned by the strategy of differential screening and RACE PCR. Its function was investigated by antisense transformation in both wheat and *Arabidopsis*. A number of transgenic plants were obtained and their response to vernalization treatment was characterized. For the plants in the 6 lines in which the antisense construct was expressed efficiently, flowering stage was significantly delayed. The developmental pattern of inflorescence was also changed in the transgenic plants of wheat and *Arabidopsis*. The upper spikes of transgenic plants were degenerated in wheat, and the florescence of transgenic plants became terminal flower in *Arabidopsis*. This suggests that the VER203 type of proteins may play an important role in floral initiation and flower development in winter wheat and *Arabidopsis*.

中字系列小偃麦遗传材料的培育、遗传分析和利用

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Breeding, Genetic Analysis and Utilization of "Zhong Series" Varieties of *Trielytrigia*

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Five "Zhong series" varieties, Zhong1, Zhong2, Zhong3, Zhong4, Zhong5, of octoploid *Trielytrigia* have been bred by hybridization of common wheat with *Elytrigia intermedium*. Many geneticists and breeders have considered these varieties as good germplasm resources possessing disease resistance as well as high seed protein quality. The breeding procedure and pedigree, morphology, biology, disease resistance, quality traits and genome constitutions of these materials have been investigated. By utilizing the "Zhong series" genetic materials, a number of commercial wheat varieties were produced. They included Long Wheat 10 with resistance to BYDV, Linkang 1, Black-grained wheat 76 with high seed protein quality, Jinyan 1 with salt tolerance, Zaoyou 504 with high quality and early maturity, Gaoyou 503 with high yield and quality, Shanxi Wheat 8007 with high quality and immunity to stripe rust, Gui Wheat 3 with early maturity and resistance to dampness, Long wheat 9 with resistance to leaf rust, Xiaoyan 33 and Jinchun 13 with high yield, good quality and disease resistance.

紫外线诱导小麦和长穗偃麦草体细胞融合产生可育株及其后代的细胞遗传学分析

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关键词 小麦;长穗偃麦草;细胞融合
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Production of Fertile Hybrid Plants Via UV Fusion between *Triticum aestivum* and *Agropyron elongatum* and Cytogenetic Analysis of the Resulted Progenies

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Protoplasts of common wheat (*Triticum aestivum* L. $2n = 42$) cv. Jinan 177 were fused with ultraviolet (UV) irradiated protoplasts of *Agropyron elongatum* ($2n = 70$) via a PEG method. Sterile hybrid plants were regenerated from the fused product and their ovaries were induced to form calli. From the resulted callus tissue, green plants were differentiated. These somaclonal plants (SF0) were investigated using chromosome and isozyme analysis. The results showed that they contained chromosomes from both donors (i.e., *Triticum aestivum* and *Agropyron elongatum*). Two of the SF0 plants grew to maturity and set seeds. The analysis of phenotype, chromosome constitution, isozyme pattern and RAPD polymorphism of the F_1 plants confirmed their hybrid nature. Collectively, these results demonstrated that fertile hybrid plants could be produced from the procedure described above. Three different phenotypes were observed in F2 progenies. The type I and II plants had higher stalks (average 75 ~ 85 cm) and big ears and grains, but plants of the former phenotype possessed fewer tillers. Type III plants had short stalks (average 55 cm) but possessed high ability of tillering. Cytogenetical analysis of F_1 plants and their successive generations showed that in F_1 to F_3 generations the chromosome numbers of root tip cells varied in the range of 36 ~ 44, and many cells contained 1-4 micro-chromosomes (mc). In PMC MI stage of the F2 plants, the chromosome configuration was mainly 17II-22II with 1-4 additional micro-chromosomes. Comparing to F_2, more chromosome configuration of 20II -21II oc-

curred in F_3, and over 70% of cells had the chromosome configurations of 21II + 1-2 mcs. A large population of the different hybrid lines have been obtained through propagation and selection in successive generations. Their agronomic traits have been studied and will be reported in a separate paper.

Key words: *Triticum aestivum*; *Agropyron elongatum*; Asymmetric somatic hybridization; Fertile hybrid plants; Cytogenetic analysis

普通小麦 F_1 杂种 *Glu*-1 基因表达过程中的共显性,基因组互作和剂量效应

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Evidence for Co-dominance, Genome Interaction and Dosage Effects in *Glu-1* Gene Expression in F_1 Seeds of Common Wheat (*Triticum aestivum* L.)

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Eighty two F1 seeds selected randomly from 33 hybrid ears of 23 crosses between 8 parental varieties were examined by 10% SDS-PAGE for HMW-glutenin expression and accumulation. The following results were obtained. 1. All tested 8 alleles co-dominated in the F1 seeds of 22 crosses. 2. In the 6 F1 seeds of the cross Pan 555 / Zheng 891, a novel subunit appeared in stead of the expected subunit 9 (contributed by Pan 555) and subunit 10 (contributed by Zheng 891), indicating that an interaction of unknown nature between the maternal and paternal genome had affected the expression of some HMW glutenin genes in the endospermic cells of the hybrid seed. 3. The subunits specified by the maternal genes accumulated twice as much as those specified by the paternal genes.

ph1b 基因对簇毛麦遗传物质导入普通小麦的影响

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关键词:*ph1b* 基因;小麦;簇毛麦
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Effect of *ph1b* gene on direct genetic transfer from *Haynaldia villosa* to *Triticum aestivum*

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Hybrid plants between *Triticum aestivum* var. "Chinese Spring" (CS), its *ph1b* mutant (CS*ph1b*) and *Haynaldia villosa* were obtained by immature embryo culture. After selfing, the two types of hybrids showed seed setting rates of 6.67% and 6.25%, respectively. The analysis of chromosome pairing behaviors at meiotic metaphase I showed that, on the average, only 1.61 chromosomes could form bivalent and trivalent in each PMC of the hybrid F_1 CS × *H. Villosa*, with the configuration $2n = 28 = 26.39I + 0.79II + 0.007III$. However, in the hybrid F_1 *CS ph1b* × *H. Villosa*, 14.43 chromosomes per PMC were involved in bivalent and multivalent formation, with the chromosome configuration of $2n = 28 = 13.55I + 5.95II + 0.55III = 0.22IV$, and, in over 56% of the PMCs, 1-4 multivalents (trivalents and quadrivalents) were produced. The observation of meiotic chromosome pairing by using genomic fluorescent *in situ* hybridization (GISH) revealed three types of chromosomal associations: wheat-wheat, wheat-*H. villosa* and *H. villosa*-*H. villosa* in PMCs for "*CS ph1b* × *H. villosa*" F_1 hybrid. The seed set of the backcross of the "*CS ph1b* × *H. villosa*" F_1 with CS was 6.67% and that of "*CS* × *H. villosa*" F_1 with CS or CS *ph1b* was only 0.45%. The chromosome number of BC_1 plants varied from 48 to 72. Robertsonian translocation chromosomes consisting of chromosome arms from wheat and *H. villosa* were detected by mitosis GISH in the BC1 plants from the backcross of "*CSph1b* × *H. villosa*" to *CS ph1b*. These results led to the conclusion that the *ph1b* gene induced a higher level of homoeologous chromosome pairing between common wheat and *H. villosa*. The transfer of desirable genes from *H. villosa* to common wheat may be facilitated by using the *ph1b* gene.

Key words: genetic transfer, GISH, *Haynaldia villosa*; *ph1b* gene; homoeologous pairing; *Triticum aestivum*

利用分子细胞遗传学方法向小麦中转移和富积优异外源基因

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关键词: 小麦; 细胞遗传学; 外源基因
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Utilization of Molecular Cytogenetics Technology in Transferring and Pyramiding Useful Alien Genes into Common Wheat

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Rich genetic resources are contained among wheat wild relatives. Cytogenetics Institute of Nanjing Agricultural University commences to transfer these genetic resources from relatives into common wheat. The hybrids between common wheat and *Haynaldia villosa*, *Leymus racemosus*, *Roegneria ciliaris*, *Roegneria Kamoji*, *Secale cereale or Thinopyrum baserabicum*, and their alien chromosome stocks such as addition, substitution, translocation and introgression lines were developed via successive pollinations and immature embryo rescues. The resulted progenies were characterized using mitotic and meiotic analysis combining with chromosome banding and *in situ* hybridization. Isozyme pattern and RFLP analysis were used to determine the homoeologous relationship between alien chromosomes and

those of common wheat. Irradiation, Ph system (homoeologous pairing control system), gametocidal chromosome effect were successfully employed to induce translocation and deletion lines. Chromosome C-banding, *in situ* hybridization, RFLP and trait-tracing were used to identify alien chromosome, chromosome segments, breakage point of translocation or deletion, and to map the introgressed genes such as those conferring resistance to powdery mildew, yellow rust, *Fusarium* head blight and take-all diseases. As the result of above effort, new genetic materials, which contained multiple useful alien genes, were developed. To improve the agronomic characters of the derived lines, back crosses or "rolling" crosses using superior varieties or lines as recurrent parents are currently being conducted.

小麦品种复壮 30 中与抗白粉病基因连锁的一个 RAPD 标记

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A RAPD Marker Linked to the Resistance Gene to Powdery Mildew in Wheat Variety-Fu Zhuang 30

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Powdery mildew is a severe fungal disease in wheat. So far 29 *Pm* genes conferring resistance to the disease in wheat have been identified. Here, we report a RAPD marker linked to a recessive *Pm* gene (tentatively designated as *Pm*30) in wheat variety - Fu Zhuang 30, which showed high resistance to all powdery mildew races recorded in China. A 628 bp fragment was specifically amplified with RAPD primer UBC405 (CTCTCGTGCG) in Fu Zhuang 30, but not in susceptible cultivars Nong Da 015 and Jing Hua No. 1. With bulk segregate analysis of 101 F_2 progenies of Nong Da 015/ Fu Zhuang 30 and 62 F_2 progenies of Jing Hua No. 1/Fu Zhuang 30 cross, we calculated that the genetic distance between the UBC405-650 RAPD marker and the *Pm*30 gene was 13 cM (LOD value of 7.4). The RAPD marker will be valuable in marker assisted selection in breeding programs aimed at transferring the *Pm*30 gene into commercial wheat varieties.

小麦和簇毛麦体细胞可育杂种植株的产生

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Production of Fertile Somatic Hybrid Plants Between *Triticum Aestivum* and *Haynaldia villosa*

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Intergeneric somatic hybridization between suspension-derive wheat (cv Jinan 177) protoplasts having only 24 ~ 28 chromosomes and callus-derived *Haynaldia villosa* potoplasts with 11-14 chromosomes was carried out by a PEG method. A high frequency of putative hybrid calli and plants were regenerated from fusion products. Their hybrid nature was identified by cytological, biochemical and 5S rDNA spacer sequence analysis. The results showed that all the analyzed regenerated calli and plants were hybrids. GISH (genomic *in situ* hybridization) analysis proved the existence of the chromosomes of both donors and the occurrence of translocation between the chromosomes of the two species in the hybrid clones. The analysis of the cytoplasmic DNA using mitochondrion and chloroplast-specific probes revealed that some degree of recombination between the organellar genome of the two species occurred. The gross morphology of hybrid plants resembled that of two donors. One hybrid was fertile and gave rise to seed, which resembled that of *Haynaldia villosa* in morphology. In conclusion, we generated fertile somatic hybrid by intergeneric somatic hybridization. The co-existence of both species' chromosomes in the hybrid clones in relation to their regeneration capacity and the production of fertile hybrid plants will be discussed in the speech.

小麦基因组中外源染色体片段的检测和小麦基因分子标记的建立

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Studies on the Detection of Alien Chromosome Fragments and Obtainment of Molecular Markers Linked to Specific Genes in Wheat

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In our laboratory, FISH technology has been applied in the detection of alien chromosome or large alien chromosome fragments (for instance, the rye chromosome fragments in translocation line of wheat). But for investigating translocations involving small segment of alien chromosomes, DNA fragment analysis based technologies such as RAPD and AFLP have been used. The latter methods also have been applied to pbtain molecular markers linked to specific genes (our focus was the *Rf3* restorer gene of G-type CMS of wheat). An alternative method for finding new molecular markers is subtractive hybridization. Our aim in using this method is to obtain high copy sequences, which are specific to rye.

小麦春化发育机制的初步研究

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Preliminary Study on the Mechanism of the Vernalization Development in Wheat

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Three wheat varieties different in development properties were treated by means of vernalization, de-vernalization and their protein extracts were analyzed by SDS-PAGE. The results showed that vernalization and de-vernalization treatment not only increased the amount of soluble proteins, but also induced some new proteins (53.2 kDa, 46 kDa) in two winter wheat varieties Mercia and Jing 841. The two new proteins also existed in spring wheat, but were absent in winter wheat before vernalization. The peroxidase and esterase isoenzyme activity also increased in the vernalization process, indicating that peroxidase and esterase activity may be required for vernalization of winter wheat. Glucose-6-phosphate isoenzyme activity changes may also be related to vernalization development of winter wheat. However, superoxide dismutase (SOD) isoenzyme activity was not changed by vernalization treatment. These results demonstrated that the vernalization was a process that involved changes in gene expression, protein synthesis and enzyme activities.

非 Robertsonian 类型小黑麦易位系的研究

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About non-Robertsonian wheat-rye chromosome translocation lines

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Plant geneticists and breeders pay great attention to the investigation of translocation lines, because it involves the study of chromosomal structure and function, and the transfer of alien chromosomal fragments (genes) into wheat. Excellent translocation lines do have direct application in breeding programs. The principles and methods of inducing chromosome translocation lines have been reviewed. The techniques used in inducing chromosomal translocations can be classified into two major types. 1. Regulating the activity of the *Ph* gene to facilitate the exchange between homoeologous chromosomes so as to create translocation lines. 2. Exploitation of irradiation, tissue

culture or gametocidal chromosome induced chromosomal breakage and reunion to obtain translocation lines. In the last decade, we obtained many wheat-rye translocation lines from regenerated pollen plants. Among the 10 translocation lines, there were 4 non-Robertsonian translocation lines. The non-Robertsonian translocation lines, were identified using a range of techniques, including C-banding, *in situ* hybridization and genome or chromosome-specific molecular markers. Based on our investigations, we conclude that the non-Robertsonian translocation lines arising from anther culture were the products of abnormal mitosis in *in vitro* cultured cells.

小麦 6B 染色体的微切割与其区域特异性 DNA 文库的构建

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Micro-dissection of Wheat Chromosome 6B and Construction of Its Region Specific DNA Libraries

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Chromosome 6B of wheat (*Triticum aestivum* L.) of cultivar "Chinese Spring" was dissected into four fragments (R1 ~ R4) by laser microbeam. Then, each of the four fragments was isolated and amplified *in vitro* via Sau3A linker-adapter-mediated PCR for two rounds. Using wheat genomic DNA as probe, southern hybridization confirmed that the chromosomal fragments were successfully amplified. A set of microsatellite primers that would amplify microsatellites in defined chromosomal locations (kindly provided by Prof. M. D. Gale of the John Innes Center) was adopted to verify the chromosomal origin of the PCR products. The results showed that the amplified products from R1 and R2 were, indeed, from the respective dissected regions. Furthermore, CISS (chromosome *in situ* suppression hybridization) was also employed to verify the location of the PCR products. The PCR products were found to hybridize to the specific regions of 6B, indicating that the products contained region-specific sequences. Following the micro-dissection, four chromosomal region-specific DNA libraries were generated by cloning the second round of the PCR products into a plasmid vector. The resulted libraries were characterized in several aspects. Approximately $2.1 \times 10^5 \sim 2.93 \times 10^5$ recombinant clones were present in each of the four libraries. The size of inserts varied from 300 to 1800 bp (with an average length of 850 bp). 43% ~ 48% of inserts hybridized to single/low copy sequences, whereas 42% ~ 47% represented medium/high copy ones. The clones in these libraries could provide a source of chromosomal region-specific probes for genetic mapping and for studying the structure of chromosome 6B in the future.

组织培养诱导外源染色体发生结构变异及其在小麦易位系创制中的利用

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Structural Changes of Alien Chromosomes Arising from Tissue Culture and Their Exploitation in Producing Novel Translocation Wheat Lines

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Fluorescence in situ hybridization was applied with total genomic DNA extracted from *D. villosum* as a probe to characterize chromosome translocations arising from tissue culture in crosses of *Triticum aestivum* × *T. durum-D. villosum* amphiploids. Chromosome translocations between wheat and *D. villosum* occurred indeed in callus cells at an average frequency of 1.9 %. Translocations existed not only in callus cells but also in regenerate plants. Three plants with translocation chromosomes were characterized among 66 regenerated plants. One of them was proved to be a reciprocal translocation with break point of wheat chromosome at about one third of a chromosome arm, and that of *D. villosum* at about one half of a chromosome arm. The break point of the other two translocations was located at, or near centromeres. These similar results from both callus cells and regenerated plants provided evidence that chromosomal translocations could take place in tissue culture. Additional chromosome structure changes (fragments, telocentrics, dicentromeres, and deletions) as well as numerical alterations (including aneuploid and polyploid) were also observed in tissue cultured cells. For 175 regenerated plants arising from immature embryos of crosses between wheat (*Triticum aestivum* L.) and 6D/6V substitution stocks, electrophoresis of glutamate oxaloacetate transaminase (GOT) isoenzymes was performed. The GOT-V2 enzyme band was absent in two plants (designated as 98R149 and 98R159, respectively). Fluorescence *in situ* hybridization with total genomic DNA extracted from *D. villosum* as a probe confirmed the occurrence of translocation between 6V chromosome and an unknown wheat one in the two regenerants mentioned above. 98R149 and 98R159 were immune to powdery mildew (*Erysiphe graminis* DC. f. sp. *tritici*) inoculation with mix races collected from Hebei Province. These results demonstrated that useful translocations might be produced via tissue culture.