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I. Review

Present status of wheat breeding and related genetic study in China

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Summary

Recent progresses in wheat breeding and breeding-oriented genetic studies in China since the second half of 1980's were reviewed. The establishment of second-line sources of resistance to stripe rust and powdery mildew, situation on scab resistance, major sources of semidwarfness, improvement of bread baking quality, reccurrent selection for various purposes, germplasm development and transfer of alien genes, and the advance of hybrid wheat development through different approaches were discussed in some details.

Introduction

China is a big wheat producing country in the world. Its annual wheat acreage and production in 1986-1990 amounted 29.56 million ha and 91.35 million t, respectively, with an average yield of 3063 kg/ha. It is mainly grown in the lower and middle reaches of the Yellow and Huai Rivers valleys, the provinces of Sichuan, Hubei, Anhui and Jiangsu along the Yangtze River, northern parts of the North China Plain, Helongjiang Province in the Northeast and Xingjiang Autonomous District in the Northwest. Nearly all the wheatlands are sown with improved cultivars developed mostly from the so-called conventional breeding. In 1990-1992 the most popular cultivars were as follows: Ji 5418, Lumai 14, Lu 215953, Lumai 15, Yumai 13, Xian 8, Yumai 17, Jimai 26, Xiaoyan 6 in the Yellow and Huai valleys; Yangmai 5, E-en1, Mianyang 15, Chuanmai 22, Mianyang 19 in the Yangtze Valley; Jing 411 in the northern parts of North China; New Kehan 9, Kefeng 3 in Helongjiang province; Ningchun 4 in Inner Mongolia and Ningxia districts; and Tang 6898 in Xingjiang district.

There are about 100 wheat breeding units in the nation which include central, provincial and prefectural levels of agricultural institutes and some universities. More than 1000 people are directly or indirectly involved in this profession including a few breeders from seed companies or private sectors. Since 1981 the major breeding units have been organised by the Ministry of Agriculture to form a cooperative network. During 1986-1990 the 36 members of the network developed 91 varieties which had passed the regional tests, and 55 of them were registered as recommended cultivars. These newly released cultivars occupied an acreage of 4.83 million ha in 1989 harvest year. This may give a general idea on the progress of wheat breeding and extension

work under ordinary conditions.

The breeding objectives vary with different wheat growing regions. In general, yield has been of primary consideration. High yield potential and good quality are aimed on the basis of yield stability. The main approaches to improve yield potential are to increase the kernel weight and kernel weight per spike, and at the same time, to improve lodging resistance and harvest index by dwarfing plant stature not at the expense of biomass production. As to yield stability, much attention has been paid to the resistance against biological stress especially disease resistance. Grain quality, bread baking quality in particular, is now put on the agenda, and is deemed as important as yield since the living standard is getting better. This is a great challenge to the breeders because for thousands of years wheat varieties raised by the Chinese farmers have never been selected against bread baking quality. Moreover, adaptation to multiple cropping systems must also

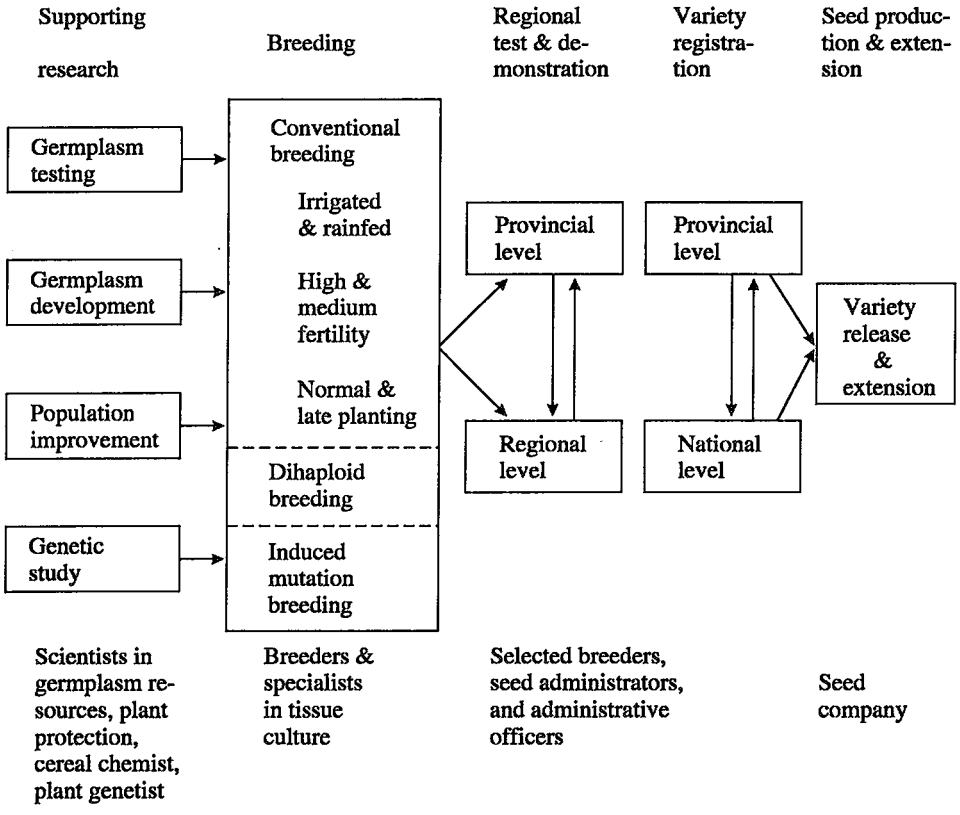

Fig. 1 A diagram of operational scheme in wheat breeding

be considered.

In regard to breeding methodology, intervarietal hybridization in the usual way supplemented by induced mutation, dihaploid breeding and wide cross. During the past 10 years, supporting researches such as testing for disease resistance, stress tolerance and quality, evaluation and development of parental stocks, population improvement and genetic studies on economically important traits have been allocated with appropriate resources. Fig. 1 illustrates the conventional procedures of wheat breeding adopted in China.

The Chinese wheat breeding program is characterized as: being small in scale but intensive (one breeding unit used to grow approximately a F_2 population of less than 200 thousand plants and 6000 F_3 and later generation progeny lines all togather annually before initial yield trial); pedigree method adopted with small spacing; emphasizing more on field observation and early generation selection; and testing the selected varieties in the farmer's field as early as possible for approval in production. So it takes 8-10 generations from crossing to the time when a variety has finished the regional test. Hereby recent advance in the following areas is briefly described.

Breeding for disease resistance

This is the main theme in wheat breeding in which three important diseases, i. e. stripe rust, powdery mildew and scab, are emphasized.

Stripe rust and powdery mildew

Stripe rust is a nation-wide disease except in the northeastern spring wheat region. From the beginning of 1950's China began to release in succession its own cultivars resistant or tolerant to stripe rust. For the nation as a whole, stripe rust has essentially been under control in the last 20 and more years except in the middle part of Shaanxi, mid-southern Gangsu and the southwestern provinces where physiological races used to variate from time to time due to special ecological conditions prevailing in these areas. Recently powdery mildew has become increasingly important as a result from the inreased application of nitrogenous fertilizers and the expansion of irrigated wheat lands. As there was pratically no breeding work in the past on powdery mildew, nearly all the varieties released for commercial production are susceptible to the disease with some exceptions from Guizhou province. When the 1BL/1RS derivatives such as Lovrin 10, Predgornaia 2, Kavkaz, etc. were first introduced from Romania in the early 1970's, they showed quite a good resistance to the prevailing races of stripe rust, leaf rust and powdery mildew as they carried *Yr9*, *Lr26* and *Pm8* resistance genes and matured normally under various heat-stressed environments as well. So all the breeders across the country gave special preference to them and used them extensively in breeding programs as a source of multiple resistance. Consequently, an overwhelming majority of varieties recently released for production in various winter and spring wheat regions were new derivatives from the above-mentioned old 1BL/1RS translocation lines. This would inevitably result in a monotonous spectrum of disease resistance which is highly vulnerable to the attack of new fungal races. It is now reported everywhere that a lot of cultivars carrying 1BL/1RS resistance genes have

Table 1. Second-line sources of resistance to the current Chinese races of stripe rust and powdery mildew

Resistance gene	Representative	New resistant variety and mode of inheritance*		
Stripe rust races (CY25, 28, 29)				
Yr1(2A)	Fenman	Luqiyou(2B)	1d	
Yr3b, 4b	Hybrid46	C39(7B)	1r	
Yr5(2BL)	*T. spelta* v. *album*	Fr84-8(2A, 3A)	2rc.	
Yr8(2B/M)	Campair	BAU233	1d	
Yr10(1BS)	Barbee			
Yr15	*T. dicoccoides* derivative			
YrSu	Suwon11			
Total 8			5	13
Powdery mildew (Beijing local races)				
Pm2(5D)	Fenman	Tx76402	—	
Pm2x(5D)	Baimian3	Little white winter	—	
Pm4a(2AL)	Khapli/8x Cc	—	—	
Pm4b(2AL)	Fr84-3	Guinong 90-21		
Pm6(2B), 2,4	C39	V2 substitution line	—	
Total 5			4	9

* : d=dominant, r=recessive, c=complementary

lost their resistance to the diseases. This has become a great threat to wheat production and a big challenge to the breeders too. Therefore wheat breeders and pathologists are anxious in looking for second-line sources of resistance which can be readily utilized in breeding programs. A disease testing group of the Beijing Agricultural University after extentive testings and genetic analyses has provided a recommendation list of new resistance genes and their carriers togather with some information on their mode of inheritance. Table 1 shows the main sources of resistance to stripe rust and powdery mildew in the comming years, at least in North China.

Within the list Luqiyou and C39 give the best performance because the former is nearly immune to stripe rust in F_1 and the latter carries three genes for powdery mildew resistance. Currently these new genes of resistance are being incorporated into the recommended varieties (Yang et al 1992, to be published). Meanwhile, Jiangsu Academy of Agricultural Sciences (hereinafter abbreviated as AAS) has identified CI12633, CI12632 and TP114 which carry *Pm2* and *Pm6* are effective in the control of powdery mildew in Nanjing area (Yiao et al 1990). Durum wheat and triticale breeders from the Institute of Crop Breeding and Cultivation, CAAS, indicate that certain durum wheats and many triticales are also good sources of resistance to powdery mildew, among them 6R of rye deserves attention (Zeng and Xin respectively, personal communications). It is reported that a common wheat line named Yangxiaohe 1-1, developed by Guizhou Agricultural College from a cross involving false wild oat, common wheat and rye, shows good resistance to powdery mildew in Sichuan and Guizhou provinces and might carry a new gene(s) (Li and Huang 1991).

Scab

Scab is an important disease in China with its own characteristics. It prevails in the middle and lower reaches of the Yangtze valley, coastal areas in southeastern China and eastern parts of Helongjiang province with a severe epidemic nearly once every four years. Since 1950's more than 100 thousand accession-times of wheat materials have been tested against scab isolates by Shanghai AAS and its partners and none is found immune or highly resistant to the disease. Only a very few landraces such as Liyang Wansuibai, Pinghu Jianzimai, Wengzhou redbeardless, etc. are found to be resistant to the extension of infection. But these landraces possess very poor agronomic traits and can hardly be used in breeding. Fortunately in 1970, a relatively resistant (to extension) variety known as Sumai 3 was developed by Suzhou Prefectural Agricultural Research Institute from a cross of two susceptible varieties (Funo/Taiwan). Since then numerous crosses were made between Sumai 3 and various improved varieties, and a series of new cultivars were developed and released for commercial production but rarely their resistance could surpass that of Sumai 3 (Liu et al 1992). Recently breeders from Fujian Agricultural College has produced some new improved wheat lines designated as Fan 60096 and the like from Jingzhou 1/Sumai 2 (a sister line of Sumai 3) whose resistance to scab is slightly better than that of Sumai 3 (Wang et al 1992). Although their difference in resistance to extension of infection is statistically significant yet such a small improvement is not enough to enhance yield stability.

There are arguments regarding the mode of inheritance in varietal resistance to scab. Most authors recognise it is polygenic in nature. Monosomic analyses of scab resistance in Sumai 3 showed that many chromosomes were involved with differential contributions (Li and Yu 1990, Yu 1990). A study of gene actions on scab resistance by generation means analysis from biparental crosses between the susceptible and a series of varieties with differenct degrees of resistance indicated that although additive effect was of primary importance, dominance should not be neglected and epistasis was unimportant (Wang et al 1992). But some authors provided evidence that scab resistance was controlled by a few major genes with a certain number of modifiers, since it usually showed in F_2 a segregation pattern of two peaks with one large and one small (Zhou et al 1987, Bai et al 1990). The discrepency may be attributed to the ways of expressing the infection indices and related also to the fact whether infection data are taken at various stages of disease development or not. If observations are taken in a dynamic pattern then the conclusion would be polygenic; but as far as the sympton of rachis infection is concerned it is of major genes inheritance (Li and Yu 1990).

A study on the components of varietal resistance to scab extention of infection through path analysis showed that yield loss depended on the number of kernels infected by the fungus and the rate of reduction in kernel weight from disease-free kernels on the infected head, the first component playing a more important role. Both of them then were affected by the number of spikelets infected which, in turn, was influenced by the number of rachis internode infected and the time of infection on the diseased head. Their relation is shown in Fig. 2.

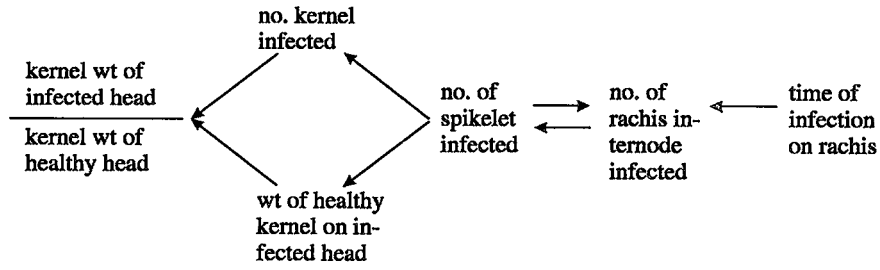

Fig. 2 Components of varietal resistance to extension of infection in wheat scab

Long time breeding practice has revealed that transgressive inheritance in scab resistance is of frequent occurrence, but the so-called "resistance" is actually varying degrees of tolerance to the extension of infection and not infection itself. Owing to lack of source of resistance to infection (note: recently *Agropyron ciliare* and *A. repens* were found to be highly resistant to scab infection by Nanjing Agric. Univ. and Sichuan Agric. Univ., independently) and a certain extent of negative corelation between scab resistance and good agronomic characters, it is difficult to achieve even a small breakthrough in the improvement of scab resistance by the conventional method. Therefore recurrent selection by using Taigu dominant male sterile gene *Ms2* or *Ta1* has been adopted in recent years for pyramiding the major and minor genes which control scab resistance. Such a project was initiated and undertaken by Nanjing Agric. Univ. and its partners. They crossed 15 selected scab resistant varieties including landraces with five improved varieties carrying *Ms2* gene to make a composite of 75 combinations. After three years of intermating a CO population was established which was grown in four locations under the same design. Phenotypic selection with an intensity of 5-10% was practised aiming first at scab resistance and paying considerable attention to major agronomic traits as well. Seeds from the selected male sterile plants were bulked with equal quantity to form the stock material for the next cycle. After three cycles of phenotypic selection comparison among the four populations from CO to C3 was made in Nanjing. The results showed that percent of infected plants in the population and the number of infected spikelets of infected plant reduded materially as the cycle advanced, with an average of 20% decrease per cycle for the latter. After two to three cycles of selection scab resistance of the population almost approached the level of Sumai 3, and spike characters except kernel weight were correspondingly improved with a gain per cycle varying from 2-8% (Jiang et al, 1992). Meanwhile, South China Agric. Univ. also conducted a similar study by crossing eight resistant parental stocks with some high yielding varieties carrying *Ms2* gene. After several cycles of selection some promising lines designated as T474, T229 and the like with acceptable scab resistance and desirable agronomic characters were developed.

Breeding for semidwarfness

Early in 1957 Xianyang Prefectural Agric. Res. Inst. using Suwon 85 (bearing *Rht1* and *Rht2*) as a source of dwarfness developed the first series of semidwarf intermediate materials among which Xiannong 39 was a good representative. Then Northwest Agric. Univ. by crossing Xiannong 39 again with a promising hybrid line produced the first series of semidwarf varieties in which Aifeng 3 was the best one and released in 1971. Since then breeders from Shandong, Sichuan, and Henan provinces had developed in succession a number of semidwarfs which were widely grown in some major wheat regions. Those varieties listed hereinbefore for the Yellow and Huai Rivers valleys and Sichuan basin are all semidwarfs. Presently about 40% of the total wheat acreage is sown with semidwarfs.

In breeding for semidwarfness development of desirable intermediate or link-up materials is very important. In addition to Xiannong 39, there was another example. Shandong Agric. Univ. was successful in developing an intermediate, link-up material called Aimengniu which was a product of Aifeng 3//Mengxian 201/Neuzucht. It combined dwarfness from Aifeng 3 and multiple disease resistance from Neuzucht, but a little bit late in maturity. Subsequently breeders in the University using this as a source of dwarfness and disease resistance in crossing bred a number of improved varieties known as Lumai series which were widely grown in Shandong province. Another experience in the breeding for semidwarfness is that selected crosses between short or mid-statured varieties may produce some semidwarfs through transgressive segregation. For example, Huixian Red (short)/Abbondanza (medium) → Taishan 4 (semidwarf); Youbao (short)/036 (tall, a Beijing selection) → semidwarf transgressive segregants; and others.

In summarizing the representative dwarf-source varieties or dwarfing genes which led to successful development of semidwarf cultivars in China in the past 20 years, five categories are recognized: (1) Suwon 86 which carries two pairs of GA3 insensitive semidwarf genes, *Rht1* and *Rht2*, located on 4B* (new designation) and 4D, respectively. (2) St2422/464 (a material of Italian origin) bearing one pair of semidwarf genes similar to that Saitama 27 with weak GA3 insensitivity designated as *Rht1s* on 4B*. (3) Huixian Red and Youbao, each carrying a pair of semidwarf genes *Rht2*. (4) Funo, Abbondanza, Mara, and other similar derivatives of Akagumughi, each carrying one or two pairs of GA3 sensitive dwarfing gene(s) designated as *Rht8* or *Rht9* and located on 2D or 7BS (Jia et al 1992). These two pairs of dwarfing genes are common in the Chinese varieties as the above-mentioned source-varieties were extensively used in breeding programs. Although their height reducing capacity is weak they may, if properly mated, manifest some auxiliary effect on dwarfing plant stature. (5) Tom Thumb carrying *Rht3* and Aibian bearing *Rht10* were used in hybrid wheat development and reccurent selection for dwarfness, respectively, by some institutes. These will be mentioned hereinafter in the text.

Breeding for dwarfness, however, does not mean the shorter the better. Dwarf stature can increase harvest index but it tends to decrease biomass production. Moreover, in the areas where heat stress is a problem in ripening, dwarf or semidwarf wheats are proned to give a sympton of presenility or "precocious senescence." Therefore the optimal plant height of a wheat variety must be justified from a joint consideration of ecological environment, cultural condition and prevalence

of disease epidemic.

Breeding for bread baking quality

Wheat in China is mainly consumed as foods such as steamed bread, oriental noodle, pitta bread, pancake, etc. Selection for bread baking properties has not been practised until early 1980's. Firstly, screening among the released varieties,existing breeding lines and germplasms available is emphasized. In 1987 and 1992 two contests for "high quality" wheats were organised respectively. Table 2 provides a comparison of major indices for bread baking quality between the top ten varieties in the two contests.

Table 2. Comparison of bread baking quality from the two contests and a third sets of wheat varieties in a different test

Year	Source of data*	Seed crude protein %, dry basis	Guten %	Zeleny sedimentation value ml	Farinogram				Bread	
					Development time min	Stability min	Mixing tolerance Bu	Valorimeter value	Volume ml	Score
1987	A	15.1	33.9	37.7	5.5	12.5	14	67	679	81
1992	B	16.3	37.1	50.0	7.6	9.5	27	68	744	83
1990-1991	C	17.5	—	50.3	19.0	28.9	3.8	90.6	937	94
	8131-1	19.6	—	41.2	13.9	12.1	13.0	83.8	922	93

A=average of top ten varieties from 16 province contest for high quality wheats.

B=average of top ten varieties from national contest for high quality wheats.

C=average of five Kansas winter wheat varieties from a study in 1990-1991 in which the Chinese "best quality" wheat 8131-1 was included as "check."

*Data provided by Inst. Crop Bread. Cultiv., CAAS.

As indicated in Table 2, there was not much improvement on the bread baking quality during the period 1987-1992. The bread baking quality of the so-called "high quality" Chinese wheats is, in general, inferior to that of the hard red winter wheats from the U. S. The former are characterized with a short dough development time, short dough stability time, less tolerance to mixing, and finally smaller loaf volume (about 150 and more ml less than the standard U. S. flour from 100 g bake). Nonetheless, a few varieties with durum parentage such as Zhongzuo 8131-1 and its sister selections possess a bread quality score approaching to that of the U. S. hard red winter wheats. This infers that A and B genomes may play a considerable role in bread baking in addition to D genome. In the present quality breeding programs good baking quality wheats from the U. S., Canada, Mexico, the former USSR, southern and eastern European countries are widely used as parents in crossing and sedimentation value is adopted as a main selection criterion for bread baking quality in

Table 3. Main HMW glutenin subunits and their frequencies in three categories of common wheat varieties

Accession	*Glu-A1*			*Glu-B1*			*Glu-D1*	
	Null	1	2	7+8	7+9	22	2+12	5+10
Landrace	88.6			84.6			94.3	3.7
Improved variety	57.4	27.6	25.0	42.0	41.9		73.7	15.7
Foreign variety	54.5	27.3		25.2	37.2	11.7	46.4	45.9

early generations.

In the past few years studies on the composition of HMW glutenin subunits of the Chinese wheats and its relation to bread baking quality have been made by several institutes, among them Hebei Agric. University's work deserves mention. The HMW glutenin subunits composition of 5071 common wheats (comprising 922 landraces, 2292 breeding lines and 1818 exogenous materials) were determined by SDS-PAGE and the corresponding Zeleny sedimentation value and other related quality indices were scored in a period of three years. They found that: (1) The main subunits variations in the three loci of homeologous chromosome 1 were: for *Glu-A1* — Null, 1, 2*; for *Glu-B1* — 7+8, 7+9, 6+8, 7, 20, 21, 22, 17+18, 14+15, 13+16, 13+19; for *Glu-D1* — 2+12, 3+12, 4+12, 5+10. Some rare or new allelic variations such as 2+10, 2.2+12, 6+9, etc. were also found. (2) The main subunits in the three loci were listed in Table 3. The frequencies of good quality subunit 5+10 in Chinese landraces and improved varieties or lines were 3.7% and 15.7%, respectively. (3) The main subunit combinations in the three loci for landrces were Null, 7+8, 2+12, with a frequency of 74.8%; for improved vareities were Null, 7+8, 2+12 (18.8%); Null, 7+9, 2+12 (16.7%); and for foreign varieties were Null, 7+9, 5+10 (14.0%); Null, 7+9, 4+12 (10.5%); Null, 7+8, 5+10 (7.5%), respectively. (4) Multiple regression analysis between glutenin subunits determined and their corresponding 12 quality indices revealed that 5+10 gave the best performance, and 2* ranked next. As judged from the 3 main bread baking quality indices, namely sedimentation value, valorimeter value and bread score, in a subset data from 126 varieties, the magnitute of effect for each subunit in *Glu-A1* was in the order of 2* >1>Null; in *Glu-D1* was 5+10>4+12>2+12; and in *Glu-B1* was 7=20>7+8>22>7+9. (5) A new *Glu-1* quality score system was suggested as follows (Mao 1992, to be published):

Score	*Glu-A1*	*Glu-B1*	*Glu-D1*
5			5+10
4	2*		
3		7, 20	
2	1	7+8	4+12
1	Null	22	2+12
0		7+9	

Wheat breeders in China have confronted for many years two problems, namely, poor or not so

Table 4. Efficiency of half-sib selection vs phenotypic selection

Yield characters	C1-C4 vs C0, % increase	
	Half-sib selection	Phenotypic selection
Kernel weight/plant	12.9-22.4**	12.0-12.3
100 kernel weight	3.1-12.9**	5.1-9.8*
No. of kernels of main spike	6.0-6.5*	3.1-6.1*

*and** represent significance at P=0.05 and P=0.01 level, respectively.

diversified genetic backgrounds and lack of superior parental materials. Henceforth they are getting more and more interests in population improvement and germplasm development.

Population improvement

There has been a small nation-wide network in this area aiming at the improvement for either single breeding objective or multiple, comprehensive breeding objectives by using Taigu dominant male sterile gene, *Ms2*. In addition to the recurrent selections for scab resistance mentioned before, many breeders have paid considerable attention to the improvement of salt tolerance and yield performance as well. Shanxi AAS in a study of half-sib selection vs phenotypic selection for 4 cycles indicated that the former gave a higher genetic gain than the latter (Table 4) (Wang et al 1991). Recently Institute of Crop Breeding and Cultivation, CAAS, has been successful in developing a genetic material called Aibai (dwarf dominant male sterile) which linked *Ms2* with *Rht10* tightly on the sams chromosome 4DS with 0.18 crossing-over unit. This provides a genetic marker for male sterility. The progeny of Aibai always segregates into one tall, male fertile: one dwarf, male sterile and the plant stature of them is quite different. Such a material will facilitate the use of *Ms2* gene because it can shift the time of recognition for male steriles from the normal to as early as shooting stage which will save much time and labor in roguing off the male fertiles. The dwarf male steriles serve readily as good reciever of any pollens available and can avoid the defect that ordinary *Ms2* carriers used to recieve pollens more frequently from the plants taller than themselves and make the reduction of plant stature very difficult.

Germplasm development

Presently all the major breeding units in China pay much attention to the development of superior parental stocks and regard it as an indispensable part of the breeding program. They are trying hard to develop germplasms with specifically valuable properties such as integrating disease resistance, dwarfness, earliness and high yield potential in combinations of any two or three of them, and also with multiple resistance of diseases and other stresses. For instance, Pangzhihua Municipal Agric. Res. Inst., Sichuan Province, has developed some long-spiked materials with 30 and more cm long and more than 30 spikelets; large-spiked and big-kerneled types with 20-28 cm long, 70-130

kernels per spike and 90-100 g per 1000 kernel weight; etc. These are very attractive to the development of super high-yielding varieities (Liu 1992, personal communication).

At the same time, various amphidiploids (including amphiploids), alien addition, substitution and translocation lines and other intermediate materials which are of value in genetic studies and breeding programs were developed by wheat geneticists. The following descriptions are cited mainly from a review by Li and Hao (1992) with a few supplementary notes.

Besides the man-made triticales, 29 different amphidiploids or amphiploids were developed from crossing wheats with their 13 wild relatives including *Aegilops caudata, Ae. comosa, Ae. crassa, Ae. aucheri, Ae. ovata, Ae. speltoides, Ae. tauschii, Ae. triuncialis, Ae. umbellulata, Ae. ventricosa, Agropyron elongatum, Ag. intermedium,* and *Haynaldia villosa.*

A series of alien addition, substitution and translocation lines were subsequently generated by either crossing some of the above amphidiploids or amphiploids with wheats or crossing wheats directly with the alien species followed by backcrossing. They are important steps in introgressing valuable alien genes. For example, Hao and He (1990) reported 14 alien addition lines each carrying an additional pairs of chromosomes from EE or XX genomes of *Ag. intermedium* by crossing octoploid wheat-wheatgrass hybrid with wheat (He et al 1989). Liu et al (1988) produced five addition/substitution lines each carrying one pair of V2, V3, V4 (addition)/V5 (substitution), V6 and V7 chromosomes, respectively, from *Haynaldia villosa.* Ma et al (1990) obtaines 3 addition lines of 1R, 4R and 5R through crossing octaploid triticale with wheat. Hu et al (1989) and Tao and Hu (1990) got 1B/1R, 1D/1R, 4D/4R, and 6D/6R substitution lines by crossing hexaploid triticale with wheat followed by anther culture and subsequent chromosome doubling. Li et al (1986) and Mu et al (1986) were successful in developing seven substitution lines with blue-grained marker gene transferred from 4Ag to 2D, 3A, 3D, 4D, 5A, 6A, and 7A, respectively, by crossing *Elytrigia (Agropyron) elongata* with wheat and backcrossing to wheat followed by subsequent γ-ray and fast neutron radiations. Most of these intermediate materials possess good resistance to one or more of the following diseases such as stripe rust, leaf rust, stem rust, powdery mildew, and barley yellow dwarf virus. Some of them are being used in breeding programs. For instance, Xin et al (1991) in cooperation with the scientists of CSIRO, Australia, have been successful in introgressing BYDV resistance (which has not been found in the genus *Triticum*) to several common wheat varieties from Zhong 4 [an amphiploid from wheat-*Thinopyron (Agropyron) intermedium* hybrid with 2n=56] and L1 (a French addition line from Taft 46 which is also an octoploid wheat-*Thinipyron intermedium* hybrid) through a combined efforts of *ph* gene-added chromosome engineering, somaclonal variation through tissue culture and conventional breeding.

In order to make alien gene transfer to wheat more effectively, severa important "tool" materials for genetic study, gene location and chromosome engineering have been established. For example, eight monosomic series of common wheats, ie, Jinghong 1, Zhong 7902, Fengkang 13, Abbondanza, Yangmai 1, Beijing 10, Gangmai 8 and Sumai 3 were produced by separate institutes. This would facilitate the use of aneuploid technic in genetic studies under different ecological conditions. Li et al (1986) created seven blue-grained monosomics (2D, 3D, 4D, 5A, 6A and 7A) and 6 self-fertile nullisomics (2D, 3A, 3D, 4D, 5A and 6A) after introgressing the blue-grained gene

of *Elytrigia elongata* to wheat. Xie et al (1991) furtherly developed 18 self-fertile nullisomics except 2B, 4B and 7D from extensive screenings in large population of the progenies of Abbondanza monosomic series. Li et al (1990) also established a new procedure — nullisomic backcrossing method — for rapid production of alien substitution lines. In the production of *Triticum-Aegilops* amphidiploids, Xu and Dong (1992) observed that two accessions of tetraploid wheats, namely *T. persicum* PS5 and *T. durum* DR14, have the ability to produce unreduced gametes when crossed with *Aegilops* species and this character was transmitted to the amphiploids and to their hybrids with common wheats. Fan et al (1990) were successful in incorporating *Ms2*, *kr* and *ph1b* genes into a "triplex." All these "tool" materials provide a sound and convenient basis for alien gene transfer in wheats.

Hybrid wheat

Presently 12 units are included in the national network in which (*T. timopheevi*) cytoplasmic male steriles (T-type) are emphasized although (*Ae. kotschyi*) cytoplasm (K-type), (*Ae. ventricosa*) cytoplasm (V-type) and others including CHA are also extensively studied. In the past five years considerable progress has been made: (1) General level of various characters of T-type A and B lines has been improved. Jiangsu AAS has successfully developed the first series of semidwarfs through the introduction of *Rht3* gene to ordinary T-type A lines and produced some hybrid wheats such as Ningai/R14 that combined semidwarfness, disease resistance and preharvest sprouting resistance with high yield potential pretty well. (2) Yielding capacity of some T-type R lines is greatly improved, for example, Ke 82-Hui 2 (Helongjiang), T 808/Zheng 65//#6609 (Henan), Yuan 8501 (Beijing), etc. approached or equalled the commercial varieties in yield. (3) A few set of K-type and V-type A, B, and R lines were developed in 1988 and hybrid wheats derived from them are now under yield trials. K-type and V-type male sterile lines have several advantages over T-type ones in that the former have more sources of fertility restorers (although the degree of fertility restoration has to be improved) than the latter and most present day cultivars bearing 1BL/1RS translocation are their maintainers. Unfortunately, most K-type A lines with 1BL/1RS translocation usually produce haploid plants or twin seedlings, because there is a *Pty* gene located on chomosome arm 1RS which tends to induce parthenogenesis. This can be overcome though screening. It is estimated that a frequency of haploids less than 5% will not influence hybrid seed production and yield performance of hybrid wheats. In addition to *Ae. kotschyi* and *Ae. ventricosa* cytoplasms, a few people are engaged in the development of hybrid wheats with sterile cytoplasms from *T. aestivum, Ae. squarrosa* and other alien species. (4) CHA is a prospective alternative for the utilization of heterosis. WL 84811 and Sc2503 have been proven to be excellent CHAs in use. Hebei Normal University and its partners were successful in using a local CHA in combination with physical means to produce hybrid seeds for a small-scaled hybrid wheat production in Hebei Province attaining a yield increase of 20% over the standard variety. Hybrid wheat combinations produced by CHA such as Ningai 13 × E-mai 9 from Jiangsu AAS and #1008 × Shaan 7859 from Shaanxi AAS and some others outyield the standard variety by more than 10-15%. Furthermore, a

few agricultural universities have synthesized some CHAs such as BAU 1, BAU 2 and XN8614 in the laboratory whose effectiveness is nearly equal to WL 84811. If they could be produced in a large scale hybrid wheats for commercial use will be greatly enhanced.

Finally, it is interesting to note that three institutes have independently found from off-seasoned growing of their breeding materials some photo-thermo-sensitive male sterile wheats which are male sterile under short day and low temperature conditions (at the reduction divisions of PMCs or early booting stage) but fertile under long day and warm temperature environments. They have now developed some agronomically desirable "dual lines" which can be used as either A line or B line by differential planting in time, and have also identified some hybrid combinations which showed strong heterosis in yield trials. For example, Hunan Agriculatural College developed three photo-thermo-sensitive male sterile lines designated as ES-3, ES-4 and ES-5 from a common wheat with alien pedigree which were male sterile under normal autumn planting at Changsa and fertile under summer planting at Kuming or late autumn planting at Changsa. The critical daylength and temperture for fertility transformation at Changsa are approximately 12 hr and 10°C, respectively. These photo-and thermo-sensitive male sterile lines have 100% sterile plants with 98-100% sterile florets as counted from the outer two florets of the spikelet (Ho et al 1992, unpublished). Scientists from Chongqing Crop Institute, Sichuan Province and Southwest Agric. Univ. also developed some similar "dual lines" either from the common wheat or from a derivative of K-type cytoplasmic male sterile line with slightly different responses to temperature (Tan et al 1992, unpublished, Fu and Ruan 1992). The mode of inheritance of this character and more exact responses to different regimes of daylength and temperature in different regions are now under study. If "dual lines" work well under a range of environments then the way to hybrid wheat production will be much broadened.

References

Bai GH et al 1990 Genetic analysis on resistance genes to scab extension in wheat (in Chinese) p171-177. In LH Zhu (ed) Advances in Researches on Inheritance to Diseases in Major Crops Jiangsu Sci-Tech Publ House, Nanjing.

Chen PD and Liu DJ 1986 Identification of *Haynaldia villosa* chromosomes in wheat alien addition lines p31-33. In ZS Li and MS Swaminathan (eds) Proc 1st Intern Symp Chromosome Engin in Plants Xian, China.

Dong YS et al 1990 Discovery and study on the germplasms for naturally occurring chromosome doubling of intergeneric hybrids of wheats (in Chinese) p171-176. In H Hu and HL Wang (eds) Plant Cell Engineeing and Breeding Beijing Polytech Univ Press, Beijing.

Dong YS et al 1990 Production and utilization of amphidiploids between *Triticum aestivum* and *Aegilops* species (in Chinese) p178-183 *ibid.*

Fan L et al 1990 Development and identification of Chinese Spring Ta1 kr, ph1b plants and their use on genetic transfer of desirable genes from *Aegilops* Sci China (Ser B) 33: 673-678.

Fu OX and Ruan RW 1992 Discovery of photo-thermo-sensitive KM-type cytoplasmic male sterile wheats and development of "dual line" method for hybrid wheat production (in Chinese) Chongqing Agric Sci and Tech 1992 (1): 1-4.

Hao S and He MY 1990 Chromosome engineering and wheat improvement (in Chinese) p125-131. In H Hu and HL Wang (eds) Plant Cell Engineering and breeding Beijing Polytech Univ Press, Beijing.

He MY et al 1989 The establishment of two sets of alien addition lines of wheat-wheatgrass Sci. China (Ser. B) 32: 695-705.

He ZM et al 1992 Photo-thermo-sensitive male sterile wheats (in Chinese) Hunan Agric College Unpublished data.

Hu H 1989 Chromosome engineering by anther culture p57-67. In A Mujeeb-Kazi and LA Sitch (eds) Review of Advances in Plant Biotechnology 1985-1988 2nd Intern Symp Genet Manipulation in Crops Mexico City, Mexico.

Huang TC (ed) 1992 Studies on Hybrid Wheats — the Progress, Problem and Prospect (in Chinese) Beijing Agric Univ Press, Beijing.

Jia JZ 1990 The chromosomal location of a dwarfing gene in wheat variety (*T. aestivum*) Huixian Hong (in Chinese) Scientia Agricultura Sinica 23: 90.

Jia JZ et al 1992 Studies on main dwarf genes and dwarf resources in Chinese wheat (in Chinese) Sci Agric Sin 25 (1): 1-5.

Jiang GL et al 1992 Studies on the development of scab resistance gene pool in wheat — A preliminary report on effects of population improvement in the scab resistance gene pool (in Chinese) Sci Agric Sin 25 (6): 30-37.

Li LY and Huang YG 1991 Genetic analysis of the resistance of two wheat lines to powdery mildew (in Chinese) Acta Agron Sin 17: 64-69.

Li YF and Yu YJ 1990a A diallel analysis on resistance index to scab in seven wheat cultivars (in Chinese) p 185-192. In LH Zhu (ed) Advances in Researches on Resistance to Diseases in Major Crops Jiangsu Sci-Tech Publ House, Nanjing.

Li YF and Yu YJ 1990b Monosomic analysis for scab resistance in four wheat varieties PHJZM, HHDTB, CYHM and YGFZ (in Chinese) p 193-196 *ibid.*

Li ZS 1986 Breeding of intergeneric hybridization between *Triticum* and *Agropyron* p179-180. In ZS Li and MS Swaminathan (eds) Proc 1st Intern Symp Chromosome Engin in Plants Xian, China.

Li ZS et al 1990 Nullisomic backcrossing method: a new method for breedinf alien substitution lines (in Chinese) p 107-111. In DJ Liu and JY Gai (ed) Proc Symp Theor Pract Plant Genet Nanjing.

Li ZS et al 1986 The establishment and application of blue-grained monosomics in wheat chromosome engineering Cereal Res Commun 14 (2): 133-137.

Li ZS and Hao S 1992 Chromosome engineering of wheat in China Critical Reviewsin Plant Science 10 (5): 471-485.

Liu BH and Yang L 1990 Development of Aibai (dwarf dominant male sterile) wheat and its application (in Chinese) p175-177. In DJ Liu and JY Gai (eds) Proc Symp Theor Pract Plant Genet Nanjing.

Liu DJ et al 1986 *Triticum durum-Hayaldia villosa* amphidiploid (in Chinese) Acta Agronomica Sinica 12: 155-162.

Liu DJ et al 1988 Transfer of *Haynaldia villosa* chromosomes into *Triticum aestivum* Proc 7th Int Wheat Genet Symp p355-361.

Liu GT and Xu MH 1990 Variation and inheritance of endosperm HMW glutenin sub units in common wheat (in Chinese) p154-157. In H D Mo et al (eds) Advances in Genetic Research on Quality Characters in Cereals Jiangsu Sci-Tech Publ House, Nanjing.

Ma CX and Wu ZS 1990 Genetic variation of HMW glutenin subunits in Chinese wheat varieties and inheritance in F1 (in Chinese) p 158-161 *ibid.*

Ma YS et al 1990 Production of wheat-rye addition lines (in Chinese) p205-207. In H Hu and H L Wang (eds) Plant Cell Engineering and Breeding Beijing Polytech Univ Press, Beijing.

Mao P 1992 The compositions of high molecular weight glutenin subunits of common wheat and their relationship to bread-making quality (in Chinese) Master thesis Hebei Agric Univ.

Mu SM et al 1986 Cytogenetic identification of blue-grained wheats p126-127. In ZS Li and MS Swaminathan (eds) Proc 1st Intern Symp Chromosome Engin in Plant Xian, China.

Shu WH et al 1990 Studies on the inheritance of disease resistance of wheat IV. A monosomic analysis of the rust resistant genes of wheat varieties Luqiyou and Yanstar (in Chinese) Acta Agron Sin 16: 289-297.

Tan CH et al 1992 Thermo-photo-sensitive male sterile wheats (in Chinese) Chongqing Crop Inst Unpublished data

Tao YZ and Hu H 1989 Recombination of R-D chromosomes in pollen plants cultured from hybrid of 6x Triticale × common wheat (in Chinese) Acta Genet Sin 16: 342-347.

Wang JF et al 1991 Studies on the application of Taigu dominant male sterile wheat — Effect of two recurrent selection schemes on population improvement of wheat (in Chinese) North China Agric Bull 6 (2): 1-6.

Wang GR et al 1969 A study on the bread baking quality characteristics of major improved varieties of wheat in China (in Chinese) Special Bull Inst Crop Breed and Cultiv CAAS.

Wang YP et al 1992 Genetical studies on the resistance to scab spread in wheat varieties (in Chinese) Acta Agron Sin 18: 373-379.

Wong YQ and Liu DJ 1989 Morphology, scab resistance and cytogenetics of intergenericc hybrid of *Triticum aestivum* L. with *Roegneria* c Koch (*Agropyron*) species (in Chinese) Sci Agric Sin 22 (5): 1-7.

Xin ZY et al 1991 Development of scommon wheat germplasm resistant to barley yellow dwarf virus by biotechnology Sci China (ser B) 34: 1055-1062.

Xu MH et al 1990 HMW glutenin subunit of common wheat in relation to bread-making quality (in Chinese) p162-165. In

H D Mo et al (eds) Advance in Genetic Research on Quality Characters in Cereals Jiangsu Sci-Tech Publ House, Nanjing.

Xu SJ and Dong YC 1989 Cytogenetic study on the formation of amphiploids in the F1 hybrids of *Triticum carthlicum* Nevski var. *darginicum* and *Aegilops tauschii* Cosson (in Chinese) Acta Agron Sin 15: 251-259.

Xu SJ et al 1990 Evaluation and utilization of resistance of amphidiploids between *Triticum* and *Aegilops* species to wheat diseases (in Chinese) Acta Agron Sin 16: 106-111.

Xu SJ and Dong YS 1992 Fertility and meiotic mechanisms of hybrids between chromosome autoduplication tetraploid wheats and *Aegilops* species Genome 35: 379-384.

Xue XZ et al 1991 Establishment of stable self-fertile nullisomic lines of the wheat cultivar "Abbondanza" (in Chinese) Acta Agron Sin 17: 417-423.

Yang TM et al 1992 Identification and utilization of diversitied sources of resistance to the rusts and mildew in China (to be published).

Yiao JX and Xiong EH 1990 Progress of the research in the inheritance of resistance to *Erysiphe graminis* f. sp. *tritici* (in Chinese) p236-248. In LH Zhu (ed) Advances in Researches on Resistance to Diseases in Major Crops Jiangsu Sci-Tech Publ House,Nanjing.

Yu YJ 1990 Genetic analysis for scab resistance in 4 wheat varieties, PHJEM, HHDTB, CYHM and YGFE (in Chinese) p197-205 *ibid.*

Zhang L et al 1992 The path analysis of the expansion resistance components to scab in wheat varieties (in Chinese) Sci Agri Sinica 25 (2): 47-52.

Zhang LQ et al 1990 Advance of studies on genetics of resistance to sab and its pathogens (a review) (in Chinese) p 165-169. In L H Zhu (ed) Advances in Researches on Resistance to Diseases in Major Crops Jiangsu Sci-Tech Publ House, Nanjing.

Zhang XY et al 1989 Studies on the nullisomic backcrossing procedures for producing alien substitution lines of common wheat (in Chinese) Acta Genet Sin 16: 420-429.

Zhao YM and Wang SJ 1990 The application of HMW glutenin subunits in the study of bread baking quality (in Chinese) Acta Agron Sin 16: 208-218.

Zhou CF et al 1987 On the problem of wheat breeding for scab resistance (in Chinese) Sci Agric Sin 20 (2); 19-25.

Zhuang QS 1983 Basic experiences in the choice of parents in wheat breeding (in Chinese) p 294-332. In S P King (ed) Chinese Wheat Varieties and their Pedigrees. Agricultural Publishing House, Beijing.

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II. Articles

Genes for susceptibility to *Ustilago nuda tritici* in bread wheat

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Summary

Genetics of resistance in Chinese Spring (C. S.) to an Indian collection of *Ustilago nuda tritici* was studied by using ditelosomic and monosomic lines. Chinese Spring was susceptible at embryonic stage and resistant at the adult plant stage. At least three hemizygous effective genes, causing susceptibility to *U. nuda tririci* at embryonic stage in C. S., were located one each on the long arm of chrosome 2A, 3A and 6B. Nullisomic condition for any one of the three genes imparted resistance at the embryonic stage to loose smut. This is the first study on genetics of resistance to the smut at embryonic stage and also the first report on nulli-condition resulting into resistance against loose smut.

Introduction

Loose smut (caused by *Ustilago nuda tritici*) is one of the serious diseases of wheat in northern India but very little work has been done on genetics and breeding of resistant wheat varieties against this smut. The nature of fungal infection, difficulty in creating artificial epiphytotic conditions and appearance of disease in the next generation are some of the major causes responsible for lack of genetic analysis of resistance to the smut and in hampering breeding of resistant varieties against it. Though disease can be contained by seed dressing with fungicides, breeding resistant varieties remains the most economic method to reduce losses.

In order to undertake genetic analysis of loose smut, it is important to note that floral infection is the rule for the transmission of this disease. The smut spores (teliospores) are blown to the flowers where they germinate and ultimately the mycelium infects embryo. This mycelium remains dormant in the seed. Next season when seed germinates the mycelium gets activated and grows with the shoot tip and causes disease at the time of ear development. In the present experiment, genetics of resistance to the disease has been studied at embryonic and adult plant stages which is being reported in this paper.

Materials and methods

The plant material used in the present study comprised of a resistant wheat variety, Chinese Spring and its aneuploid lines, viz., monosomics and ditelosomics. Susceptible varieties namely, NP4,

Timagalen and Sonalika were used as checks. The inoculum used was a mixture of unidentified isolates (collection from Northern India) maintained on a susceptible cultivar, Sonalika.

Chinese Spring, its monosomic lines, ditelosomic lines and susceptible cultivars including Sonalika (maintainer for inoculum) were inoculated in 1988–1989 with the smut spores collected from susceptible heads of Sonalika by the method described by Agrawal et al (1963) and Mathur and Kohli (1963). Six ear heads were inoculated repeatedly at an interval of 48 h till completion of flowering. On maturity the inoculated seeds obtained from each ear head were divided into two lots. One lot of seeds was used for detecting loose smut mycelium in the embryo by "whole embryo count test" and the other lot of seeds was sown in the field during 1989–90 to study disease reaction at the adult plant stage.

Ten dried inoculated seeds from each ear head were tested for embryo infection by whole embryo count test as suggesged by Mortan (1961). The processed embryos were examined under Wild Stereobinocular Microscope at 12, 25 and 50 × magnifications. The presence of blue stained knotted mycelium in the embryo indicated the loose smut infection.

Results and discussion

Results of inoculation of experimental material are summarised in Table 1 and 2. NP4, Timagalen and Sonalika showed very good infection to the embryos and so also expression of the disease at the adult plant stage (Table 1).

Chinese Spring exhibited embryonic infection but the disease was not expressed. Reaction of ditelosomic lines (Table 2) revealed that susceptibility was determined by three genes present on the long arm of chromosomes 2A, 3A and 6B.

The absence of any one of the genes resulted in resistance of the embryo to the fungus strain used. Monosomic 2A, 3A and 6B (Table 2) behaved exactly like the disomic. Obviously, the genes are hemizygous effective for susceptibility but nullisomic condition as caused in ditelosomic lines imparted resistance. It can thus be suggested that susceptibility of embryo infection in Chinese Spring against the inoculum used was controlled by at least three genes hemizygous effective to cause susceptibility but in nulli-condition resulted in resistance of the embryo against the fungus.

This is for the first time that genes have been identified for susceptibility of embryo to infection of the smut. Since disease has not appeared in the adult plant, it is quite evident that the genes for infection and expression of the disease are different. So far this distinction has not been made.

In the present study all the aneuploid lines of Chinese Spring have shown adult plant resistance (Table 2). Effect of absence of a single gene at a time did not cause any smutted ear heads. This study shows that inheritance of disease expression is complex in Chinese Spring. It is, therefore, suggested that there may be at least two or more genes (may be interacting) conditioning resistance to disease expression. However, the exact picture can be drawn by conventional genetic analysis.

Chinese Spring with respect to the Indian inoculum used was found to be susceptible to

Table 1. Susceptibility of embryo and adult plant of some varieties to *U. nuda tritici.*

Variety	Embryo test			Field test		
	No. of embryos examined	No. of infected embryos	Embryo infection %	No. of adult plants observed	No. of diseased plants	Disease incidence %
Chinese Spring	60	34	56.66	50	00	00.00
Timagalen	56	46	82.15	67	53	79.10
NP4	48	44	91.66	46	41	89.08
Sonalika	60	53	88.33	54	46	83.33

Table 2. Susceptibility of embryo and adult plant of Chinese Spring aneuploids to *U. nuda tritici.*

C. S. aneu-ploids	Embryo test	Field test	C. S. aneu-ploids	Embryo test	Field test	C. S. aneu-ploids	Embryo test	Field test
Monosomics:								
2A	S	R	2B	S	R	1D	S	R
3A	S	R	3B	S	R	2D	S	R
4A	S	R	4B	S	R	5D	S	R
5A	S	R	5B	S	R	7D	S	R
7A	S	R	6B	S	R			
Ditelosomics:								
1AS	S	R	1BS	S	R	1DS	S	R
1AL	S	R	1BL	S	R	1DL	S	R
2AS*	R	R	2BS	S	R	2DS	S	R
2AL	S	R	2BL	S	R	2DL	S	R
3AS*	R	R	3BL	S	R	3DS	S	R
3AL	S	R	4BS	S	R	3DL	S	R
4AL	S	R	4BL	S	R	4DS	S	R
5AL	S	R	5BL	S	R	5DL	S	R
6AS	S	R	6BS*	R	R	6DS	S	R
6AL	S	R	6BL	S	R	6DL	S	R
7AS	S	R	7BS	S	R	7DS	S	R
7AL	S	R	7BS	S	R			

*: critical lines S: susceptible R: resistant

infection but resistance to the expression of the disease. This is in contrast to the reports from Poland (Heinrich 1970) and Ireland (Dhitaphichit et al 1989) where it gets infected and develops disease. Obviously, the races in India and Poland or Ireland are expected to be different. Infection and expression of disease are genetically governed and hence this differential behaviour of Chinese Spring can be used to distinguish pathogenic races. So far, pathogenic races are differentiated on the basis of disease expression on differential cultivars.

Location of genes on specific chromosome arms for conferring susceptibility at embryonic level should prove to be of use in identifying other markers together with RFLP markers. Such a study can be of great use to wheat breeders for incorporating resistance to this disease which otherwise is difficult to deal with. In addition, for other characters, Chinese Spring with a number of factors determining resistance to the smut can also be more successfully used in hybridization programme.

References

Agrawal RK, Rao MV and Joshi AB (1963) Inheritance of loose smut resistance in an inter-varietal cross of *Triticum aestivum* L. Ind J Genet and Pl Breed 23 (2): 135–140.

Dhitaphichit P, Jones P and Keane EM (1989) Nuclear and cytoplasmic gene control of resistance to loose smut (*Ustilago tritici* (Pers.) Rostr.) in wheat (*Triticum aestivum*) Theor and Appl Genet 78 (6): 897–903.

Heinrich J (1970) Studies of genetic resistance of wheat to loose smut [*Ustilago tritici* (Pers.) Rostr]. Hodowla Roslin Aklimatyzaeja i Nasiennictwo 14 (5): 393–404.

Mathur HC and Kohli SP (1963) Inheritance of resistance to loose smut in *Triticum aestivum.* Ind J Genet and Pl Breed 23 (3): 256–258.

Mortan DJ (1961) Trypan blue and boiling lactophenol for staining and clearing barley tissues infected with *Ustilago nuda.* Phytopathol 51: 27–29.

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Transfer of yellow rust resistance to Unnath Kalyan Sona

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Summary

A yellow rust resistant gene was transferred from three Indian wheat cultivars BW 1052, BW 1109, HUWL 9032 into a hexaploid wheat cultivar Unnath Kalyan Sona after fifth backcrossing. The concurrent restoration of stem rust resistance in Unnath Kalyan Sona was attributed to the transfer of an undesignated stem rust resistance gene from the donor parents to the recipient parent. This gene was suggested to be linked with the undesignated *Yr* gene which was transferred.

Introduction

Unnath Kalyan Sona, an improved variety of Kalyan Sona developed by Kochumadhavan et al (1988), was resistant to stem and leaf rusts under the conditions of Wellington in South India. This variety possesses *Sr24 + Lr24*, a linked gene complex derived from an Australian wheat cultivar TR 380-14* 7/3 Ag 14. However, this variety was found susceptible to stem rust early in the 1990's at Wellington. The variety was also found highly susceptible to yellow rust. Therefore, to the background of this wheat cultivar, an attempt was made to transfer an undesignated yellow rust resistant gene from three hexaploid Indian wheat cultivars.

Materials and methods

Crosses between Unnath Kalyan Sona and three Indian wheat cultivars, namely, BW 1052, BW 1109 and HUWL 9032, which carry the undesignated yellow rust resistant genes, were made at Regional Station, The Directorate of Wheat research, Wellington, The Nilgiris, South India. The place is a 'hot spot' of wheat diseases, where all the three rusts are prevalent throughout the year under natural conditions. The resistant hybrids were successively backcrossed to recurent parent five times.

Results and discussion

Crosses between Unnath Kalyan Sona and the three Indian wheat cultivars were highly successful. Screening for rust resistance were made under natural conditions. The lines developed after five successive backcrosses showed completely resistant to yellow rust (Table 1). The lines also

Table 1. Rust reactions in Unnath Kalyan Sona and its newly constituted lines with three Indian wheat cultivars.

Parents/Cross	Rust reactions		
	Stem rust	Leaf rust	Yellow rust
Unnath Kalyan Sona (UKS)	40S	F	40-60S
BW 1052	TR	80S	F
BW 1109	TR	60S	F
HUWL 9032	TR	60S	F
UKS/BW 1052	TR	F	F
UKS/BW 1109	TR	F	F
UKS/HUWL 9032	TR	F	F

S=susceptible; R=resistant; T=trace infection; F=free.

exhibited complete resistance to stem rust, although the original Unnath Kalyan Sona was susceptible to the same rust (Table 1).

It is not known what caused the breakdown of *Sr24* gene in Unnath Kalyan Sona, despite the fact that the linked leaf rust resistant gene *Lr24* is still effective. Crossing over between the two genes was ruled out since there is close linkage between them (McIntosh 1988). Susceptability of a resistant line may be due to spread of new races with matching pathogenicity. However, under the same experimental conditions at Wellington, the only stem rust race 40-1 (62G29-1) that knocks down *Sr24* did not show any symptoms on the original Australian hexaploid wheat donor from which the linked gene complex *Sr24* + *Lr24* was derived. It has also been shown that under Wellington conditions, the leaf rust resistant gene *Lr24* is epistatic over *Sr24*. Therefore, only resistance showed by *Lr24* will be seen (Kochumadhavan et al 1988). However, the lines developed after the backcrosses showed completely resistance to stem rust and leaf rust along with yellow rust resistance.

Restoration of stem rust resistance in Unnath Kalyan Sona after crossing with three Indian Wheat cultivars (resistant to yellow and stem rust at Wellington) was probably due to transfer of an additional unidentified and undesignated stem rust resistant gene from donor parents to the recepient parent, and this gene is combination with *Sr24* provided the durable stem rust resistance in the newly developed lines. It was already reported that rust resistance provided by single genes is not completely effective and that durable rust resistance is provided only by combination of a few resistant genes (Roelfs 1988, McIntosh 1988, Roelfs et al 1992). Singh and McIntosh (1986) reported that durable stem rust resistance in wheat cultivar Kenya Plume is due to eight genes. Since the constituted lines developed resistance simultaneously to yellow rust and stem rust, the undesignated yellow rust resistant gene and undesignated stem rust resistant genes might be linked to each other.

References

Kochumadhavan K, Tomar SMS and Nambisan PNN (1988) Transfer of rust resistance genes into commercial cultivars of wheat. Annual Wheat News Letter 34: 54-55.

McIntosh RA (1988) The role of specific genes in breeding for durable stem rust resistance in wheat and triticale. In: Breeding Strategies for resistance to the rusts of wheat (eds NW Simmonds and S Rajaram), CIMMYT Report, pp 1-9.

Roelfs AP (1988) Resistance to leaf and stem rusts in wheat. In: Breeding strategies for resistance to the rusts of wheat (eds NW Simmonds and S Rajaram), CIMMYT Report, pp 10-22.

Roelfs AP, Singh RP and Saari EE (1992) Rust diseases of wheat. Concepts and methods of disease management. CIMMYT Report, p 81.

Singh RP and McIntosh RA (1986) Genetics of resistance to *Puccinia graminis tritici* and *Puccinia recondita tritici* in Kenya Plume wheat. Euphytica 35: 245-256.

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Rust resistance and chromosome pairing in *Triticum* × *Aegilops* crosses

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The wild relatives of wheat have served as a good source of resistance to wheat rusts (Sharma and Gill 1983, Knott 1989). It has been observed that *Aegilops* species with C, U and M genomes are a good source of resistance to leaf and stripe rusts (Dhaliwal et al 1991). Keeping this in view, leaf rust resistant accessions of *Aegilops triuncialis* (UC) and *Ae. ovata* (UM) were crossed with a hexaploid spring wheat cultivar WL711. The transfer of desirable characters from the donor species depends upon the extent of homoeologous chromosome pairing between the genomes of two species. This article reports the rust resistance and meiotic chromosome pairing in two intergeneric crosses.

Triticum aestivum cv. WL711 (a widely adapted and agronomically superior Indian spring wheat cultivar) was crossed as female with *Ae. triuncialis* (Acc No. 3549) and *Ae. ovata* (Acc No. 3547) during 1990-91. The rust reactions of the parents are given in Table 1. The F_1's of the two crosses were planted at Ludhiana in October 1991. The spikes of hybrid plants were fixed in 6:3:1, Carnoy's solution, for analysis of meiosis. The spikes were removed to 70% ethanol and the anthers were dissected and squashed in acetocarmine. Meiotic metaphase of more than 50 PMC's were scored for chromosome pairing in each cross.

The number of florets pollinated for the two crosses WL711 × *Ae. triuncialis* and WL711 × *Ae. ovata* were 40 and 100, respectively, and the per cent seed set 25 and 9, respectively. Ozgen (1983) found 32% crossability in the cross *T. aestivum* × *Ae. triuncialis* and Claesson et al (1990) obtained 2% seed set in a *T. aestivum* × *Ae. ovata* cross. However, Claesson et al (1990) observed much higher crossability with *Ae. triuncialis* (67%) and *Ae. ovata* (40%) when *T. aestivum* was used as male parent rather than the female parent.

The percentage germination of the crossed seeds for the crosses with *Ae. triuncialis* and *Ae. ovata* was 50.0 and 44.4, respectively. Ozgen (1983) had obtained 26.8 to 100 per cent germination in the crosses of seven *Aegilops* species with *T. aestivum.* The hybrid plants of both the intergeneric crosses were free from leaf rust whereas leaf rust score was 90S on the hexaploid parent WL711 (Table 1). This indicated that the leaf rust resistance gene(s) from both the *Aegilops* species were dominant in the WL711 background. The accessions of *Ae. triuncialis* and *Ae. ovata* used in the intergeneric crosses were susceptible to the M race of yellow rust (Table 1). These lines were also susceptible to yellow rust under field conditions at Gurdaspur. However, F_1 hybrid of WL711× *Ae. ovata* (Acc. 3547) was resistant to yellow rust. The hybrid plants of both the crosses were completely male and highly female sterile. The pollination of 50 spikes of each cross with pollen from WL711, however, gave 11 backcross seeds in the cross WL711 × *Ae. triuncialis* and 12 seeds in the cross WL711 × *Ae. ovata.*

There was low meiotic pairing in both the crosses (Table 2) with only rod bivalents and no

Table 1. Reactions of *T. aestivum* cv. WL711, one accession each of *Ae. triuncialis* and *Ae. ovata* and their crosses with WL711 to the two rusts

Parent	Seedling reaction to pathotype*					Field Reaction 1990-91 Gurd-aspur	1990-91 Lud-hiana	1991-92 Gurd-aspur	1991-92 Lud-hiana
Leaf rust									
	77	77A-1	77-1	77-2	104-1				
Ae. triuncialis (Acc. 3549)	0;2	0;	0;	0;	0	F	F	F	F
Ae. ovata (Acc. 3547)	1-N1	0;N1	0;1-	0;1	2	—	F	F	F
T. aestivum cv. WL711	3+4–	3+4–	33+	3+4–	—	100S	90S	80S	90S
F WL711 × Acc 3549	—	—	—	—	—	—	—	—	F
F WL711 × Acc 3547	—	—	—	—	—	—	—	—	F
Yellow rust									
	K	38	M						
Ae. triuncialis (Acc 3549)	0;	0;	3			F	—	40S	F
Ae. ovata (Acc 3547)	0;	0;	3+			—	—	20S	F
T. aestivum cv. WL711	4	4	0;			80S	40S	60S	40S
F_1 WL711 × Acc 3549	—	—	—	—	—	—	—	—	20S
F_1 WL711 × Acc 3547	—	—	—	—	—	—	—	—	F

F: free from rust, S: susceptible, N: Severe necrosis.

*0; to 2: Resistant; 3 to 4: Susceptible [Seedling reactions recorded on 0; to 4 scale, + or – were added to indicate slightly higher (more susceptible) or lower reaction than the mean of the class. The range of infection types produced on different plants by a given race in presented without spaces or dashes between the figures.

Table 2. Meiotic chromosome pairing in F_1 of the intergeneric crosses of *T. aestivum* cv. WL711 with two *Aegilops* species

Cross	Chromosome number	No. of PMCs	Chromosome pairing I	II	III
WL711 × *Ae. triuncialis* (Acc 3549)	35	51	31.88 (22-35)*	1.53 (0-5)	0.02 (0-1)
WL711 × *Ae. ovata* (Acc 3547)	35	57	33.98 (29-35)	0.51 (0-3)	0.00 (0)

*Figures in parentheses indicate the range.

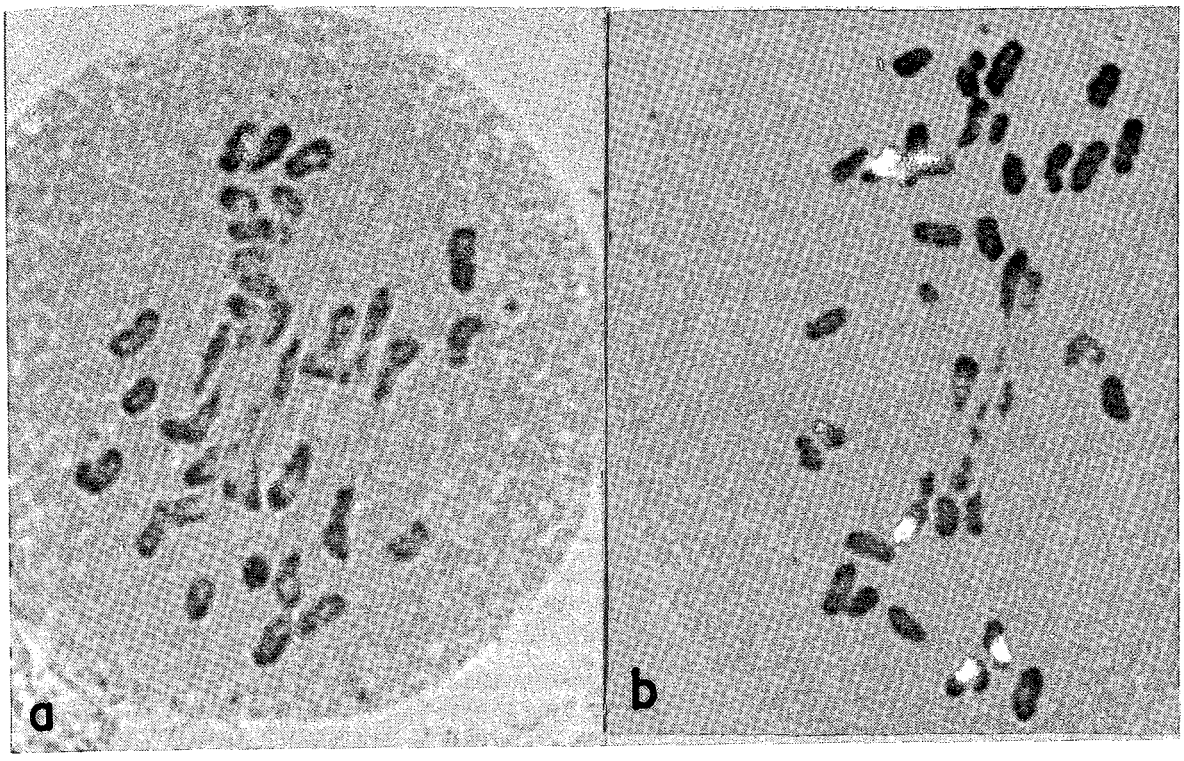

Fig. 1. Chromosome pairing in intergeneric *Triticum* × *Aegilops* crosses: a) Chromosome pairing in a PMC of *T. aestivum* × *Ae. triuncialis* hybrid showing 22I+5II+1III. (b) Chromosome pairing in a PMC of *T. aestivum* × *Ae. ovata* hybrid showing 29I+3II.

ring bivalents or multivalents except that one trivalent was observed in the cross WL711 × *Ae. triuncialis* (Fig. 1). In the previous studies also (Bochev and Ganeva 1981, Abu Bakar and Kimber 1982, Claesson et al 1990) only rod bivalents were observed though ring bivalents and multivalents were observed in very low frequency. In the present study, the average number of bivalents was 1.53 in the cross *T. aestivum* × *Ae. triuncialis.* However, the average frequency of rod bivalents was 5.21 in the study by Claesson et al (1990) whereas it ranged from 1.87 to 5.06 in the study by Bochev and Ganeva (1981). According to Claesson et al (1990) higher frequency of pairing in some studies may be attributed to the presence of pairing promotors or genes suppressing the *Ph* gene in some of the populations of *Ae. triuncialis.* The average number of rod bivalents in the cross *T. aestivum* × *Ae. ovata* was 0.51 only. The results are comparable with that of Abu Bakar and Kimber (1982), McGuire and Dvorak (1982) and Claesson et al (1990). The low pairing may indicate that the *Ph* gene is not suppressed by *Ae. ovata* genomes.

The low homoeologous pairing in the two crosses shows that the chances of transfer of leaf rust resistance to *T. aestivum* are low. Since the genes suppressing *Ph* gene are absent in the two donor species, there is need to use a system to induce homoeologous pairing to facilitate transfer of rust resistance from these accessions. Keeping this in view, monosomic 5B plants of *T. aestivum* cv. Chinese Spring have been crossed with rust resistant accessions of the two *Aegilops* species.

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References

Abu Bakar M and Kimber G (1982) Chromosome pairing regulators in the former genus *Aegilops*. Z Pflanzenzucht 89: 130-138.

Bochev B and Ganeva G (1981) Cytological studies on alloplasmatic intergeneric hybrids. In: Vtora Nats Kon ferentsiya Tsito genetika sofia, Bulgaria; Bulgarian Academy of Sciences, pp 227-233.

Claesson L, Kotimaki M and Von Bothmer R (1990) Crossability and chromosome pairing in some interspecific *Triticum* hybrids. Hereditas 112: 49-55.

Dhaliwal H S, Harjit Singh, Gupta S, Bagga P S and Gill K S (1991) Evaluation of *Aegilops* and wild *triticum* species for resistance to leaf rust (*Puccinia recondita* f. sp. *tritici*) of wheat. Int J Tropic Agr. 9: 118-122.

Knott DR (1989) The wheat rusts-breeding for resistance. Springer-Verlag, Berlin, Heidelberg, New York pp 162-181.

Mc Guire P and Dvorak J (1982) Genetic regulations of heterogenetic chromosome pairing in polyploid species of the genus *Triticum* sensulato. Can J Genet Cytol 24: 57-82.

Ozgen M (1983) Hybrid seed set in wheat × *Aegilops* crosses. Wheat Inf Serv 56: 9-11.

Sharma HC and Gill BS (1983) Current Status of wide hybridization in wheat. Euphytica 32: 17-31.

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Studies on enzymatic activities and leakage of solutes from wheat leaf during an early course of differential brown rust pathogenesis

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Summary

During progressive brown rust pathogenesis of wheat leaf (cv. WL 711) both susceptible (race 77) and resistant (race 63) -interactions increased the activity of polyphenoloxidase initially upto 36 h stage after inoculation. But later on the activity declined in general towards 72 h stage of infection, however, the decline in activity was comparatively more in case of the susceptible-interaction due to race 77. The catalase activity was also found to be increased at the initial phases of the infection in both cases but the susceptible-interaction reflected comparatively higher activity profile. However, during later phases of infection the activity declined in the susceptible-interaction whereas it continued to increase in the resistant one. Moreover, the leakage of amino acids was noted to be comparatively more in the susceptible-interaction while that of UV-absorbing materials was lesser during this mutual interacting host-pathogen complexity.

Introduction

The changes in enzymatic activities of peroxidase and phenolase or phenol oxidases have been multifacially reported in relation to patho-physiological processes during infection of plants (Farkas and Kiraly 1958, Seevers et al 1971, Johnson and Cunningham 1972, Bell 1981, Saini et al 1988). Along with enzymatic activities the changes in membrane permeability are thought to be the initial indicative results of host pathogen infection process (Hoppe and Heitefuss 1974, Wheeler 1978, Saini et al 1990). Considering this important aspect of metabolic activities during plant infection process the present study was undertaken with an objective to record changes in enzymatic activities of polyphenoloxidase and catalase along with observing of leakage pattern of amino acids and UV-absorbing materials from wheat leaves. This was studied not merely in relation to general infection process but also in relation to differential (resistant-race 63 and susceptible-race 77) rust race-interaction during an early course of brown rust pathogenesis upon *Puccinia recondita* inoculation.

Materials and methods

Present study was carried out on wheat (*Triticum aestivum* L.) cv WL 711 interacting differentailly when inoculated with two races of the brown rust pathogen, *Puccinia recondita*, viz. race 63-the resistant interaction (rare uredosori formation) and race 77-the susceptible interaction (heavy uredosori formation). The winter wheat crop was grown and maintained in the screen house in earthen pots in the mixture of sand and farm yard manure in ratio of 3:1 with soil pH 8.3. The pathogen races were multiplied, maintained and used to inoculate second and third leaf of 25 day old plants. Plant leaves were sampled for analysis at 0 h (just before inoculation) 12 h, 24 h, 36 h, 48 h and 72 h after inoculation as per our earlier report (Saini et al 1988).

For estimating enzyme activities 1 g of leaf tissue sample was extracted by homogenizing in 10 ml of cold 0.1 M phosphate bufer, pH 7.0 containing 1 mM cysteine hydrochloride and 0.1% ascorbic acid in a chilled pestle mortar using acid washed sand as an abrasive. The homogenate was filtered through four layers of cheese cloth and subsequently the filtrate was centrifuged at 15,000 g for 20 min in a refrigerated centrifuge at 4°C. The supernatant obtained was used for enzyme activity assays.

Polyphenoloxidase (EC 1.10.3.2) was assayed following the method of Taneja and Sachar (1974). The reaction mixture contained 2.0 ml of 1% catechol solution as substrate, 0.2 ml of enzyme extract and rest of 0.05 M phosphate buffer pH 6.6 in a final volume of 4 ml. Boiled enzyme extracts served as the control. The change recorded at 430 nm was expressed for enzyme activity as change in absorbance per mg of protein per hour. For estimation of catalase (EC 1.11.1.6) activity the assay method of Beers and Sizer (1962) was followed. During activity assay the sample cuvette contained in a final volume of 3.0 ml; 0.1 ml of 0.2 tissue extract, 0.5 ml of 0.2 M sodium phosphate buffer pH 7.6, 0.3 ml of hydrogen peroxide and rest of distilled water. The enzyme activity was expressed as the change in absorbance per min per mg protein when measured at 240 nm.

For estimation of leakage of amino acids and UV-absorbing materials equal number of leaves (32) weighing almost equal (4.0 g) were taken. Each leaf was cut into four equal pieces and were put into 100 ml of deionized water in a 250 ml capacity conical flask and were shaken at 70 strokes per min for 8 hours in a water bath at 37°C. The leaves were then filtered out and in the filtrate the concentration of amino acids was determined as glycine equivalents by taking 0.5 ml of the filtrate as per the method of Barnett and Naylor (1966). For UV-absorbing materials the O.D of the filtrate was read at 270 nm.

Results and discussion

The enzymatic activities (Table 1) of polyphenoloxidase and catalase was found to be increased as a result of progressive rust infection upto 36 h in both susceptible (race 77) and resistant (race 63) - interactions. However the increase was more in susceptible-interaction due to race 77. Toward later stages (ie at 48 h and 72 h), polyphenoloxidase activity declined which was found to be more

Table 1. Enzymatic activities of polyphenoloxidase and catalase in wheat leaves inoculated with *Puccinia recondita.*

Hours after inoculation	Resistant (race 63)-interaction		Susceptible (race 77)-interaction	
	Polyphenoloxidase activity	Catalase activity	Polyphenoloxidase activity	Catalase activity
0	43.99 ± 0.98	10.44 ± 0.29	43.99 ± 0.98	10.44 ± 0.29
12	42.00 ± 1.96	11.35 ± 0.27	90.61 ± 1.52	15.05 ± 0.31
24	117.45 ± 1.06	14.06 ± 0.69	133.79 ± 2.21	20.21 ± 0.68
36	144.51 ± 7.13	16.22 ± 0.29	140.80 ± 1.78	22.80 ± 0.99
48	106.23 ± 3.67	14.62 ± 0.31	91.75 ± 2.23	17.27 ± 0.14
72	118.70 ± 2.29	18.70 ± 0.52	76.27 ± 0.98	18.62 ± 0.71

in the susceptible-interaction. The activity of catalse also declined toward 48 h stage but again reflected an increasing trend toward final stage of 72 h. The overall activity profile remained higher in case of race 77-interaction as shown in Table 1.

Higher levels of polyphenoloxidase activity in resistant-interaction particularly toward later stages of infection account for such pathophysiological conditions due for easier disease progression and its establishment. As the activity of this enzyme in diseased or wounded tissues has been shown to be accompanied by enhanced levels of phenolics which have been thought to be metabolically implicated in disease resitance phenomenon (Farkas and Kiraly 1958, Farkas and Kiraly 1962, Kosuge 1962, Rohringer and Samborski 1967, Bell 1981, Goodman et al 1986). The higher activity profile of catalase in the susceptible-interaction signifies the differential race pathogenesis as the increased levels of catalase endangered by virulent isolets have been shown to reduce the efficacy of natural defence of the bean plants against *Pseudomonas phaseolicola* through supression of peroxidase activity by destroying its substrate hydrogen peroxide. Thus creating a redox potential environment believed to favour the maintenance of phenolics in their reduced state which are considered less active as antimicrobial substances than their quinone forms (Rudolph and Stahmann 1964). In general occurence of the lowered activity of polyphenoloxidase and the elevated activity of catalase in the susceptible-interaction due to race 77 and that too particularly toward later stages of progressive rust infection characterizes the general nature of infection in this system.

Comparatively less leakage of amino acids in case of resistant-interaction (Table 2) may be due to immediate defensive host response toward the pathogen by trying to make less available the metabolites for the growth of the pathogen or may be due to such related changes as result of attempted infection. Alternatively, the comparatively increased leakage in case of susceptible-interaction may either be due to adaptive cointeraction or due to disintegration of the host cellular membranes (Saini et al 1989, Saini et al 1990). The higher losses of the metabolites particularly at later stage of *Uromyces* infection of the *Phaseolus* leaves might be due to presence of fungal mycelium and spores (Hoppe and Heitefuss 1974). The decreased leakage of UV-absorbing materials through successive stages of infection in susceptible-interaction (Table 2) probably be

Table 2. Leakage pattern of amino acids and UV-absorbing materials from wheat leaves inoculated with *Puccinia recondita.*

Hours after inoculation	Resistant (race 63)-interaction		Susceptible (race 77)-interaction	
	Amino acids leaked[1)]	UV-absorbing materials leaked[2)]	Amino acids leaked[1)]	UV-absorbing materials leaked[2)]
0	1.99 ± 0.07	0.351 ± 0.004	1.99 ± 0.07	0.351 ± 0.004
12	0.49 ± 0.01	0.312 ± 0.002	1.54 ± 0.03	0.321 ± 0.002
24	1.78 ± 0.02	0.297 ± 0.003	2.71 ± 0.02	0.306 ± 0.002
36	1.50 ± 0.20	0.391 ± 0.001	1.61 ± 0.03	0.312 ± 0.003
48	2.15 ± 0.23	0.331 ± 0.002	3.67 ± 0.04	0.325 ± 0.004
72	1.90 ± 0.06	0.321 ± 0.002	3.55 ± 0.03	0.313 ± 0.002

1) mg/g f wt. 2) optical density at 270 nm.

taken for their degradation first and then consumption of such substances for anabolic purpose by the growing pathogen to make them less available to leak. The physiological changes during progressive rust infection signifies at least partial correlations to susceptible and resistant pathogenesis of wheat leaves during its early course upto 72 h stage.

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References

Barnett NM and Naylor AW (1966) Amino acids and protein metabolism in bermuda grass during water stress. Plant Physiol 47: 1222-1230.

Beers RF and Sizer IW (1952) A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J Biol Chem 195: 133-140.

Bell AA (1981) Biochemical mechanism of disease resistance. Ann. Rev. Plant Physiol. 32: 21-81.

Farkas GL and Kiraly Z (1958) Enzymological aspects of plant diseases. I. Oxidative enzymes. Phytopath Z 13: 251-272.

Farkas GL and Kiraly Z (1962) Role of phenolic compounds in the physiology of plant diseases and disease resistance. Phytopath Z 44: 8-150.

Goodman RN, Kiraly Z and Wood KR (1986) Secondary metabolites. In: The biochemistry and physiology of plant disease. (Ed.) Kiraly Z and Wood KR pp 211-214. University of Missouri Press, Missouri.

Hoppe HH and Heitefuss R (1974) Permeability and membrane lipid metabolism of *Phaseolus vulgaris* infected with

Uromyces phaseoli. I. Changes in efflux of cell constituent. Physiol Plant Pathol 4: 5-9.

Johnson LB and Cunningham BA (1972) Peroxidase activity in healthy and rust infected wheat leaves. Phytochem 11: 547-551.

Kosuge T (1969) The role of phenolics in host response to infection. Ann Rev Phytopath 7: 195-222.

Rohringer R and Samborski DJ (1967) Aromatic compounds in the host-parasite interaction. Ann Rev Phytopath 5: 77-86.

Rudolph K and Stahmann MA (1964) Interaction of peroxidases and catalases between *Phaseolus vulgaris* and *Pseudomonas phaseolicola* (halo blight of bean). Nature 204: 474-475.

Saini RS, Chawla HKL and Wagle DS (1988) Leakage of electrolytes and phenols and altered activity profile of peroxidase and its isoenzymes from wheat leaves inoculated with *Puccinia recondita.* Biol Plant 30: 387-392.

Saini RS, Chawla HKL and Wagle DS (1989) Lipoxygenase activity, total lipid content and leaf mass leaching pattern of wheat leaves inoculated with brown rust, *Puccinia recondita.* Biol Plant 31: 207-212.

Saini RS, Chawla HKL and Wagle DS (1990) Catabolic activity of two phosphoric diester hydrolases in wheat leaves inoculated with brown rust, *Puccinia recondita.* Biol Plant 32: 313-318.

Seevers PM, Daly JM and Catedral FF (1971) The role of peroxidase isoenzymes in resistance of wheat stem rust disease. Plant Physiol 48: 353-360.

Taneja SR and Sachar RC (1974) Induction of polyphenoloxidase in germinating wheat seeds. Phytochemistry 13: 2695-2702.

Wheeler H (1978) Disease alteration in permeability and membranes. In Plant disease: An advanced treatise. Ed Horsfall, JG and Cowling EB Vol. III pp 327-347, Academic Press Inc, New York.

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Effect of Septoria nodorum blotch on yield and size of processed wheat seed

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Needs in wheat, a major bread cereal, have conditioned the intensification of its production by introducing intensive cultural practices. Such intensive wheat growing is creating problems in seed production because of many diseases that directly or indirectly effect sanitary seed condition. Detrimental effects of fungus *Leptosphaeria nodorum* should be underlined, which cause septoria nodorum glume blotch and attacks all above-ground parts of a plant including seeds through spikes.

The effects of Septoria nodorum blotch in seed production of wheat is manifested not only in lower yield due to reduced photosynthesis, but also in poorer quality of the seeds—primarily its sanitary condition and the size. Such problems encountered in seed production are the main reason why we decided to investigate the effects of Septoria nodorum blotch on yield, size and sanitary condition of the produced seed.

Materials and methods

Field trial experiments were set following block design in randomized blocks with five replications with the plot size of 10 m^2 using a variety "Super Zlatna" for the treatments, shown as follows, at Botinec for two years, 1989 and 1990.

1. Natural infection — plots exposed to natural conditions of growing (no artificial infection or fungicide application for disease control)
2. Artificial infection — plots exposed to natural conditions of growing with artificial infection of a population of isolates of *Leptosphaeria nodorum* fungus
3. Fungicide application — plots exposed to natural conditions of growing with artificial infection of a population of *Leptosphaeria nodorum* and one treatment of a fungicide

All field trials were carried out under conditions of artificial infection made at two intervals with collected, selected and tested isolates of *Leptosphaeria nodorum* from Croatia region. Artificial infection was made on 8th, 15th and 22nd of May, 1989 and 15th and 22nd of May, 1990. First application was made with a population of selected isolates at flag-leaf stage (47 of Zadoks scale), and repeated at full heading stage (59 of Zadoks scale) (Zadoks et al 1974, Eyal et al 1987). One treatment of a fungicide was applied with a backpack sprayer on 9th of May, 1989 and 15th of May, 1990 at the growth stage of 51 (early heading stage).

For processing we used the line "Kamas" (processing machine) that has screens with perforations of 2.5 mm. Calibration was made with an average seed sample of 1 kg from each treatment on a calibrator with perforations of 2.2 mm and 2.8 mm (Strona and Uboženko 1970).

Deep freezing blotter method was used for determining the percentage of infection of

Leptosphaeria nodorum into kernels (Mathur and Lee Lilvia 1978).

Results and discussion

Yield in each treatment is presented in Table 1. The great effect of Septoria nodorum blotch was shown on the yields of natural seeds with reduction in 37% in 1989 and 14% reduction in 1990 in the plot of artificial infection. Unfavourable climate conditions for development of this disease were the reason for that the yield in 1990 was increased tremendously than that in 1989. The fungicide treatment alleviated the effect of the disease on yield. These results agree with those reported in the previous investigations (Korić 1980, 1987a). The results also indicate that the effect of this disease on yield could not be completely eliminated with one treatment at early heading stage, maybe because climatic conditions were favourable for the development of the disease under natural conditions. This disease also reduced the yield of processed seed to 52% and 81% without and with the treatment of a fungicide in relation to the check, respectively, in 1989. Percentage of discarded seeds was 23% higher than that of the check when wheat was severely infected with Septoria nodorum blotch. One treatment of a fungicide resulted 11% lesser discarded seeds than those in the check. Such reduction in discarded seeds can easily be explained by the effect of fungicide which protect upper leaves from disease attack, thus providing normal vegetative condition of the plant and kernel formation.

Table 2 gives the yield classified by a share of each grade for each treatment in year 1989. Percentage of grades that passed through opening lesser than 2.2 mm (discarded seed) was far

Table 1. Effect of Septoria nodorum blotch on yield (kg/10 m^2)

	Artificial infection	Fungicide application	Natural infection Check
Year 1989			
Natural seed	3.78	4.93	5.96
Processed seed*	2.59	4.05	4.97
Discarded seed	1.16	0.84	0.94
Other impurtities	0.03	0.04	0.05
% of seed infected	71	49	31
Year 1990			
Natural seed	8.0	9.35	9.25
Processed seed*	6.0	7.60	7.55
Discarded seed	1.85	1.57	1.55
Other impurities	0.15	0.18	0.15
% of seed infected	57	27	7

* Screen peforations 2.5 mm

Table 2. Effect of septoria nodorum blotch on quality of seed in each grade (%) in 1989*

Seed grade (mm)	Artificial infection	Fungicide application	Natural infection
<2.2	5.5	1.8	1.0
2.2-2.5	27.5	21.7	16.0
2.5-2.8	49.5	49.9	42.2
>2.8	17.5	26.6	40.8

* analysis was made with 1 kg of natural seed

greater in the plots of artificial infection than in other two treatments. A similar ratio was observed with a share of grade 2.2 mm. Share of grades with screen perforations 2.5 mm was more or less identical in all treatments, whereas the one with perforations 2.8 mm was the markedly highest in the check. Based on this investigation and all others carried out up to now in our Institute, we concluded that Septoria nodorum blotch affects kernel size and hence, directly seed quality.

Comparing the results of Table 1 and 2, ie, the results obtained after processing and grading mean sample (1 kg) of the same seed, virtually the same ration of high and low-quality seed was obtained for all the treatment examined, when kernels smaller than 2.5 mm were classified as low-quality, and those above 2.5 mm as high quality seed. Significantly different rations in seed grades over 2.5 mm were observed among the treatments, namely 67%, 76.5% and 83% in artificial infection, fungicide application and natural infection, respectively, which were much higher percentages than smaller seed grades of 2.5 mm. Also is a well-known fact that kernel size above 2.5 mm provided the highest seed capacity, ie, the highest productivity which is a result of kernel size or absolute seed weight.

Results of testing sanitary condition of the seed for each treatment showed that endosperm infection with *Leptosphaeria nodorum* were from 7 to 71% which depends on year and treatments (Table 1). That is the same as the investigation before (Korić 1987b). Percentage of *Leptosphaeria nodorum* grain infection was significantly higher in artificially infected plants than those in the check plot. With one fungicide application, infection was somewhat less severe, but still very high, 49% and 27% in 1989 and 1990, respectively, and far from Croatia's regulations in which only seeds up to 3% of infection can be marketed as commodity.

References

Eyal Z, Scharen AL Prescott JM, and Ginkel M van (1987) The Septoria Diseases of Wheat: Concepts and methods of disease management Mexico, DF: CIMMYT

Korić B (1980) Cemical control of septoria nodorum blotch in wheat. Glasnik zaštite bilja vol 9 (5): 196-202 (in Croatian).

Korić B (1987a) Investigation of effects some fungicides in control of Leptosphaeria nodorum causal septoria nodorum blotch. Proceedings of Yugoslav conference for application of pesticides. 201-203 (in Croatian).

Korić B (1987 b) Investigation of seed-boorn infection with *Leptosphaeria nodorum*. Rachis, vol. 6 (1): 44-45.

Mathur SB and Lee Silvia, LN (1987) A quick method for screening wheat seed samples for Septoria nodorum. Seed Science and Technology 6: 925-926.

Strona IG and Uboženko, AG (1970) Importance of bigness seed in seed production. Selekcija i semenovodstvo 1: 48-51 (in Russian).

Zadoks JC, Chang TT and Konzak CF (1974) A decimal code for the growth astages of cereals. Weed Research 14: 415-421.

Wheat Information Service
Number 76: 35 – 38 (1993)

Genetic divergence in bread wheat (*Triticum aestivum* L. em Thell) under sodic soil conditions

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Genetic divergence as measured by Mahalanobis D^2 statistics was studied under sodic soil conditions for yield and its component traits in ninety-nine bread wheat lines obtained from different parts of India. The lines were grouped into 10 different clusters. The clustering pattern of lines did not follow their geographical origins, although some clusters consisted of lines having a common geographical origin. Grain yield, ear-bearing tillers and plant height were the potent variables which could be used as parameters in selecting genetically divergent parents in crossing programme for breeding high yielding wheat varieties for salt-affected soils.

Genetic divergence is dependent on geographical diversity as well as phenotypic components of varieties, and its quantitative assessment could provide a rational basis for the selection of parents for any breeding programme. The main objective of the present study was to identify and classify the available wheat materials into genetically different, distinctive groups so as to enable the breeders to efficiently plan the hybridization programme. The present investigation was, thus, aimed at assessing the genetic divergence among ninety-nine bread wheat lines under sodic soil conditions. The lines were of diverse geographical origin, and included indigenous one, Kharchia, grown locally in remote parts of Rajasthan State of India, lines developed through single plant selections of advanced generations of diverse crosses and commercially grown high yielding varieties for normal soils.

Materials and methods

Ninety-nine lines of wheat were selected from 10 States and Union Territory of India, viz., Bihar, Delhi, Gujarat, Haryana, Madhya Pradesh, Maharashtra, Punjab, Rajasthan, Uttar Pradesh, and West Bengal.

The experiment was laid out in a randomized block design with two replications with single row-plots of 6 m length and 30 cm inter-row spacing under sodic stress (average pH 9.3) in the winter season of 1990-91 at the experimental farm of Central Soil Salinity Research Institute, Karnal. Data were recorded for grain yield based on whole plot (5 m row) and for number of ear-bearing tillers per plant on the basis of 5 m length (finally averaged to one m length), while random 10 plants were chosen from each row-plot for recording of characters like grain weight per ear, grain number per ear, 1000 grain weight, plant height, ear length and number of spikelets per spike. The analysis of divergence using Mahalanobis D^2 statistics and subsequent grouping of genotypes following Tocher's method were carried out as described by Rao (1952).

Table 1. Contribution of grain yield and its component traits to the genetic diversity under sodic stress conditions.

Character	Percent contribution
Grain yield (g/5 m row)	28.02
Grain no. per ear	3.31
Grain wt. (g/ear)	4.36
1000 grain wt. (g)	4.00
Ear-bearing tillers in 1 m row	15.80
Plant height (cm)	29.90
Ear Length (cm)	3.90
No. of spikelets/ear	10.65

Results and discussion

The analysis of variance revealed highly significant differences for all the characters studied, thereby indicating existence of genetic diversity among the lines under sodic stress conditions. The grain yield, plant height, number of ear-bearing tillers and number of spikelets per spike contributed, as a whole, more than 80 per cent of the genetic diversity (Table 1).

Mahalanobis D^2 analysis was conducted and all the 99 lines were grouped in 10 clusters (Table 2). Clusters formed on the basis of D^2 values were such that lines belonging to the same cluster had, on average, smaller D^2 values than those belonging to the other clusters. The so formed clusters were not entirely based on the geographical diversity of the material, because genetic drift and intense natural and human selection for diverse adaptive gene complexes could also cause considerable genetic diversity (Murty and Arunachalam 1966). Similar trends were observed in *Pennisetum typhoides* (Singh 1979a) and in *Brassica juncea* L. (Singh 1979b) when they were grown under salt affected soils.

Cluster VI consisted of lines that were tall in stature and considered to be salt tolerant. Kharchia 65 is a well known salt tolerant variety used in hybridization programme for development of high yielding salt tolerant genotypes. Accompanying with Kharchia 65 were KRL 3-4, KRL 4-1, KRL 4-4, KRL 4-10 and KRL 9 which were the improved Kharachia derivative lines. This cluster had a high grain yield (Table 3) and had the highest plant height. Cluster VIII consisted of high yielding salt tolerant lines including KRL 1-4 which is the only one specifically released variety for salt-affected areas. WH 157 is also a high yielding and salt tolerant variety. The other entries of this cluster were also good yielders under sodic stress as was indicated by the highest cluster mean grain yield. Contrary to the cluster VI, this cluster was comprised of dwarf types. Cluster IV is also worth consideration as the cluster mean grain yield was quite high (137.68 g) and it had high tillering ability. The clusters having salt sensitive lines were I and IX, as was indicated by their extremely low grain yields. Though cluster I had a high tillering ability, it had less number of grains per ear and also showed low grain weight per ear. This could be possibly due to sterility occurring under salt stress, as already reported by Singh and Rana (1983) in Triticale where poor

Table 2. Distribution of 99 wheat varieties/lines in 10 clusters under sodic stress conditions.

Cluster	Number of genotypes	Varieties/Strains
I	3	Jobner 609, Jobner 612, Jobner 617
II	13	CPAN 1988, Raj 3077, Lok 1, SNH 9, Sonalika, BW 1022, CSW 7, DL 3301, CSW 16, WJ 71, CSW 59, Jobner 666, Jobner 2030
III	14	PBW 65, HD 2385, M 3096, M 3098, CSW 9, MP 845, MP 847, CSW 26, HS 223, HS 280, WH 534, MACS 2496, CSW 51, Raj 3733
IV	11	AKW 65-1, NI 5439, CSW 27, Sujata, Swati, WJ 126, CSW 36, Jobner 601, Jobner 673, Raj 3730, Raj 3732
V	10	Raj 3221, HD 2329,PDW 220, GW 1021, Raj 6392, K 8704, WJ 13, WJ 128, CSW 48, DL 157
VI	7	Kharchia 65, KRL 3-4,KRL 4-1, KRL 4-4, KRL 4-10, KRL 9, WJ 75
VII	12	KRL 2-22, PYT 3, Raj 1972, CSW 12, HD 2501, CSW 79, HP 1659, KS 13, WJ 127, CSW 50, CSW 53, CSW 56
VIII	9	KRL 1-4, WH 157, M 3097, BW 1052, HP 1529, KRL 12, CSW 57, CSW 58, KRL 13
IX	5	WJ 24, WJ 42, WJ 44, WJ 60, WJ 69
X	15	KRL 4-8, BAU 2276, SNH 9, CSW 11, KRL 10, VL 682, HD 2557, N 9343, HD 2499, WJ 78, CSW 46, CSW 49, CSW 54, CSW 55, Raj 3732

Table 3. Cluster means of eight characters in wheat varieties/lines under sodic stress conditions.

Character	Character means in different clusters									
	I	II	III	IV	V	VI	VII	VIII	IX	X
Grain yield (g/5 m row)	66.83	124.25	99.43	137.68	71.20	135.25	89.58	161.83	55.40	108.57
Grain no. per ear	23.65	31.05	43.71	40.21	36.72	33.05	42.80	42.00	24.48	36.00
Grain wt. (g/ear)	0.67	1.15	1.32	1.30	1.34	1.44	1.57	1.63	0.88	1.30
1000 grain wt. (g)	27.65	37.12	30.39	32.58	36.03	43.61	36.87	38.84	35.51	36.26
Ear-bearing tillers in 1 m row	145.33	120.89	87.46	133.91	76.60	93.58	74.19	101.28	95.90	91.93
Plant height (cm)	69.89	75.32	72.54	79.97	65.03	87.23	75.56	75.96	91.67	78.29
Ear length (cm)	8.11	8.12	9.71	8.17	7.62	8.57	8.96	9.19	9.80	9.38
No. of spikelets/ear	15.33	14.19	18.29	15.76	15.40	15.28	15.90	16.85	17.53	16.89

Table 4. Intra- and inter-cluster distances of wheat varieties/lines under sodic stress conditions based on eight characters.

Cluster	Cluster									
	I	II	III	IV	V	VI	VII	VIII	IX	X
I	1.19	3.74	5.36	4.45	4.69	6.03	5.91	6.46	4.41	4.79
II		1.72	4.37	2.26	2.95	2.73	3.49	3.57	4.34	2.73
III			1.94	3.35	3.57	4.64	2.59	3.22	4.46	2.18
IV				1.87	3.52	3.29	3.15	2.76	4.76	2.53
V					1.62	3.87	2.41	3.88	5.02	2.90
VI						1.81	3.03	2.75	4.40	2.61
VII							1.64	2.40	4.80	1.93
VIII								1.45	5.47	2.31
IX									1.52	3.53
X										1.49

correlation was observed between grain weight and number of ear-bearing tillers per plant in sodic soils.

Table 4 gives the intra- and inter-cluster distances between cluster centroids. The first and second largest distances were between cluster I and VIII (6.64) and cluster I and VI (6.03), as the former cluster consisted of poor yielders and the later of high yielders under sodic stress.

References

Murty BR and Arunachalam V (1966) The nature of genetic divergence in relation to breeding system in crop plants. Indian J Genet 26A: 188-189.

Rao CR (1952) Advanced statistical methods in biometrical research. (Ed.) John Wiley and sons, Inc, New York: 357-363.

Singh KN (1979a) Genetic diversity and performance of some bajra genotypes tested under saline sodic conditions. Indian J Heredity II (1): 71-76.

Singh KN (1979b) Genetic divergence in Indian mustard in sodic soil conditions. Indian J Genet 39 (3): 439-443.

Singh KN and Rana RS (1983) Genetic variability and path analysis in triticale grown in alkali soil. Indian J Agric Sci 53 (1): 21-24.

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III. Genetic Stock

A report of the wheat field research in Yugoslavia

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This is a short report of the field research work in Yugoslavia (including the present regions of Croatia, Bosnia-Hercegovina and Makedonija) carried out in 1991. It was a second year's project on 'Comparative Ecological Genetics on Phylogeny of Wheat, Barley and Their Wild Relatives in Northeastern Region of the Mediterranean Sea' sponsored by the Ministry of Education, Science and Culture, Japan (Grant-in-Aid for International Scientific Research Program: Field Research No. 0204137). It was a cooperative work between Professor S. Borojević, Faculty of Agriculture, University of Novi Sad, and the Japanese members. The field research members were as follows:

Yoshihiko Furuta, Professor, Faculty of Agriculture, Gifu University
Shoji Ohta, Instructor, Plant Germ-plasm Institute, Faculty of Agriculture, Kyoto University

Table 1. Schedule and route of the present field research in Yugoslavia in 1991

Date	Route	Date	Route
June 9	Leaving from Itami (Japan)	July 3 - 4	Novi Sad
10	Arrival at Beograd (Serbia)	5	Novi Sad-Kumanova (Makedonija)
12	Beograd-Novi Sad (Vojvodina)	6	Kumanova-Skopje-Ohrid
17	Novi Sad-Beograd	7	Ohrid-Kičevo
18	Beograd-Titograd (Montenegro)	8	Kičevo-Gevgelija
19 - 21	Titograd and suburbs	9	Gevgelija-Štip
22	Titograd-Dubrovnik (Croatia)	10	Štip-Skopje
23	Dubrovnik-Mostar (Bosnia-Hercegovina)	11	Skopje-Priština (Kosovo)
24	Mostar-Split (Croatia)	12	Priština-Novi Sad (Vojvodina)
25	Split-Sibenik	13 - 18	Novi Sad
26	Sibenik-Senj	19	Beograd (Serbia)
27	Senj-Opatija	20	Leaving from Beograd
28 - July 2	Opatija-Rijeka-Split-Mostar (Bosnia-Hercegovina)-Sarajevo-Novi Sad (Vojvodina)		

Table 2. A summarized list of the present collection in Yugoslavia

Plant species	No. of samples[1)]						
	Vj	Mn	Cr	BH	Mk	Ks	Total
Cultivated wheats		**3**	**21**	**43**	**41**	**7**	**115**
Triticum monococcum				14			14
Triticum dicoccum			3	9			12
Triticum durum			5			5	10
Triticum turgidum					17		17
Triticum aestivum		3	13	20	24	2	62
Wild wheat and *Aegilops* species	**1**	**30**	**32**	**16**	**71**	**1**	**151**
Triticum boeoticum					2		2
Aegilops biuncialis		4	7		23	1	35
Aegilops comosa					2		2
Aegilops cylindrica	1			1	2		4
Aegilops ovata		8	9	6	10		33
Aegilops triaristata		11	7	5	15		38
Aegilops triuncialis		2	9	4	17		32
Aegilops uniaristata		5					5
Rye, barley and their wild relatives	**1**	**3**	**23**	**23**	**37**	**10**	**97**
Secale cereale		1	7	4	27	10	49
Hordeum vulgare			8	17	3		28
Hordeum bulbosum			2	1	4		7
other wild *Hordeum* spp.	1	2	6	1	3		13

1) Vj: Vojvodina, Mn: Montenegro, Cr: Croatia, BH: Bosnia–Hercegovina, Mk: Makedonija, Ks: Kosovo.

The schedule and the route of the field research were summarized in Table 1. It consisted of two trips. The first trip covered the Dinara mountain region and the coastal region of the Adriatic Sea in Montenegro, Croatia and Bosnia-Hercegovina where the cultivation of einkorn and emmer wheats was once reported (Schiemann 1956, Borojević 1956, Pavićević 1982). The second one was carried out in Makedonija and Kosovo. During the trips, a total of 637 samples belonging to more than eight families were collected (Table 2). They consisted of five species of cultivated wheats, rye, barley, oat, their wild relatives, other Gramineae species, and so on.

Table 2. (Continued)

Plant species	No. of samples[1)]						
	Vj	Mn	Cr	BH	Mk	Ks	Total
Other Triticeae species		**6**	**6**		**30**		**42**
Haynaldia villosa		6	6		15		27
Taeniatherum spp.					15		15
Oat and its wild relatives		**8**	**18**	**11**	**4**		**41**
Avena sativa			3	4			7
other *Avena* spp.		8	15	7	4		34
Other Gramineae species	**2**	**17**	**40**	**24**	**56**	**1**	**140**
Brachypodium spp.		1	3				4
Bromus secalinus				4			4
other *Bromus* spp.	2	8	23	3	46	1	83
Lolium temulentum			4	14	1		19
Trachynia distachya		5	7	2	6		20
others		3	3	1	3		10
Other plant species		**4**	**11**	**10**	**26**		**51**
Caryophyllaceae spp.			4	4	9		17
Compositae spp.					4		4
Cruciferae spp.				1			1
Leguminosae spp.		1	1	1	3		6
Liliaceae spp.		2	4		2		8
Papaveraceae spp.				3	3		6
Ranunculaceae spp.			1		2		3
Rosaceae spp.		1		1			2
others			1		3		4
Total	**4**	**71**	**151**	**127**	**265**	**19**	**637**

In the Dinara mountain region, 14 accessions of einkorn wheat (*Triticum monococcum* L.) and 12 of emmer wheat (*T. dicoccum* Schübl.) were successfully collected. A plant of einkorn grew in a six-rowed barley field at Volujac, Bosnia-Hercegovina. The owner of the field cultivated barley for animal feed and he did not recognize einkorn wheat. The spikelets of emmer were found in the two samples of local farmer's oat seed stocks for animal feed. Morphologically, the obtained emmer spikelets were divided into three accessions. To the contrary, a pure cultivation of einkorn and eight fields where emmer and einkorn grew together were found at the village of Sovići, Bosnia-Hercegovina. A seed sample in which emmer and einkorn mixed was also obtained from a local farmer's stock at the village. He grew it for animal feed and called einkorn and emmer as '*pir*'.

A total of 17 accessions of *T. turgidum* L. were collected in Makedonija. The members found several fields of *T. turgidum* around the Lake Ohrid. A local farmer, who worked in the *turgidum* field with his family, told us that the crop is not '*pšenica*' (bread wheat) but '*kremenka*' and that he makes bread from '*kremenka*'. Another field of '*kremenka*' was found at 2 km west from Demir Kapija on the way to Skopje in Makedonija.

Two samples of wild einkorn (*T. boeoticum* Boiss.) and 149 samples of seven *Aegilops* species were collected. *T. boeoticum* was collected in Makedonija. The collection of *Aegilops* species consisted of 35 samples of *Ae. biuncialis* Vis., two of *Ae. comosa* Sibth. et Sm., four of *Ae. cylindrica* Host, 33 of *Ae. ovata* L., 38 of *Ae. triaristata* Willd., 32 of *Ae. triuncialis* L. and five of *Ae. uniaristata* Vis.

All the samples collected in the present field work were divided into two parts: one for University of Novi Sad and the other for the Japanese members. The latter is preserved at Plant Germ-plasm Institute, Kyoto University.

Acknowledgement

The Japanese field research members really appreciate the kind help and arrangement of Prof. S. Borojević and Mr. M. Dimitrijević, Faculty of Agriculture, University of Novi Sad, during their staying in Yugoslavia.

References

Borojević S (1956) A note about the "New dates for recent cultivation of *Triticum monococcum* and *Triticum dicoccum* in Yugoslavia". Wheat Inf Serv 4: 1.

Pavićević Lj (1982) Some positive traits of domestic strains of diploid and tetraploid wheat. Genetika 14: 1-11.

Schiemann E (1956) New dates for recent cultivation of *Triticum monococcum* and *Triticum dicoccum* in Jugoslavia. Wheat Inf Serv 3: 1-3.

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IV. Proceedings of 23rd Japanese Wheat Genetics Symposium

The 23rd Japanese Wheat Genetics Symposium was held in November 28-29, 1992 at Nonoichi, Ishikawa, Japan, under organization by Dr. Takiko Shimada (Research Institute of Agricultural Resources, Ishikawa Agricultural College). Eighty-three wheat researchers gathered together, and exchanged sincere but warm discussions and information. The attendants had an opportunity to observe the experimental demonstration of transformation of wheat tissues by means of particle gun in Dr. Shimada's laboratory. The symposium is partly sponsored by Kihara Memorial Foundation.

The followings are the program and abstract papers of the symposium.

November 28, 1992

Symposium "Recent progress of wheat breeding in Japan"

Chairman: S. Nonaka (Ishikawa Agr. Col.)

Y. Taniguchi, M. Fujita and K. Ujihara (Kyushu Natl. Agr. Exp. Stn.) The simple method for testing grain fading tolerance and mechanism of fading in wheat.

H. Seko (Natl. Agr. Res. Ctr.) Quality breeding of wheat in Australia.

S. Taya (Chugoku Natl. Agr. Exp. Stn.) Influences of differences in the ultrastructure of hard- and soft-textured endosperms on their milling properties in wheat.

T. Nakamura (Tohoku. Natl. Agr. Res. Stn.) Studies on waxy loci in wheat.

Exploration report

Y. Furuta[1] and S. Ohta[2] (1. Fac. Agr., Gifu Univ.; 2. Plant Germplasm Inst., Kyoto Univ.) Exploration of Triticeae in Yugoslavia.

Business Session I

Y. Ogihara (Kihara Inst. Biol. Res., YCU) Establishment of DNA clone bank of wheat.

Reception

November 29, 1992

Presentation I

Chairman: Y. Furuta (Fac. Agr., Gifu Univ.)

Zh. Atanassov[1], C. Nakamura[1], T. Yoshizawa[2] U, H. Kato[1], K. Murai[3], and C. Kaneda[1] (1. Fac. Agr., Kobe Univ.; 2. Fac. Agr., Kagawa Univ.; 3. Sumitomo Chemical Co.) Effect of *Fusarium* mycotoxins on wheat response to Fusarium head blight.

K. Kato, K. Nakagawa and H. Kuno (Fac. Agr., Kochi Univ.)
Chromosomal location of the genes for vernalization response, *Vrn2* and *Vrn4*, in common wheat, *Triticum aestivum* L.

N. Nakata, M. Tomita and Y. Yasumuro (Fac. Agr., Tottori Univ.)
Origin of midget chromosome present in rye cytoplasmic Chinese Spring.

K. Murai[1], H. Hirohara[1] and K. Tsunewaki[2] (1. Sumitomo Chemical Co.; 2. Fac., Agr., Kyoto Univ.) Effect of *Aegilops crassa* cytoplasm on agronomic characters of F_1 hybrid.

J. Fujigaki and T. Tozu (Tokyo Agr. Univ.) Reproductive barriers between *Hordeum* and *Secales* species.

Presentation II

Chairman: T. R. Endo (Nara Univ.)

H. Tsujimoto (Kihara Inst. Biol. Res., YCU) Synthesis of telomere repetitive sequences at broken chromosome ends in common wheat.

Y. Mukai (Osaka Kyoiku Univ.) Structure and evolution of tetraploid wheats revealed by *in situ* hybridization.

S. Yanagawa and Y. Ogihara (Kihara Inst. Biol. Res., YCU) Interoganellar translocation of chloroplast genes into mitochondria.

Y. Ogihara, M. Furuya and T. Sasakuma (Kihara Inst. Biol. Res., YCU) Structure analyses of tripartite genomes in *Aegilops triuncialis* complex.

S. Nasuda[1], T. R. Endo[2] and K. Tsunewaki[1] (1.Fac. Agr., Kyoto Univ.; 2. Nara Univ.) Determination of the positions of RFLP loci on the cytological map of common wheat chromosomes using terminal deletion lines of Chinese Spring wheat.

Business Session II

H. Tsujimoto (Kihara Inst. Biol. Res., YCU) On the editorial board of WIS.

Presentation III

Chairman: H. Tsujimoto (Kihara Inst. Biol. Res., YCU)

H. Ishihara[1], Y. Furuta[1], K. Nishikawa[2] and N. Watanabe[1] (1. Fac. Agr., Gifu Univ.; 2. Aichi Sangyo Univ.) Comparative phylogenetic study in amylase isozymes in Gramineae: Why are these enzymes nice marker for phylogenetical approach in common wheat?

S. Sekiguchi[1], J. Ono[1] and T. Taira[2] (1. Nippon Flour Mills Co.; 2. Osaka Pref. Univ.) Detection of HMW glutenin genes by DNA hybridization and breadbaking quality of amphidiploid synthesized between *Aegilops squarrosa* and *Secale* cereale.

H. Nakamura and H. Yoshida (Natl. Agr. Res. Ctr.) Relation between wheat seed storage protein subunits and noodle viscoelasticity.

K. Noda and N. Kawakami (Kihara Inst. Biol. Res., YCU) Embryonic mRNA related to seed dormancy in wheat.

Visit to Research Institute of Agricultural Resources, Ishikawa Agricultural College.

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The simple method for testing grain fading tolerance and mechanism of fading in wheat

Y. Taniguchi, M. Fujita and K. Ujihara

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Wheats are harvested during the rainy season in Japan, thus grain colors are often faded by rainfall and the grain quality is deteriorated. In this study, we developed a simplified method for the evaluation of grain fading in wheat cultivars by rainfall and selected the tolerance gene sources. Hear after, it will be released the cultivars of high grain fading tolerance by those method and gene sources, and search possibility of breeding cultivar which has tolerance for rain damage in respect of flour quality thorough the study of grain fading tolerance.

Evaluated method of grain fading tolerance

It was investigated a method that steep the ears of wheat in distilled water in order to make artificial faded grain.1) In this method, mature ears were cut at the sheltered field against rainfall. These ears were dryed in the sun for two days and they were stored by a low temperature for two weeks, and they were steeped in distilled water for 8, 16, 24, 48 hours in order to fade grains. After being dried up, these ears were threshed, and the faded degree of threshed grain were classified into six gropes by observation and were measured by a color meter (L*a*b*). By using this method, it confirmed the varietal differences in grain fading tolerance. In faded grain color, it was found that the value of L* (luminosity) increased, the value of a* (reddish) decreased and the value of ΔE*ab (difference of color between normal grain) increased. The value of b* (yellowish) also decreased, but the correlation between these degrees of decrease and faded degrees by observation was low. It was found that faded degrees had highly correlated with the value of ΔL* and ΔE*ab, this indicated that faded degrees could be evaluated by the value of ΔL* and ΔE*ab. Artificial faded grain color by this method had slightly higher value of a* than naturally faded grain color, but directions of both color changes were almost same (Fig. 1). These results indicated that this method could be used generally for to evaluate of grain fading tolerance. the grains from ears cut before maturing stage had lower faded degrees than grains from ears cut at maturing stage, and grains from ears cut after maturing stage had higher faded degree than its. The grains from ears which was not dryed up after cutting had not faded too mutch. The grains from ears which had been wet in field without shelter from rainfall had higher faded degree.

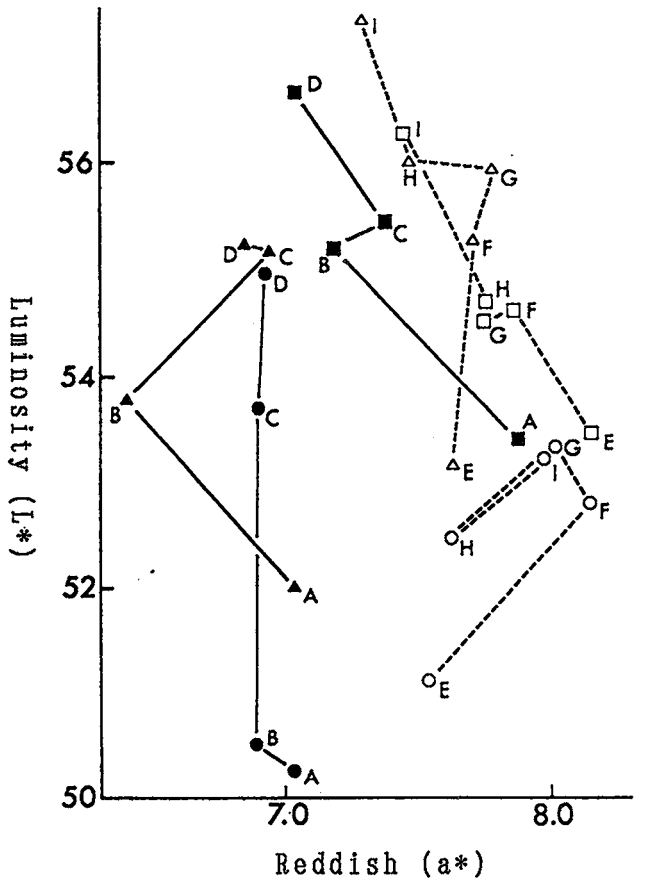

Fig. 1. In artificial and nature faded grain, Change of grain color with progress of fading (1991/92)

£ nature fadded grain (A cut at maturing stage, B-D cut after maturity)

: artificial faded grain (E control F-I watered for 8h, 16h, 24h, 48h)

: Asakaze komugi, £ : Akadaruma, ¡: Touzan 28

Mechanism of fading

It investigated to clearly the mechanism of fading why they looked as faded color. In this examination, threshed grains (20g) from ears were cut at maturing stage time and were steeped in distilled water (100mℓ) for 24 hours, then these grains and color of water were measured by a color meter, and glassiness of grains were also measured. As a result, it was observed that there were the varietal differences in the concentration of exuded color in water, and it was associated that this exudation was related with fading of grain, but no correlation between concentration of exuded color and degree of fading was observed (Table 1). However, we need another examination in consideration of original grain color, because it seems that there was no correlation by the differences of original grain color among varieties. the glassiness of steeped grain had been lower than glassiness of original (Table 2). Generally, mealy grain looked pale color, therefore it was thought that decrease of glassiness was related to the fading.

Table 1. Faded degree of grain and concentration of exuded color in water (1991/92)

Cultivar	value of color meter			
	steeped grain		water	
	ΔL*	Δa*	ΔL*	Δa*
Asakaze komugi	2.51	0.49	5.0	0.32
Saikai 161	3.59	0.53	3.9	–0.04
Touzan 28	1.21	–0.16	3.7	–0.23
Aka daruma	2.29	0.42	3.6	–0.15
Shiro mansaku	2.56	0.00	3.0	–0.32
Yamaguchi komugi	2.79	0.39	3.2	–0.47
Aoba komugi	4.99	0.60	4.3	–0.08
Kitakami komugi	3.02	0.67	2.4	–0.66
Shiro daruma	1.83	0.06	4.0	–0.30

Table 2. Glassiness of steeped grain (1990/91-1991/92)

Cultivar	Steeped grain (%)	Normal grain (%)
1990/91		
Kitakami komugi	61	24
Kantou 69	62	18
Garnet	94	62
Chikushi komugi	32	0
1991/92		
Asakaze komugi	47	5
Aoba komugi	95	61

Table 3. Flour quality of faded grain (1991/92)

Cultivar	ΔE*ab	flour yield (%)	Ash content (%)	Protein content (%)	Reflex rate R455 (%)	Reflex rate R554 (%)	Maximum viscosity (B. U.)
Faded grain							
Nourin 61	3.23	59.6	0.39	9.1	59.8	76.6	925
Shirogane komugi	4.02	65.0	0.33	9.9	61.0	77.2	945
Asakaze komugi	4.04	63.2	0.34	9.2	60.8	76.7	870
Nishikaze komugi	4.04	63.4	0.33	9.4	58.8	76.1	965
Daichinominori	3.85	58.0	0.38	9.1	59.7	77.2	860
Average	3.84	61.8	0.35	9.3	60.0	76.8	913
Normal grain							
Nourin 61		64.1	0.40	8.7	60.1	76.9	940
Shirogane komugi		66.0	0.37	9.4	60.6	76.9	935
Asakaze komugi		64.8	0.34	10.2	61.1	76.7	925
Nishikaze komugi		63.4	0.35	10.3	58.5	75.2	985
Daichinominori		62.2	0.38	9.7	58.9	76.6	920
Average		64.1	0.37	9.7	59.8	76.4	941

*Except for flour yield, measurements did to use fraction of flour with low ash. R455: Whiteness R554: Brightness

Relation between faded degree and flour quality

The stock harvested at maturing stage in field were steeped in water for 24 hours immediately after harvest, and after dried up by sunlight, they were steeped in water again for 24 hours. After dried up by sunlight, stock were threshed and these grain were milled by brabender junior testmill. In all cultivars, flour yield of faded grains were lower than normal grains (Table 3). Then faded grains had lower ash content and protein content and better brightness of flour than normal grain. It was cousidered that these fluctuation were caused by low flour yield. Maximum viscosity became lower, but the degree of decrease make no problem in practical use. Therefore in this experiment, it was proved that only flour yield had become by fading.

Reference

Taniguchi Y, M Fujita and K Ujihara Evaluation of grain fading tolerance of wheat by rainfall (point of lecture). Japan J Breed 42 (supple 2): 392-393.

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Three waxy (Wx) proteins in common wheat (*Triticum aestivum* L.)

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Nullisomic analysis of waxy (Wx) protein of *Triticum aestivum* L. cv. "Chinese Spring" using two dimensional polyacrylamide gel electrophoresis revealed that three *Wx* loci, *Wx-A1*, *Wx-B1* and *Wx-D1*, located in chromosome arms 7AS, 4AL and 7DS produce three distinct Wx proteins, Wx-A1, Wx-B1 and Wx-D1 respectively. Wx-A1 possess higher molecular weight and more basic isoelectric-point (pI) than the other two. Wx-B1 and Wx-D1 possess the same molecular weight but a slightly different pI range. Owing to the detection of these three Wx proteins, we were able to identify the expression of three *Wx* genes in wheat endosperms and to find three types of mutants among Japanese wheat cultivars, first type was lacking for Wx-A1, second type lacking for Wx-B1 and third type lacking for both Wx-A1 and Wx-B1. These results suggested the possibility of breeding a waxy wheat.

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Effect of *Fusarium* mycotoxins on wheat response to *Fusarium* head blight

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Fusarium head blight of wheat and other cereals caused by some *Fusarium* species is one of the most serious disease problems worldwide since this disease causes large economic losses and mostly because the different strains of this fungus produce a variety of toxic metabolites (mycotoxins) that can affect human health and animal productivity (Snijders 1990). There are many problems that should be solved in *Fusarium* head blight. However, little is known about the genetic system of resistance to this disease and especially about the role of *Fusarium* mycotoxins in determining strains' pathogenicity, plant response and correlation between them.

Two wheat lines which showed good field-resistance in the experimental field of the Institute for Wheat and Sunflower, Bulgaria were crossed with four susceptible Bulgarian cultivars. These six wheat lines, their F_1's and Nobeoka Bozu, a Japanese resistant variety, were used in our investigations. Before the experiment, thirty strains from seven *Fusarium* species were examined for their abilities to produce mycotoxins on rice and wheat cultures and six *Fusarium* strains from three different species were selected according to the different types and amounts of mycotoxins produced on rice culture. The species differentiation in their mycotoxin productivity was in accordance with the previous reports (Ichinoe et al 1985).

Plants were grown in a glasshouse and fifteen spikes per combination were artificially

Table 1. Kinds and mean amounts of mycotoxins accumulated in the infected wheat grains

Strain	mycotoxin (μg/g dry weight)						
	DON	3-ADON	15-ADON	3,15-ADON	NIV	F-X	ZEA
F. graminearum							
ARC-13	46.05	8.10	0.10	0.11	tr	nd	tr
KU-1368	12.69	2.38	nd	tr	nd	nd	nd
KU-1615	0.14	nd	nd	nd	57.21	nd	nd
F. culmorum							
ARC-2124-1	0.14	nd	nd	nd	17.46	tr	2.68
SHIN-996	0.97	0.03	nd	nd	0.09	nd	nd
	NEOS	T-2	HT-2	TOL			
F. acuminatum							
ARC-2050-2	0.40	2.54	4.88	12.55			

nd: not detected; tr: traces

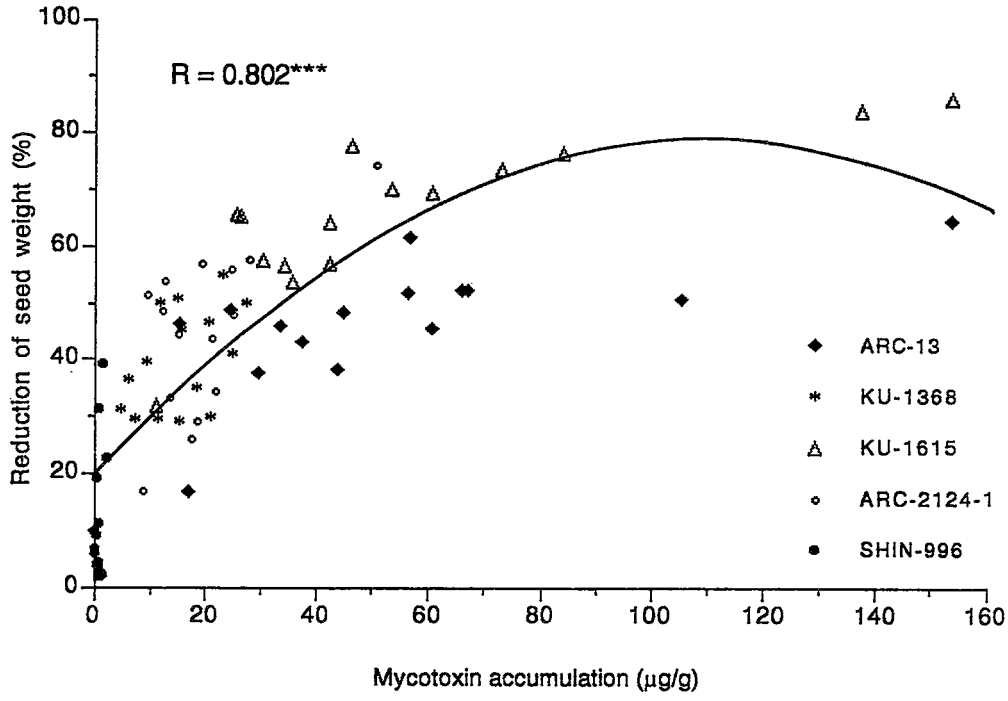

Fig. 1. Multiple regression between reduction of seed weight and amount of mycotoxins accumulated in wheat grains infected by five *Fusarium* strains

inoculated at anthesis with each *Fusarium* strain by spraying 2 ml suspension containing 10^{5-6} macro-conidia/ml. Three criteria were used in evaluating wheat response to *Fusarium* head blight, ie, reduction of seed set, reduction of seed weight and mycotoxin accumulation in the infected grains. Mycotoxins were extracted with (3:1) acetonitrile/water, purified by Florisil column and analysed by GCMS and HPLC. Different mixtures of mycotoxins were detected in the grains infected by different *Fusarium* strains (Table 1), as Wang and Miller reported (1988).

A good accordance was observed between the strains' mycotoxin productivity detected on rice culture (data not shown) and mycotoxins accumulated in the infected wheat grains (Table 1). *Fusarium* strains that showed higher mycotoxin productivity on rice culture, such as ARC-13 and KU-1615 produced higher amounts of the same types of mycotoxins in the infected grains than the other low productive strains.

A significant correlation was found between the reduction of seed weight and the amount of mycotoxins accumulated in wheat grains infected by five *Fusarium* strains (Fig. 1). This might be expected, since the higher percentage of the heavily damaged or mouldy seeds could directly reflect on the reduction of seed weight. When the data were evaluated individually for each *Fusarium* strain, it was found that the correlation coefficients were higher in the higher mycotoxin productive strains such as KU-1615 and ARC-13 than in the lower productive strains such as SHIN-996 and KU-1368.

Table 2. Correlation between the total mycotoxin accumulation in wheat grains in the field and glasshouse conditions

Wheat line	Level of natural infection (μg/g dry weight)	Level of artificial infection* (μg/g dry weight)
Nobeoka Bozu	0.09	11.73
KM-34/90	0.29	21.65
Charodejka	0.48	25.55
KM-10/90	0.69	47.09
Trakia	0.84	42.95
IT-10179-4-85	1.17	48.43
IP-211-50-37	3.51	110.45

*mean of three *F. graminearum* strains

$r = 0.98$, $P < 0.001$

Several wheat lines were examined under natural conditions in Kasai experimental field of Sumitomo Chemical Co. (Table 2). The results obtained based on the concentration of mycotoxins accumulated showed a significant correlation between natural and artificial conditions of infection, suggesting validity of the method applied in estimating wheat response to *Fusarium* head blight. The observation that the resistant lines apparently reduced the mycotoxin level in the grains, whereas susceptible lines did not and that the reaction of F_1's was intermediate in most of the cases (data not shown) suggested a possible role of mycotoxin degradation in determining wheat response against *Fusarium* and a polygenic nature of resistance to the disease.

References

Ichinoe M et al (1985) Trichothecenes-producing *Fusarium* in barley and wheat in Japan. In: Trichothecenes and Other Mycotoxins, (ed: J. Lacey), pp. 21-32. John Wiley and Sons Ltd.

Snijders CHA (1990) Aspects of resistance to Fusarium head blight caused by *Fusarium culmorum* in wheat. Ph. D. thesis, University of Wageningen, the Netherlands.

Wang YZ and Miller JD (1988) Effects of *Fusarium graminearum* metabolites on wheat tissue in relation to Fusarium head blight resistance. J. Phytopathology 122, 118-125.

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Chromosomal location of the genes for vernalization response, *Vrn2* and *Vrn4*, in common wheat, *Triticum aestivum* L.

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Although five genes are known to give spring growth habit in common wheat, their chromosomal location has been established only for three genes, namely *Vrn1*(5A), *Vrn3*(5D), and *Vrn5*(7B). Using monosomic series of 'Chinese Spring', Maystrenko (1980) concluded that *Vrn2* and *Vrn4* was located on 2B and 5B chromosomes, respectively. On the other hand, Hoogendoorn (1985) suggested that *Vrn* gene on 5B chromosome is not *Vrn4*, but *Vrn2*. Such a confusion seems to be resulted from the fact that 'Chinese Spring' itself carries *Vrn3*. In other words, in F_2 population of the crosses between *Vrn2* carrier and CS monosomics, winter type should segregate at 1/16 and at a few percent in non-critical and critical crosses, respectively. Accordingly it is difficult to distinguish the two types of segregation ratio, and thus careful analysis is necessary to know their chromosomal location.

From these considerations, the monosomic series of winter wheat varieties, 'Dwarf A' and 'Nanbukomugi', were used to locate *Vrn2* and *Vrn4*, respectively. The seeds of these lines were kindly supplied by Dr. A. J. Worland, Cambridge Laboratory, UK, and Dr. K. Mukade, Tohoku National Agricultural Experiment Station, Japan, respectively.

Segregation ratio of spring and winter types fitted well to 3:1 in most of F_2 populations derived from monosomic F_1 plants of crosses between 'Dwarf A' monosomics and 'Triple Dirk(B)', which carried singly *Vrn2* among five *Vrn* genes. On the other hand, segregation ratio didn't fit to the expected ratio in the cross with 'Dwarf A' monosomic for 5BL-7BL chromosome, winter type being much less (99:8) than expected. This result indicated that *Vrn2* must be located on either 5BL or 7BL chromosome.

Similarly segregation ratio of spring and winter types was examined in F_2 populations derived from monosomic F_1 plants of crosses between 'Nanbukomugi' monosomics and 'Triple Dirk(F)', which carried singly *Vrn4* among five *Vrn* genes. In the cross with 'Nanbukomugi' monosomic for 5D, winter type segregated much less (126:13) than expected, indicating that *Vrn4* was located on 5D chromosome.

References

Hoogendoorn J (1985) The physiology of variation in the time of ear emergence among wheat varieties from different regions of the world. Euphytica 34: 559-571.

Maystrenko OI (1980) Cytogenetic study of the growth habit and ear-emergence time in wheat (*Triticum aestivum* L.). Proc. XIV Int. Congr. Genet. 1 (No.2): 267-282.

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Origin of midget chromosome present in the rye cytoplasmic Chinese Spring

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We found a diminutive chromosome in the rye cytoplasmic Chinese Spring ((cer)-CS) and named it "Midget chromosomes" (Nakata et al 1986). This chromosome has essential genes responsible for endosperm development and fertility in the rye cytoplasmic line and was derived from rye chromosome 1R (Nakata et al 1988). This study was conducted to locate the origin of the midget chromosome on chromosome 1R by producing deletion lines for 1R. The deletions of 1R were induced by the action of the gametocidal gene of chromosome $4S^1$ of *Aegilops longissima* (Endo 1985).

Materials and methods

(cer)-CS with 1R and without the midget chromosome was obtained by the cross between (cer)-CS and Imperial Rye chromosome 1R addition Chinese Spring(CS) line. For induction of deletion 1R, (cer)-CS with 1R was crossed with chromosome $4S^1$ addition CS line. The seeds of the $4S^1$ addition line were kindly provided by Dr. T. R. Endo, Nara University. The structural changes of 1R were examined in the F_2 progenies, because all the vaible F_2 plants with rye cytoplasm have the essential genes for endosperm development which located on 1R. Chromosome 1R was recognized by the double bands on the end of short arm and the large band on the end of long arm by C-banding, and carried the *Sec3* gene for high molecular seed storage protein at the region near the interstitial C-band of the long arm (Gustafson et al 1990). Therefore, the presence or absence of the C-band and *Sec3* gene were examined for identification of structural changes of 1R. Seed proteins for the *Sec3* gene were fractionated by SDS-PAGE.

Results and discussion

Plump and shrivelled seeds were segregated in the F_2. Only the plump seeds have chromosome 1R or structurally changed 1R carrying the essential genes for endosperm development. These plump seeds were examined for inspecting the structural changes of 1R.

The expression of the *Sec3* gene was recognized in 109 seeds out of 145 plump seeds of F_2 examined. Sixty-one of them germinated and grew. These plants were examined by C-banding with respect to chromosome 1R. The intact 1R was monosomic or disomic in 51 plants. An isochromosome of the long arms of 1R was found in three plants. Monotelosome or ditelosomes of long arm of 1R were found in three plants. The translocated chromosome composed of the short arm of 1B and the long arm of 1R was found in three plants. The translocated chromosome composed of the long arms of 4A and 1R was found in one plant (Fig. 1A-E). Thus, all of 61 plants

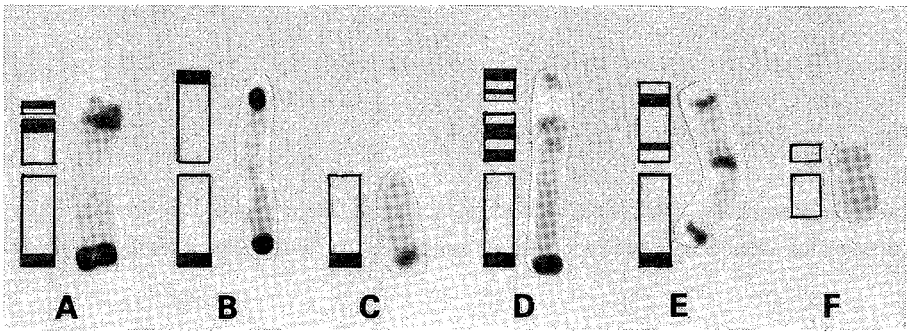

Fig. 1. Normal chromosome 1R and its structural changes found in the F_2 progeny of the hybrid between (cer)-CS with 1R and $4S^1$ addition lines of CS. A: Normal 1R, B: Isochromosome of the long arms, C: Telosome of the long arm, D: Translocated chromosome 1BS/1RL, E: Translocated chromosome 4AL/1RL, F: Deletion of terminal regions of the both arms.

having *Sec3* gene had the long arm of 1R though ten of 61 plants did not have the short arm of 1R. This indicates that the long arm of 1R as well as the midget chromosome has the essential genes for endosperm development.

Out of 26 plump seeds showing no subunit of the *Sec3* gene, five plants germinated and grew. However, the karyotypes of only two plants were identified by C-banding. The two plants had one small subterminal chromosome in addition to 42 chromosomes of wheat (Fig. 1F). This chromosome was similar in size to the D genome chromosomes and must have the essential genes for endosperm development although it did not carry the *Sec3* gene. This chromosome was presumed to be derived from 1R by the deletion of a large part of the terminal regions of both arms. This indicates that the loci of the essential genes for endosperm development is between the centromere and the *Sec3* locus of the long arm. The midget chromosome must be derived from the region near the centromere on the long arm of chromosome 1R, because the chromosome was telocentric.

References

Endo TR (1985) An *Aegilops longissima* chromosome causing chromosome aberrations in common wheat. Wheat Inf Serv 60: 29.

Gustafson JP, Butler E an cIntyre (1990) Physical mapping of a low-copy DNA sequence in rye (*Secale cereale* L.). Proc Natl Acad Sci USA 87: 1899-1902.

Nakata N, Yasumuro Y and Sasaki M (1986) Midget chromosome found in common wheat cv. Chinese Spring with rye cytoplasm. Wheat Inf Serv 63: 39-41.

Nakata N, Asami H, Yasumuro Y and Sasaki M (1988) Role and transmission of midget chromosome found in the rye cytoplasmic Chinese Spring line. Proc 7th Int Wheat Genet Symp 391-396.

Wheat Information Service
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Effects of *Aegilops crassa* cytoplasm on agronomic characters of F_1 hybrids

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We have demonstrated that the male sterility in the alloplasmic line of *Triticum aestivum* cv. Norin 26 with *Aegilops crassa* cytoplasm was induced by long-day treatments of 15 hours or longer light period, and proposed a new system for hybrid seed production using this conditional male sterility (Murai et al 1991). In contrast to the system using *T. timopheevi* cytoplasm, the present system requires only the male sterile and pollinator lines, so that it is called "two-line system" for hybrid wheat production. In this report, we examine the genetic effects of the *Ae. crassa* cytoplasm on the F_1 hybrids.

Materials and Methods

Five alloplasmic lines, *Ae. crassa*/11*Norin 26 (Tsunewaki 1980), *Ae. crassa*/6*Shirasagi-komugi, *Ae. crassa*/6*Junrei-komugi, *Ae. crassa*/6*Fujimi-komugi and *Ae. crassa*/5*Asakaze-komugi, and five cultivars, Norin 61, Nichirin-komugi, Ushio-komugi, Sakigake-komugi, and Orofen, were used as the male sterile and restorer lines, respectively. Twenty-three F_1 hybrids were produced by hand-pollination. They were space-planted at the Kasai Experimental Farm of Sumitomo Chemical Co, Hyogo, Japan, and compared their agronomic characters and heterosis on grain weight/plant with the F_1 hybrids having the corresponding normal (wheat) cytoplasm.

Results and Discussion

Average performances on agronomic characters of the 23 F_1 hybrids with the *Ae. crassa* cytoplasm and with the normal (wheat) cytoplasm are shown in Table 1. Two F_1 hybrids, Junrei-komugi × Sakigake-komugi and Fujimi-komugi × Nichirin-komugi, were not available for this investigation because of their low germinability and a small number of seeds obtained, respectively. The average of five (or four) pollinators is given in the table. The analyses of variance revealed that the effects of the *Ae. crassa* cytoplasm were significant on five characters, grain number/ear, grain weight/plant, 1000-grain weight, selfed and open-pollinated seed fertilities (Table 2). Fujigaki and Tsunewaki (1976) reported that the *T. timopheevi* cytoplasm hastened heading date and increased plant height and ear number/plant in Japanese cultivars. However, our results indicated that the effects of the *Ae. crassa* cytoplasm were not significant on these characters. The over-all averages

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Table 1. Average performances on agronomic characters of the F_1 hybrids with the *Ae. crassa* (Ms) and *T. aestivum* (N) cytoplasms

Cross combination[1]	Heading date(day)		Plant ht.(cm)		Ear no. /plant		Grain no. /ear		Grain wt. /plant(g)		1000-grain weight(g)		Test weight(g)		Seed fertility (%) Selfed		Seed fertility (%) Open-poll.		Heterosis (%)[2] Standard		Heterosis (%)[2] Mid-parent	
	Ms	N	Ms	N	Ms	N	Ms	N	Ms	N	Ms	N	Ms	N	Ms	N	Ms	N	Ms	N	Ms	N
Norin 26 × Poll.	4.16	4.16	85	80	16.0	15.2	33.0	37.7	19.7	21.6	38.1	37.7	705	715	82.4	90.9	83.3	91.0	12.4	23.3	30.7	43.2
Shirasagi × Poll.	4.18	4.16	84	86	15.7	14.4	31.6	41.2	20.0	23.5	41.0	39.4	718	717	80.6	91.5	78.6	91.7	13.7	34.0	12.9	33.4
Junrei × Poll.	4.15	4.19	91	85	19.4	19.4	34.5	40.1	27.5	30.6	41.5	38.2	708	713	83.3	93.7	82.5	91.1	57.0	74.4	30.8	51.8
Fujimi × Poll.	4.16	4.15	84	83	16.5	15.7	29.8	41.5	19.8	25.9	41.1	39.6	720	719	78.2	93.2	81.3	93.6	12.9	47.8	−4.3	34.5
Asakaze × Poll.	4.15	4.14	73	76	14.9	16.6	31.6	39.5	18.3	25.5	38.9	38.7	711	712	78.9	92.5	78.3	92.6	4.5	45.4	−1.8	37.1
Average	4.16	4.16	83	82	16.5	16.3	32.1	40.0	21.1	25.4	40.1	38.7	712	715	80.7	92.4	80.8	92.0	20.1	45.0	13.7	40.0

1) Norin 61, Nichirin-komugi, Ushio-komugi, Sakigake-komugi and Orofen were used as pollinator, and the average of five pollinators (four pollinators for Junrei-komugi and Fujimi-komugi) is given.

2) Standard and mid-parent heterosis (%) are given by percentages of grain weight/plant over the check cultivar (Norin 61) and the average (mid-parent) of the female and male parents with the wheat cytoplasm, respectively.

Table 2. The results of analyses of variance for the agronomic characters of the F_1 hybrids

Source of variation	d.f.	Mean square								
		Heading date	Plant height	Ear no. /plant	Grain no. /ear	Grain wt. /plant	1000-grain weight	Test weight	Seed fertility Selfed	Seed fertility Open-poll.
Main plot										
Rep.	1	2.46	14.88	15.37	179.48**	18.90	3.48	1448.10*:	0.04	37.33
Line (L)	22	24.90**	282.00**	19.79	19.20	57.87	20.28**	192.32	108.60**	67.98**
Error	22	2.83	13.68	18.22	17.27	50.45	4.76	96.42	22.69	21.76
Subplots										
Cytoplasm (C)	1	1.84	30.53	1.51	1414.18**	432.61**	39.26**	148.79	3098.36**	2914.31**
L × C	22	8.45**	41.78	14.76	23.70	30.52	6.53	209.75	62.86**	57.36**
Error	23	2.73	29.25	20.83	15.51	57.89	4.27	146.01	15.16	13.05

* and **: Significant at the 5% and 1% level, respectively.

of both the selfed and open-pollinated seed fertilities of the F_1 hybrids with the *Ae. crassa* cytoplasm were 11% lower than those of the F_1's with the wheat cytoplasm. The decreases in grain number/ear and grain weight/plant of the F_1 hybrids with the *Ae. crassa* cytoplasm were associated with their lower fertility than that of the F_1's with the wheat cytoplasm. A significant increase in 1000-grain weight of the F_1 hybrids with the *Ae. crassa* cytoplasm is probably due to the compensatory effect of the decreased seed fertility. No significant effect of the cytoplasm was detected on test weight, indicating that the grains of the F_1 hybrids with the *Ae. crassa* cytoplasm were not shriveled. In spite of the decreased fertility (80% fertility), the F_1 hybrids with the *Ae. crassa* cytoplasm showed, on the average, 20% and 14% heterosis on grain weight/plant over the check cultivar (Norin 61) and the average (mid-parent) of the female and male parents with the wheat cytoplasm, respectively. Fig. 1 shows ears and grains of the F_1 hybrids between Junrei-komugi × Nichirin-komugi and Norin 26 × Nichirin-komugi, which showed the highest standard and mid-parent heterosis, respectively.

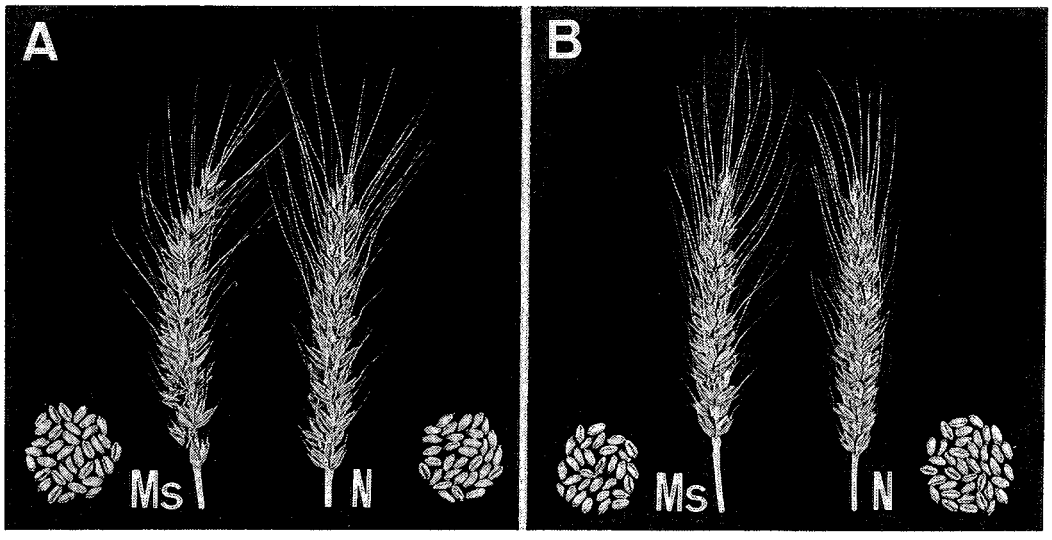

Fig. 1. Ears and grains of the F_1 hybrids between Junrei-komugi × Nichirin-komugi (A) and Norin 26 × Nichirin-komugi (B), which showed the highest standard and mid-parent heterosis, respectively. Ms and N indicate the F_1 hybrids with the *Ae. crassa* and wheat cytoplasm, respectively.

References

Fujigaki J and Tsunewaki K (1976) Basic studies on hybrid wheat breeding. VII. Characteristics of the male sterile lines of common wheat cultivars. Jpn J Breed 26: 179-186.

Murai K, Hirohara H and Tsunewaki K (1991) A new system for hybrid wheat production using *Aegilops crassa* cytoplasm. Wheat Inf Serv 72: 91-94.

Tsunewaki K (1980) Genetic diversity of the cytoplasm in *Triticum* and *Aegilops*. Japan Society for the Promotion of Science, Tokyo.

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Reproductive barriers between *Hordeum* and *Secale* species

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Intergeneric as well as interspecific hybridizations have been attempted to reveal phylogenetical relationships among genera in the tribe Triticeae and a number of successful hybrids have been produced (Sears 1948, Sakamoto 1973, Sharma and Gill 1983). These hybrids have potential values to widen genetic variation for cereal breeding. The hybrid between *Hordeum* and *Secale* was firstly reported by Brink et al (1944), then several successful hybrids have been reported (Fedak 1985 for review). There still remain problems to be solved concerning reproductive barriers between the two genera.

In the present report, several combinations of crosses between *Hordeum* and *Secale* species were conducted to estimate crossability between the two genera. Abortive process of the hybrid seeds between them was studied cytohistologically. Then the reproductive barriers were discussed.

Five Japanese two-rowed cultivars; *H. vulgare* cv. Hatakaze, Golden Melon, Satsuki-nijo, Nitta-nijo, Akagi-nijo, and five wild *Hordeum* species; *spontaneum* (two accessions), *murinum*, *stebbinssii*, *glaucum*, *bulbosum* (4x, two acces.) were used as female parents. Six different species of *Secale*; *africanum*, *kuprijanovii*, *segetale* (two acces.), *vavilovii* (two acces.), *ancestrale*, *cereale* (two acces.) were employed as male.

The female parents were emasculated about two days before anthesis and pollen grains were applied to the stigmas directly from anthers of the male parent. One day after pollination, one drop of 100 ppm solution of giberellic acid (GA_3) was applied to each floret. After surface sterilization of the immature caryopses they were cultured directly on modified Murashige and Skoog medium.

Some of the fertilized caryopses were harvested at 3, 6, 9, 12 and 15 days after pollination and they were studied on the paraffin sections.

In total 5,058 caryopses from 19,623 florets pollinated in 24 cross combinations were scored by their size and shape as fertilized ones with rye pollen, because the self-fertilized ones became plump and large but the crossed ones much smaller in size and slender in shape at ten to 14 days after pollination. Japanese two-rowed cultivars used in this experiment could be crossed with rye species, though the rates of obtaining the fertilized caryopses ranged from 1.5% for Akagi-nijo × *S. vavilovii* to 60.1% for Hatakaze × *S. vavilovii.* When Hatakaze was used as the female parent in the crosses, it always showed fairly good crossability, 58.2%, 60.1% and 55.3%, with different rye species. Conversely Nitta-nijo showed low crossability (1.5 - 17.9%) and wild *Hordeum* species had much less crossability (0.0 - 4.5%) with rye.

Considerable genetic variation is known to exist for crossability among cultivars as well as wild species in the same genus (Sharma and Gill, 1983). In wheat two genes, *Kr1* on 5BL and *Kr2* on 5AL, were reported to control effectively the crossability with rye (Krolow 1970, Lange and

Riley 1973, Sitch et al 1985), and rye carries a single gene for crossability (Tanner and Falk 1981). *Kr1* and *Kr2* repress crossability independently and the wheat cultivars carrying the both genes have low or no crossability with rye, while non-carriers show high crossability and those carrying one of the two are intermediate. The present result showed similar pattern in the crossability, thus it is suggested that *Hordeum* species also have two crossability genes interacting against *Secale* pollen.

Through the immature caryopsis culture only four caryopses from Satsuki-nijo × *S. africanum* generated shoots and roots. However, one died before and another one after transplanting into soil. Finally two hybrid plants grew up, though those were weak at the early seedling stage and showed leaf chlorosis. It was clearly showed that response to the immature caryopsis culture is quite different among different cross combinations and is independent of crossability.

Cytohistological study on seed abortion revealed that after fertilization of barley egg cell with rye pollen the zygote developed up to globular embryo stage, while in endosperm nuclear divisions occurred but cytokinesis did not follow. Abnormal nuclear divisions and nucellus enlargement were also observed. At nine to 12 days after pollination the embryo and endosperm seemed to degenerate.

There are several reproductive barriers such as prevention of fertilization, hybrid break-down, hybrid weakness or inviability, hybrid sterility, etc. (Hadley and Openshaw 1980). The present result suggests the crossability of *Hordeum* species is controlled by two genes against rye. The cytohistological study and the hybrids rescued by the immature caryopsis culture clearly show that fertilization occurs and the zygotes grow up to globular stage, but gradually break down in the course of development. Especially endosperm development is quite abnormal, eg, no cytokinesis occurs following the nuclear divisions. It seems to be a main cause of the seed abortion. The hybrids were weak and showed leaf chlorosis. This suggests complementary gene actions might occur in the hybrids.

Wheat Information Service
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Synthesis of telomere repetitive sequences at the broken ends in common wheat chromosomes

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Telomeres of chromosomes possess special repetitive DNA sequences. In higher plants, Richard and Ausubel (1988) have first cloned the sequence from *Arabidopsis thaliana* and revealed that they mainly consisted of repeats of 5'-CCCTAAA-3'. *In situ* hybridization using the sequences of *Arabidopsis* made signals on the chromosomal termini of tomato, barley and wheat, indicating high conservation of the sequences among the species of higher plants (Ganal et al 1991, Wang et al 1991, Murata et al 1992).

In yeast and human, the telomere repetitive sequence are known to be synthesized by telomerase but not by ordinary DNA polymerase. The numbers of the repeats are retained as a metabolic balance between the synthesis by the telomerase and the loss of terminal bases that results from every semiconservative DNA replication (reviewed by Blackburn 1991).

The purpose of this study is to investigate synthesis of the telomere sequences at the broken ends of chromosomes. For this study, common wheat is a suitable material because 'gametocidal genes' that induce chromosome breakage only in a specific developmental stage are available (Tsujimoto and Tsunewaki 1985, reviewed by Endo 1990). It is possible therefore to obtain broken chromosomes at various periods after the chromosomes had been broken. *In situ* hybridization probed by the telomere repetitive sequences to these broken chromosomes will indicate when, in the developmental stage, the broken ends acquire the telomere sequences.

Hybridization using the biotin-labeled probe of the *Arabidopsis* telomere sequences (pAtT4) and subsequent amplification by an antibody detected prominent signals at all the telomeres of the normal chromosomes and also the broken ends of the telocentric and deletion chromosomes that had passed through more than one generation since the appearance. However, no complete signals such as found in normal telomeres were not observed at the broken ends that had been produced during the former gametogenesis and thus passed through only the stages of gametogenesis, fertilization, embryogenesis and root development (Fig. 1). These findings clearly indicate that a certain time or stage is required for synthesis of the telomere repetitive sequences with a complete length. Nevertheless, because the broken ends without complete telomere sequences had been already healed, restoration of the normal complement of telomere sequences is not necessary for healing of broken ends.

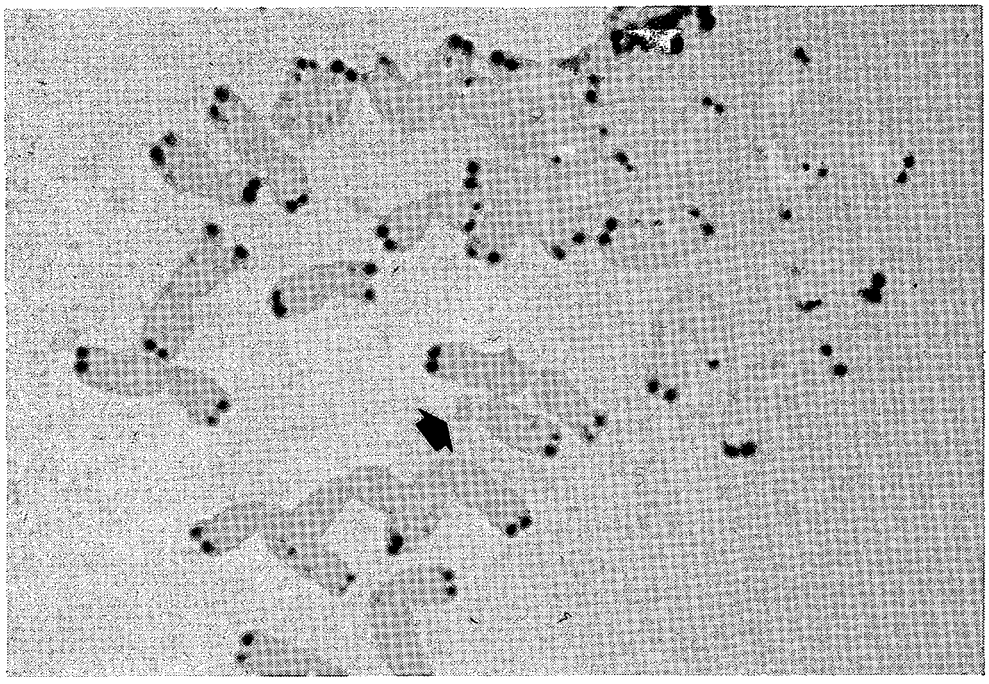

Fig. 1. *In situ* hybridization to the chromosomes of an F_2 plant between Norin 26 and disomic addition line with chromosome 3C carrying a gametocidal gene. The arrow indicates the broken ends without complete telomere sequences.

References

Blackburn EH (1991) Structure and function of telomeres. Nature 350:569-573.

Endo TR (1990) Gametocidal chromosomes and their induction of chromosome mutations in wheat. Jpn J Genet 65:135-152.

Ganal MW, Lapitan NLV, Tanksley SD (1991) Macrostructure of tomato telomeres. Plant Cell 3:87-94.

Murata M, Nakata N, Yasumuro Y (1992) Origin and molecular structure of the midget chromosomes in a common wheat with rye cytoplasm. Genome (in press).

Richard EJ, Ausubel FM (1988) Isolation of higher eukaryotic telomere from *Arabidopsis thaliana*. Cell 53:127-136.

Tsujimoto H, Tsunewaki, K (1985) Gametocidal genes in wheat and its relatives. II. Suppression of chromosome 3C gametocidal gene of *Aegilops triuncialis*. Genome 27:178-185.

Wang S, Lapitan NLV, Tsuchiya T (1991) Characterization of telomere in *Hordeum vulgare* chromosomes by *in situ* hybridization. I. Normal diploid barley. Jpn J Genet 66:313-316.

Wheat Information Service
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Genome structure of tetraploid wheats revealed by fluorescence *in situ* hybridization using total genomic DNA probes

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Introduction

Tetraploid wheats can be classified into two groups: the emmer wheats (genome = AABBDD) and the timopheevi wheats (AAGG). Both tetraploids received their A genome from the einkorn wheats. Although the origin of B and G genomes is under discussion, *Aegilops speltoides* is most suitable for both B and G genome donors (Tsunewaki et al 1980, Dvořák and Zhang 1990). In this study the relationship of tetraploid wheats and their putative diploid species was analyzed with respect to the genome structure by fluorescence *in situ* hybridization (FISH) using total genomic DNA probes of the diploid species. This paper provides molecular cytogenetic evidence for translocation between different genomes.

Materials and methods

Four tetraploid species, *Triticum dicoccoides* (AABB), *T. dicoccum* (AABB), *T. araraticum* (AAGG) and *T. timopheevi* (AAGG) and two diploid species, *T. monococcum* (AA) and *Ae. speltoides* (SS) were used in the present study. The genomic *in situ* hybridization (GISH) analysis was carried out as described by Mukai and Gill (1991) and Mukai et al (1993). Total genomic DNA from *T. monococcum* or *Ae. speltoides* was used as a probe. The other total genomic DNA was used as a blocking DNA at eight times the probe concentration. The sites of hybridization of biotinylated probe were detected using avidin-FITC.

Results and discussion

FISH analysis using total genomic T. monococcum DNA

The FISH patterns of *T. dicoccoides* and *T. dicoccum* showed that the A genome chromosomes were labeled by yellowish green fluorescence, while the B genome chromosomes revealed little fluorescence. In both species one translocation between genomes was detected. The distal 34% of the long arm of chromosome 4A was derived from a B genome chromosome.

The FISH patterns of *T. araraticum* and *T. timopheevi* showed that the biotin label was seen on all A genome chromosomes and two G genome chromosomes (Fig. 1). Three translocations between two genomes were detected. Chromosomes involved in the translocation were 6A, 1G and 4G. In chromosome 6A, one third of the short arm and a satellite including NOR came from the G

genome. Chromosome 1G showed an intercalary translocation. A small segment of the A genome chromatin is inserted in the interstitial region of the 1G short arm. The other A genome chromatin is present in the distal part of the 4G short arm.

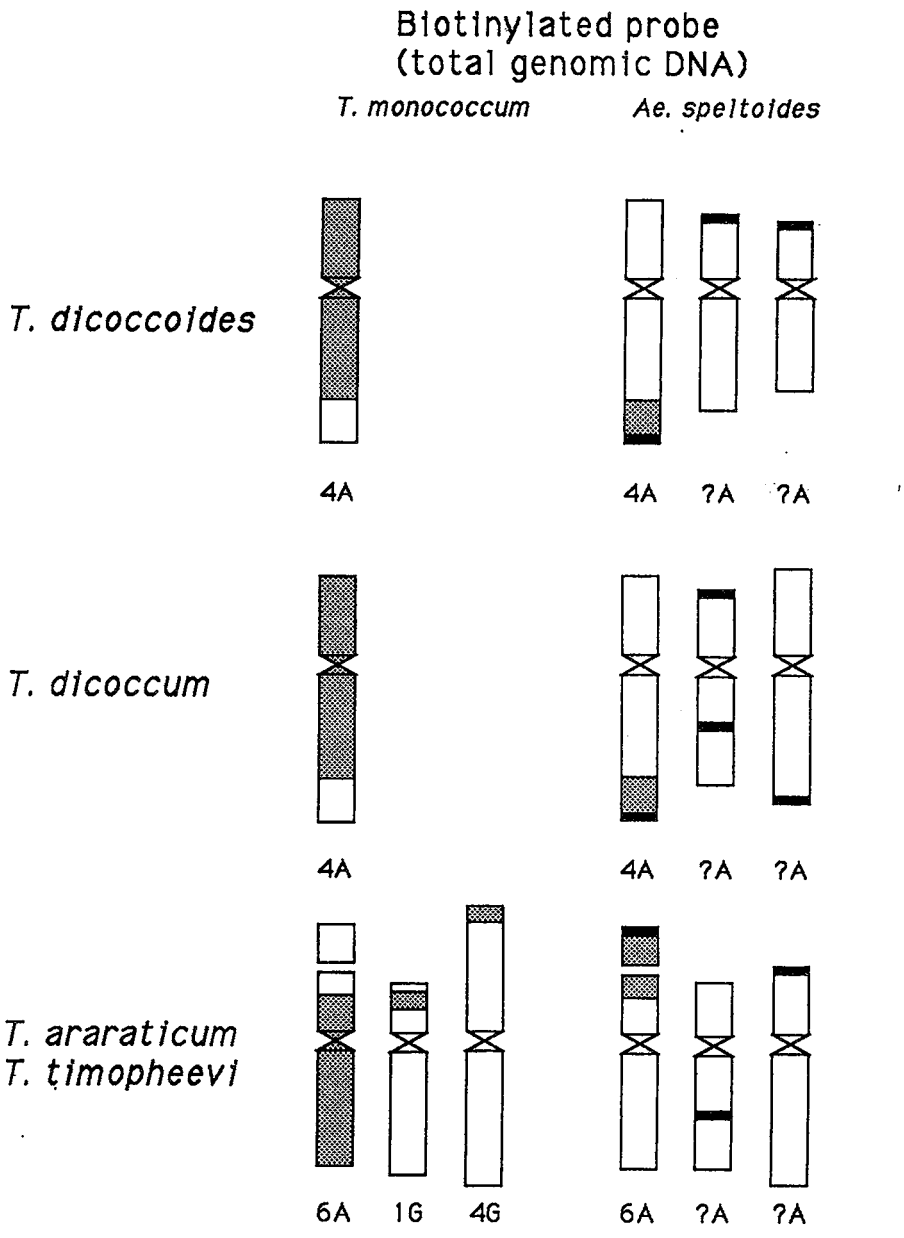

Fig. 1. The FISH patterns of intergenomic translocations in tetraploid wheats using total genomic DNA probes. Dotted and solid regions represent dispersed and localized labeling, respectively.

FISH analysis using total genomic Ae. speltoides DNA

In all tetraploid wheats, the B or G genome chromatin was easily distinguished from the A genome chromatin, because the *Ae. speltoides* probe was preferentially hybridized to the B or G genome chromosomes. No hybridization sites were observed in any chromosomes of the diploid wheat. Three A genome chromosomes of *T. dicoccoides* showed terminal translocation: one is on the long arm and two are on the short arm. In *T. dicoccum*, two chromosomes showed terminal translocation in the long arm. One chromosome carried two hybridization sites at terminal position in the short arm and in the middle of the long arm.

The FISH pattern of *T. araraticum* showed three kinds of chromosome translocations. The satellite and the proximal 30% of the short arm of chromosome 6A were labeled. Two other A genome chromosomes had only a localized telomeric or interstitial ISH sites.

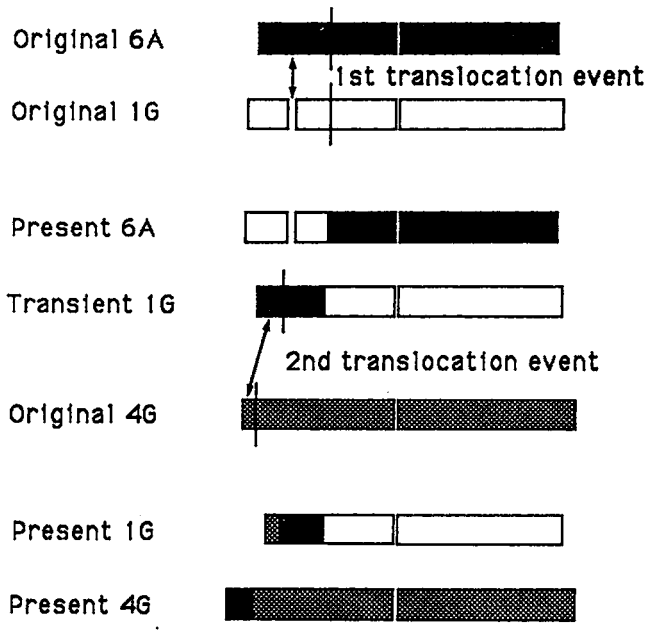

Fig. 2. Sequence of translocations between chromosomes 6A, 1G and 4G in the timopheevi wheats.

Nonhomoeologous translocation between chromosomes 6A, 1G and 4G in the timopheevi wheats

As for the present structures of chromosomes 6A, 1G, and 4G and their origins, we can speculate a hypothesis involving two translocation events, as shown in Fig. 2. The most possible course of events is a reciprocal interchromosomal translocation of the original 1G short arm including the satellite and the original 6A short arm and then a reciprocal translocation between transient 1G, distal to the 1GS-6AS breakpoint, and part of the original 4G short arm. The presence of a tiny segment of the G genome chromatin in two other A genome chromosomes can be explained by transposition, but its mechanism is unknown. Further molecular evidence of these intergenomic translocations, as Liu et al (1992) pointed out, will be achieved by the development of RFLP-based genetic map.

Acknowledgments

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References

Dvořák J and Zhang HB (1990) Variation in repeated nucleotide sequences sheds light on the phylogeny of the wheat B and G genomes. Proc Natl Acad Sci 87: 9640-9644.

Liu CJ, Atkinson MD, Chinoy CN, Devos KM and Gale MD (1992) Nonhomoeologous translocations between group 4, 5 and 7 chromosomes within wheat and rye. Theor Appl Genet 83: 305-312.

Mukai Y and Gill BS (1991) Detection of barley chromatin added to wheat by genomic *in situ* hybridization. Genome 34: 448-452.

Mukai Y, Friebe B, Hatchett JH, Yamamoto M and Gill BS (1993) Molecular cytogenetic analysis of radition-induced wheat-rye terminal and intercalary chromatin specifying resistance to Hessian fly. Chromosoma 102: 88-95.

Tsunewaki K, Mukai Y and Endo TR (1980) General discussion and conclusion In: Genetic Diversity of the Cytoplasm in *Triticum* and *Aegilops*, (ed.: K. Tsunewaki), pp. 159-209. Japan Soc Prom Sci, Tokyo.

Wheat Information Service
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Interorganellar translocation of chloroplast genes into mitochondria

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Mitochondria genome of angiosperm is known to show some characteristic features such as; 1) plasticity of genome structure, 2) promiscuous DNAs, and 3) RNA editing. In order to analyze the promiscuous DNAs in the mitochondria genome of common wheat, interorganellar translocation of chloroplast genes into mitochondria genome was investigated.

Fourteen cosmid clones which cover the entire part of master circle DNA of common wheat mitochondria (Quetier et al 1985) were amplified, and their DNAs were extracted. Chloroplast and mitochondrial DNAs were also extracted from young seedling of common wheat, *Triticum aestivum* cv. Chihoku as controls. Those DNAs were digested with *SalI* and supplied for Southern hybridization. DNA probes corresponding to 33 chloroplast genes out of 56 protein coding genes, including rRNA genes were prepared with digestion of the appropriate restriction endonucleases and/or PCR amplification after the computer search of rice chloroplast DNA (Hiratsuka et al 1989). Rice chloroplast genes used in the present study are listed in Table 1.

Table 1. List of rice chloroplast genes which have been identified their function.

Photosynthesis genes		
Rubisco	(1/1)	*rbcL*
H^+-Atpase subunit	(6/6)	*atpA, atpB, atpE, atpF, atpH, atpI*
Photosystem I	(3/5)	*psaA, psaB, psaC, psaI, psaJ*
Photosystem II	(10/13)	*psbA, psbB, psbC, psbD, psbE, psbF, psbH, psbI, psb(J), psbK, psbL, psbM, psbN*
Cytochrome b/f complex	(2/4)	*petA, petB, petD, petG*
Genetic system genes		
30S ribosomal proteins	(5/13)	*rps2, rps3, rps4, rps7, rps8, rps11, 3'-rps12, 5'-rps12, rps14, rps15, rps16, rps18, rps19*
50S ribosomal proteins	(4/10)	*rpl2, rpl14, rpl16, rpl20, rpl21, rpl22, rpl23, rpl32, rpl33, rpl36*
rRNAs	(2/4)	*5S rRNA, 4.5S rRNA, 23S rRNA, 16S rRNA*

Probes used for southern hybridization and/or PCR analysis are underlined.

Table 2. List of chloroplast genes found in the mitochondrial genome of common wheat

Photosynthesis genes	
H+-Atpase subunit	*atpA, atpB, atpE, atpF, atpH, atpI*
Photosystem I	*psaA, psaC, psaI*
Photosystem II	*psbA, psbB, psbC, psbD, psbE, psbF, psbH, psb(J), psbK, psbL, psbM, psbN*
Cytochromen b/f complex	*petA, petD*
Genetic system genes	
30S ribosomal proteins	*rps2, rps7, rps11, rps12, rps18*
50S ribosomal proteins	*rpl2, rpl16, rpl20, rpl36*
rDNA	*23S rDNA, 16S rDNA*

Translocated chloroplast genes are underlined.

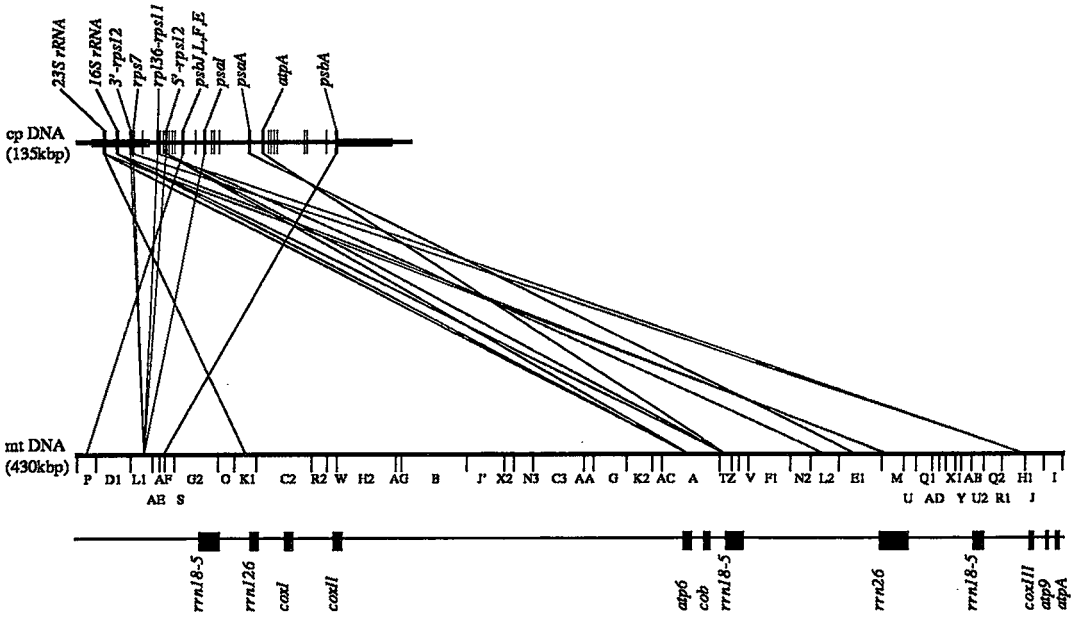

Fig. 1. Homologous portions of wheat mt DNA with chloroplast genes

Southern hybridization analysis of cosmid DNAs using these DNA probes showed that homologous sequences with 14 out of 33 chloroplast genes were detected in the cosmid clones of mitochondrial DNA. The chloroplast genes whose homologous sequences were found in mitochondrial DNA are listed up in Table 2. It is noteworthy that *rbc*L gene whose homologous sequence was found in the mitochondria genome of maize (Lonsdale et al 1983) and rice (Moon et al 1988) was not detected in wheat mitochondrial DNA. Physical map of homologous portions in mitochondrial DNA with chloroplast genes is shown in Fig. 1. Translocation units were almost corresponded to each chloroplast gene, because no gene clusters of chloroplast DNA were found in wheat mitochondria genome. These lines of evidence suggest that translocation mode of chloroplast genes in wheat group differed from those of rice and maize. Furthermore, it should be emphasized that translocation hot spots in the mitochondrial DNA were found: Fragment L_1 contains five kinds of homomogous sequence, and T harbors three homologous sequences. On the other hand, fragments C_2 to AC carry no homologous sequences with chloroplast genes (Fig. 1).DNA sequencing around the translocated region is required to estimate translocation mechanism(s).

Acknowledgments

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References

Hiratsuka J, Shimada H, Whittier R, Ishibashi T, Sakamoto M, Mori M, Kondo C, Honji Y, Sun CR, Meng BY, Li YQ, Kanno A, Nishizawa Y and Hirai A (1989) The complete sequence of rice (Oryza sativa) chloroplast genome: Intermolecular recombination between distinct tRNA genes accounts for a major plast DNA inversion during the evolution of the cereals. Mol Gen Genet 217: 185-194.

Lonsdale DM, Hodge TP, Howe CJ and Stern DB (1983) Maize mitochondrial DNA contains a sequence homologous to the ribulose-1,5-bisphosphate carboxylase large subunit gene of chloroplast DNA. Cell 34: 1007-1014.

Moon E, Kao T-H and Wu R (1988) Rice mitochondrial genome contains a rearranged chloroplast gene cluster. Mol Gene Genet 213:247-253.

Quetier F, Lejeune B, Delorme S and Falconet D (1985) Molecular organization and expression of the mitochondrial genome of higher plants. In: Encyclopedia of Plant Physiology 18. Ed. Doce R and Days DA. pp. 25-36. Springer-Verlag.

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Structure analysis of mitochondria genomes in interspecific hybrids related to *Aegilops triuncialis*

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Mitochondria genome of angiosperm is known to show some characteristic features such as; 1) plasticity of genome structure, 2) promiscuous DNA, and 3) RNA editing. In order to analyze structural alterations of mitochondria genomes in interspecific hybrids related to *Aegilops triuncialis* (CCUU as genome formula), Restriction Fragment Length Polymorphism (RFLP) analysis of mitochondrial DNAs in *Ae. triuncialis* complex was carried out. *Ae. triuncialis* complex was used for the study, because various combinations of interspecific hybrids (natural hybrids, ancestral species, artificially synthetic strains and alloplasmic lines) were obtained.

Table 1. Plant materials used in the study

Plant source		Genome formula (Haploid)	Chloroplast type*	Abbrev.
Ae. caudata	var. *polyathera*	C	2a(C)	cdt1
	var. *typica*	C	2a(C)	cdt2
Ae. umbellulata	var. *typica*	U	3(U)	umb1
	var. *typica*	U	3(U)	umb2
Ae. triuncialis	var. *typica*	CU	2a(C)	trn1
	var. *persica*	CU	2a(C)	trn2
	eu-triuncialis	CU	2a(C)	trn3
	orientalis	CU	3(U)	trn4
	orientalis	CU	2a(C)	trn5
	eu-triuncialis	CU	2b(#)	trn6
	eu-triuncialis	CU	3(U)	trn7
	T1**	CU	3(U)	trn8
	Synthetic	CU	2a(C)	S-trn1
	Synthetic	CU	2a(C)	S-trn2
(*caudata*)-compactum		ABD	2a(C)	J02
(*umbellulata*)-Jones Fife		ABD	3(U)	F03
T. compactum	var. *humboldii*	ABD	7(B)	Com
T. aestivum	cv. Jones Fife	ABD	7(B)	JF
	cv. Chinese Spring	ABD	7(B)	CS
	cv. Chihoku komugi	ABD	7(B)	Chihoku

* Plasma type is given in parentheses.

** T1 is obtained from Dr. I. Panayotov, Bulgaria.

Table 2. The gene probes used in the study

Gene	Source of DNA	Fragment	
Mitochondrial gene			
atpA	Pea	1.5kb	*Eco* RI / *Hind* III
atp6	*Oenothera*	1.5kb	*Nhe* I
atp9	*Oenothera*	0.65kb	*Bam* HI / *Mlu* I
cox I	*Oenothera*	0.66kb	*Bam* HI / *Eco* RI
cox II	Pea	1.9kb	*Eco* RI
cox III	*Oenothera*	1.1kb	*Eco* RI / *Pst* I
cob	Wheat	1.7kb	*Bam* HI / *Eco* RI
rrn26S	Wheat	5.1kb	*Bam* HI / *Sal* I
rrn18S-5S	Wheat	3.2kb	*Bam* HI / *Sal* I
nad3 / rps12	Wheat	1.4kb	*Bgl* II
nad5 ab	Wheat	5.6kb	*Hind* III
orf25	Wheat	0.8kb	*Mlu* I / *Bam* HI

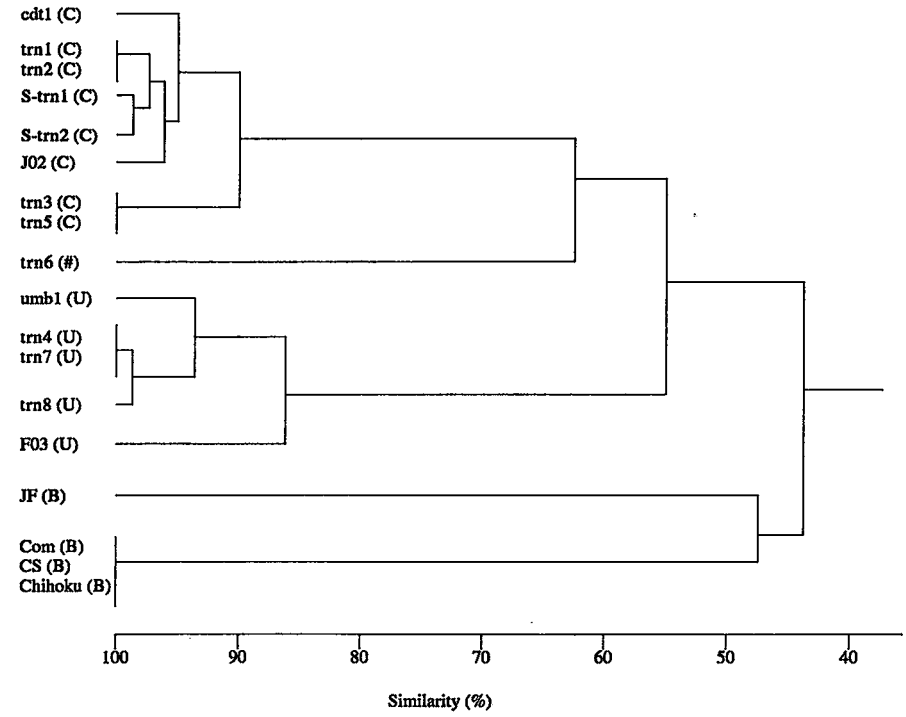

Fig. 1. A dendrogram showing the phylogenetic relationships among *Ae. triuncialis* complex based on RFLPs of mitochondrial genes

Total DNAs were isolated from the young seedlings of 20 strains of *Ae. triuncialis* complex (Table 1) according to the CTAB method (Murray and Thompson 1980). These DNAs were digested with *Bam*HI and *Hin*dIII, and hybridized with twelve mitochondrial genes as probes (Table 2) to carry out RFLP analysis. RFLP analysis of total chloroplast DNAs from five strains of *Ae. triuncialis* as well as common wheat was also conducted.

Chloroplast DNAs of five strains were classified into three groups, namely C type, U type and the third type (Murai and Tsunewaki 1986), the two of which revealed the identical fragment pattern with those of *Ae. caudata* (C type) and *Ae. umbellulata* (U), respectively (Ogihara and Tsunewaki 1988). No ancestral strains corresponding to the third type of *Ae. triuncialis* was found, so far examined.

Based on the fragment patterns produced from Southern hybridization in combination of twelve mitochondrial gene probes with two restriction enzymes, namely 24 probe-enzyme combination, similarity among eighteen strains was calculated. Using the similarity value, a dendrogram showing genetic relationships among eighteen strains were constructed according to the UPGMA (Nei 1975) as shown in Fig. 1. Mitochondrial DNAs of *Ae. triuncialis* were classified into three major groups, namely C type, U type and the third type. The mitochondrial DNA of the third type of *Ae. triuncialis* (#) was much closer to the C type rather than the U type. These lines of evidence support the data obtained from RFLP analysis of chloroplast DNAs. But intraspecific variations were found in both mitochondria DNAs of C and U groups. It is striking that mitochondrial DNA of Jones Fife was diversed from other mitochondrial DNAs of B type (Fig. 1), although the fragment patterns of chloroplast DNA of Jones Fife was identical to that of other common wheat. It is noteworthy that mitochondrial genomes of interspecific hybrids were different from those of original strains: Fragment patterns of trn1-trn2,S-trn1, S-trn2, and J02 differed from those of cdt1. Simultaneously, those of trn4-trn7, and trn8 were different from that of umb1. Cdt1 and umb1 were the cytoplasm donors of hybrid species of *Ae. triuncialis* including synthetic strains and alloplasmic lines. This suggests that mitochondrial DNAs were possibly changed during and/or after interspecific hybridization. These lines of evidence indicate the plasticity of mitochondria genome in wheat complex.

References

Murray HG and Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. Nucl Acid Res 8: 4321-4325.

Murai K and Tsunewaki K (1986) Molecular basis of genetic diversity among cytoplasms of *Triticum* and *Aegilops* species. IV CtDNA variation in *Ae. triuncialis*. Heredity 57: 335-339.

Nei M (1975) Molecular population genetics and evolution. North Holland.

Ogihara Y and Tsunewaki K (1988) Diversity and evolution of chloroplast DNA in *Triticum and Aegilops* as revealed by restriction fragment analysis. Their Appl Genet 76: 321-332.

Wheat Information Service
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Comparative phylogenetic study in amylase isozymes in Gramineae: Why are these enzymes nice marker for phylogenetical approach in common wheat?

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Amylase plays a key role in metabolism of the plants, especially during germination of starchy seeds. The molecular variants of this enzyme have brought about the nice markers for phylogenetical approaches in wheat, because they are genetically polymorphic and can be detected easily by polyacrylamide isoelectrofocusing (Nishikawa and Nobuhara 1971, Nishikawa 1983, Nishikawa et al 1992). On the other hand, rice seed at germinating stage had only few isozyme bands or less variable in comparison with wheat seed. While corn is intermediate in the number of isozyme bands and variability. That is, there are apparent interspecific differences in some traits of amylase isozymes in family Gramineae, which is dynamically differentiates (Takeoka 1959) and contains several important cereals. This brought us the vivid interests in differentiation of amylase isozyme genetic system in the course of evolution of this large family.

Materials and methods

Seeds of common wheat, oat, finger millet, corn and rice in three conditions, dry, germinating and developing stages were used. The following treatments were applied to the crude extracts of amylase from respective seeds; (1) incubation in 70°C hot water for inactivating β-amylase, (2) dialyze in acetate buffers (pH 5.0-3.0) for investigating the critical pH of α-amylase tolerance to acidity, (3) pappain or 2-mercaptoethanol treatment for activating the latent β-amylase. The isozymes were separated by the thin layer polyacrylamide isoelectrofocusing.

Results and discussion

Table 1 shows the characteristics of both α- and β- amylase isozymes in common wheat. It is apparent from Table 1 that there are genetical, physiological and biochemical differentiation of these isozymes in wheat. Such differentiation of the isozymes would be expected in the other cereals. So, five crops were compared with one other in regard to these characteristics. The results obtained are shown in Table 2. Hexaploid wheat had 15 bands of β-amylase in dry seed, oat and corn having a single band of β-amylase. Whereas millet and rice had none of β-amylase band in dry seed. By the treatment of pappain, 14 bands of β-amylase isozymes were induced in wheat, and eight bands in corn. But none was induced in oat and rice. Unfortunately, millet was out of analysis. Three to 22 bands of β-amylase isozyme were found in germinating seed of these five cereal plants. In wheat, 12 bands of out of these found during germination also occurred in developing seed, five

Table 1. Properties of amylase in wheat

	α-amylase[1]		β-amylase[2]
	malt	green	
Gene location	long arm of	long arm of	
(homoeologous chromosome)	group 6	group 7	groups 4 and 5
Active stage			
germinating	1-10 days	3-12 days	0-12 days
developing	non	little	1 week-dry seed
Adsorption to	strong	very weak	
intact starch granale[3]			
Isoelectric point	6.2-7.2	4.3-5.9	
Critical pH	3.6	3.0	

[1] Nishikawa and Watanabe (1988), [2] Ainsworth et al (1983), [3] Sargeant and Walker (1978)

Table 2. Comparison of amylase isozymes among five cereals

		Number of isozyme bands or limmiting pH				
		wheat	oat	millet	corn	rice
Dry seed						
β-amylase		15(4.0-6.0)*	1(4.2)	0	1(3.8)	0
β-amylase induced by pappain		14(4.4-6.0)	0	na**	8(3.8-4.8)	0
Germinating seed						
β-amylase		22(4.0-6.3)	8(3.9-4.5)	4(4.2-4.6)	5(3.8-5.5)	3(4.6-4.8)
α-amylase	Malt	15(6.3-7.0)				
	?		10(3.9-4.8)	6(4.3-5.0)	12(3.6-5.2)	4(4.6-4.8)
	Green	9(4.1-4.6)				
Developing seed						
β-amylase		12(4.0-5.5)	5(4.1-4.7)	na	2(3.8)	0
α-amylase		3(4.1-4.5)	5(4.1-4.7)	na	2(3.6-4.5)	3(4.6-4.7)
Limmiting pH						
β-amylase		3.0	3.0	na	3.6	3.6
α-amylase	Malt	3.6				
	?		4.0	5.0	3.6	4.0
	Green	3.0				

* : pH zone in which bands distribute in the gel, ** na: non-analysis

bands in oat and two bands in corn, respectively. Beta-amylase isozyme bands in wheat and oat were tolerant to the stronger acidity (pH3.0), whereas the critical pH for those in corn and rice was 3.6.

As previously reported, 15 malt type bands and nine green type bands of α-amylase isozyme were detected in germinating seed of common wheat. Ten, six, twelve and four α-amylase isozyme bands were detected in germinating seed of oat, millet, corn and rice, respectively. Three very faint bands occurred in the early stage of developing seed in wheat. Oat, corn and rice had five, two and three faint bands in the young developing seed, respectively. Malt type and green type of α-amylase isozymes in wheat are different in several characteristics as shown in Table 1. The critical pH of acid tolerance was 3.6 for malt type isozymes and 3.0 for those of green type in wheat, respectively. Alpha-amylase isozymes in oat, millet, corn and rice were inactivated at pH 4.0, 5.0, 3.6 and 4.0, respectively. These pH data were not sensitive enough for clear discrimination into two types of isozymes. However, Gonokami et al (1992) reported clear discrimination of α-amylase isozymes of wheat into malt and green types by use of differential adsorption ability of the intact raw starch granules. They also could detect four of eleven bands of corn and two of three bands in rice as malt type. The result suggests that malt type isozymes tend to distribute in the higher pH zone in these materials as in the case of wheat.

It is evident from the informations presented above, that differentiation of α-amylase isozymes into malt type and green type have taken place in the very early stage of, or before differentiation of Gramineae, that is the dual genetic system of two types of α-amylase isozymes, which was confirmed at first in wheat and its relatives (Nishikawa et al 1989, Furuta et al unpublished) is a characteristics common to the whole family. One more important aspect is that there are more or less intraspecific genetic variations in amylase isozymes in this family, especially in *Triticinae*. In addition, common wheat and oat, hexaploid species, have triplicate genetic system. Interspecific variation and polyploidy no doubt result in the considerable genetic variation of α- and β-amylase isozymes. Nishikawa and Nobuhara (1971), Nishikawa et al (1975, 1980, 1988) analyzed the genetic variation of those isozymes in di- and tetraploid wheat as well as hexaploid wheat. In addition, diploid ancestral species of tetra- and hexaploid wheat were also analyzed. Based on these results, phylogeny of common and tetraploid wheat was discussed (Nishikawa et al 1980, 1992). Some of α-amylase loci were analyzed and mapped on the chromosomes of homoeologous groups 6 and 7 (Nishikawa et al 1981). On the other hand, the fewer studies comparable to those in wheat were reported in oat, corn and rice and of course in millet.

Huang et al (1992) compared DNA base sequences of α-amylase among rice, barley and wheat, and classified the genes into two families or three subfamilies and discussed about phylogeny of this enzyme in Gramineae. The present results at the enzyme level well consistent with data at DNA sequence level.

References

Ainsworth CC, Gale MD and Baird S (1983) The genetics of β-amylase isozymes in wheat. 1. Allelic variations among hexaploid wheat. Theor Appl Genet 66: 39-49.

Gonokami Y, Nishikawa K and Furuta Y (1992) Adsorption of malt type α-amylase to raw starch. Japan J Breed 42 (suppl 2): 316-317.

Huang N, Stebbins GL and Rodriguez RL (1992) Classification and evolution of α-amylase genes in plants. Proc Natl Acad Sci USA 89: 7526-7530.

Nishikawa K (1983) Species relationship of wheat and its putative ancestors as viewed from isozyme variation. Proc 6th Intern Wheat Genet Symp Kyoto: 59-63.

Nishikawa K and Nobuhara (1971) Genetic studies of α-amylase isozymes in wheat. I. Location of genes and variation in tetra- and hexaploid wheat. Japan J Genet. 46: 345-353.

Nishikawa K and Watanabe Y (1988) Change in activity of alpha-amylase in developing and germinating wheat seed. Proc 7th Intern Wheat Genet Sympo, Cambridge, 1: 597-602.

Nishikawa K, Furuta Y and Ban T (1989) The dual genetic systems for α-amylase isozymes of germinating seed of wheat and its relatives. Isozyme Bulletin 22: S113.

Nishikawa K, Furuta Y and Goshima (1975) Genetic studies of α-amylase isozymes in wheat. II. Reconstituted AABB tetraploid, *Aegilops squarrosa* and their synthetic hexaploid. Jpn J Genet 50: 409-416.

Nishikawa K, Furuta Y and Wada T (1980) Genetic studies of α-amylase isozymes in wheat. III. Intraspecific variation in *Aegilops squarrosa* and birthplace of hexaploid wheat. Jpn J Genet 55: 325-336.

Nishikawa K, Furuta Y, Hina Y and Yamada T (1981) Genetic studies of α-amylase isozymes in wheat. IV. Genetic analysis in hexaploid wheat. Jpn J Genet 56: 385-395.

Nishikawa K, Furuta Y, Hiraku T and Takayanagi M (1988) Variation and chromosome arm location of the genes for gibberellic acid induced isozymes (Genetic studies of α-amylase isozymes in wheat V.). Japan J Breed 38: 187-197.

Nishikawa K, Furuta Y, Yamada T and Kudo S (1992) Genetic studies of α-amylase isozymes in wheat. VII. Variation in diploid ancestral species and phylogeny of tetraploid wheat. Jpn J Genet 67: 1-15.

Sargeant JB and Walker TS (1978) Adsorption of wheat alpha-amylase isozymes to wheat starch. Starke 30: 160-163.

Tateoka T (1959) The family Gramineae (in Japanese). Meibundo (Tokyo) pp. 150.

Wheat Information Service
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Detection of HMW glutenin genes by DNA hybridization and breadbaking quality of amphidiploid synthesized between *Aegilops squarrosa* and *Secale cereale*.

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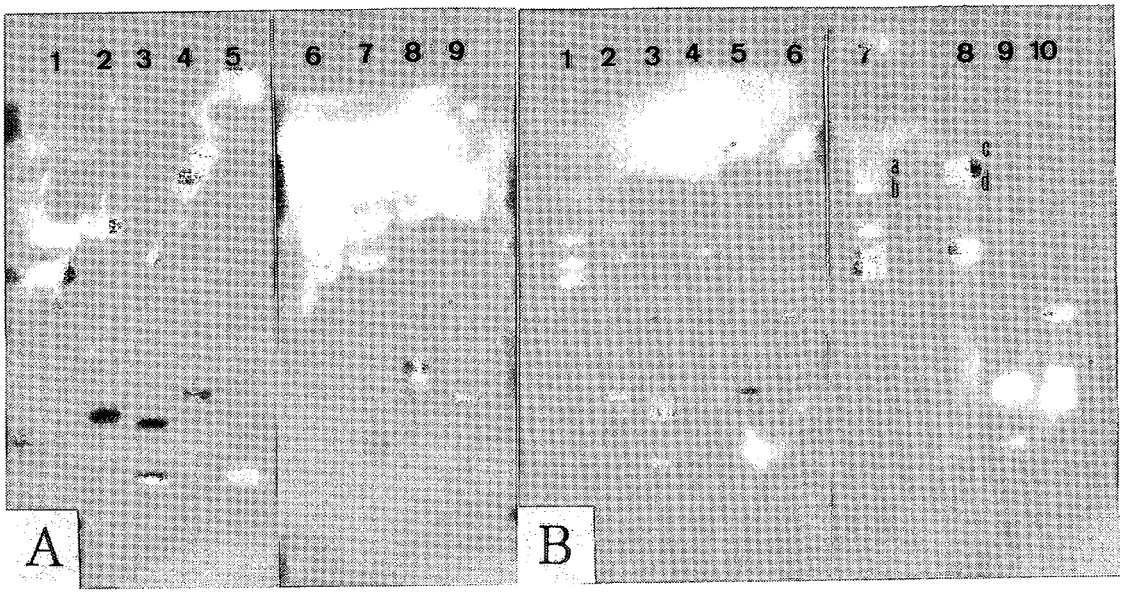
We have synthesized a new amphidiploid having genomes DDRR between *Aegilops squarrosa* var. typica and *Secale cereale* cv. Prolific (Kawakubo and Taira 1992). High molecular weight (HMW) glutenin subunits are governed by genes located on long arms of homoeologous group 1 chromosomes in hexaploid wheat (Boyd et al 1967, Payne et al 1979) and all three HMW glutenin loci are *Glu-A1, Glu-B1, and Glu-D1* (Payne and Lawrence 1983). Since alleles at these three loci have shown differential effects on bread-baking quality in hexaploid wheat, the structure of HMW glutenin genes of genomes D and R, and the flour quality of amphidiploid were investigated in this study.

Identification and comparison of genomic DNA restriction-fragment of HMW glutenin genes between amphidiploid and its direct parents

Amino acid sequence of protein encoded by a HMW glutenin cDNA fragment (777bps) isolated from hexaploid wheat cv. Park consisted of repetitive units of two motif, PGQGQQ and GYYPTSLQQ, which were typical to the amino acid sequence of common region in HMW glutenin proteins. Enzyme-digested genomic DNA fragments from F_3 progeny of amphidiploid and its direct parents, tetraploid *Ae. squarrosa* (genomes DDDD) and *S. cereale* (RRRR), were probed with the cDNA fragment. The hybridization patterns of DNA restriction fragments were as follows (Fig. 1): in *Eco*RI digestion, five bands were found in amphidiploid, two bands in *Ae. squarrosa* and three bands in *S. cereale*; in *Bam*HI digestion, five bands were found in amphidiploid, two bands in *Ae. squarrosa* and three bands in *S. cereale*; in *Hin*dIII digestion, five bands were found in amphidiploid, two bands in *Ae. squarrosa* and four bands in *S. cereale*. In particular, the band c in *Eco*RI digestion of *S. cereale* (Fig. 1B, lane 8) had slightly large molecular weight than the band a of amphidiploid (lane 7), and the band d (lane 8) was the same as the band a. It seems that the band c in *S. cereale* splits into two fragments such as bands a and b in amphidiploid.

Comparison of HMW glutenin proteins of amphidiploid and its parents

Glutenin proteins deposited in endosperm were extracted and separated by SDS PAGE (Fig. 2). The distribution of HMW glutenin proteins in diploid seeds was different from that in tetraploid seeds in both parental lines. Origin of protein bands of amphidiploid was considered as follows: band a was derived from either *S. cereale* or *Ae. squarrosa*; bands b, c, e and f from *S. cereale*; band d from *Ae. squarrosa.*



Flg 1. Hybridization of the HMW glutenin cDNA to restriction enzyme-digested DNA from amphidiploid and its direct parents. A. Lanes 1, 2, 3, 7 and 9 are in *Ae. squarrosa* (genomes DDDD) and lanes 4, 5, 6 and 8 are in amphidiploid (DDRR). Lanes 1, 6 and 7 are *Eco*RI-digestion, lanes 2, 4, 8 and 9 are *Bam*HI-digestion and lanes 3 and 5 are *Hin*dIII-digestion. B. Lanes 1, 2, 3 and 7 are in amphidiploid and lanes 4, 5, 6, 8, 9 and 10 are in *S. cereale* (RRRR). Lanes 1, 4, 7 and 8 are *Eco*Ri-digestion, lanes 2, 5 and 9 are *Bam*HI-digestion, and lanes 3, 6 and 10 are *Hin*dIII-digestion.

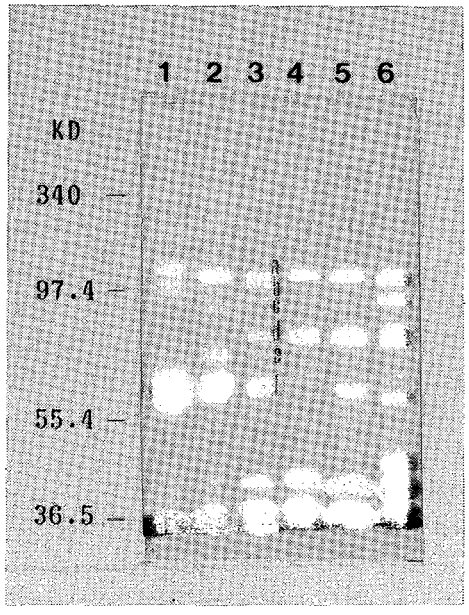


Fig. 2. SDS PAGE of glutenins. Lane 1 molecular weight standards, lane 2 *S. cereale* (2X), lane 3 *S. cereale* (4X), lane 4 amphidiploid, lane 5 *Ae. squarrosa* (2X), lane 6 *Ae. squarrosa* (4X), lane 7 common wheat (Hard Red Spring).

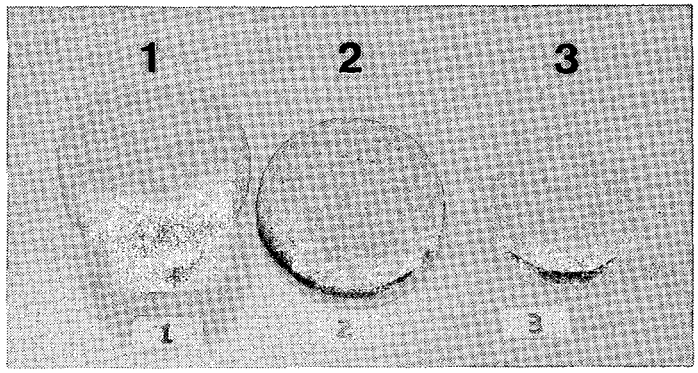


Fig. 3. Baking test for flours. 1 common wheat (Hard Red Spring), loaf volume of 175 ml; 2 amphidiploid, 85 ml; 3 *S. cereale*, 66 ml.

Baking tests

Bread was baked with 30 g of flour by straight dough procedure. Because of bad weather condition at harvesting time, amphidiploid flour was low quality, and thus the dough was sticky and runny. Nevertheless, a loaf of amphidiploid was increased by 29% in volume compared with that of rye, although amphidiploid showed 49% in volume of bread wheat (Fig. 3). Wet gluten (3%) was obtained from amphidiploid flour, but no gluten was detected in rye flour.

These results showed that the amphidiploid in which *Glu-1D* gene of glutenin subunits was introduced from *Ae. squarrosa* had improved bread-baking quality in comparison with rye.

References

Boyd WJR, Lee JW, and Wrigley CW (1967) The control of wheat gluten synthesis at the genome and chromosome level. Experientia 23: 332-333.

Kawakubo J and Taira T (1992) Intergeneric hybrids between *Aegilops squarrosa* and *Secale cereale* and their meiotic chromosome behavior. Plant Breeding 109: 108-115.

Payne PI, Law CN, and Mudd EE (1979) Control by homoeologous group 1 chromosome of the high molecular weight subunits of glutenin: A major protein of wheat endosperm. Theor. Appl. Genet. 58: 113-120.

Payne PI, and Lawrence GJ (1983) Catalogue of alleles for the complex gene loci, *Glu-A1*, *Glu-B1* and *Glu-D1* which code for high molecular weight subunits of glutenin in hexaploid wheat. Cereal Res. Commun. 11: 29-35.

Wheat Information Service
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Relation between wheat seed storage protein subunits and noodle viscoelasticity

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Summary

A comparison was made of the electrophoretic patterns of Wheat seed storage proteins for Kanto 107, a wheat (*Triticum aestivum* L.) line with good noodle viscoelasticity, and Norin 61, a wheat cultivar with standard viscoelasticity. A protein band with molecular weight of 53 kD was detected in Kanto 107, but not in Norin 61. A band with molecular weight of 129 kD was found in Norin 61, but not in Kanto 107. When Kanto 107 was cultivated in winter cropping on drained paddy fields, viscoelasticity was found to decrease, the 53 kD subunit disappeared from the electrophoretic pattern and the 129 kD subunit appeared. These subunits thus appear to relate to viscoelasticity.

Introduction

Wheat (*Triticum aestivum* L.) seed storage protein consists of glutenin, gliadin, globulin and albumin. Glutenin and gliadin account for about 80% total seed storage protein content, and this feature is closely related to bread making quality (Payne et al 1984), flour hardness (Nakamura et al 1990) and Chinese noodle making quality (Huang et al 1988). Japanese noodle making quality may also possibly be associated with seed protein composition. In this study, a comparison was made of electrophoretic patterns between a wheat line and a cultivar differing in noodle making quality, so as to determine the relation between seed storage protein subunits and noodle viscoelasticity.

Materials and methods

Kanto 107, a wheat line with good noodle viscoelasticity and Norin 61, a cultivar with standard viscoelasticity were used, Four Kankei lines obtained by crossing with Kanto 107 as a maternal parent were also used. The materials were cultivated three times in 1988 to 1991 in an upland and a paddy field double-cropped with rice. Flour (5 mg) was suspended in the sample buffer (2% sodium dodecyl sulfate (SDS), 10% glycerol, 5% 2-mercaptoetanol and 0.0625 M Tris-HCL pH 6.8) and shaken for 2 hr. The sully was heated at 95 °C for 3 min and centrifuged at 15,000 ×g for 3 min. The supernatant was subjected to SDS-poly acrylamide gel electrophoresis (PAGE). Noodles (10 g) were boiled in water for 20 min. Noodle viscoelasticity was estimated by the sensory test of the Ministry of Agriculture, Forestry and Fishries noodle quality test manual. Protein content was analyzed by the Kjeldahl method.

Results and discussion

Fig. 1 shows SDS-PAGE patterns of seed storage proteins from Norin 61 (lane 1-4) and Kanto 107 (lane 5-8). A subunit with molecular weight of 53 kD was detected in Kanto 107 cultivated in an upland field, but not in Norin 61 cultivated in upland and paddy fields. In contrast, a subunit with molecular weight of 129 kD was found in Norin 61 in upland and paddy fields, but not in upland Kanto 107. Kanto 107 in the upland field produced noodles with good viscoelasticity while Norin 61 of low viscoelasticity was produced. According to the experiments conducted in 1989-1990, when Kanto 107 was cultivated in a paddy field, the 53 kD subunit disappeared and the 129 kD subunit appeared. These viscoelasticity appeared to decrease. However, in the experiment in 1991, protein subunits and viscoelasticity of Kanto 107 cultivated in the paddy field were identical to those of Kanto 107 in the upland field. The protein content in 1991 was slightly higher than that in 1989 and 1990, but the relationship between quantity and quality of protein is not yet clear. Furthermore, investigation was made in wheat Kankei lines. When the 53 kD subunit was present, but not the 129 kD subunit, viscoelasticity was relatively high.

The 53 kD and 129 kD subunits thus appear to relate with noodle viscoelasticity, though a definite conclusion requires further research.

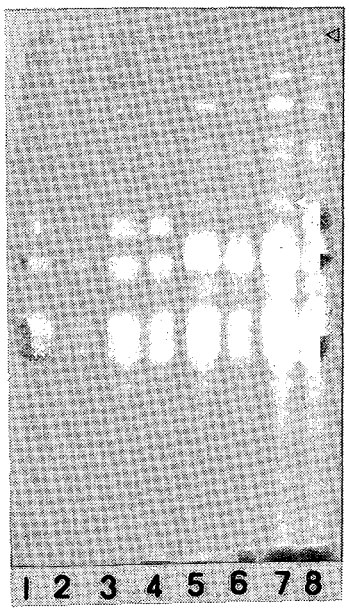

Fig. 1. SDS-PAGE patterns of Norin 61 (lane 1-4) and Kanto 107 (5-8). Lane 1 to 4; Norin 61 in upland and paddy fields in 1989 to 1990, respectively. Lane 5 to 8; Kanto 107 in the same as Norin 61.

Table 1. Result of quality test on Kanto 107, Norin 61 and Kankei lines cultivated under different conditions.

Cultivar or Line	Field condition	Harvesting time	129kD subunit	53kD subunit	Noodle viscoelasticity	Protein content (%)
Kanto 107	Upland	1989	–	+	23.0	11.0
do	do	1990	–	+	23.0	10.4
do	do	1991	–	+	22.5	10.3
Kanto 107	Paddy	1989	+	–	18.0	6.4
do	do	1990	+	–	17.0	7.7
do	do	1991	–	+	21.0	8.1
Norin 61	Upland	1989	+	–	18.0	12.0
do	do	1990	+	–	18.0	11.0
do	do	1991	+	–	18.5	10.3
Norin 61	Paddy	1989	+	–	18.0	6.8
do	do	1990	+	–	18.0	7.9
do	do	1991	+	–	17.5	9.0
Kankei W376	Upland	1991	+	+	19.0	10.9
do	Paddy	1991	+	+	18.0	7.7
Kankei W378	Upland	1991	–	+	23.0	9.5
do	Paddy	1991	–	+	21.0	7.7
Kankei W385	Upland	1991	–	+	21.0	10.4
do	Paddy	1991	–	+	21.0	7.1
Kankei W386	Upland	1991	–	+	21.0	10.1
do	Paddy	1991	–	+	21.0	7.9

Paddy field; Winter cropping on drained paddy field

+; Present, –; Absent

Viscoelasticity; full score = 25.0

References

Huang S and Morrison WR (1988) Aspects of proteins in Chinese and British common (hexaploid) wheats related to quality of white and yellow Chinese noodles. J Cereal Sci. 8: 177-187.

Nakamura H, Sasaki H, Hirano H and Yamashita A (1990) A high molecular weight subunit of wheat glutenin seed storage protein correlates with its flour quality.

Payne PI, Holt LM, Jackson EA and Law CN (1984) Wheat storage proteins; their genetics and their potential for manipulation by plant breeding. Phil Trans R Soc Lond B304. 359-371.

Wheat Information Service
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Embryonic mRNA related to seed dormancy in wheat

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Grain dormancy is a major factor involved in the resistance of preharvest sprouting of wheat. Among the characteristics such as seed color, endogenous germination inhibitors and pericarp structure, which have been studied in relation to dormancy, response of the embryos to abscisic acid (ABA) is the one that differentiate clearly dormant grains from nondormant ones (Stoy and Sundin 1976). Embryos of the dormant grains respond to ABA and do not germinate in ABA solution, but those of nondormant grains do. Embryo responsiveness to ABA also decrease in concert with the loss of grain dormancy during afterripening (Walker-Simmons 1987, Noda and Kanzaki 1988). In a field, preharvest sprouting is induced when grains absorb water at low temperatures. Imbibition at low temperature is the primary factor in breaking dormancy and resulted in preharvest sprouting. Noda et al (1992) reported that severe chilling of grains also changed the embryo responsiveness to ABA.

Besides the above physiological studies, plant mutants have the potential to provide insight into the mechanism of seed dormancy. Seeds of ABA-deficient mutants from maize (vp), Arabidopsis (aba) and tomato (sit) exhibit viviparous germination or non-dormancy (Robichaud et al 1980, Koornneef et al 1982, Karssen et al 1987). Additionally, ABA-insensitive mutants of Arabidopsis (abi3) and maize (vp1) produce non-dormant seeds (Koornneef et al 1984, Robichaud et al 1980). These findings suggest that responsiveness of the embryo to ABA plays an important role in developing and maintaining seed dormancy.

To identify factors related to responsiveness of seed embryos to ABA, we tried to obtain non-dormant mutant lines of wheat after mutagenized seeds of pre-harvest sprouting resistant and dormant Kitakei-1354 with EMS. Three lines (EH 47-1, EH 47-2-5, EH 47-2-6) of non-dormant mutants were established (Table 1). All three lines were susceptible to pre-harvest sprouting in the field. Also, mature seed embryos from these lines showed only weak responsiveness to 10 μM ABA, compared with the wild type Kitakei-1354 (Table 2).

Table 1. Germination rates of whole seeds of Kitakei-1354 and three mutant lines in water, and of their embryos in ABA (10 μM).

Line	Whole seed + water	Embryo + ABA
Kitakei-1354	0.0 ± 0.0*	24.4 ± 5.9
EH47-1	100.0 ± 0.0	100.0 ± 0.0
EH47-2-5	100.0 ± 0.0	100.0 ± 0.0
EH47-2-6	100.0 ± 0.0	100.0 ± 0.0

*Mean ±SE

Table 2. Expression of mRNA for polypeptide e in the embryos of Kitakei-1354, three mutant lines and non-dormant wheat Chihokukomugi (Chihoku) during seed development and upon imbibition in water and 10 μM ABA

	Developing Seed		Dormant Seed (60 DPA)		Afterripened Seed (120 DPA)	
Line	30 DPA	60DPA	Water 48h	ABA* 36h	Water 48h	ABA* 36h
Kitakei	++	+	+	++	–	–
Chihoku	–	–			ND	ND
EH 47-1	–	–			ND	ND
EH 47-2-5	–	–			ND	ND
EH 47-2-6	–	–			ND	ND

*: embryo

ND: not determined

We compared the embryonic mRNAs of Kitakei-1354 and the three mutant lines. Poly (A)$^+$RNA was extracted from embryos at 30 and 60 days post-anthesis (DPA) seeds. mRNAs were translated in a wheat germ cell-free system and the polypeptide products were compared using two-dimensional polyacrylamide gel electrophoresis. Several mRNAs were expressed differently in the Kitakei-1354 and mutant lines. One polypeptide designated as e was not expressed in embryos from the mutant lines, but existed in Kitakei-1354. This mRNA is also up-regulated by ABA in embryos from dormant, but not afterripened Kitakei-1354 seeds (Table 2). We conclude that polypeptide e is a candidate for the factor that positively regulates dormancy in wheat seeds.

References

Karssen CM, Groot SPC and Koornneef M (1987) Hormone mutants and seed dormancy in Arabidopsis and tomato. In: Developmental Mutants in Higher Plants. H Thomas and D Grierson, eds. Cambridge University Press, Cambridge, UK, pp 119-133.

Koornneef M, Jorna MI, Brinkhorst-van der Swan DLC and Karssen CM (1982) The isolation of abscisic acid (ABA) deficient mutants by selection of induced revertants in non-germinating gibberellin sensitive lines of Arabidopsis thaliana (L.) Heynh. Theor Appl Genet 61: 385-393.

Koornneef M, Reuling G and Karssen CM (1984) The isolation and characterization of abscisic acid insensitive mutants of Arabidopsis thaliana. Physiol Plant 61: 377-383.

Noda K and Kanzaki K (1988) Fluorescence staining technique for evaluating embryo sensitivity to abscisic acid of sprouting resistant wheat cultivars. Jpn J Breed 38: 301-308.

Noda K, Kawabata C and Kawakami N (1992) Wheat grain imbibition at low temperatures and embryo responsiveness to ABA. In: 6th Int.Symp. on Pre-Harvest sprouting in Cereals. in press.

Robichaud CS, Wong J and Sussex IM (1980) Control of in vivo growth of viviparous embryo mutants of maize by abscisic acid. Develop Genet 1: 325-330.

Stoy V and Sundin K (1976) Effects of growth regulating substances in cereal seed germination. Cereal Res Commun 4: 157-163.

Walker-Simmons M (1987) ABA levels and sensitivity in developing wheat embryo of sprouting resistant and susceptible. cultivars. Plant Physiol 84: 61-66.

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V. Information

1. Second International Triticeae Symposium

(June 20-24, 1994 in Logan, Utah, United States of America)
Programs:
Plenary lectures will be delivered by invited speakers. Scientific papers may be presented either orally or as posters by all participants. Lectures and papers will be offered in the following areas: Taxonomy and Systematics, Cytogenetics and Genetics, Molecular Biology and Biotechnology, Physiology, Pathology and Entomology, Germplasm Resources and Breeding. Field trips to the USDA Living Collection of Perennial Triticeae and other experimental plots will be arranged. If sufficient interest is expressed, a 3-day post-symposium tour to Yellowstone National Park and/or Teton National Park will be organized for an additional cost.

Facilities:
The Symposium will be held in the facilities on the campus of Utah State University, Logan, Utah. The USDA-Agricultural Research Service Forage and Range Research Laboratory and Utah State University will serve as hosts.

First call for papers is available now and second circular with registration forms will be sent to those responded or request for further information. Please contact:

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Logan, UT 84322-6300
USA

2. The 8th International Wheat Genetics Symposium

(July 20-25f, 1993 in Beijing, China)
More than 400 participants are expected to attend The VIII IWGS at Beijing.
Scientific program: I. Evolution and genome relationships of Triticeae, II. Cytogenetics, transfer of alien genetic materials, and genetic resources, III. Molecular genetics and biotechnology, IV. General genetic analysis, gene mapping and marker systems, V. Genetics of resistance to pathogens and pests, VI. Genetics of tolerance to environmental stress, VIII. Genetical Approaches to breeding and the application of new breeding technologies.

Registration Fee

	Before May 1	After May 1
Participant	US$320	US$380
Student	US$160	US$190
The accompanied	US$50	US$80

Excursions

Beijing Zoo, Arts and Craft Museum and Old Observatory, Forbidden City, Summer Palace, The Temple of Heaven and Silk Fashion Sore, and The Ming Tombs and the Great Wall.

Post symposium tour

PT-1: Xian-Guilin-Guangzhou ; July 27-Aug. 2 ; US$765
PT-2: Xian-Shanghai ; July 27-31 ; US$622
PT-3: Chengdu-Lahsa-Guangzhou ; July 27-Aug. 3 ; US$1195

Correspondence

For scientific program

Prof. Shouyi Chen
Secretary-General of 8th IWGS
Institute of Genetics, Chinese Acady of Science
Beijing 100101, China, FAX 861-491-4896

For further informatin regarding registration, hotel accomodation, visa and social program

Secretary of 8th IWGS
CICCST
Friendship Hotel
Beijing 100086, China
Phone: 861-831-3335, FAX: 861-831-6091

3. XV International Botanical Congress, Tokyo

(August 28-September 3, 1993 at Congress Center of Pacifico, Yokohama, Japan)

Congress date:

August 22, 1993 13:00	Registration open
August 23-27, 1993	Nomenclature session
August 28, 1993 15:00	Opening ceremonies
August 29-September 3, 1993	Scientific program
September 3, 1993 14:00	Closing ceremonies

Third circular is now available from:

K. Iwatsuki, Secretary XV IBC,
Botanical Gardens, University of Tokyo,
3-7-1 Hakusan, Bunkyo-ku, Tokyo 112, Japan
Telephone: (81)3-3814-2625, FAX: (81)3-3814-0139

4. IV International Workshop on Rice Molecular Biology

An internatinal workshop will be held on rice molecular biology at Yokohama, Japan, on September 4-5, 1993, in cooparation by Department of Agriculture, Forestory and Fishery of Japan, and IRRI.

The detailed informations will be obtained from:

Dr. Korehiro Nakagahra
Division of Genetic Resources
National Institute of Agricultural Resources
Kannondai, Tsukuba-shi, Ibaragi, Japan
Phone (81)298-38-7431

5. 1st B-chromosome conference

Place: Miraflores de la Sierra, Madrid, Spain.
Date: September 21-25, 1993.
Scientific program:

1. Polymorphisms and geographic distribution.
2. Transmission: non-Menderian heredity.
3. Genetic structure and organization.
4. Phenotypic effects.
5. Population dynamics.

The meeting will start with five simultaneous Workshops in which delegates contributing to one of the topics mentioned above will jointly prepare the framework of the discussion to be developed in the Plenary Session. Each Workshop will be moderated by a chairperson who will also present an up-to-date resume of their topic in the Plenary Session. There will be no lectures, and meeting will therefore be highly interactive with all delegates expected to participate in the discussion. Before each Plenary Session delegates may discuss particular topics concerning the contributions in the Poster Session.

Reservation and payment should be made by the deadline of May 15th, 1993. For details:

Carlos García de la Vega

Unidad de Genética, Departomento de Biología, Facultad de Ciencias, Edificio de Biología, Universidad Autónoma de Madrid, 28049 Madrid, Spain. Fax: (1) 397 8344.

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VI. Editorial Remarks

Most of wheat researchers must be anxious for travelling China coming summer when The 8th International Wheat Genetic Symposium will be held in Beijing. The symbol mark of the symposium is printed on the cover of the present volume. Also, Dr. Q. S. Zhuang and Dr. Li Zhensheng, Chinese Academy of Sciences, timely contributes the present issue of WIS by writing a review article, which helps us to understand the present status of wheat breeding researches in China.

For articles for Information, including meeting notice, book advertisement, or announcement, you can use facsimile to send the manuscripts through the number; 45-715-0022 (Country code of Japan is 81).

The next issue (No. 77) will be published in October, 1993. Your further contributions will be very appreciated.

Explanation of the picture on the cover

The symbol mark of the 8th International Wheat Genetics Symposium which will be held on July 20–25, 1993 in Beijing, China

WIS No.76

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