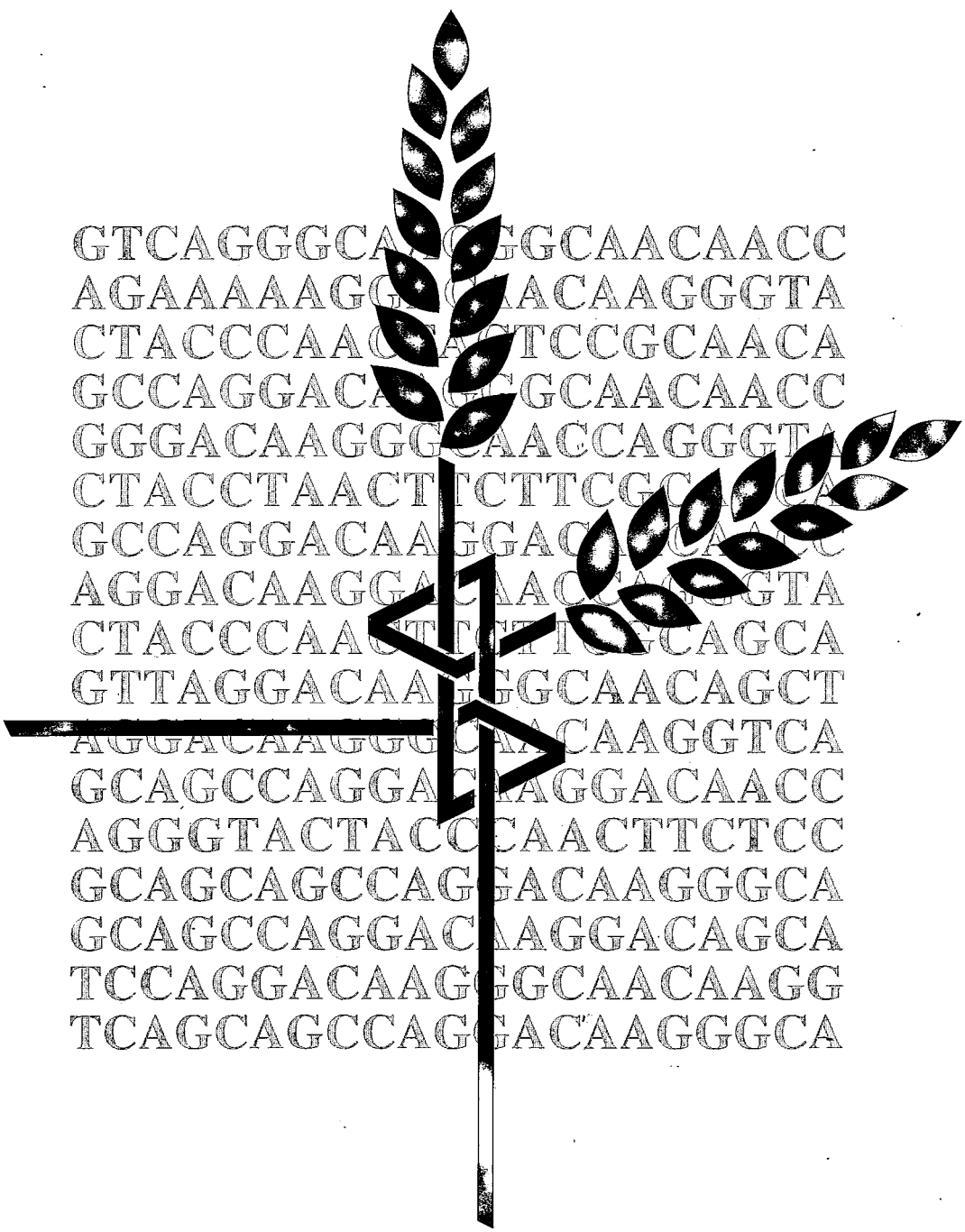
The International Journal for Wheat Genetics and Breeding

WIS

Wheat Information Service

2001 No.92

ISSN 0510-3517



Kihara Memorial

Yokohama Foundation for the Advancement of Life Sciences

Wheat Information Service (WIS) was founded in 1954 to exchange information about genetics and breeding of wheat among researchers in the world, and now published twice a year by Kihara Memorial Yokohama Foundation for the Advancement of Life Sciences. This journal includes Research Articles (original paper reviewed by the editorial board), Research Information, Proposals, Gene Catalogues, Invited Reviews, Records of Scientific Meeting and any other information useful for wheat geneticists and breeders. The editors welcome to receive any information on wheat researches, such as meetings, books, jobs, etc (e-mail: yamabosi@yokohama-cu.ac.jp).

Finance

Kihara Memorial Foundation supports the publishing expenses, and the donations from the readers partly support the editing and mailing expenses. The editors appreciate donations from readers (2,000 Japanese Yen per year; ca. US $20). Only the International Postal Money Order, credit cards (Visa or MasterCard) or cash are acceptable for donation. Because of expensive commission, we can not receive bank transfer and check. A special beautiful picture card will be issued for the donator.

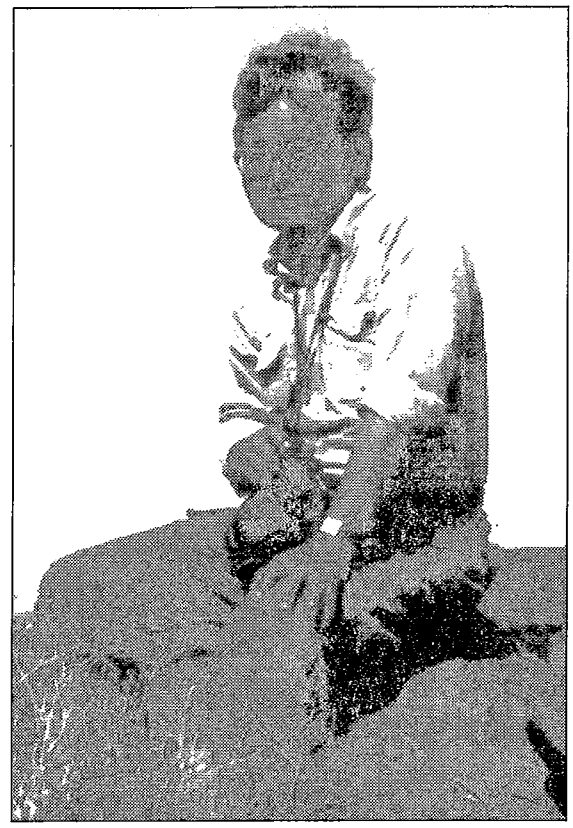
Subscription

For subscription, mail your name, address, and e-mail address (if available) to the Business Office of WIS. We hope your donation for editing and mailing expenses.

Instruction to Authors

The manuscript should be type in English on one side of the 216 x 280 mm or 210 x 297 mm paper (A4 size) with double spacing throughout, allowing for 30mm margins. The manuscript should start from title, author(s), address of institutions, e-mail address (if available) of corresponding author, 3-5 key words, summary of less than 200 words (only for Research Article), and should be followed by text of the paper, arranged in the following sequence: Introduction, Materials and methods, Results, Discussion, Acknowledgments and References, Table, Figures (ready to print), and Legends of the figures. Reference should be cited in the text using name-date system. Where there are three or more authors, only the first author's name should appear, followed by *et al.* Where several references are cited at the same point in the text, these should be arranged in chronological order. The reference list should be typed with double spacing and arranged in alphabetical order following the style as seen in this issue of WIS. The gene symbols and scientific name of species should be either underlined or italicized. Nomenclature of genes and chromosomes should follow the rule in 'Catalogue of gene symbols for wheat' (McIntosh *et al.*: 9th Int. Wheat Genet. Symp., 1998). Limitation of the printing pages is five for 'Research Articles' and two for 'Research Information'.

Original manuscript and its copy should be submitted to the Business Office of WIS in Kihara Memorial Foundation (See the back cover). Authors will have an opportunity to examine the first proof. After acceptance, submission of either diskette or attached file of e-mail of the final revision is required. Microsoft Word (Win or Mac), Write Now (Mac), Claris Works (Mac) for the text and Excel (Win or Mac) for Tables are the most preferable software for submission. The corresponding author of Research Articles, Research Information, Invited Reviews and Proposals will receive 50 free offprint.



Dr. Masatake Tanaka

We are deeply saddened that Dr. Masatake Tanaka, Professor Emeritus of Crop Evolution at Kyoto University, passed away on February 13th, 2001, at the age of 80.

He graduated from the Genetics Laboratory, Faculty of Agriculture, Kyoto University in 1947. From that time on, he was a member of the staff of Kyoto University, becoming professor of the Plant Germ-plasm Institute that was founded in 1971.

Tanaka made contributions to the genome analysis of wheats and their closely related genus *Aegilops*, to the analysis of dwarf genes found in wheats, and to the cytogenetic studies on the differentiation of two wild tetraploid wheats, *Triticum dicoccoides* and *T. araraticum*. He has also contributed to the compilation of the useful catalogue of genetic resources of *Triticum* and *Aegilops* in 1983 based on his active exploration and collection of those genera in the eastern Mediterranean countries (1959), in Transcaucasia (1966), in the northern highlands of Mesopotamia (1970) and in the eastern Turkey (1976). Furthermore, as chairperson of the Local Organizing Committee, he successfully carried out the Sixth International Wheat Genetics Symposium at Kyoto in 1983. Throughout his career, he devoted enormous time and effort to teaching his many students.

After retirement from Kyoto University, he moved to Yokohama to be the director of Kihara Institute for Biological Research (1984-1986) and then the managing director of Kihara Memorial Yokohama Foundation for the Advancement of Life Sciences (1986-1993). As the editor in chief of Wheat Information Service (1986-1993), he made special efforts in improvement of the journal.

His death will be profoundly felt among wheat geneticists around the world, and he will be missed especially by his many friends and colleagues in Japan.

Sadao Sakamoto
Faculty of Intercultural Communication, Ryukoku University, Ohtsu, Japan

Wheat Information Service
Number 92: 1–4 (2001)
Research article

Interlocus interaction of chlorina mutant genes *cn-A1* and *cn-D1* in near-isogenic lines of spring wheat Novosibirskaya 67 (*T. aestivum*)

S. F. Koval, S. I. Baiborodin and V. D. Fedotova

Institute of Cytology and Genetics, Russian Academy of Sciences, Siberian Department, Novosibirsk, 630090, Russia

Summary

Genetic analysis of the near-isogenic lines ANK-32A (*cn-A1*) and ANK-32B (*cn-D1*) of spring soft wheat Novosibirskaya 67 is described. The data on interlocus interaction of these genes and their dosage effects are demonstrated. Chlorina genes are suggested as morphological markers of monosomics for seventh group chromosomes.

Key words: near-isogenic lines, aneuploids, chlorina, chloroplasts

Introduction

Mutations *cn-A1* (7AL), *cn-B1* (7BL), and *cn-D1* (7DL) are useful genetic markers, therefore a detailed study of their expression is necessary. A dose effect of *chlorina-1* (7AL) mutation in different combinations of the mutant gene and normal homoeologs was described (Pettigrew et al. 1969; Pettigrew and Driscoll 1970). Other authors (Washington and Sears 1970) believe that *chlorina-1* mutation is corrected phenotypically in a hemizygous state. However, they report that this gene in the line *chlorina-214* (marker of 7D chromosome) caused pale color of sprouts is hemizygous, and chlorina being homozygous. These works employed mutant Chinese Spring plants, so one cannot exclude that the dose effect observed stemmed from a specific genetic environment, in particular, different genetic background that might include other accompanying mutations. The interlocus interaction of mutant alleles of homologous chromosomes have not received enough attention too. To solve this problem, we have bred near-isogenic lines (NIL) marked with this trait and studied their progeny in the crosses with monosomics. The goal of this work was to study the interlocus interactions of two genes, *cn-A1* (7AL) and *cn-D1* (7DL), which mark the near-isogenic lines ANK-32A and ANK-32B, respectively.

Materials and methods

Allelic states of certain genes in Novosibirskaya 67 (*Triticum aestivum* L.), were characterized in our previous publication (Koval 1999). Description of breeding of the set of BC_9 NIL and their complete catalogue is available in Koval (1997). The lines ANK-32A and ANK-32B, according to this catalogue, are marked with chlorina trait. They display yellow-greenish sprouts and retarded growth during the first half of the vegetation period. By the beginning of spike emergence (occurring 10 days later compared with the control), their color is already indistinguishable from the recurrent parent. The NIL ANK-30A, marked with the gene for enlarged glume *Egl* (7AL) was also used in the experiments.

The lines of Chinese Spring marked with the alleles *cn-A1* (7A) and *cn-D1* (7D) were kindly provided by Dr. O.P. Mitrofanova (Mitrofanova 1991), Institute of Plant Industry, St. Petersburg, Russia. ANK-32A received the marker trait (*cn-A1*) from *chlorina-1* line; the mutant (AH-215 x BC_6 Chinese Spring) was the

donor of the marker trait (*cn-D1*) for ANK-32B. The genes were relocated employing monosomics, mono7A and mono7D from the monosomic series Milturum 553 (Tsilke and Zharkov 1981). The aneuploids were kindly provided by Prof. R.A. Tsilke, Agrarian University, Novosibirsk, Russia.

The F_1 hybrids of aneuploids with the carriers of chlorina markers in a hemizygous state failed to differ from the plants carrying the normal allele. All the plants displaying chlorina phenotype that appeared in the progeny of monosomic F_1 hybrids were disomics. Therefore, the segregation in the progeny of an F_1 monosomic was assessed according to the progenies of F_2 plants. Occurrence of chlorina plants among the F_3 plants of a given family indicated that the initial F_2 plant carried the marker gene. Such plants were united into one class with the plants exhibiting chlorina phenotype in F_2. The absence of plants with chlorina phenotype in F_3 indicated the wild type allele in the initial F_2 plant.

To study the chloroplast structure with electron microscope, the basal part of the first leaf plate was fixed with 2.5% glutaric aldehyde in 0.1% phosphate buffer (pH 7.4) over 2 h, post-fixed in 1% osmium tetroxide for 1 h, and embedded in Araldite resin. Ultrathin sections were produced using an Ultracut ultramicrotome (Leica, Switzerland), stained with uranyl acetate and lead citrate, and examined under a JEM 100S electron microscope (JEOL, Japan). Morphometric analysis was carried out in 50 chloroplast negatives of each line; using test grid, the numbers of dots within the entire chloroplast and only within its membrane structures (granum and stroma thylakoids) were calculated. The relative volume of the chloroplast membrane structure was calculated by the formula $Vv = P_i/P_{cl}$, where Vv is the relative volume of the membrane structures, P_i number of dots within the chloroplast membrane structures, and P_{cl} the number of dots within the entire chloroplast. The values obtained were processed using the Statistica software package.

Results and discussion

The F_1 hybrids of the NILs with their recurrent parent were green, while a 3 : 1 (green : chlorina) segregation was observed in F_2 (Table 1). Both monosomic and disomic F_1 plants were green in the crosses, mono 7A x ANK-32A and mono 7D x ANK-32B. Assessing of the F_2 plants according to their F_3 progenies has demonstrated the lack of segregation in these crossing combinations. The inefficiency of marker alleles of the both loci in heterozygous as well as in hemizygous states indicates the dose effect. This allowed us to create morphologically marked monosomics 7A and 7D carrying the alleles *cn-A1* and *cn-D1* in the critical chromosomes. The phenotype of hemizygotes in these lines are marked with green color, while euploids display chlorina phenotype.

Disagreement of our observations on the inefficiency of one dose of these markers with the previously obtained results (Pettigrew et al. 1969; Pettigrew and Driscoll 1970; Washington and Sears 1970) may be connected with the employment of Chinese Spring mutants that, possibly, have different genetic background, including, in particular, additional mutations able to enhance the marker's effect. The genotype of Chinese Spring itself could enhance the phenotypic manifestation of one dose of genes *cn-A1* and *cn-D1*. Sufficient number of backcrosses in our lines (BC_9) guaranteed the absence of modifier genes, while involvement of different cultivars (Novosibirskaya 67, Milturum 553) in the genetic analysis excluded the mistakes connected with the peculiarities of the tester's genetic environment.

We have determined the linkage of gene *cn-A1* and the gene coding for enlarged glume *Egl* and located in 7AL (Arbuzova et al. 1996). BC_9 NIL ANK-30A (Koval 1997, 1999) was used as a parent. The segregation of the F_2 population of the cross between ANK-32A (chlorina, short glume) and ANK-30A (green, enlarged glume) is shown in Table 2. Among 271 F_2 plants, 55 displayed chlorina phenotype, while 216 were green ($\chi^2 = 3.20$). The segregation with

Table 1. Location of markers in near-isogenic lines ANK-32A and ANK-32B

Crossing combination	Total no. of F_2	No. of green plants	No. of chlorina plants	χ^2 (3:1)
ANK-32A x N 67	292	230	62	2.21
ANK-32B x N 67	206	156	50	0.057
mono7A x ANK-32A	58	2	56	14.37
mono7D x ANK-32B	73	0	73	

N 67 - Novosibirskaya 67

Table 2. Segregation of the marker gene in ANK-32A (*cn-1A*) x ANK-30A (*Egl*) hybrid

Phenotye	Enlarged glume		Short glume	
	Observed	Calculated	Observed	Calculated
Green	188	152.4	28	50.8
Chlorina	40	50.8	15	16.9

regard to the glume length was 228 : 43 (χ^2 = 12.06). The linkage between these genes amounted to 36.1 ± 3.8%, χ^2 = 6.72 being for the segregation classes 9 : 3 : 3 : 1.

In the crossing combination (ANK-32A x ANK-32B), all the F_1 plants displayed chlorina phenotype. The F_2 plants segregated into following four classes ; green, chlorina, pale chlorina (semilethal) and xantha (lethal)(Table 3). The green and chlorina-type plants exhibited normal fertility. The number of xantha plants corresponded to a dihybrid segregation pattern and represented a phenotypic manifestation of recessive homozygotes for both loci. The progeny of all the F_2 plants were grown to examine the further segregation. Analysis of the F_3 progeny has demonstrated that the group of F_2 green plants contained heterozygotes which segregate chlorina at a ratio of 3 : 1 (χ^2 = 0.09); the group of F_2 chlorina contained plants doubleheterozygous, segregating xantha at the ratio 15 : 1 (χ^2 = 4.46). All the F_2 plants belonging to pale chlorina group were heterozygous for either of loci (3 : 1, χ^2 = 3.98). The phenotypic classes that we observed in the (*cn-A1* x *cn-D1*) cross suggested a dose effect and a complementary interaction between the two loci, *cn-A1c* and *cn-D1c*.

The chlorophyll deficiency is increased by excess doses of the gene also in case they belong to one and the same locus. One plant displaying pale chlorina phenotype emerged spontaneously in F_3 progeny of the hybrid (mono7A x ANK-32A). The progeny of this plant segregated as 4 chlorina plants (2n=42 chromosomes) : 25 pale chlorina plants (2n=43 chromosomes) : 3 xantha plants (2n=44 chromosomes). Thus, the emergence of pale chlorina and xantha phenotypes depends not on the interaction of alleles from different loci, but exclusively on excess doses of the mutant allele.

Electron microscopic examination has demonstrated that the increase in the gene dose causes the decrease in the volume of photosynthetic membrane structures and the number of thylakoids per granum (Table 4). The chloroplasts of Novosibirskaya 67 display a typical structure: they are of oval shape and their medium density matrix contains numerous ribosomes and well developed photosynthetic membranes (Fig. 1 a). Occurrence of starch grains in stroma is common as well as certain amount of plastoglobuli (osmophilic globules). In the chloroplasts of the NILs ANK-32A and ANK-32B (two doses of the mutant gene), the volumes of the membrane structures and the number of thylakoids per granum are equally decreased; however, they yet contain starch grains and plastoglobuli (Fig. 1b, c). The volume of the photosynthetic membranes and number of thylakoids continue to decrease in pale chlorina plants (three doses of the gene; Fig. 1d), whereas complete degradation of the granum structures occurred in xantha plants (four doses of the gene; Fig. 1e).

Nullisomics of soft wheat display green color;

Table 3. Segregation of the F_2 progeny in ANK-32A x ANK32B hybrid

Segregation	Total no. of plants	Green(AABB, AaBB, AABb)	Chlorina (AAbb, aaBB, AaBb)	Pale chlorina (aaBb, Aabb)	Xantha (aabb)	χ^2 (15:1)
Observed	1078	293	511	222	52	3.74
Calculated	1078	336.9	404.3	269.5	67.4	–

Table 4. Morphometric analysis of the chloroplast ultrastructure in near-isogenic lines ANK 32A, ANK-32B, and their hybrids

Trait	Near-isogenic lines			F_2 hybrids	
	N-67	ANK-32A (2)†	ANK-32B (2)	Pale chlorina (3)	Xantha (4)
Relative membrane volume	0.343±0.046	0.230±0.059	0.231±0.049	0.154±0.031	0.124±0.001
No.of thylakoids per granum	4-25	3-10	3-11	1-2	0

†Figure in parenthesis indicates dose of mutant gene(s)
Difference significant at P<0.001, N 67: Novosibirskaya 67,

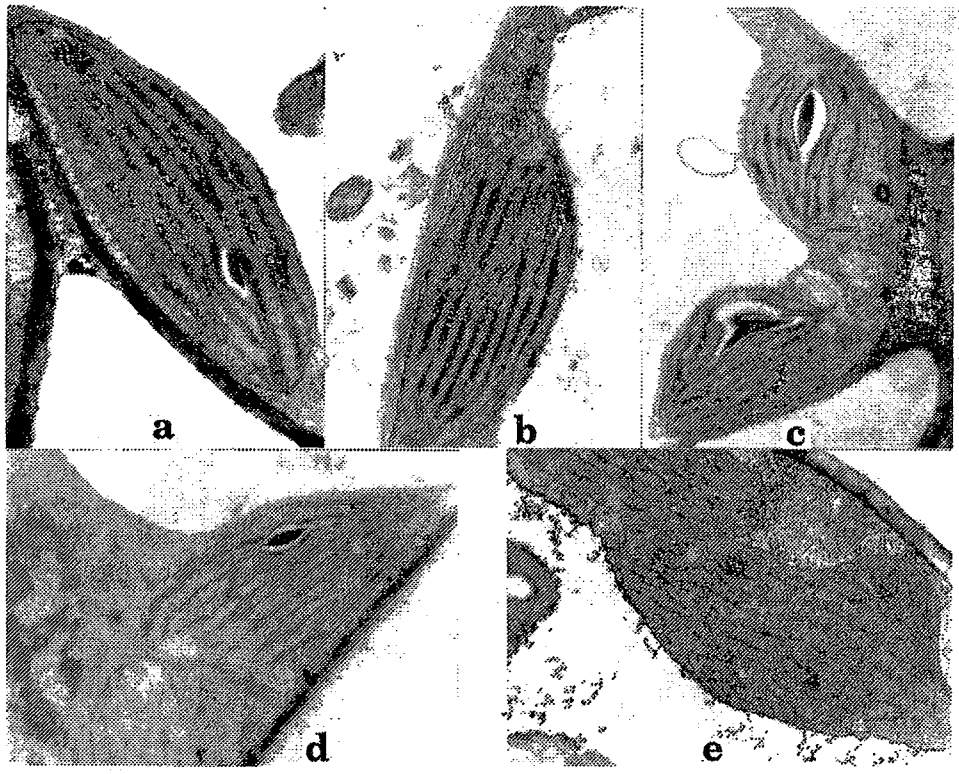


Fig.1. Changes in the chloroplast structure depending on the dose of *cn* alleles (x 16400). a: dominant homozygote (Novosibirskaya 67), b: homozygote for *cn-A1* (ANK-32A), c: homozygote for *cn-D1* (ANK-32B), d: three doses of mutant alleles with pale chlorina phenotype (F_2 ANK-32A x ANK-32B), e: recessive homozygote for both loci with xantha phenotype (F_2 ANK-32A x ANK-32B).

therefore, the chlorina phenotype is not a result of either deletion or halt in gene function. An increase in the number of *cn* alleles, either of both loci or of the same one, in the genotype enhances the phenotypic manifestation of the trait. Similar effect is observed in case of a decrease in the number of the corresponding normal loci as a result of aneuploidy involving other chromosomes (Pettigrew and Driscoll 1970). One may suggest that *cn* alleles produce a defect protein, which compete with the products of the wild type alleles at the same metabolic stage.

Chlorina mutants (7AL) exhibit not only the decreased chlorophyll content, but also the decreased content of carotenoids (Pettigrew et al. 1969), whose synthesis is not connected with green pigment production. These data combined with the damage of chloroplast ultrastructure suggest that the mutant *cn* alleles impair the assemblage of thylakoids and grana in chloroplasts rather than the synthesis of chlorophyll. As a result, the sites for chlorophyll fixation on the membranes appear to be in deficiency. Then, either retroinhibition of the pigment synthesis, or oxidation of the pigments unbound to the membrane takes place. The latter suggestion is in accordance with the decrease in a chlorophyll amount caused in chlorina mutants by high light intensity (Klindworth et al. 1995). A weaker (compared to chlorophyll) decrease in the level of the carotenoids resistant to oxidation also confirms the oxidation hypothesis.

References

Arbuzova VS, Efremova TT, Laikova LI, Maystrenko OI and Popova OM (1996) The development of precise genetic stocks in two wheat cultivars and their use in genetic analysis. Euphytica 89: 11-15.

Klindworth DL, Williams ND and Duysen ME (1995) Genetic analysis of chlorina mutants of durum wheat. Crop Sci 35: 431-436.

Koval SF (1997) The catalogue of near-isogenic lines of Novosibirskaya 67 common wheat and principles of their use in experiments. Russ J Genet 33: 995-1000.

Koval SF (1999) Near-isogenic lines of spring common wheat Novosibirskaya 67 marked with short and long glumes. Wheat Inf Serv 88: 37-42.

Mitrofanova OP (1991) Creation of the bank of soft wheat marker genes. 1. Analysis of chlorophyll mutations. In: Koval SF (ed) Near-isogenic lines of cultivated species Novosibirsk: ICG SO AN SSSR, 47-57 (in Russian).

Pettigrew R and Driscoll CJ (1970) Cytogenetic studies of a chlorophyll mutant of hexaploid wheat. Heredity 25(3): 650-655.

Pettigrew R, Driscoll CJ and Rienits KG (1969) A spontaneous chlorophyll mutant in hexaploid wheat. Heredity 24(3): 481-487.

Tsilke RA and Zharkov NA (1981) Comparative study of productivity components of monosomics and disomics of wheat Milturum 553. In: Scientific methodical questions of increasing breeding efficiency of commercial plants. Novosibirsk, SO VASKhNIL 7: 44-48 (in Russian).

Washington WJ and Sears ER (1970) Ethyl methanesulfonate-induced chlorophyll mutations in *Triticum aestivum*. Can J Genet Cytol 12(4): 851-859.

Wheat Information Service
Number 92: 5–8 (2001)
Research article

Esterase, gliadins and RAPD variations among Sichuan wheat cultivars

You-Liang Zheng, Yu-Qing Chen, Yu-Ming Wei, Yong-Hong Zhou, Zhi-Qing Zhang, Deng-Cai Liu, Xiu-Jin Lan and Ze-Hong Yan

Triticeae Research Institute, Sichuan Agricultural University, Dujiangyan City 611830, Sichuan, P.R. China

Summary

The genetic diversity among 40 Sichuan elite wheat cultivars, which had once popularized above 66,700 ha per year during recent 50 years, was evaluated by esterase isozyme, gliadins and RAPD markers. These wheat cultivars showed a considerably low level of variability for esterase isozyme, while had greater genetic variability for gliadins and RAPD markers. Only seven different esterase patterns were found, while 32 out of 40 cultivars (80%) had identical esterase patterns. There were 38 different gliadin patterns present in 40 Sichuan wheat cultivars, while 36 out of 40 cultivars (90%) did not have identical gliadin patterns with others. In RAPD analysis, a total of 183 bands were amplified using 55 primers in the 40 cultivars. Ninety-three out of 183 bands (50.8%) were polymorphic, with 1.7 polymorphic bands per primer on an average. The RAPD-based genetic similarity (RAPD-GS) among Sichuan wheat cultivars ranged from 0.804 to 0.991, with an average of 0.887.

Key words: esterase, genetic diversity, gliadins, wheat

Introduction

Choice of parents for developing base populations is crucial in breeding of line cultivars because it largely predetermines the outcome of subsequent selection steps and affects the optimum allocation of resources in breeding programs. Evaluation of genetic diversity levels among adapted, elite germplasm can give breeders much information for selecting less related parents in breeding program. It also can provide predictive estimates of genetic variation among segregation progeny for pure line cultivar development (Manjarrez-Sandoval et al. 1997) and may estimate the degree of heterosis in progeny of some parental combinations (Cox and Murphy 1990). Genetic diversity in wheat has been evaluated using markers such as quantitative morphological traits (Zeven and Schachl 1989), isozymes (Cox et al. 1988), gliadins (Cox et al. 1985) and DNA markers (Devos and Gale 1992; He et al. 1992; Vaccino et al. 1993; Chen et al. 1994; Plaschke et al. 1995).

In China, Sichuan is the largest spring wheat (*Triticum aestivum* L.) planting region. In Sichuan, prior to 1950, landraces were widely cultivated, but its yield was much low (Yen 1999). During 1950s, landraces were replaced by modern cultivars step by step. From then on, the wheat production of Sichuan was largely increased (Yen 1999). During 1990s, Sichuan wheat changed more vulnerable to biological and environmental stress because the same parental genotypes (i.e. Fan 6 and Mianyang 11) were used for breeding cultivars (Yu 1998). It becomes a very important work to evaluate the genetic diversity among Sichuan wheats (Yu 1998; Yen 1999). To date, the isozyme, gliadin and RFLP variations in Sichuan wheat landraces had been investigated (Yang et al. 1992; Ward 1998; Wei et al. 2000), but no information was available on the genetic variation in Sichuan wheat cultivars. The objective of this paper is to describe the genetic variations for esterase, gliadins and RAPDs present in Sichuan wheat cultivars.

Materials and methods

Plant materials: In recent 50 years, more than 120 wheat cultivars had been cultivated in Sichuan (Yu 1998). The genetic diversity among 40 elite Sichuan wheat cultivars (Table 1), which had once popularized above 66,700 ha per year, were evaluated in this study.
Electrophoresis: According to the procedure described by Yang et al. (1992), esterase isozymes were extracted from young shoots of 5 day-old seedling and fractionated by polyacrylamide gel electrophoresis.

Gliadin proteins were extracted from single seeds with a solution of 70% (V/V) ethanol and 0.01% (W/V) methyl green, and fractionated by a standard acid-polyacrylamide gel electrophoresis (APAGE) at pH#3.1 according to the procedure of Cooke (1987).
DNA extraction and PCR amplification: Genomic DNA was extracted from young leaves following the procedure described by Sharp et al. (1988). The primers used as random primers in the PCR were purchased from Operon Technologies. The PCR volume was 25 µl and contained 10 ng genomic DNA as template, 1U *Taq* DNA polymerase, 100 nM primer, 100 µM each of dATP, dCTP, dGTP and dTTP, and 1 x PCR buffer. DNA amplification was performed in a Biometra UNO II DNA Thermal Cycler programmed for 45 cycles of 1 min at 94°C, 1 min at 36°C, 2 min at 72°C. PCR products were separated on 1.0% agarose gels and visualized by ethidium bromide staining.
RAPD data scoring and analysis: For each cultivar x primer combination, the presence (1) or absence (0) of an amplified fragment was treated as an independent character without consideration of the quantitative aspects of the results, i.e. band intensity. The data matrix was then used to calculate genetic similarity index (GS)

$$GS=2N_{ij}/(N_i+N_j)$$

where N_{ij} is the number of RAPD bands in common between genotypes i and j, and N_i and N_j are the total number of RAPD bands observed for genotypes i and j, respectively. Based on the 1–GS matrix, a dendrogram showing the genetic relationships between genotypes was constructed by a UPGMA method (Sneath and Sokal 1973) using computer software NTSYS (Rohlf 1993).

Table 1. Forty Sichuan elite wheat cultivars used in this study

Cultivar	Release date	Cultivar	Release date
80-8	1980	Fan 6	1969
Abbandanza‡	1956	Fan 7	1969
Aerai	1966	Jinda 2905	1936
Ardito‡	1937	Mentana‡	1937
Chengdu-guangtou†		Miannong 3	1986
Chuanfu1	1982	Miannong 4	1986
Chuanmai 10	1989	Mianyang 11	1976
Chuanmai 16	1965	Mianyang 12	1978
Chuanmai 18	1965	Mianyang 15	1981
Chuanmai 19	1975	Mianyang 19	1981
Chuanmai 20	1977	Mianyang 20	1984
Chuanmai 21	1978	Mianyang 21	1984
Chuanmai 22	1981	Mianyang 26	1991
Chuanmai 24	1984	Shannong 205	1956
Chuanmai 28	1988	Shuwan 761	1976
Chuannomgmai1	1990	Shuwan 831	1981
Chuanyu 12	1987	Wuyimai	1951
Chuanyu 6	1976	Yaanzao	1962
Chuanyu 8	1980	Yiyuan 2	1995
Datouhuang	1962	Yumai 4	1988

†Chengdu-guangtou is a famous Sichuan wheat landrace.
‡ Cultivar introduced from Italy.

Results and discussion

Esterase variations: Only seven esterase patterns were observed among 40 Sichuan wheat cultivars, while 32 out of 40 cultivars (80%) had identical esterase pattern. The seven different zymograms were described in Fig. 1. The esterase zymograms were similar with each other. Esterase pattern I was the most frequent pattern appeared in Sichuan wheat cultivars. A total of 16 esterase bands, including seven polymorphic bands, were observed in seven zymograms. In the seven polymorphic bands, four

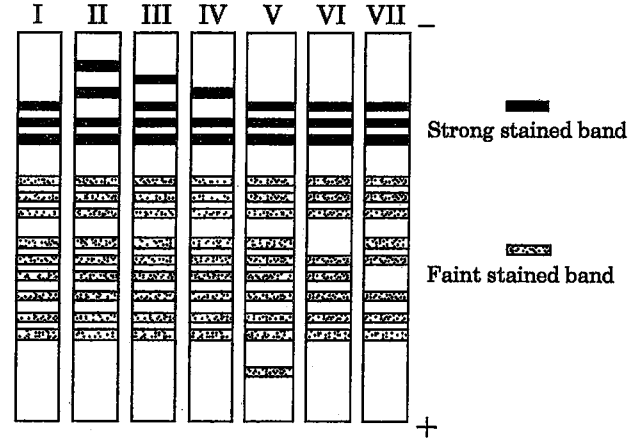


Fig. 1. Seven zymograms observed in 40 Sichuan wheat cultivars.

bands were strong stained, and three bands were faint. Yang et al. (1992) also reported that the esterase variations among Sichuan wheat landraces were much low. These results suggested that low level of esterase variability was present in Sichuan wheats.

Gliadin variations: Fig. 2 shows the representative gliadin patterns of some Sichuan wheat cultivars. There were 38 different gliadin patterns among 40 Sichuan wheat cultivars. Thirty-six cultivars (90%) had unique gliadin pattern, while Fan 6 and Fan 7, and Miangyan 19 and Mianyang 20 had identical gliadin patterns. Fan 7 was the selected line from Fan 6 (Yen 1999), and also Mianyang 20 from Mianyang 19 (Yu 1998), and therefore it is reasonable that they had identical gliadin patterns.

Wei et al. (2000) found that there were 35 different gliadin patterns among 89 Sichuan wheat landraces, while only 18 landraces had unique gliadin patterns. In this study, 36 out of 40 (90%) Sichuan modern wheat cultivars had unique gliadin patterns. In comparison with Sichuan wheat landraces, larger gliadin variability was present in Sichuan modern wheat cultivars.

Sozinov et al. (1987) had identified a block of gliadin bands (*Gld1B3*) as a marker for the presence of 1RS chromosome segment from rye in wheat. Using *Gld1B3* as marker, it was found that nine cultivars (22.5%) (Chuanmai 19, Chuanmai 21, Chuannongmai 1, Miannong 3, Miannong 4, Mianyang 21, Shuwan 761, Yiyuan 2 and Yumai 4) carried the 1RS chromosome segment.

RAPD polymorphisms: Fifty-five 10-mer arbitrary primers were used for PCR amplification of the genomic DNA of 40 Sichuan wheat cultivars, among which PCR products of 32 primers (58.2%) showed polymorphism. A total of 183 bands were amplified using 55 primers in the 40 cultivars. Ninety-three out of 183 bands (50.8%) were polymorphic, among which 1 to 11 polymorphic bands were amplified by each primer, with 1.7 polymorphic bands per primer on an average. The similar results were reported in other wheat genotypes by He et al. (1992) and Sun et al. (1998).

All the 183 bands were used to calculate genetic similarity (GS) among 40 Sichuan wheat cultivars. The GS value ranged from 0.804 to 0.991, with the mean of 0.887. The highest genetic similarity was found between Yaanzao and Datouhuang, while the lowest genetic similarity was observed between Shannong 205 and Yiyuan 2.

RAPD makers allows the rapid, efficient resolution of high levels of polymorphism among closely related lines of common wheat (He et al. 1992;

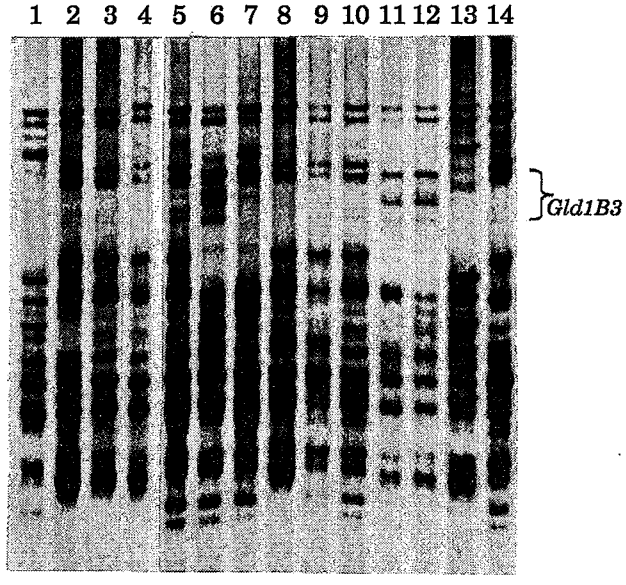


Fig. 2. Gliadin APAGE patterns of the international standard cultivar Marquis (lane 1) and some Sichuan wheat cultivars.
Chuanfu 1 (2), Fan 6 (3), Fan 7 (4), Mianyang 11 (5), Chuannongmai 1 (6), Jinda 2905 (7), 80-8 (8), Aerai (9), Shuwan 831 (10), Shuwan 761 (11), Yumai 4 (12) Chuanyu 6 (13), Chuanyu 8 (14). Chuannongmai 1, Shuwan 761 and Yumai 4 had the gliadin marker *Gld1B3* of 1RS.

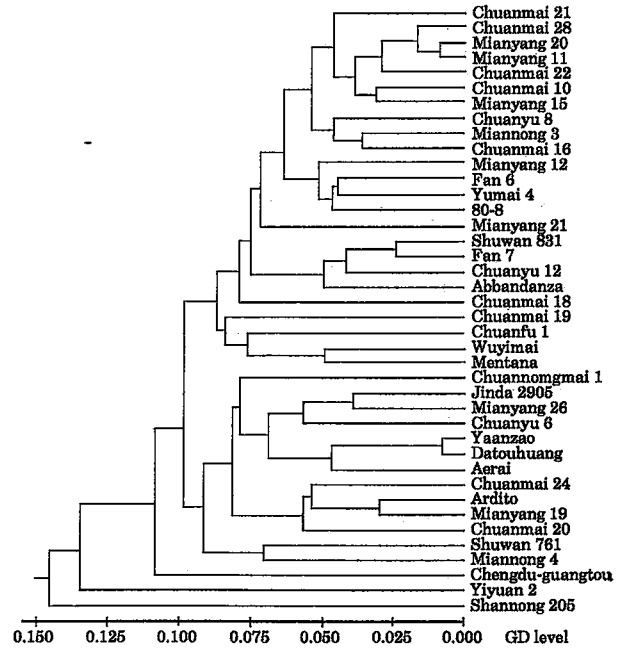


Fig. 3. Dendrogram resulting from cluster analysis of the RAPD based genetic distance matrix among 40 Sichuan wheat cultivars.

Sun et al. 1998). The genetic diversities (GD) among 40 Sichuan wheat cultivars were obtained from 1–GS matrix. The genetic relationships among 40 Sichuan wheat cultivars were analyzed by a UPGMA method (Fig. 3). The results showed that all 40 cultivars could be distinguished by RAPD markers, including closely related sister lines (such as Fan 6 and Fan 7). It was similar to the results reported by He et al. (1992) and Sun et al. (1998). Three cultivars (i.e. Shannong 205, Yiyuan 2 and Chengdu-guangtou) were less related with other cultivars, and divergent from the other cultivars. Two subgroups were evident for the remaining 37 cultivars, with the first subgroup including 24 cultivars. The cultivars were more closely related with others among the first subgroup, while more diverse among the second subgroup. Three cultivars introduced from Italy (Abbandanza, Ardito and Mentana) were distributed within different subgroups because they were included in the different pedigree of some Sichuan wheat cultivars (Yu 1998; Yen 1999).

Acknowledgments

The authors are thankful to the Science and Technology Committee, and Education Committee of Sichuan Province, China for their financial supports.

References

Chen HB, Martin JM, Lavin M and Talbert LE (1994) Genetic diversity in hard red spring wheat based on sequence -tagged-site PCR markers. Crop Sci 34: 1269-1632.

Cooke RJ (1987) The classification of wheat cultivars using a standard reference electrophoresis method. J Nat Agric Bot 17: 273 –281.

Cox TS, Lookhart GL and Walker DE (1985) Genetic relationships among hard red winter wheat cultivars as evaluated by pedigree analysis and gliadin ployacrylamide gel electrophoretic patterns. Crop Sci 25: 1058-1063.

Cox TS and Murphy JP (1990) The effect of parental divergence on F_2 heterosis in winter wheat crosses. Theor Appl Genet 79: 241-250.

Cox TS, Murphy JP and Harrell LG (1988) Isoelectric focusing patterns of kernel isozymes from 80 North American winter wheat cultivars. Can J Plant Sci 68: 65-72.

Devos KM and Gale MD (1992) The use of random amplified polymorphic DNA markers in wheat. Theor Appl Genet 84: 567-572.

He S, Oham H and Machenzie S (1992) Detection of DNA sequence polymorphisms among wheat varieties. Theor Appl Genet 84: 573-578.

Manjarrez-Sandoval P, Carter TE, Webb DM and Burton JW (1997) RFLP genetic similarly estimates and coefficient of parentage as genetic variance predictors for soybean yield. Crop Sci 37: 698-703.

Plaschke J, Ganal MW and Roder MS (1995) Detection of genetic diversity in closely related bread wheats using microsatellite markers. Theor Appl Genet 91: 1001-1007.

Rohlf FJ (1993) NTSYS-pc version 1.80. Distribution by Exeter Software, Setauket, New York.

Sharp PJ, Kresis M, Shewry P and Gale MD (1988) Location of β-amylase sequences in wheat and its relatives. Theor Appl Genet 75: 286-290.

Sneath PHA and Sokal RR (1973) Numerical taxonomy. Freeman, San Francisco.

Sozinov AA, Novoselskaya AY, Lushnikova AA and Bogdanov YF (1987) Cytological and biochemical analysis of bread wheat variants with 1B/1R substitutions and translocations in the karyotype. Tsitologiya i Genetika 21: 256-261.

Sun Q, Ni Z, Liu Z, Gao J and Huang T (1998) Genetic relationships and diversity among Tibetan wheat, common wheat and European spelt wheat revealed by RAPD markers. Euphytica 99: 205-211.

Vaccino P, Accerbi M and Corbelli M (1993) Cultivar identification in *T. aestivum* using highly polymorphic RFLP probes. Theor Appl Genet 86: 833-836.

Ward RW, Yang ZL, Kim HS and Yen C (1998) Comparative analyses of RFLP diversity in landraces of *Triticum aesitvum* and collections of *T. tauschii* from China and southwest Asia. Theor Appl Genet 96: 312-318.

Wei YM, Zheng YL, Liu DC, Zhou YH and Lan XJ (2000) Gliadin and HMW-glutenin variations in *Triticum turgidum* L. ssp. *turgidum* and *T. aestivum* L. landraces native to Sichuan, China. Wheat Inf Serv 90: 13-20.

Yang ZL, Luo MC and Yen C (1992) The study on esterase and peroxidase isozymes in landraces of wheat from Sichuan. J Sichuan Agric Univ 10: 105-112.

Yen C (1999) History and prospect of study on wheat breeding of fifty years in Sichuan. J Sichuan Agri Univ 17: 108-113.

Yu Y (1998) Sichuan wheats. Sichuan Science and Technology Press, Chengdu.

Zeven AC and Schachl R (1989) Groups of bread wheat landraces in Austrian Alps. Euphytica 41: 235-246.

Wheat Information Service
Number 92: 9–11 (2001)
Research article

The possible candidate of *Vrn-B1* in wheat, as revealed by monosomic analysis of *Vrn* gene carried by Triple Dirk (B), the former *Vrn2*

Kazunari Iwaki[1], Kiyokazu Nakagawa[2] and Kenji Kato[1]*

[1]Faculty of Agriculture, Okayama University, 1-1-1 Tsushima-Naka, Okayama 700-8530, Japan
[2]Faculty of Agriculture, Kochi University, 200 Monobe-Otsu, Nankoku, Kochi 783-8502, Japan

Summary

Chromosomal location of the former *Vrn2* was analyzed by using monosomic lines of a winter wheat, Dwarf A. Segregation ratio of spring and winter type plants fitted well to an expected ratio of 3:1 in most cross combinations. On the contrary, for the cross with mono $5B^L$-$7B^L$ line, segregation of winter type was significantly less than expected, 8/107, and thus was concluded that *Vrn2* should be located on $5B^L$-$7B^L$ chromosome.

Key words: monosomic analysis, *Vrn-B1*, vernalization, wheat

Introduction

Vernalization requirement is one of the most important traits for the adaptation to cold winter, and wheat cultivars are classified into two groups, spring and winter types, according to their requirement. Genetic analysis of vernalization requirement revealed the involvement of four (or five) major *Vrn* genes, formerly termed as *Vrn1*, *Vrn2*, *Vrn3*, *Vrn4* and *Vrn5* (reviewed by Flood and Halloran 1986). Cytological analysis located *Vrn1* and *Vrn3* on the long arm of 5A and 5D, respectively (Law et al. 1975). Based on the result of RFLP mapping, synteny between homoeologous chromosomes and orthologous genes for vernalization requirement (*Vrn1*, *Vrn3*, *Sp1*, *Sh2*) has been confirmed in wheat, rye and barley (Nelson et al. 1995; Dubcovsky et al. 1998). Then gene nomenclature has been changed as *Vrn-A1*, *Vrn-D1*, *Vrn-R1* and *Vrn-H1*(McIntosh et al. 1998). Another *Vrn* gene is located on 5B chromosome (Hoogendoorn 1985a), and is termed as *Vrn-B1* (McIntosh et al. 1998).

However, there have been two conflicting reports about *Vrn* gene on 5B chromosome. *Vrn2* was located on 5B by Hoogendoorn (1985b), while *Vrn4* was located on 5B by Maystrenko (1980). Reflecting such confusion, McIntosh et al. (1998) summarized that the genes formerly designated as *Vrn4* and *Vrn2* are probably the same, or allelic, and then two genes are designated as *Vrn-B1* in the new nomenclature system. However, independence of *Vrn2* and *Vrn4* was clearly shown by test cross between four near-isogenic lines (NILs) (Gotoh 1979; Kato unpublished), and by the existence of cultivars carrying either *Vrn2* or *Vrn4* (Kato et al. 1988). Considering the importance of *Vrn2* in spring type wheat cultivated in the Mediterranean countries, chromosomal location of *Vrn2* and *Vrn4* should be clarified to solve such a confusion.

Therefore, in the present study, chromosomal location of *Vrn2* was analyzed by using monosomic

* Corresponding author: Kenji Kato, Fax. +81-86-251-8388, E-mail:kenkato@cc.okayama-u.ac.jp

series of Dwarf A.

Materials and methods

Triple Dirk (B), Pugsley's near-isogenic line carrying *Vrn2*, was crossed with a set of monosomic lines of a winter type wheat cultivar Dwarf A which was kindly supplied by Dr. A. J. Worland, John Innes Centre, UK. Monosomic F_1 plants were selfed for each cross combination. F_2 populations were sown on 18th September and grown in a glasshouse of Kochi University, Japan, under a regime of 24 hour day-length without heating. They were sown in soil-filled containers at a spacing of 2 cm (between hills) x 3.5 cm (between rows). The number of days from sowing to flag leaf unfolding or the number of vegetative rosette segregants was determined. Plants successfully unfolded flag leaf were regarded as spring type, and those remained vegetative rosette as winter type.

Results and discussion

Frequency distribution of the number of days from sowing to flag leaf unfolding was shown in Fig. 1, for F_2 populations of two cross combinations between Triple Dirk (B) and monosomics $5B^L$-$7B^L$ (Fig. 1a) and $5B^S$-$7B^S$ (Fig. 1b). Days from sowing to flag leaf unfolding ranged from 45 to 136 in both populations, and these segregants were regarded as spring type

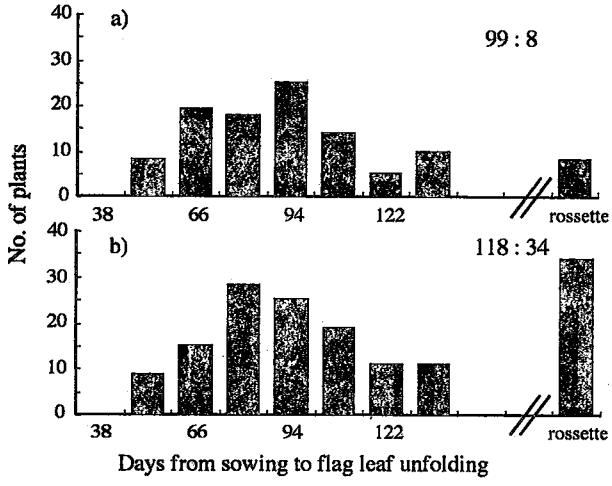


Fig. 1. Frequency distribution of the days from sowing to flag leaf unfolding in the F_2 populations of two cross combinations between Triple Dirk (B) and monosomics $5B^L$-$7B^L$ (a) and $5B^S$-$7B^S$ (b).

which carried *Vrn2* in homozygous, heterozygous or hemizygous condition. On the contrary, eight and thirty-four plants did not unfold flag leaf within 150 days after sowing, and they were regarded as winter type.

Segregation ratio of spring and winter type plants fitted well to an expected ratio of 3:1 in most cross combinations, though monosomics for 2A and 3A were not tested (Table 1). Significantly deviated segregation ratio was observed in the cross with monosomics 7A and $5B^L$-$7B^L$ ($P<0.01$). However, the deviation was caused by over segregation of winter type in the former. Segregation of winter type was significantly less than expected, 8/107, and thus was concluded that *Vrn2* should be located on $5B^L$-$7B^L$ chromosome.

Reciprocal translocation between 5B and 7B chromosome is known in European wheat cultivars

Table 1. Segregation of spring and winter types in F_2 population of Dwarf A monosomic lines X Triple Dirk(B)

Monosomic chromosome	No. of plants		Chi-square 3:1
	Spring type	Winter type	
1A	74	35	2.94
2A	-	-	-
3A	-	-	-
4A	92	42	2.88
5A	58	20	0.02
6A	80	29	0.15
7A	87	49	8.82 **
1B	125	36	0.60
2B	63	10	4.97
3B	27	9	0.00
4B	73	35	3.16
$5B^L$-$7B^L$	99	8	17.52 **
6B	116	25	3.97
$5B^S$-$7B^S$	118	34	0.56
1D	90	28	0.10
2D	21	1	4.91
3D	112	27	2.31
4D	86	32	0.28
5D	98	34	0.04
6D	99	32	0.02
7D	124	43	0.05

** Significant at 1% level.

including Dwarf A (Lange et al. 1987), and thus monosomic lines of Chinese Spring should be preferable for the analysis of genes on groups 5 and 7 chromosomes. However, in F_2 population of the cross combinations between CS monosomics and *Vrn2* or *Vrn4* carrier, frequency of winter type segregants will be 1/16 in non-critical cross and a few percent in critical cross, respectively. Accordingly it is difficult to distinguish the two types of segregation ratio. From these reasons, monosomic lines of Dwarf A were used in the present study, and *Vrn2* was successfully identified on either $5B^L$ or $7B^L$.

For vernalization response, five genes are known in common wheat, three genes in barley, and one gene in rye, respectively. Synteny of group 5 chromosomes is well known among these crop species, and *Vrn* genes on 5A, 5D (wheat), 5H (barley), and 5R (rye) are regarded as orthologous gene of *Vrn-1* (Plaschke et al. 1993; Nelson et al. 1995; Laurie et al. 1995). Additional recessive gene for spring growth habit has been located on 5A chromosome of *T. monococcum*, and proved to be on translocated segment from 4AL (Dubcovsky et al. 1998). Its synteny with barley *sh* gene on 4H chromosome was confirmed, and these two genes are regarded as orthologous gene of *Vrn-2*. Besides these two orthologous genes, *Sh3* on 1H (Takahashi and Yasuda 1971) and *Vrn5* on $7B^S$ (Law 1966) have been reported. However, no vernalization response gene have been located on $7B^L$. It was therefore suggested that *Vrn2* should be equivalent to *Vrn-B1* located on $5B^L$. Further analysis is now going on to locate *Vrn2* on RFLP map of $5B^L$, by converting RFLP markers to PCR markers.

Most of attentions have been so far paid to orthologous genes of *Vrn-1* on group 5 chromosomes, since orthologous genes corresponding to *sh* (4H) and *Sh3* (1H) are not known in common wheat. However, as mentioned above, the second gene corresponding to *sh* has been identified in *T. monococcum* (Dubcovsky et al. 1998), and digenic segregation of growth habit has been reported in *Aegilops tauschii* (Goncharov and Chikida 1995). Even in common wheat, local landraces carrying *Vrn* gene(s), other than *Vrn-A1*, *Vrn-B1*, *Vrn-D1* and *Vrn4*, have been identified in various areas (Iwaki and Kato 1998). Detailed analysis of these landraces might lead to the identification of orthologous genes, *Vrn-3* and *Vrn-2*, on groups 1 and 4 chromosome.

Acknowledgments

We would like to thank Dr. A. J. Worland, John Innes Centre, UK, for kindly supplying the seeds of monosomic lines of Dwarf A.

References

Dubcovsky L, Lijavetzky D, Appendino L and Tranquilli G (1998) Comparative RFLP mapping of *Triticum monococcum* genes controlling vernalization requirement. Theor Appl Genet 97: 968-975.

Flood RG and Halloran GM (1986) Genetics and physiology of vernalization response in wheat. Adv Agr 39: 87-125.

Goncharov NP and Chikida NN (1995) Genetics of growth habit in *Aegilops squarrosa* L. Russian J Genet 31: 343-345.

Gotoh T (1979) Genetic studies on growth habit of some important spring wheat cultivars in Japan, with special reference to the identification of the spring genes involved. Jap J Breed 29: 133-145.

Hoogendoorn J (1985a) A reciprocal F_1 monosomic analysis of the genetic control of the time of ear emergence, number of leaves and number of spikelets in wheat (*Triticum aestivum* L.). Euphytica 34: 545-558.

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

Iwaki K and Kato K (1998) Geographical variation of *Vrn* genotype in wheat caused by the selection for adaptability to winter coldness. Proc 9th Int Wheat Genet Symp 4: 39-41.

Kato K, Ikoma H and Hayashi K (1988) Geographical distribution of the genes for vernalization response and its implication for the adaptability of wheat. Proc 7th Int Wheat Genet Symp: 533-539.

Lange W, Linde-Laursen I, Larsen J, Ljungberg A and Ellerstrom S (1987) Cytogenetic analysis of structural rearrangements in three varieties of common wheat, *Triticum aestivum*. Theor Appl Genet 73: 635-645.

Laurie DA, Pratchett N, Bezant JH and Snape JW (1995) RFLP mapping of five major genes and eight quantitative trait loci controlling flowering time in a winter x spring barley (*Hordeum vulgare* L.) cross. Genome 38: 575-585.

Law CN (1966) The location of genetic factors affecting a quantitative character in wheat. Genetics 53: 487-498.

Law CN, Worland AJ and Giorgi B (1975) The genetic control of ear emergence time by chromosomes 5A and 5D of wheat. Heredity 36: 49-58.

Maystrenko OI (1980) Cytogenetic study of growth habit and ear-emergence time in wheat (*Triticum aestivum* L.). Proc XIVth Int Congr Genet 1: 267-282.

McIntosh RA, Hart GE, Devos KM, Gale MD and Rogers WJ (1998) Catalogue of gene symbols for wheat. Proc 9th Int Wheat Genet Symp 5: 1-235.

Nelson JC, Sorrells ME, Van Deyne AE, Lu YH, Atkinson M, Bernard M, Leroy P, Faris JD and Anderson JA (1995) Molecular mapping of wheat: Major genes and rearrangements in homoeologous groups 4, 5 and 7. Genetics 141: 721-731.

Plaschke J, Bner A, Xie DX, Koebner RMD, Schlegel R and Gale MD (1993) RFLP mapping of genes affecting plant height and growth habit in rye. Theor Appl Genet 85: 1049-1054.

Takahashi R and Yasuda S (1971) Genetics of earliness and growth habit in barley. Proc 2nd Int Barley Genet Symp: 388-408.

Wheat Information Service
Number 92: 12–16 (2001)
Research article

Suppression of rust resistance genes from distantly related species in *Triticum durum-Aegilops* amphiploids

M. Aghaee-Sarbarzeh, H. S. Dhaliwal, P. Chhuneja and Harjit-Singh

Department of Genetics and Biotechnology, Punjab Agricultural University, Ludhiana 141 004, India

Summary

To transfer resistance gene(s) from non-progenitor diploid wild species, *Aegilops caudata* (CC) and *Ae. umbellulata* (UU), amphiploids were developed from their crosses with susceptible *Triticum durum* cultivars. These amphiploids were subsequently backcrossed to hexaploid wheat cv. Chinese Spring (CS). Most of the gene(s) conditioning leaf rust and stripe rust resistance from C and U genomes under the field condition were suppressed by A and/or B genomes of *Triticum durum* in the amphiploids whereas only a few of the resistance genes were expressed in the amphiploids at the seedling stage. Differential suppression of the genes in the amphiploids at seedling stage indicated the selective specificity of the suppression system. Recovery of resistant plants in F_2, F_3 and backcross generations of all amphiploids with CS at seedling stage and under field conditions indicated the absence of suppressor genes in Chinese Spring for these resistance genes.

Key words: *Triticum aestivum, Aegilops caudata, Ae. umbellulata,* rust resistance, suppression

Introduction

Related wild progenitor and non-progenitor species of wheat represent a large reservoir of useful variability that can be exploited for wheat improvement. Wide hybridization has contributed significantly to germplasm enhancement of bread wheat. Many agronomically important traits, including resistance to diseases and pests, and abiotic stresses have been transferred from related species and genera into wheat (Knott and Dvorak 1976; Sharma and Gill 1983; Gale and Miller 1987; Jiang et al. 1994; Friebe et al. 1996) and exploited commercially. The alien resistance genes are useful only when they are expressed in the cultivated background. Genetic suppression of disease resistance of related species by D genome has frequently been reported (Kerber 1983; Bai and Knott 1992; Dhaliwal et al. 1993; Innes and Kerber 1994; Ma et al. 1997).

Studies at the Punjab Agricultural University (PAU), Ludhiana has shown that among less closely related wild species, diploid *Aegilops* species with C, U and M genomes are excellent sources of resistance to leaf rust and stripe rust (Dhaliwal et al. 1993; Harjit-Singh and Dhaliwal 2000). Therefore, a study was initiated to transfer the rust resistance gene(s) from these species into hexaploid wheat. The present paper reports the suppression of resistance gene(s) of C and U genomes of *Ae. caudata* and *Ae. umbellulata*, respectively, by gene(s) on A and/or B genomes of durum wheat.

Materials and methods

The plant materials used in this investigation are listed in Table 1. To transfer leaf rust and stripe rust resistance from diploid *Aegilops* species with C or U genomes to hexaploid wheat, amphiploids were

developed between these species and susceptible *T. durum* cultivars. To synthesize amphiploids, the coleoptiles of 4-5 day-old F_1 seedlings, of these crosses, were treated with 0.25% colchicine in 2% DMSO solution for four hours (Gill et al. 1988). These amphiploids were used as the bridge for the transfer of leaf and stripe rust resistance genes into cultivated hexaploid wheat. To induce homoeologous chromosome pairing these amphiploids were first crossed with *T. aestivum* cv. Chinese Spring carrying the *Ph*^{I} gene from *Ae. speltoides* (Chen et al. 1994; Aghaee-Sarbarzeh et al. 2000) and further backcrossed to Chinese Spring.

The parents, F_1's, amphiploids, and the derivatives of their crosses with Chinese Spring (CS) were scored for field reaction to leaf rust and stripe rust by recording terminal disease severity and response to individual rust pathotypes. The disease severity under field conditions was recorded as percentage of leaf area covered by rust following modified Cobb's scale as developed by Peterson et al. (1948). According to this scale, at 100 % disease severity, the actual leaf area covered by rust pustules is 33 %. The response of individual plant/entry was recorded as follows; 0: resistant, no infection, R: resistant, necrotic areas with or without minute uredia, MR: moderately resistant, small uredia surrounded by necrotic areas, MS: moderately susceptible, medium sized uredia with no chlorosis, S: susceptible, large uredia without necrosis or chlorosis, X: intermediate, variable size uredia, some with necrosis or chlorosis. In addition, these materials were also evaluated for seedling resistance to individual pathotypes of leaf rust and stripe rust. First leaves of five to seven day-old seedlings were inoculated with urediospores mixed with talc using lancet needle (Nayar et al. 1997). The inoculated seedlings were incubated for 24 hours at 20±1°C for leaf rust and 8-9°C for stripe rust at 100 % relative humidity. Subsequently, plants were placed on benches in the glass house at temperature around 20-25°C for leaf rust and 15-20°C for stripe rust. About 11 to 15 days after inoculation, infection types were recorded according to a modification of 0-4 scale (Knott 1989) given by Stakman et al. (1962) as follows; 0: immune, no visible infection, 0;: no uredia, hypersensitive flecks present, 1: resistant, minute nonsporulating uredia surrounded by distinct necrotic areas, 2: resistant to moderately resistant, small uredia with slight sporulation surrounded by chlorotic or necrotic areas, 3: moderately resistant to moderately susceptible, medium sized sporulating uredia, chlorotic areas may be present, 4: susceptible, large sporulating uredia with no chlorosis or necrosis, X: resistant, heterogeneous, variable size uredia distributed over leaves. Variations were indicated by the use of – (less than the average for the class) and + (more than the average for the class) as superscripts.

Table 1. Plant materials along with their genome constitution used in the present study

No.	Line	Genome constitution†
1	*Triticum aestivum* cv. Chinese Spring	AABBDD
2	*Triticum aestivum* cv. Chinese Spring with *Ph*^{I} [CS(*Ph*^{I})]	AABBDD
3	*Triticum durum* cv. Bijaga Yellow	AABB
4	*Triticum durum* cv. Malvi Local	AABB
5	*Triticum durum* cv. A 206	AABB
6	*Triticum durum* cv. WH 868	AABB
7	*Triticum durum* cv. WH 890	AABB
8	*Aegilops umbellulata* Acc. 13749	UU
9	*Aegilops umbellulata* Acc. 3732	UU
10	*Aegilops caudata* Acc. 3556	CC
11	Amph.[*T. durum* cv. Bijaga Yellow-*Ae umbellulata* Acc. 13749]	AABBUU
12	Amph.[*T. durum* cv. Malvi Local -*Ae umbellulata* Acc. 13749]	AABBUU
13	Amph.[*T. durum* cv. WH 890-*Ae. umbellulata* Acc. 3732]	AABBUU
14	Amph.[*T. durum* cv. A 206- *Ae. caudata* Acc. 3556]	AABBCC
15	Amph.[*T. durum* cv. WH 868- *Ae. caudata* Acc. 3556]	AABBCC

†After Kimber and Tsunewaki (1988)

Results and discussion

The accession 13749 of *Ae. umbellulata* was resistant under field conditions as well as at seedling stage to pathotype N of stripe rust and pathotypes 77-4, 77-5, 104B, and 104-2 of leaf rust (Table 2). However, the durum wheat cultivars, Bijaga Yellow (BY) and Malvi Local (ML) were susceptible to both the rusts under the field conditions. The cultivar BY was resistant to races 77-4 and 77-5 of leaf rust and race N of stripe rust, but susceptible to races 104B and 104-2 of leaf rust, whereas, the durum wheat cv. ML, showed susceptibility to all the pathotypes of rusts , except 104B of leaf rust, at seedling stage (Table 2).

The amphiploid of BY with Acc. 13749 was susceptible to both the rusts under field conditions. At seedling stage it was susceptible to 77-5 and 104-2 races of leaf rust, and race N of stripe rust (Table 2). The resistance of the amphiploid to race 77-4 may be from either of the parents, or combination of resistance genes from both of them. However, resistance to race 104B, due to gene(s) from *Ae. umbellulata*, was expressed in the amphiploid. As the hexaploid wheat, CS was also susceptible to race 104B, this race can, therefore, be used as the discriminating race to screen the segregating generations for rust resistance of *Ae. umbellulata*. The amphiploid of ML with *Ae. umbellulata* Acc. 13749, was also susceptible to both the rusts under the field conditions and at seedling stage to race 77-5 of leaf rust and race N of stripe rust, but was resistant to races 77-4 and 104-2, to which ML was susceptible. The two races, 77-4 and 104-2 can therefore be used for screening derivatives at seedling stage.

Susceptibility of the amphiploids and their F_1 hybrids with CS (*Ph*^{1}) indicated the presence of suppressor gene(s) on A and /or B genomes of the durum wheat cultivars for the resistance genes of *Ae. umbellulata* Acc. 13749. Resistance of the donor species, *Ae. umbellulata* Acc. 13749, to all the races, and differential reaction of the amphiploid to different pathotypes of leaf rust at seedling stage (Table 2) indicate that at least two different genes for rust resistance may be present in the U genome of *Ae. umbellulata* Acc. 13749. For instance, in amphiploid Bijaga Yellow-*Ae. umbellulata* Acc. 13749, one of the genes was effective against race 104B, which was expressed in the amphiploid, and the other effective against races 104-2 was suppressed in the amphiploid due to gene(s) on A and/or B genome of durum wheat. This may be attributed to selective specificity of the suppression system. Differential specificity of suppressor genes has already been reported. Nelson et al. (1997) reported a locus designated as *SuLr23*, from *T. tauschii*, which suppressed *Lr23* of durum wheat in an amphiploid of these two species. However, the gene *SuLr23* could not suppress other *Lr* gene in the F_1 hybrid of the amphiploid and hexaploid wheat. Susceptibility of the amphiploid, Bijaga Yellow-*Ae. umbellulata*, to the race N of stripe rust and 77-5 of leaf rust, to which both the parents were resistant, may be attributed to gene interaction in the amphiploids.

The *Ae. caudata* Acc. 3556 showed consistent resistance to prevalent races of leaf rust and stripe rust under the field conditions (Dhaliwal et al. 1993 ; Harjit-Singh et al. 1998) and to pathotypes 77A-1, 77-1, 77-2, 77-4 and 77-5 of leaf rust at seedling stage (Table 2). *T. durum* cv. A 206 and WH 868 were susceptible to these rusts under the field conditions but resistant to pathotypes 77-5 and 104B at seedling stage. The amphiploids of these two cultivars with *Ae. caudata*, and their F_1 hybrids with CS (*Ph*^{1}) were also susceptible under the field condition as well as to individual pathotypes of leaf rust, 77-2 and 77-4, to which the durum cultivars were susceptible.

Susceptibility of the amphiploids and their F_1 hybrids with CS (*Ph*^{1}) under the field conditions as well as to individual pathotypes of leaf rusts at seedling stage, clearly indicated suppression of resistance gene(s) of *Aegilops caudata* also by gene(s) of A and/or B genomes of durum wheat. Suppression of rust resistance genes by the A or B genome of wheat has already been reported (Kerber 1983; Ma et al. 1997). Innes and Kerber (1994) reported suppression of resistance to leaf rust in amphiploid of susceptible durum wheat and resistant *Ae. squarrosa*.

Recovery of resistant plants in the F_2 and subsequent backcross generations (BC_1 and BC_2) of crosses of amphiploids involving *Ae. caudata* with CS, due to segregation of the resistance gene(s) from the suppression gene(s) of durum wheat suggests the absence of suppression gene(s) in CS. Furthermore, it also indicates the absence of suppressor genes in the D genome of CS for resistance gene(s) of *Ae. caudata*.

T. durum cv. WH 890 was also susceptible to both the rusts under field conditions and to most of the pathotypes of leaf rust and pathotype P of stripe rust (Table 2). However, in contrast to the previous amphiploids involving *Ae. umbellulata* and *Ae. caudata* , the resistance gene(s) of *Ae. umbellulata* Acc. 3732 was expressed in the amphiploid *T. durum* cv. WH 890-*Ae. umbellulata* Acc. 3732 under field conditions whereas at the seedling stage the amphiploid was susceptible to all the individual pathotypes of leaf rust, except race 77-3. *Ae. umbellulata* Acc. 3732 was resistant to all the races

of leaf rust (Table 2), however, the amphiploid was resistant only to race 77-3 suggesting that *Ae. umbellulata* accession carried at least two different genes. One of the genes effective at seedling stage against race 77-3 was probably also expressed in the adult plants, the other gene(s) which were suppressed

Table 2. Rust reaction of *T. durum*, *Aegilops* species, their amphiploids, and derivatives of crosses of amphiploids with *T. aestivum* cv. Chinese Spring

No.	Line	Field reaction†		Seedling reaction against individual race‡								
		Leaf rust	Stripe rust	Leaf rust								Stripe rust
				77A-1	77-1	77-2	77-3	77-4	77-5	104B	104-2	N/P
1	*T. aestivum* cv. Chinese Spring	20S	60S	3^{+}	-	3^{+}	-	3^{+}	3^{+}	3^{+}	3^{+}	3^{+}
2	*T. aestivum* cv. Chinese Spring CS(*Ph*^{I})	220S	60S	3^{+}	-	3^{+}	-	3^{+}	3^{+}	3^{+}	3^{+}	3^{+}
3	*Aegilops umbellulata* Acc. 13749	0	0	-	-	-	-	0;	2^{-}	0;	0;	0
4	*T. durum* cv. Bijaga Yellow	20S	60S	-	-	-	-	0;	0;	3	3	;
5	BY-*Ae. umbellulata* amphiploid	80S	60S	-	-	-	-	2^{-}	$3\text{-}3^{+}$	0;	3	3
6	F1 CS(*Ph*^{I}) x amphiploid (No.5)	40S	5S	-	-	-	-	-	$3\text{-}3^{+}$	-	-	-
7	*T. durum* cv. Malvi Local	90S	40S	-	-	-	-	3	3^{+}	0;	3	3
8	ML -*Ae. umbellulata* amphiploid	40S	60S	-	-	-	-	2^{-}	3^{+}	0;	2^{-}	3
9	F1 CS(*Ph*^{I}) x amphiploid(No.8)	40S	5S	-	-	-	-	-	$3\text{-}3^{+}$	-	-	-
10	*T. durum* cv. A 206	90S	40S	$3^{+}\text{-}4$	-	$4^{-}\text{-}4$	-	$3^{+}\text{-}4^{-}$	0;	0;	3	3
11	*Ae. caudata* Acc. 3556	0	0	0;	0-0;	0;	-	0	0	-	-	-
12	Amph.[*T. durum- Ae. caudata*]	5S-80S	5S	-	-	3^{+}	0-0;	3	3^{+}	3	3	3
13	F1 CS(*Ph*^{I}) / amphiploid	20S	40S	-	-	-	-	-	-	-	-	-
14	F2 CS(*Ph*^{I}) / amphiploid	0-20S	0-20S	-	-	-	-	-	-	-	-	-
15	F3 CS(*Ph*^{I}) / amphiploid	0-90S	0-60S	-	-	0;-3	-	-	-	-	-	-
16	BC1 CS(*Ph*^{I}) / amphiploid // CS	0-40S	0-20S	-	-	-	-	-	-	-	-	-
17	BC1F2 CS(*Ph*^{I}) / amphiploid // CS	0-80S	0-10S	-	-	-	-	0;-3	-	0;-3	0;-3	0;-3
18	BC2 CS(*Ph*^{I}) / amphiploid // CS^{2}	0-20S	0-5S	-	-	-	-	-	-	-	-	-
19	*T. durum* cv. WH 868	40S	40S	3^{-}	$3^{+}\text{-}4^{-}$	3^{+}	-	X^{+}	0;	0;	3	3
20	*Ae. caudata* Acc. 3556	0	0	0;	0;	0;	-	0	0	-	-	-
21	Amph.[*T. durum- Ae. caudata*]	20-60S	5-20S	-	-	4^{-}	-	3	$2\text{-}3^{-}$	3	3	3
22	F1 CS(*Ph*^{I}) / amphiploid	40S	60S	-	-	-	0-0;	-	-	-	-	-
23	F2 CS(*Ph*^{I}) / amphiploid	0-10S	0-20S	-	-	-	-	-	-	-	-	-
24	F3 CS(*Ph*^{I}) / amphiploid	0-20S	0-90S	-	-	0;-3	-	-	-	-	-	0;-3
25	BC1 CS(*Ph*^{I}) / amphiploid // CS	0-80S	0-20S	-	-	-	-	-	-	-	-	-
26	BC1F2 CS(*Ph*^{I}) / amphiploid // CS	0-80S	0-5S	-	-	-	-	0;-3	-	0;-3	0;-3	-
27	BC2 CS(*Ph*^{I}) / amphiploid // CS^{2}	0-90S	0-10S	-	-	-	-	-	-	-	-	-
28	*T. durum* cv. WH 890	20S	20S	-	X^{+}	$3\text{-}3^{+}$	3-4	3^{+}	2	3^{+}	3^{+}	3
29	*Ae. umbellulata* Acc. 3732	0	0	0;	0-0;	0;	0	0;	0	0;	0;	0;
30	Amph. [*T. durum-Ae. umbellulata*]	0	0	-	-	3	0-0;	3^{+}	3^{+}	3^{+}	3^{+}	3
31	F1 CS(*Ph*^{I}) / amphiploid	0	-	-	-	-	-	-	-	-	-	-
32	F2 CS(*Ph*^{I}) / amphiploid	0-40S	0-20S	-	-	-	-	-	-	-	-	-
33	F3 CS(*Ph*^{I}) / amphiploid	0-60S	0-20S	-	-	-	-	-	-	-	-	-
34	BC1 CS(*Ph*^{I}) / amphiploid // CS	0-10S	0-5S	-	-	0;-3	-	0;-3	-	0;-3	0;-3	0;-3
35	BC1F2 CS(*Ph*^{I}) / amphiploid // CS	0-90S	0-20S	-	-	0;-3	-	0;-3	-	0;-3	0;-3^{+}	0;-3
36	BC2 CS(*Ph*^{I}) / amphiploid // CS^{2}	0-90S	0-10S	-	-	0;-3	-	0;-3^{+}	-	0;-3^{+}	0;-3	0;-3

† 0: Free, S: Susceptible.

‡ 0-2: Resistant, 3-4: Susceptible; X: Mesothetic (heterogeneous).
Variations are indicated by the use of + (more than the average for the class) and - (less than the average for the class) as superscripts. Dashes indicate data not recorded.

at the seedling stage may or may not have expressed at the adult plant stage. Recovery of plants in segregating generations of crosses of amphiploids with CS that were resistant to races to which durum wheat, amphiploid, and hexaploid wheat were susceptible (Table 2) further confirms the suppression of some of the resistance gene(s) of *Ae. umbellulata* by the A and/or B genomes of *T. durum* parents and that the Chinese Spring did not carry any suppressor system for resistance gene(s) of *Ae. umbellulata*.

In conclusion, the study unequivocally demonstrated the suppression of leaf and stripe rust resistance of the C and U genomes of *Aegilops* species by the A and/or B genomes of the *T. durum* parents in the synthetic amphiploids. The study further showed that the suppression system of *T. durum* suppressing the expression of resistance genes from *Aegilops* species had selective specificity as it suppressed only some of the resistance gene(s) and not the other. The impediment of suppression of resistance to interspecific gene introgression can, however, be overcome by selecting the recipient wheat stocks lacking the suppression system.

Acknowledgments

The first author is grateful to the Agricultural Research, Education and Extension Organization (AREEO), Ministry of Agriculture, Government of Islamic Republic of Iran for grant of scholarship to study in India. Financial support made by the United States Department of Agriculture under U.S.- India Fund is acknowledged.

References

Aghaee-Sarbarzeh M, Harjit-Singh and Dhaliwal HS (2000) *Ph*^{1} gene derived from *Aegilops speltoides* induces homoeologous chromosome pairing in wide crosses of *Triticum aestivum*. J Hered 91: 417-421.

Bai DP and Knott DR (1992) Suppression of rust resistance in bread wheat (*Triticum aestivum*) by D-genome chromosomes. Genome 35: 276-282.

Chen PD, Tsujimoto H and Gill BS (1994) Transfer of *Ph*^{1} gene promoting homoeologous pairing from *Triticum speltoides* into common wheat and their utilization in alien genetic introgression. Theor Appl Genet 88: 97-101.

Dhaliwal HS, Harjit-Singh, Gill KS and Randhawa HS (1993) Evaluation and cataloguing of wheat germplasm for disease resistance and quality. In: Damania AB (ed) Biodiversity and wheat improvement. John Wiley & Sons Pub: 123-140.

Friebe B, Jiang J, Raupp WJ, McIntosh RA and Gill BS (1996) Characterization of wheat-alien translocations conferring resistance to disease and pest: Current status. Euphytica 91: 59-87.

Gale MD and Miller TE (1987) The introduction of alien genetic variation into wheat. In: Lupton FGH (ed) Wheat breeding: Its scientific basis. Chapman and Hall, UK: 173-210.

Gill RS, Dhaliwal HS and Multani DS (1988) Synthesis and evaluation of *T. durum-T. monococcum* amphiploids. Theor Appl Genet 76: 912-916.

Harjit-Singh and Dhaliwal HS (2000) Intraspecific genetic diversity for resistance to wheat rusts in wild *Triticum* and *Aegilops* species. Wheat Inf Serv 90: 21-30.

Harjit-Singh, Grewal TS, Dhaliwal HS, Pannu PPS and Bagga PPS (1998) Sources of leaf rust and stripe rust resistance in wild relatives of wheat. Crop Improv 25: 26-33.

Innes RL and Kerber ER (1994) Resistance to wheat leaf rust and stem rust in *Triticum tauschii* and inheritance in hexaploid wheat of resistance transferred from *T. tauschii*. Genome 37: 813-822.

Jiang J, Friebe B and Gill BS (1994) Recent advances in alien gene transfer in wheat. Euphytica 73: 199-212.

Kerber ER (1983) Suppression of rust resistance in amphiploids of *Triticum*. Proc 6th Int Wheat Genet Symp: 813-814.

Kimber G and Tsunewaki K (1988) Genome symbols and plasma types in the wheat group. Proc 7th Int Wheat Genet Symp: 1209-1210.

Knott DR (1989) The wheat rusts: Breeding for resistance. Springer-Verlag, Berlin, Germany.

Knott DR, and Dvorak J (1976) Alien germplasm as a source of resistance to disease. Ann Rev Phytopath 14: 211-235.

Ma H, Singh RP and Mujeeb-Kazi A (1997) Resistance to stripe rust in durum wheat, A-genome diploids, and their amphiploids. Euphytica 94: 279-286.

Nayar SK, Prashar M and Bhardwaj SC (1997) Manual of current techniques in wheat rusts. Res Bull No.2. Regional Station, DWR, Flowerdale, Shimla, India.

Nelson JC, Singh RP, Antrique JE and Sorrells ME (1997) Mapping genes conferring and suppressing leaf rust resistance in wheat. Crop Sci 37: 1928-1933.

Peterson RF, Campbell AR and Hannah AE (1948) A diagrammatic scale for estimating of rust intensity of leaves and stems of cereals. Can J Res Series C 26: 496-500.

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

Stakman EC, Stewart DM and Loegering WQ (1962) Identification of physiological races of *Puccinia recondita* var. *tritici*. Miss Agri Expt Sta Sci J Series paper 4691.

Wheat Information Service
Number 92: 17–19 (2001)
Research article

Influence of leaf extract of bermuda grass (*Cynodon dactylon* L.) on the germination and seedling growth of wheat

S. M. Alam[1], S. A. Ala[2], R. Ansari[1] and M. A. Khan[1]

[1]Nuclear Institute of Agriculture, Tandojam, Pakistan
[2]Department of Botany, University of Jamshoro, Pakistan

Summary

Bermuda grass (*Cynodon dactylon* L.) was evaluated for its effect on germination and seedling growth of wheat, cv. Pavon. Increasing leaf water extract of bermuda grass significantly decreased the germination, shoot and root growth and the maximum percent decreases in these parameters at the highest residue extract level (2%) were 39, 68 and 83, respectively. It was concluded that this weed does not seem to be beneficial to the growth of wheat.

Key words: bermuda grass, leaf extract, weed, wheat

Introduction

Bermuda grass commonly known as *Cynodon dactylon* L. is a perennial creeping grass and grows as a weed in cultivated and non-cultivated lands, bunds and channels. It interferes with the growth of crop and reduces their yield. Seed germination, root and top growth of barley were inhibited when growing in soil that had previously contained bermuda grass residues (Horowitz and Friedman 1971). Castro et al. (1984) in laboratory experiments showed that root aqueous extracts of bermuda grass inhibited germination of rice seeds and the growth of aerial parts. The shoot and root litters or their aqueous extracts and soil collected from bermuda grass patches significantly reduced the germination, early growth biomass, moisture and chlorophyll content of wheat, barley and maize crops (Hussain and Khan 1988). Meissner et al. (1989) and Montemurro (1988) have reported that growth pattern of young carrot, cucumber, lettuce, maize, squash, onion, radish, sunflower and tomato plants were affected when grown in *Cynodon dactylon* infested soil. The present investigation was therefore, conducted to envisage the effect of aqueous leaf extract of bermuda grass on germination and seedling growth of wheat.

Materials and methods

Fresh leaves of bermuda grass (*Cynodon dactylon* L.) were collected, washed with water and dried in an oven at 75 °C for 48 hr. Samples were ground in a Wiley mill to pass through a 20 mesh screen. Ground samples were stored in plastic bottles at room temperature. Five levels (0, 0.5, 1.0, 1.5 and 2.0%) of dried ground residue were kept in distilled water for 24 hr at room temperature in 250 ml conical flask. The extracts were then filtered into 100 ml beakers using Whatman filter No. 42. Wheat (cv. Pavon) seeds were surface sterilized with 1% sodium hypochlorite solution for 2 minutes and then washed thoroughly with distilled water. Ten healthy seeds of wheat cultivar were placed on the surface of 0.8% agar containing 5 ml aqueous extract of each treatment in glass bowls. The bowls with only 0.8% agar were

treated as control. The bowls were covered with petri-dishes and incubated at 28 °C for 5 days. A 16 hour-light and 8 hour-dark period was also provided. Treatment at each extract level was replicated four times in a complete randomized block design. The number of seeds germinated were counted. Shoot and root length were measured. The experiment was repeated and the results were expressed in terms of the averages of duplicate trials. The data has been analyzed and presented in Table 1 and Table 2.

Results and discussion

Germination of wheat seeds significantly reduced as the concentration of the extract increased, and the magnitude of effect was more pronounced at concentration above 1 %, where it was markedly reduced (Table 1). The reduction percent in germination at 2 % level of extract was 39 % compared to control. This shows that aqueous extract from *Cynodon dactylon* residue contains some germination inhibiting chemicals resulting in the reduced germination of wheat seeds. Many investigators have suggested phenolics as the cause of inhibition of metabolic process during germination (Williams and Hoagland 1982; Blum et al. 1984; Kuiters 1989). Phenolic compounds generally caused inhibition in germination due to the interference with IAA metabolism, synthesis of protein and ion uptake by the plants (Castro et al. 1984; Rice 1984; Einhellig 1986; Hussain and Khan 1988). The seedling growth of both shoot and root of wheat significantly decreased with the increasing concentration of leaf extract used. The percent decreased in shoot and root length at the highest extract concentration were 68 and 83, respectively (Table 1). The reduced growth and development of the receiver plants in these studies demonstrated that allelochemicals released from the residues or produced by microorganisms during decomposition, affected wheat growth. This result confirmed the findings of others (Castro et al. 1984; Diaz and Kogan 1985; Weller et al. 1985; Hussain and Khan 1988; Montermurro 1988; Seetha et al. 1990).

In nature, the crop and weed residues or their parts might release water-soluble phytotoxins into the environment, which accumulate to the extent of toxicity to affect the wheat species tested. Hussain and Khan (1988) reported that aqueous leaf extract of bermuda grass contains ferulic, p-coumaric, vanillic, p-hydroxybenzoic, caffeic and syringic acids. Similarly, Habib and Rehman (1988) found caffeicchlorogenic, isochlorogenic, ferulic, o-coumaric, p-coumaric acids and scopoletin as the component of bermuda grass. All of these phenolic compounds have also been shown to possess allelopathic effect. This supports the assumption that extract phototoxicity of bermuda grass in this study might be due to the presence of phenolic compounds, all of them are water-extractable, strong and recognized as allelopathic agents (Rice 1988). Incorporating leaf extract as 2 % inhibited the root length more than the shoot. Roots of the species are in direct contact with residue of *Cynodon dactylon* when incorporated and subsequently are exposed to allelochemicals which in turn may have direct or indirect effect on the root system. Other researchers reported that plant residues caused injury, if the residue as in contact with or in the immediate vicinity of plant roots (Bhowmik and Doll 1982; Rice 1984). The present findings, therefore, reveal that *Cynodon dactylon* L. water extract releases toxic compounds which were inhibitory to the growth of wheat crop.

Table 1. Effect of different levels of Bermuda grass leaf aqueous extract on germination and shoot and root lengths of wheat

Leaf aqueous extract (%)	Germination (%)	Shoot length (cm)	Root length (cm)
0.0	98 a†	4.52 a	7.25 a
0.5	90 a (-8.2)‡	4.34 a (-4.0)	5.52 b (-23.9)
1.0	90 a (-8.2)	3.19 b (-29.4)	4.51 c (-37.8)
1.5	76 b (-22.5)	2.05 c (-54.7)	3.73 d (-48.6)
2.0	60 c (-38.8)	1.45 d (-67.9)	1.25 e (-82.8)

†Means followed by the same letters in a column do not differ significantly at 5 % level by Duncan's multiple range test.

‡Figures in the parentheses show decrease percent over control

Table 2. Analysis of variance

	Degree of freedom	Sum of squares	Mean square	F-value	Prob.
Germination					
Total	19	3841.00			
Rep-1	3	96.20	32.07	1.74	0.21
Rep-2	4	3524.00	881.00	47.88	0.00
Error	12	220.80	18.40		
Shoot length					
Total	19	29.64			
Rep-1	3	0.09	0.03	1.43	0.28
Rep-2	4	29.30	7.32	348.76	0.00
Error	12	0.25	0.02		
Root length					
Total	19	79.37			
Rep-1	3	0.17	0.06	2.76	0.09
Rep-2	4	78.94	19.74	939.81	0.00
Error	12	0.25	0.02		

References

Bhowmik PC and Doll JD (1982) Corn and soybean response to allelopathic effects of weed and crop residues. Agron J 74: 601-606.

Blum U, Dalton BR and Rawlings JO (1984) Effects of ferulic acid and some of its microbial products on radicle growth of cucumber. J Chem Ecol 10: 1169-1191.

Castro PRC, Rodrigues JD, Rabelo JC, Viega RFA, Lima GPP, Jureidini P and Denbandai IM (1984) Allelopathic action of some weed extracts on rice (*Oryza sativa* L. cv. IAC-195). Agric Luiz de Queiro. 41: 369-381.

Daiz MV and Kogan AM (1985) The allelopathic effect of perennial weeds and pasture species on the growth of plum, apple and vine plantlet. Simiente 55: 33-36.

Einhelling FA (1986) Mechanisms and modes of action of allelochemicals. In: Putnam AR and Tang CS (ed) The science of allelopathy. John Wiley and Sons, New York : 171-188,

Habib SA and Abdul Rehman AA (1988) Evaluation of some weed extracts against doddar on alfalfa (*Medicago sativa*). J Chem Ecol 14: 443-452.

Horowitz M and Friedman T (1971) Biological activity of substerranean residues of *Cynodon dactylon* L., *Sorghum halepense* and *Cyperus rotundus* L. Weed Res 11: 88-93.

Hussain F and Khan TW (1988) Allelopathic effects of Pakistani weed, *Cynodon dactylon* L. Pers. Pak J Weed Sci Res 1: 8-17.

Kuiters AT (1989) Effect of phenolic acids on germination and early growth of herbaceous woodland plants. J Chem Ecol 15: 467-479.

Meissner R, Nel PC and Beyers EA (1989) Allelopathic effect of *Cynodon dactylon* infested soil on early growth of certain crop species. Appl Plant Sci 3: 125-126.

Montemurro P (1988) Weed control in lettuce. Inform-Fitopatol 38: 17-21.

Rice EL (1984) Allelopathy: 2nd ed. Academic Press, Orlando Florida, USA.

Seetha RM, Babu RC, Sheriff MM, Perumal PKP, Seetha RM, Chandra BR, Mossa SM and Pallikonda PRK (1990) Allelopathic potential of shoot and root leachates of certain weed species. J Agron Crop Sci 164: 81-84.

Weller SC, Shroch WA and Monaco TJ (1985) Common bermuda grass interference in newly planted peach (*Prunus persica*) trees. Weed Sci 33: 50-56.

Williams RD and Hoagland RE (1982) The effect of naturally occuring phenolic compound on seed germination. Weed Sci 30: 206-212.

Wheat Information Service
Number 92: 20–24 (2001)
Research article

Transfer of *Agropyron elongatum*-derived rust resistance genes *Sr24* and *Lr24* into some Indian bread wheat cultivars

M. K. Menon[1] and S. M. S. Tomar[2]

[1]IARI Regional Station, Wellington, The Nilgiris - 643231, India
[2]Division of Genetics, Indian Agricultural Research Institute, New Delhi-110012, India

Summary

Agropyron elongatum-derived linked rust resistance genes *Sr24* and *Lr24* were transferred into nineteen elite Indian bread wheat (*Triticum aestivum* L.) cultivars, namely, Sonalika, Kalyansona, NI 5439, C 306, WH 147, Lok-1, HD 2329, HD 2285, J 24, HD 2009, VL 421, UP 262, WL 711, HUW 234, PBW 226, HD 2402, HI 1077, HS 240 and WH 542 through backcrossing. Gene *Lr24* conferred total resistance to leaf rust, whereas a new virulence for *Sr24*, namely, 40-1 rendered the gene ineffective against stem rust. The chromosome segment carrying genes *Sr24* and *Lr24* imparted enhanced resistance to Barley Yellow Dwarf Virus (BYDV). The segment was also associated with terminal clubbiness of spikes.

Key words: *Triticum aestivum*, bread wheat, leaf rust resistance, backcrossing, cultivar

Introduction

Extensive investigations on near-isogenic lines of wheat (*Triticum aestivum* L.) cultivar Thatcher and wheat stocks carrying known specific genes for resistance to rusts have revealed that resistance genes *Sr24, Sr26, Sr27, Sr31, Sr32, Sr36, SrAgi* and *Lr9, Lr19, Lr24, Lr28, Lr32, Lr37* (Tomar and Menon 1998) are highly effective against stem and leaf rust pathogens in India. The lines carrying these genes were screened under natural and artificially created rust epiphytotics at Wellington, an important hotspot for rusts in the southern hills of peninsular India. The weather conditions in these hills are very congenial for host and pathogens and the rusts are highly destructive throughout the year. A wide spectrum of stem and leaf rust pathotypes are prevalent in these hills (Bahadur 1986; Nayar et al. 1988). The authors have taken up an elaborate and comprehensive backcross program since 1983 to introgress alien genes conferring resistance to leaf and stem rusts into widely adapted and popular Indian bread wheat.

The tightly linked genes *Sr24-Lr24* originating from *Agropyron elongatum* (now *Lophopyrum elongatum*) confer resistance to stem and leaf rusts. Because of their linkage with red kernel color in cultivar Agent they could not be utilized in Indian subcontinent where the red grains were not commercially acceptable. However, one of the white seeded recombinant line TR 380-14*7/3Ag#14 (McIntosh and Partridge pers comm) proved to be a valuable stock for developing backcross lines. This article deals with the introgression of *Agropyron elongatum*-derived linked genes *Sr24* and *Lr24* into 19 Indian bread wheat cultivars, which are highly susceptible to leaf rust.

Materials and methods

The experimental material in the present study

[2] Corresponding author

comprised a white seeded donor parent TR 380-14*7/3Ag#14 carrying the genes *Sr24* and *Lr24* and 19 elite Indian bread wheat cultivars. They were Sonalika, Kalyansona, NI 5439, C 306, WH 147, Lok-1, HD 2329,

Table 1. Rust reactions of improved Indian bread wheat cultivars carrying *Agropyron elongatum*-derived genes *Sr24* and *Lr24*

Stocks	Genes present	Reaction to		
		Stem rust	Leaf rust	Stripe rust
Non recurrent parent				
TR 380-14*7/3Ag#14	*Sr24-Lr24, Sr?*	15R, MR	F	5MR
Recurrent parent/improved cultivar				
Sonalika		60S	80S	60S
Sonalika*7//TR 380-14*7/3Ag#14	*Sr24-Lr24*	40S	F	60S
Kalyansona		80S	80S	90S
Kalyansona*7//TR 380-14*7/3Ag#14	*Sr24-Lr24*	60S	F	90S
NI 5439		90S	90S	100S
NI 5439*6//TR 380-14*7/3Ag#14	*Sr24-Lr24*	70S	F	100S
C 306		90S	90S	F
C 306*7//TR 380-14*7/3Ag#14	*Sr24-Lr24*	70S	F	F
WH 147		90S	90S	90S
WH 147*6//TR 380-14*7/3Ag#14	*Sr24-Lr24*	70S	F	90S
Lok-1		70S	80S	80S
Lok-1*7//TR 380-14*7/3Ag#14	*Sr24-Lr24*	50S	F	80S
HD 2329		80S	90S	90S
HD 2329*7//TR 380-14*7/3Ag#14	*Sr24-Lr24*	70S	F	90S
HD 2285		30MS	100S	30S
HD 2285*7//TR 380-14*7/3Ag#14	*Sr24-Lr24*	30MR	F	30S
J 24		90S	100S	100S
J 24*7//TR 380-14*7/3Ag#14	*Sr24-Lr24*	70S	F	100S
HD 2009		40S	60S	100S
HD 2009*7//TR 380-14*7/3Ag#14	*Sr24-Lr24*	30S	F	100S
VL 421		60S	90S	80S
VL 421*7//TR 380-14*7/3Ag#14	*Sr24-Lr24*	50S	F	80S
UP 262		50S	50S	50S
UP 262*7//TR 380-14*7/3Ag#14	*Sr24-Lr24*	40S	F	50S
WL 711		100S	100S	90S
WL 711*7//TR 380-14*7/3Ag#14	*Sr24-Lr24*	80S	F	90S
HUW 234		20MS, S	100S	F
HUW 234*6//TR 380-14*7/3Ag#14	*Sr24-Lr24*	15MS, S	F	F
PBW 226		20S	90S	F
PBW 226*6//TR 380-14*7/3Ag#14	*Sr24-Lr24*	10S	F	F
HD 2402		30S	100S	F
HD 2402*6//TR 380-14*7/3Ag#14	*Sr24-Lr24*	15MS, S	F	F
HI 1077		30MS, S	50S	40MS
HI 1077*5//TR 380-14*7/3Ag#14	*Sr24-Lr24*	20MR, MS	F	40MS
HS 240	*Sr31-Lr26-Yr9*	5R, MR	70S	F
HS 240*7//TR 380-14*7/3Ag#14	*Sr31-Lr26-Yr9, Sr24-Lr24*	F-TR, MR	F	F
WH 542	*Sr31-Lr26-Yr9*	10R, MR	80S	F
WH 542*6//TR 380-14*7/3Ag#14	*Sr31-Lr26-Yr9, Sr24-Lr24*	F-TR, MR	F	F

Sr31 is highly effective, but *Lr26* not effective in india; *Yr9* highly effective only at Wellington.
R, MR = Resistant and moderately resistant, pustules of stem rust appear very late at maturity.

HD 2285, J 24, HD 2009, VL 421, UP 262, WL 711, HUW 234, PBW 226, HD 2402, HI 1077, HS 240, and WH 542. Cultivars HS 240 and WH 542 carry Petkus rye-derived resistance genes *Sr31*, *Lr26*, *Yr9* and *Pm8*. The recurrent parents were highly susceptible to leaf rust and most of them showed high susceptibility to stem rust except HS 240 and WH 542. Since *Sr24* and *Lr24* are tightly linked dominant genes, five to seven backcrosses were given in quick succession within a short span of two to three years, raising three experimental crops in a year, under high natural incidence of rusts, and the genotypes phenotypically similar to their recurrent parents in all respects and carrying resistance to stem rust and leaf rust were constituted after three generations of selfing at F_3 generation.

All the improved lines were screened for stem rust and leaf rust under artificial epiphytotic conditions at adult plant stage. Rust reactions were scored following standard practices. Only 10 backcross lines were subjected to seedling test with 40A and 40-1 pathotypes of stem rust under glass house conditions at temperature ranging from 27 to 30 °C. The data on Barley Yellow Dwarf Virus (BYDV) incidence under natural condition was also recorded in percent infection over three years.

Results and discussion

Improved backcross lines exhibited total resistance to leaf rust at adult plant stage (Table 1). This indicates that the gene *Lr24* had been introgressed into susceptible recurrent parents. However, thirteen backcross lines exhibited susceptibility to stem rust and six lines showed moderately resistant to moderately susceptible reaction to stem rust. Of the seven, five recurrent parents, namely, HD 2285, HUW 234, PBW 226, HI 1077 and HD 2402 appear to carry unknown factor(s) for low reaction (10S to 30MS) to stem rust. Cultivars HS 240 and WH 542 and their improved version showed a high degree of stem rust resistance by the presence of *Sr31* derived from rye.

Sawhney and Goel (1981), Patil and Deokar (1996) reported that *Sr24* conditioned seedling resistance to 19 prevailing and virulent Indian stem rust pathotypes. *Sr24* displayed a high degree of resistance to stem rust for over 25 years at Wellington, a hotspot for stem rust. However, virulence for *Sr24* has been reported from South Africa (Le Roux and Rijkenberg 1987). Its resistance was also overcome by a new Indian pathotype 40-1 (Bharadwaj et al. 1990) of stem rust. TR 380-14*7/3Ag#14 is resistant to stem rust by different factor(s) from *Sr24*. Only 10 backcross lines and their recurrent parents were tested in seedling stage with two pathotypes 40A and 40-1 of stem rust (Table 2). All the tested lines except WH 542*6/TR 380-14*7/3Ag#14 and WH 542, showed 3+ or 4 infection type to 40-1 in seedling stage. This indicates that the gene *Sr24* is indeed overcome by this virulence. The seedling reaction to 40A pathotype of stem rust confirms the presence of *Sr24*. The line WH 542*6/TR 380-14*7/3Ag#14 and its recurrent parent WH 542 exhibited ;1 and ;1,2 reactions in seedling stage to 40A and 40-1 pathotypes by the presence of *Sr31* (Goel et al. 1994) respectively. Thus, based on the pattern and type of reactions to stem and leaf rusts tested in seedling it is presumed that the segment carrying *Sr24-Lr24* has been introgressed into the backcross lines.

Bread wheat cultivars carrying *Sr24* and *Lr24* are widely grown in Australia, North America and South Africa. In India too, three cultivars viz., Vidisha, Vaishali and HW 2004 (C 306*7/TR 380-14*7/3Ag#14), carrying *Sr24* and *Lr24* have been released commercially in recent years. Backcross line HW 2004

Table 2. Seedling reaction of backcross improved lines carrying *Sr24-Lr24* and their recurrent parents to two pathotypes of *Puccinia graminis tritici*

Backcross lines/parents	Pathotypes	
	40A	40-1
NI 5439	4	4
NI 5439*6/TR 380-14*7/3Ag#14	1N	3+
C 306	3+	4
C 306*7/TR 380-14*7/3Ag#14	;1	4
Lok-1	3+	4
Lok-1*7/TR 380-14*7/3Ag#14	;	4
HD 2329	3+	4
HD 2329*7/TR 380-14*7/3Ag#14	0;	4
WL 711	4	4
WL 711*7/TR 380-14*7/3Ag#14	;1+	4
HUW 234	3+	3+
HUW 234*6/TR 380-14*7/3Ag#14	;1	3+
PBW 226	;1, 2	3+
PBW 226*6/TR 380-14*7/3Ag#14	;1	3+
HD 2402	1,2+	3+
HD 2402*6/TR 380-14*7/3Ag#14	;1+	3+
HI 1077	3C	3+
HI 1077*5/TR 380-14*7/3Ag#14	;1, 2	3+
WH 542	;1	;1, 2
WH 542*6/TR 380-14*7/3Ag#14	;1	;1, 2
Agra Local (check)	4	4

Table 3. Mean infection (%) of BYDV affected plants in some of the Indian bread wheat cultivars and backcross lines carrying *Sr24-Lr24* over three seasons

Cultivar/backcross lines	Frequency of infected plants (%)	Height (cm)		Seed fertility (%)	
		Normal plant	Affected plants	Normal earhead	Affected earhead
Kalyansona	16	90	45	100	10.6
HD 2329	12	80	43	100	9.09
WL 711	18	95	48	100	8
Kalyansona*6//TR 380-14*7/3Ag#14	3	92	44	100	10
HD 2329*7//TR 380-14*7/3Ag#14	2	80	42	100	9
WL 711*7//TR 380-14*7/3Ag#14	2	95	47	100	9
Sonalika (check)	0	90	0	100	0

has been released for cultivation under dryland conditions of Central India. This backcross line has yielded significantly higher than that of its recurrent parent C 306, particularly under high incidence of leaf rust. The plains of Central India are the route of migration of stem rust and leaf rust uredospores to the main wheat belt of northern plains. Since the rainfed crop in central plains is early sown, the inoculum built up on susceptible cultivars poses a major threat to the timely and late sown crop of central and northern plains. To check rust infection in this secondary source, cultivation of HW 2004 is the only economic and effective answer. Sawhney and Goel (1983) reported that *Lr24* conferred effective seedling resistance to all prevalent and virulent leaf rust pathotypes in India. Though virulences for *Lr24* have been reported from North America (Gough and Merkle 1971; Long and Kolmer 1989), South America (Singh 1991) and South Africa (Pretorius et al. 1990), this gene is still very effective in India.

The authors observed that the chromosome segment with *Sr24* and *Lr24* enhanced resistance to Barley Yellow Dwarf Virus (BYDV) in backcross lines, Kalyansona*6/TR 380-14*7/3Ag#14, HD 2329*7/ TR 380-14*7/3Ag#14 and WL 711*7/TR 380-14*7/3Ag#14. The recurrent parents viz., Kalyansona, HD 2329 and WL 711 are susceptible to BYDV. This disease is the most widely distributed viral disease on cereals and can cause serious economic losses (Zillinsky 1983); wheat, barley, oats, rye, triticale and grasses are hosts of BYDV. Affected plants were stunted (reduced height, short leaves with excessive tillering and had pale yellow leaves, most of the spikes were sterile and had delayed anthesis). The frequency of BYDV affected plants was 12-18% in HD 2329, Kalyansona and WL 711 compared to backcross lines carrying *Sr24-Lr24* (2-3%) (Table 3). Variability for resistance to BYDV seems to be scanty among wheat germplasm (Fedak 1998) and must be sought in tertiary gene pool. Cultivar Sonalika identified as resistant in present study should be screened at multilocation where BYDV infection is ensured. The authors also observed varying degree of terminal clubbiness of spikes which seems to be associated with the resistance imparted by *Sr24* and *Lr24*; however, cultivars with least or minimum clubbiness were selected to eliminate the risk of shattering of seeds in the final variety constituted.

Acknowledgments

The authors express their deep gratitude to Drs. R.N. Sawhney, Indian Agricultural Research Institute, New Delhi, India (now superannuated) and R. A. Mclntosh, Plant Breeding Institute, University of Sydney, Australia for the supply of donor used in the present study. Our grateful thanks are also due to Mr. S. Bojan for providing technical assistance.

References

Bahadur P (1986) Physiologic specialization in wheat rust. In: Joshi LM, Singh DV and Srivastava KD (ed) Problems and progress of wheat pathology in south Asia. Malhotra Publishing House, New Delhi: 69-91.

Bhardwaj SC, Nayar SK, Prashar M, Kumar J, Menon MK and Singh SB (1990) A pathotype of *Puccinia graminis* f. sp. *tritici* on *Sr24* in India. Cereal Rusts and Powdery Mildews Bull 18: 35-38.

Fedak G (1998) Procedures for transferring agronomic traits from alien species to crop plant. Proc 9th Int Wheat Genet Symp 1: 1-7.

Goel LB, Kumar J and Nagarajan S (1994) Results of the coordinated experiments; 1993-94. Crop Protec (Pathology) : 38-46.

Gough FJ and Merkle OG (1971) Inheritance of stem and leaf rust resistance in Agent and Agrus cultivars of

Triticum aestivum. Phytopath 61: 1501-1505.
Le Roux J and Rijkenberg FHJ (1987) Pathotypes of *Puccinia graminis* f. sp. *tritici* with increased virulence for *Sr24*. Plant Disease 71: 1115-1119.
Long DL and Kolmer JA (1989) A North American system of nomenclature for *Puccinia recondita* f. sp. *tritici*. Phytopath 79: 525-529.
Nayar SK, Nagarajan S and Bahadur P (1988) Virulence distribution pattern of *Puccinia recondita* f. sp. *tritici* in India during 1983-86. Pl Dis Res 3: 203-210.
Patil VV and Deokar AB (1996) Host parasite interactions between lines and varieties of wheat with known *Sr* genes and races of stem rust. Cereal Rusts and Powdery Mildew Bull 24: 91-97.
Pretorius ZA, Le Roux J and Drijenpondt SC (1990) Occurrence and pathogenicity of *Puccinia recondita* f. sp. *tritici* on wheat in South Africa during 1988. Phytophylactica 22: 225-228.
Sawhney RN and Goel LB (1981) Race-specific interaction between wheat genotypes and Indian cultures of stem rust. Theor Appl Genet 60: 61-166.
Sawhney RN and Goel LB (1983) Effectiveness of newly described leaf rust resistance genes against Indian cultures of standard races and biotypes of leaf rust in wheat. Wheat Inf Serv 56: 34-36.
Singh RP (1991) Pathogenicity variations of *Puccinia recondita* f. sp. *tritici* and *P. graminis* f. sp. *tritici* in wheat growing areas of Mexico during 1988 and 1989. Plant Disease 75: 790-794.
Tomar SMS and Menon MK (1998) Adult plant response of near-isogenic lines and stocks of wheat carrying specific *Lr* genes against leaf rust. Indian Phytopath 51: 61-67.
Zillinsky FJ (1983) Common disease of small grain cereals. A guide to identification. CIMMYT, Mexico.

Wheat Information Service
Number 92: 25–26 (2001)
Research information

Identification of hybrid necrosis genes among some bread wheat accessions

Rajiv K. Sharma, S. Chowdhury and A. P. Sethi

Division of Genetics, Indian Agricultural Research Institute, New Delhi 110012, India

Hybrid necrosis in wheat causing seedling lethality is caused by two dominant complementary genes *Ne1* and *Ne2* (Zeven 1966). These genes are widely distributed in wheat germplasm throughout the world (Zeven 1973; Tsunewaki and Nakai 1974). The presence of these genes limits the hybridization between the *Ne1* and *Ne2* carriers (Hermsen 1963). Therefore, cataloguing the information about necrosis genes in different wheat strains is necessary for formulating effective hybridization programs. Thousands of new genotypes are developed every year necessitating this cataloguing to be a continuous work.

A total of 462 F_1's, involving 208 accessions of common wheat as parents, formed the experimental material for this study. The parental stocks included testers like Kalyansona (*ne1Ne2)* and C306 (*Ne1ne2*) (Srivastava and Singh 1988). The parents used in the study were categorized (Table 1) as having *Ne1* or *Ne2* as per the hybrid necrosis shown by respective F_1 seedlings. The information generated will help breeders choose their hybridization material successfully.

Table 1. Distribution of hybrid necrosis genes among some bread wheat accessions

Ne1Ne1ne2ne2	*ne1ne1 Ne2Ne2*	*ne1ne1ne2ne2*
HD 2733, HD 2590, SK 282†, ESWYT 22‡, ESWYT 13, ESWYT 40	Naya 2285, Naya 2329, HD 2380, HD 2402, HD 2643, HD 2745, HD 2710, HD 2747, PBW 476, PS-484, T-2447, HW 2045, VC-CP-200, DL-69, UP 2504, HD 2329, HD 2285, HD 2009+*Lr24* (HW 2011), WR 956, HD 2775, HD 2705, WR686, HD 2721, HW 2001A, HW 2031	PBW 343,HD 2687, ESWYT14, ESWYT42, ESWYT49, HUW520, PBW 466, CL 428, DL 9

†PASTOR/3/SERI*3//RL6010/4*YR
‡19TH ESWYT:
ESWYT22: ATTILLA/3/HUI/CARC//CHEN/CHTO/4/ATTILA
ESWYT13: Pfau/Weaver
ESWYT40: Debeira
ESWYT14: CHIL/2*STAR
ESWYT42: OASIS/SKAUZ//4*BCN
ESWYT49: PARUS

References

Hermsen JGTh (1963) Hybrid necrosis as a problem for the wheat breeders. Euphytica 12: 1-16.

Srivastava PSL and Singh SR (1988) Identification of genes for hybrid necrosis in wheat. Indian J Genet 48: 267-269.

Tsunewaki K and Nakai Y (1974) Necrosis genes in common wheat varieties from the USSR and the East Mediterranean region. Wheat Inf Serv 39: 19-30.

Zeven AC (1966) Geographical distribution of genes causing hybrid necrosis in wheat. Euphytica 15: 281-284.

Zeven AC (1973) Sixth supplementary list of wheat varieties classified according to their genotype for hybrid necrosis and geographical distribution of *Ne*-genes. Euphytica 22: 618-632.

Wheat Information Service
Number 92: 27–29 (2001)
Research information

Loose smut resistant lines in wheat and triticale with combined resistance to Karnal bunt, rusts, powdery mildew and leaf blight

D. P. Singh[1], A. K. Sharma[1] and A. S. Grewal[2]

[1]Crop Protection Programme, Directorate of Wheat Research, P.B.No.158, Karnal 132 001, India
[2]Department of Plant Pathology, P.A.U. Ludhiana 141 004, India

Loose smut caused by *Ustilago segetum* (Pres.) Russel var. *tritici* (Jensen) is one of the major diseases of wheat in northern India and is responsible for about 1-5 % yield losses every year, on an average (Joshi et al. 1988). The disease is totally seedborn and can easily be controlled by seed treatment with systemic fungicides like carboxin, carbendazim and tebuconazole (Chatrath et al. 1969; Bahadur and Sinha 1978; Sinha and Singh 1996). However, chemical usage has its own drawbacks and till date the adoption of these fungicides by the farmers is not up to the desired level, mainly due to the high cost of fungicides coupled with other issues like non availability of fungicides at proper time, unsuitable packing quantity and above all unfitness of treated left over seeds for consumption. The issues of health and environmental hazards are also drawing attention and pressing for restricted uses of pesticides in agriculture. Keeping these problems in view, efforts were made by some workers in past to identify resistant lines for loose smut (Aujla et al. 1990; Srivastava et al. 1992; Beniwal et al. 1998). However, such lines were not utilized extensively by the breeders since resistance to loose smut alone does not carry much significance in wheat since resistance to diseases like rusts and leaf blight is on the first priority of the wheat breeders. The present study was, therefore, done with an object to evaluate the wheat and triticale lines possessing resistance to other major diseases like rusts, Karnal bunt, powdery mildew and leaf blight, against loose smut, under artificially inoculated conditions so as to identify resistant sources that carry resistance to loose smut along with resistance to other important diseases.

The confirmed resistant lines of wheat and triticale to one or more diseases like rusts, Karnal bunt, powdery mildew and moderately resistant to leaf blight, were tested for their resistance to loose smut disease under artificially inoculated conditions. In all, 132 lines of wheat and triticale, were tested under artificially inoculated conditions. Out of these, 50 were resistant to rusts, 60 to Karnal bunt, 14 to leaf blight and 8 to powdery mildew since the past few years (5 or more years) at multilocation hot spot testing. The lines were sown in 2 m long rows during November and five ear heads of each entry were inoculated at growth stage '59' of Zadoks' scale (Zadoks et al. 1974) artificially with loose smut teliospores using modified 'Go-go' method (Joshi et al. 1988), during 1997-98 crop season, at Ludhiana. The inoculated ear heads were tagged and harvested separately. The seeds obtained from such inoculated ear heads were planted during next crop season i. e. 1998-99 and record on smutted and healthy ear heads was taken on tiller basis after ear emergence. The percent loose smut infection was calculated. The entries having 0-5 % loose smut infection, under artificially inoculated conditions were categorized as resistant whereas those showing >5 % infection were identified as susceptible to loose smut.

Out of total 132 wheat and triticale lines, 53 were found free from loose smut whereas two were having 1-3 % loose smut infection. The details of these

This work was done under All India Coordinated Wheat Improvement Project, Contribution No. 60/DWR/2000-01. E-mail: dwr@vsnl.com, Fax: +91-184-251390

promising entries are presented in Table 1. The inoculation and establishment of infection of loose smut in the seeds harvested during 1997-98 was quite perfect and some of the susceptible entries like HD 2556 and HW 1065 showed loose smut expression up to 98 % in field during 1998-99 crop season.

For the first time, an interesting observation on the possible correlation of resistance between two major seedborne diseases namely Karnal bunt and loose smut was made. Out of 60 Karnal bunt resistant entries tested against loose smut, 32 (53 %) were turned resistant to loose smut also indicating possibilities of a positive correlation between the Karnal bunt and loose smut resistance. Both these seedborne pathogens infect the floret and host resistance plays important role in seed infection and transmission of pathogen through infected seed.

However in case of leaf diseases like rusts, leaf blights and powdery mildew, no such strong correlation of resistance to the loose smut was observed and only 12 entries out of 46 (19 %) were resistant to loose smut. Amongst wheat and triticale entries, the level of resistance was also better in triticale lines in comparison to the wheat and 13, out

Table 1. Loose smut resistant lines with combined resistance to rusts, leaf blight, powdery mildew and Karnal bunt in wheat and triticale

Entry	Loose smut (%)	Entry	Loose smut (%)
Resistant to :			
A. Stem and leaf rusts (1979-97)		PAF 82/90	0
HW 888	0	SCP(Dwarf mutant)–XW 568	0
ISWRN (W 240)	0	Victoria-1	0
B. Leaf and stripe rusts (1979-97)		HD 2281	0
MPO 615(d)†	0	HD 4564	0
C. Stem, leaf and stripe rusts (1979-97)		HI 8073	0
DLRRL 5	0	JU 69(d)	0
DLRRL 16	0	MPO 215(d)	0
DLRRL 32	0	Karnal bunt (1987-97)	
DLRRL 34	0	HS 240	0
UPT 78274(T)‡	0	RAJ 1707	0
JNIT 519(T)	0	RAJ 1710(d)	0
DT 8(T)	0	WEIBULT-RING	0
DT 17(T)	0	PG'S' GGO-VZ 380x5V5xCR 'S'(d)	0
JNIT 169(T)	0	DWL 5010	0
DT 31(T)	0	DWL 5023(d)	0
DT 33(T)	0	WH 805(d)	0
DT 38(T)	0	PBW 225	0
TL 2735(T)	0	RAJ 911(d)	0
TL 2736(T)	0	RAJ 2071	0
HPT 5(T)	0	SUN Bird 'S'	3
D. Karnal bunt (1985-97)		HD 2383	0
WL 6975	0	Karnal bunt (PAU) (1990-98)	
USDA 203	0	WG 2780	0
FAT	0	E. Powdery mildew (1980-97)	
HARLAN JR 3471	0	JNIT 42(T)	0
Teremas Branee 8189	0	NP 200 (*T.dicoccum*)	0
VEE'S'/MJI	0	TL 2597(T)	0
GA 'S'	1	F. Moderately resistant to Leaf blight (1986-97)	
KRK 5	0	Wuhani/310 B-0	0
SCA'A'	0		

†(d): *T. durum*, ‡(T): Triticale

of 22 (59 %) lines of triticale tested, were resistant to loose smut. In case of wheat only 38 % entries were resistant to loose smut. One line of *Triticum dicoccum* (NP 200) was tested and found free from loose smut, whereas most of the *T. durum* entries were also resistant. These resistant lines to loose smut with combined resistance to other major diseases are therefore recommended for the use in breeding for disease resistance in wheat and triticale. This is of further importance since resistance to only loose smut is of not much use and so far these are not being preferred as donor parents, in spite of the fact that these have been listed by earlier workers (Aujla et al. 1990; Beniwal et al. 1998).

References

Aujla SS, Grewal AS, Nanda GS and Sharma Indu (1990) Identification of stable resistance in wheat to loose smut. Indian Phytopath 43: 90-91.

Bahadur P and Sinha VC (1978) Efficacy of Bavistin for controlling loose smut of wheat. Pesticides 12: 31-32.

Beniwal MS, Karwasra SS, Gupta A, Chhabra ML and Singh R (1998) Stable sources of resistance to loose smut of wheat. Ann Biol 14: 231-232.

Chatrath MS, Renfro BL, Nene YL, Grover RK, Roy MK, Singh DV and Gandhi SM (1969) Control of loose smut of wheat with carboxin and benomyl. Indian Phytopath 22: 183-187.

Joshi LM, Singh DV and Srivastava KD (1988) Manual of wheat diseases. Malhotra Publishing House, New Delhi 75.

Sinha VC and Singh DP (1996) Raxil (tebuconazole) in the control of loose smut of wheat. Indian J Mycol Pl Pathol 26: 297-281.

Srivastava KD, Singh DV, Aggarwal R, Bahadur P and Nagarajan S (1992) Occurrence of loose smut and its sources of resistance. Indian Phytopath 45: 111-112.

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

Wheat Information Service
Number 92: 30–31 (2001)
Genetic stocks

Seeds of newly-synthesized allopolyploids of *Aegilops* and *Triticum* and of their parental plants

Hakan Ozkan[1] and Moshe Feldman[2]

[1]Department of Field Crops, Faculty of Agriculture, University of Cukurova, Adana 01330, Turkey
[2]Department of Plant Sciences, The Weizmann Institute of Science, Rehovot, 72100, Israel

During the course of our studies on the effect of allopolyploidy on genome evolution in wheat, we produced a large number of different allopolyploids. Single plants used as parents were bagged and selfed so that the genotype of the synthetic allopolyploids can be traced to specific parental plants. The allopolyploids were produced by colchicine treatment and following treatment, plants were grown in the greenhouse until maturity and all spikes were bagged. Three allopolyploids were derived from spontaneous formation of unreduced gametes on F_1 plants that were not treated with colchicine. All in all, twenty-three different allopolyploids were obtained (Table 1) of which 10 are "Natural", i.e., they have a genomic combination that exists in nature, and the remainder 13 are "Non-Natural" having a genomic combination that does not exists in nature (Table 1). Chromosome number was determined in all the newly synthesized allopolyploids and only those having the expected euploid chromosome number were harvested. Small samples of seeds, S_2, S_3, and S_4 of all these 23 allopolyploids and of their parental plants are available upon request from the senior author (Hakan Ozkan).

Table 1. List of newly-synthesized allopolyploids of *Triticum*, *Aegilops* and *Secale* that were recently produced by us

Cross combination	Genomic constitution of newly synthesized allopolyploids
"Natural" allopolyploid combinations	
Tetraploids (2n=4x=28)	
Ae. sharonensis (TH01) x *Ae. umbellulata* (TU04)	S^lS^lUU
Ae. longissima (TL02) x *Ae. umbellulata* (TU02)	S^lS^lUU
Ae. longissima (TL05) x *T. urartu* (TMU06)	S^lS^lAA
Ae. sharonensis (TH02) x *T. monococcum* ssp. *aegilopoides* (TMB02)	$S^lS^lA^mA^m$
Hexaploids (2n=6x=42)	
T. turgidum ssp. *carthlicum* (TTH01) x *Ae. tauschii* (TQ27)	BBAADD
T. turgidum ssp. *carthlicum* (TTH01) x *Ae. tauschii* (TQ17)	BBAADD
T. turgidum ssp. *durum* (TTR04) x *Ae. tauschii* (TQ27)	BBAADD
T. turgidum ssp. *durum* (TTR16) x *Ae. tauschii* (TQ27)†	BBAADD
T. turgidum ssp. *durum* (TTR19) x *Ae. tauschii* (TQ27)†	BBAADD
T. turgidum ssp. *dicoccoides* (TTD20) x *Ae. tauschii* (TQ27)	BBAADD

(continued on the next page)

Table 1. (Continued)

Cross combination	Genomic constitution of newly synthesized allopolyploids
"Non-Natural" allopolyploid combinations	
Tetraploids (2n=4x=28)	
Ae. speltoides (TS86) x *Ae. caudata* (TD01)	SSCC
Ae. longissima (TL01) x *Ae. tauschii* (TQ27)	S^lS^lDD
T. urartu (TMU38) x *Ae. tauschii* (TQ27)	AADD
Ae. bicornis (TB01) x *Ae. tauschii* (TQ27)	S^bS^bDD
Hexaploids (2n=6x=42)	
T. turgidum ssp. *durum* (TTR16) x *Ae. speltoides* (TS01)	BBAASS
T. turgidum ssp. *durum* (TTR19) x *Ae. sharonensis* (TH01)	$BBAAS^lS^l$
Octoploids (2n=8x=56)	
T. aestivum (TAA01) x *Ae. speltoides* (TS01)	BBAADDSS
Ae. speltoides (TS42) x *T. aestivum* (TAA01)	SSBBAADD
T. aestivum (TAA01) x *Ae. longissima* (TL01)	$BBAADDS^lS^l$
T. aestivum (TAA01) x *Ae. longissima* (TL02)	$BBAADDS^lS^l$
T. aestivum (TAA01, *ph1 ph1*) x *Ae. longissima* (TL01)	$BBAADDS^lS^l$
T. aestivum (TAA01) x *S. cereale* (SC01)	BBAADDRR
Ae. variabilis (TKE02) x *T. turgidum* ssp. *dicoccoides* (TTD20) †	SSUUBBAA

All amphiploids were produced by colchicine treatment of F_1 seedlings, except for the three marked with superscript,†.
†Amphiploid obtained by spontaneous formation of unreduced gametes in F_1 plants.

Wheat Information Service
Number 92: 32–38 (2001)
Record

The joint meeting of the 5th Japanese Society of Molecular Biology of Triticeae and the 27th Japanese Wheat Genetics Symposium, October 7–9, 2000

Introductory remarks

Yoshihiko Furuta (Dept Agr, Gifu Univ; furutay@cc.gifu-u.ac.jp)

The 5th Japanese Society of Molecular Biology of Triticeae and the 27th Japanese Wheat Genetics Symposium were held at Gifu University in October 7 to 9, 2000. The Local Organizing Committee consisting of Y. Furuta (Chairman) and N. Watanabe was in charge of its organization and management. The joint meeting involved two special lectures, four sessions of 32 research papers including open-discussion in total, business session, and postmeeting excursion of *Lycoris* plants-observation in university garden. Total number of the attendants were 95 belonging to 26 different institutions, including eight researchers or students from four foreign countries. The titles of the three oral sessions were; (1) the forefront of chromosome research in cereals (seven papers), (2) recent topics and prospects in wheat and barley breeding (seven papers), (3) general presentation (eleven papers). In poster session by young researchers or students, seven papers were presented. The abstracts or titles of all research papers are presented below. In the evening of October 7, a free talking mixer session was held. The banquet was held in traditional "ayu (Japanese river fish, *Plecoglossus altivelis*) riverside restaurant" in the evening of October 8. During these parties, we have nice time and exchange some information. In the business session, one agenda was discussed and agreement was made as follows; site of the 28th Japanese Wheat Genetics Symposium was decided to be Obihiro Chikusan University and S. Sawada and H. Miura, professors there, addressed their willingness to become its host. T. Sasakuma who is a member of Bio-resources Committee of Science Council of Japan, reported progress and plan of "Mugi-net" and official germplasm maintaining organization of wheat. Finally, H. Tsujimoto, editor of Wheat Information Service (WIS), reported activity of publication of WIS.

Special lecture

Y. Niimi and M. Murayama (Gifu Univ)
Allelic variation of the dopamine receptor *D4* gene polymorphic region in dogs in relation to behavior character among varieties of dog

H. Nagano (Gifu Univ)
Making bread by using yeast and bacteria based on the observation of histomorphological analysis of traditional or local breads collected from all over the world

1. Symposium on the forefront of chromosome research in cereals (Organizer: Y. Mukai)

Y. Mukai (Osaka Kyoiku Univ; ymukai@cc.osaka-kyoiku.ac.jp)
Recent progress in wheat chromosome research

The development of FISH techniques over the past decade has contributed to our understanding of genome organization and chromosome structure. FISH methods have been applied to the field of wheat molecular cytogenetics and precise visualization of agronomically important genes is a routine now by

FISH using lambda and BAC clones. Recent new technologies, in situ PCR and DNA fiber FISH, improved the detection sensitivity and resolution on chromosomes or DNA molecules. We have characterized many large-insert clones of wheat containing the centromeric regions and the structure of centromere is being unraveled. The important problems and prospects for wheat chromosome studies have been discussed here.

S. Taketa (Fac Agr, Kagawa Univ; staketa@ag.kagawa-u.ac.jp)
Recent advances in chromosome research of the genus *Hordeum*

The genus *Hordeum* is classified into 31 species and includes ploidy series ranging from diploid to hexaploid. Recent molecular cytogenetic analyses on cultivated and wild barleys were summarized with special reference to the physical mapping of various repetitive DNA sequences. These analyses supported the distinction of the four basic genomes (H, I, X and Y) in the genus. Genomic in situ hybridization unequivocally revealed the presence of *H. marinum* genome in three polyploid species. On the basis of chromosomal distribution of repetitive DNA sequences, the phylogenetic relationships of the *Hordeum* species are discussed.

T. Morikawa* and M. Hayasaki (Graduate School Agr Bio Sci, Osaka Pref Univ; *morikawa@plant.osakafu-u.ac.jp)
Molecular cytogenetics of oats

Wild and cultivated hexaploid oats share the same genomes (AACCDD) and display a considerable level of interspecific variation in both plant and chromosome morphology. The GISH and FISH were utilized to detect the inter- and intraspecific genomic compositions in oats using total genomic DNA of *Avena eriantha* (a C-genome diploid) and 18S-5.8S-26S rDNA as probes. Intergenomic translocations between A/D and C-genome chromosomes were frequently observed in hexaploid and tetraploid by GISH. The FISH data also indicated the intraspecific variation of rDNA major sites of *A. canariensis* and *A. agadiriana* chromosomes. These observations indicate that the genome of this genus continues to evolve via chromosomal rearrangement.

M. Tomita (Dept Agrobiol, Fac Agr, Tottori Univ; tomita@muses.tottori-u.ac.jp)
Recent advances in chromosome research of rye and *Thinopyrum*

A new class of multigene family designated 2.8 kb family have been identified in rye, *Secale cereale*, by using a highly repetitive 89 bp probe initially obtained by a genomic subtraction method. Their 2,817 bp consensus sequence determined from E genomic DNA clones did not share any identity with known sequences. A 726 bp cDNA derived from the 2.8 kb family was isolated and the gene constitution including 3 exons and 2 introns was characterized. As much as 10,000 copies of the 2.8 kb multigene family were distributed on all rye chromosomes. On the other hand, a retrotransposon-like clone pTi28 found in *Thinopyrum intermedium* enabled to detect *Thinopyrum* chromosomes in the wheat background by their enrichment in the telomeric regions.

M. Yamamoto (Kansai Women's Coll; ymaki@user.center.osaka-u.ac.jp)
FISH from chromosomes to DNA

Fluorescence in situ hybridization on extended DNA fibers from interphase nuclei has been developed as high-resolution FISH. Molecular combing has enabled direct mapping to purified BAC or lambda DNA clones. We have achieved the direct visualization of gene organization in agronomically important genes of wheat and rye. Our results have indicated a spatial resolution of 1 kb between adjacent targets and detection sensitivity of a target of as small as 800 bp. The fiber FISH technique is very useful for determining the size of target DNA sequences, the order of genes or clones and their distances in a large chromosome region.

M. Murata (Res Inst Bioresour, Okayama Univ; mmura@rib.okayama-u.ac.jp)
Construction of chromosome-specific DNA libraries by laser-microdissection

We are constructing chromosome arm-specific DNA libraries in wheat by using laser-microdissection. Telocentric chromosomes in ditelosomic lines were chosen as targets, scratched off and picked up with glass needles adjusted to a micromanipulator. The microdissected chromosomes were then harvested into a PCR tube, and their DNA was amplified using DOP-PCR. In order to evaluate the efficiency of our microdissection procedure, we attempted to amplify

DNA from only one telocentric chromosome, and compared with the results from two, and four microdissected fragments. In all cases, distinct DNA amplification could be observed. Sequencing analysis revealed that a relatively high proportion of low-copy sequences are involved in the PCR fragments. This suggests the present microdissection procedure is effective in creating painting probes and in generating region-specific DNA markers.

T. Wako[1], A. Houben[2], R. Furushima-Shimogawara[3], B. M. Turner[4] and K. Fukui[5]
([1]Natl Inst Agrobiol Resour, [2]Adelaide Univ, [3]Tokyo Sci Univ, [4]Dept Medicine, Univ Birmingham, [5]Dept Biotech, Graduate School Engineer, Osaka Univ)

Three dimensional analysis of histone acetylation and phosphorylation on mitotic chromosomes in cereals

Histone acetylation and phosphorylation affect chromatin conformation and regulate various cellular function. Changes in acetylation of H4 at lysine 5 (K5) and 16 (K16), and phosphorylation of H3 at serine 10 (S10) during mitosis have been examined by three-dimensional microscopy. Telomeric region was enriched acetylated H4 at K16 throughout mitosis. Centromeric region was enriched acetylated H4 at K5 when chromosomes were decondensed and phosphorylated H3 at S10 when chromosomes were condensed. Nucleolar organizing regions were acetylated at K5 between prophase to anaphase. We propose that H4 acetylation and H3 phosphorylation define functional chromatin domains throughout the cell cycle.

2. Symsium on recent topics and prospects in wheat and barley breeding (Organizer: T. R. Endo)

T. Nakamura[1]*, P. Vrinten[1] and K. Hayakawa[2]
([1]Dept Crop Breed, Tohoku Natl Agr Expt Stan, [2]Cereal Res Cent, Nisshin Flour Milling Co; *tnaka@tnaes.affrc.go.jp)

Waxy Wheat: Production, properties and mutations

Partial waxy lines were used to produce both hexaploid (common) and tetraploid (durum) waxy wheat lines. The starch of these lines lacks amylose, and this change in composition drastically alters starch properties. Our primary application tests indicate that blending waxy and regular wheat flour improves texture and resistance to starch retrogradation in products such as noodles, bread and Chinese dumplings. The mutations in a hexaploid waxy wheat line produced by our group were analyzed and all three alleles were found to carry deletions. Although the three *Wx* genes in waxy wheat are nonfunctional, amylose was present in the pericarp starch of waxy wheat, and a second GBSS isozyme (GBSSII) was detected in pericarp starch. A GBSSII cDNA was isolated, and expression analysis indicated GBSSII mRNA was present in leaf, culm, and pericarp tissue, but was not detected in endosperm tissue.

Ali Masoudi-Nejad (Lab Plant Genetics, Div Applied Biosci, Kyoto Univ; amasoudin@kais.kyoto-u.ac.jp)

Wheat storage protein: present and future

Wheat seed storage proteins have been studied extensively for their pivotal role in determining nutritional and bread-making quality of flour. Because of their high proline and glutamine content they are called prolamin. Wheat prolamins are synthesized on the endoplasmic reticulum in the developing endosperm. They are classified as glutenin and gliadins, which are controlled by the genes located on the long arms of the homoeologous group 1 chromosomes (*Glu-1*) and those on the short arms of groups 1 and 6 chromosomes (*Gli-1* and *Gli-2*), respectively. The last few decades witnessed a rapid advance in our knowledge on the wheat storage proteins, especially through the progress in the basic sciences like biochemistry and molecular biology. Numerous gene sequences coding for glutenin and gliadin have been isolated, cloned and characterized. This has allowed more deep understanding of their structure, function and evolution. In this talk I review the past and our current knowledge about glutenin and gliadin from different point of view incorporating the results of recent developments in molecular genetics and biochemistry. I also discuss works undergoing in our laboratory on sequencing of an omega-gliadin gene and its deletion mapping.

T. Ban (Biol Resour Div, Japan Intern Res Cent Agr Sci (JIRCAS); tomohiro@affrc.go.jp)

The status and future prospect of studies on resistance to Fusarium head blight in wheat

In recent years, considerable emphasis has been laid on the improvement of resistance to Fusarium head

blight in wheat. It is essential to study the genetics of the resistance, including the identification of the genes for resistance to FHB in several gene pools, so that different genes can be combined to improve the overall resistance of wheat. Furthermore, the genetics of resistance to FHB to develop FHB-resistant wheat has been required to examine, indicating the relationship between the characteristics of FHB and resistance mechanisms in wheat. The emphasis laid on the genetics and breeding of resistance to FHB was examined.

Kaz. Noda[1]*, N. Kawakami[2], E. Himi[1], S. Utsugi[1] and A. Yanagisawa[3] ([1]RIBR, Okayama Univ, [2]Dept Agr, Meiji Univ, [3]Kitami Agr Exp Stan; *knoda@rib.okayama-u.ac.jp)

Recent studies on preharvest sprouting in wheat

Physiological studies on seed dormancy indicated that embryo sensitivity to ABA played an important role in the mechanism of seed dormancy. Our genetic analysis of embryo sensitivity to ABA showed that chromosome 4 of wheat, especially long arm of 4A, carried gene(s) for embryo sensitivity to ABA. Recent studies on ABA insensitive (ABI) mutants of *Arabidopsis* showed that *ABI1* and *ABI2* were protein phosphatase, *ABI3* homologous to *Vp1* of maize was a transcription factor and *ABI4* and *ABI5* were transcription factors similar to *apetala2* and *bZIP* respectively. *ABI3*, *ABI4* and *ABI5* were suggested to be in the same ABA signal pathway that were different from the pathway constituted of *ABI1* and *ABI2*. *Vp1* was expressed earlier during seed development than the period of dormancy formation. At present, wheat genes similar to *ABI4* and *ABI5* are candidates responsible for the development of wheat seed dormancy.

M. Furusho (Fukuoka Agric Res Cent; furusho@farc.pref.fukuoka.jp)

Breeding barley for yellow mosaic disease (BaYMV) resistance, present and future

Barley yellow mosaic disease caused by barley yellow mosaic virus (BaYMV) is the most important disease in Japan as well as in Europe, China and South Korea. Utilization of resistant cultivars to this disease is the most efficient method to prevent agronomic losses. Although some resistance genes have been identified, evaluation of the resistant genetic stocks and detection of other resistance genes are essential because the virus strains were characterized in the current studies. This study showed the breeding barley for BaYMV, analyzing the new resistance gene(s), production of lines with two resistance genes and future for the resistance breeding.

T. Makino (Dept Plant Breed, Natl Agr Res Cent; makinot@narc.affrc.go.jp)

A dominant semi-dwarf mutant for hybrid breeding and its genetic analyses in barley

A dominant semi-dwarf mutant was induced by sodium azide treatment in barley. This mutant was found as homozygous genotype in M1 generation. Genetic analyses indicated that this mutant is controlled by single incomplete dominant gene. It was suggested that this mutation event occurred in a single apical cell with somatic chromosome pairing. Effect of mutant gene on shortening spike length was small and on heading date was none. Expression of the gene was different from that of other mutants previously reported (Falk 1994; Kurauchi et al. 1996, 1998).

3. General presentation

Talaat A. Ahmed, H. Tsujimoto and T. Sasakuma* (Kihara Inst Biol Res and Graduate School Integrated Sci, Yokohama City Univ; *sasakuma@yokohama-cu.ac.jp)

Genetic basis of heterosis as revealed by QTL analysis in hexaploid wheat

66 F_8 recombinant inbred lines (RILs) derived from a cross between *Triticum aestivum* cv. Chinese Spring (CS) and *T. spelta* var. *duhamelianum* (Sp) were backcrossed to CS. Six quantitative traits were phenotyped on the RILs and backcrossed lines to examine heterosis for mid-parents. QTL analysis was conducted by using heterosis data as well as an RFLP map based on the RILs. 23 QTLs for heterosis were detected. Among them the heterozygotes of 15 QTLs (65.2 %) were superior to their respective homozygotes. Epistasis analysis was also conducted for all possible interactions of marker pairs, and all traits showed significant interactions. These results suggest that both dominance and epistasis are the genetic sources for heterosis in hexaploid wheat.

K. Hori, H. Tsujimoto and T. Sasakuma* (Kihara Inst Biol Res and Graduate School of Integrated Sci, Yokohama City Univ; *sasakuma@yokohama-cu.ac.jp)

Comparative QTL analysis of hexaploid wheat

We produced the recombinant inbred lines (RILs) between artificial synthetic wheat ABD-4 (*T. carthlicum* x *Ae. squarrosa*) and *T. aestivum* cv. Chinese Spring. For construction of a linkage map, we detected 121 RFLP markers and investigated genotype segregation in 35 loci. We analyzed quantitative trait loci (QTLs) on 11 morphological and 5 physiological traits. The single marker analysis detected several QTLs on each trait. Comparison of the linkage map and detected QTLs with those analyzed in another set of RILs between CS and *T. spelta* will reveal genotype specificity and universal performance of QTLs in hexaploid wheat.

Y. Morisaki and Y. Furuta* (Dept Agr, Gifu Univ; *furutay@cc.gifu-u.ac.jp)

Properties of seed fertility between central vs lateral spikelets in *Hordeum bulbosum*

The wild barley species, *Hordeum bulbosum* has some specific characteristics, 1) induction of haploid plant in both, wheat and barley by bulbosum-chromosome elimination in hybrid zygotes after fertilization with pollen of this species, 2) presence of autotetraploid as well as diploid, 3) vegetative propagation by bulbs formed in lowest internodes, 4) complete seed sterility in spite of normal or fertile anthers in lateral spikelets. This report deals with comparative anatomical and external morphological analysis and crossability with respect to complete female sterility in lateral spikelets in *Hordeum bulbosum*.

K. Kakeda (Fac Bioresour, Mie Univ; kakeda@bio.mie-u.ac.jp)

The two-locus self-incompatibility in *Hordeum bulbosum*

Self-incompatibility in *Hordeum bulbosum* (2x) is controlled by two multiallelic loci, *S* and *Z*. Molecular analysis of the *Hordeum* thioredoxin-like (*HTL*) gene that is homologous to the gene closely linked to the *S* locus of the grass *Phalaris coerulescens* (Aveneae) confirmed that *HTL* is also linked to the *S* locus in *H. bulbosum*. Further linkage analyses using RFLP markers mapped near the *S* or *Z* locus of rye showed that the chromosomal region encompassing each locus would be conserved between *Hordeum* and *Secale*. Recent progress toward the cloning of *S* and *Z* genes from *H. bulbosum* using the AMF method is reported.

M. Takaoka* and H. Hirano (Kihara Inst Biol Res, Yokohama City Univ; *tmotoko@yokohama-cu.ac.jp)

Proteome analysis of wheat endosperm

The wheat seed proteins are known to have different properties from the other cereal and the legume seed proteins in various aspects. The diagonal gel electrophoresis using non-dissociated condition in the first dimension and dissociated condition in the second dimension showed that there are few proteins which have interdisulfide bonds in the seeds. Even the high molecular weight subunits of glutenin have only intradisulfide bonds in vivo, but not interdisulfide bonds. The solubility of wheat seed proteins is also characteristic. We are interested in proteomic analysis of the functions of all wheat proteins with such unique properties in the seeds. As the first step of the analysis, a number of the seed proteins were separated by two-dimensional gel electrophoresis and characterized peptide mass-fingerprinting.

R. Ohno, S. Takumi, N. Mori and C. Nakamura* (Lab Plant Genetics, Fac Agr, Kobe Univ: *nakamura@kobe-u.ac.jp)

Molecular cloning and characterization of *cor* and related gene/protein families in wheat

We have isolated several new cor (cold-responsive) members of wheat using a cDNA library constructed from a cold-acclimated Ukrainian winter-hardy common wheat cv. Mironovska 808. One novel clone wcor14 encodes a 14kDa acidic (pI=4.71) and hydrophilic protein which is specifically induced by low temperature. Wcor14 had a putative chloroplast transit peptide of 51 amino acids at the N-terminal end. We have also isolated four other cor-related cDNA clones, designated as wdhn13, wrab19 and wrab17, and wlt10. Protein for WCOR14 and WDHN13 were purified for the production of antibodies.

W.Y. Yuan, M. Tomita*, Z.Y. Pei and Y. Yasumuro (Dept Agrobiol, Fac Agr, Tottori Univ; *tomita@muses.tottori-u.ac.jp)

Chromosomal localization of rRNA genes of *Dasypyrum villosum* by dual-color FISH and C-banding

Chromosomal locations of rRNA genes and a subtelomeric tandem repetitive sequence (383 bp), which is a homologue of p380 (De Pace et al. 1992), were analyzed by C-banding and sequential dual-color FISH in *Dasypyrum villosum*. The 18S-5.8S-25S rDNA loci probed with a wheat clone pTa71 were localized on 1VS and the 5S rDNA loci probed with a wheat clone pTa794 were visualized on 5VS, according to the C-banding pattern of Friebe et al. (1987). Homologues of the p380 family were localized on subtelomeric regions of both arms of *Dasypyrum* chromosomes except for 7V.

K. Kato and Y. Watanabe (Fac Agr, Okayama Univ)
Genetic differentiation in common wheat, revealed by the analysis of several complementary genes for worldwide collection of wheat landraces

Hybrid weakness, such as necrosis, grass clump dwarfness and partial pollen sterility, is known to be controlled by complementary genes (*Ne*, *D*, *Ki* genes) in common wheat. The frequency of *D2* proved to be ca. 30%-40% in areas from Turkey to western part of China. It was high in Europe (ca. 50%), and extremely low in eastern part of China and Japan (ca. 6%). Similar geographical variation was also observed for *Ki* genes. It was therefore suggested wheat spread to the Far East had been genetically differentiated from those in Central Asia and western areas.

K. Murai[1]* and Y. Ogihara[2] ([1]Dept Biosci, Fukui Pref Univ, [2]Kihara Inst Biol Res, Yokohama City Univ; *murai@fpu.ac.jp)
Genetic system of photoperiod-sensitive cytoplasmic male sterility in wheat

Triticum aestivum cv. Norin 26 (N26) with *Aegilops crassa* cytoplasm shows photoperiod-sensitive cytoplasmic male sterility (PCMS); this alloplasmic line exhibits homeotic transformation of stamens into pistil-like structure (pistillody) when grown under the long-day conditions (>15 h light). On the other hand, wheat cultivar, Chinese Spring (CS) has fertility-restoring gene, *Rfd1*, on the long arm of chromosome 7B, which prevents inducing pistillody caused by *Ae. crassa* cytoplasm. Alloplasmic lines of CS ditelosomic 7BS ((c)-CSdt7BS) lacking *Rfd1* exhibits complete pistillody under any light conditions, whereas CSdt7BS with wheat cytoplasm has normal stamens. These results indicate that *Ae. crassa* cytoplasm causes male sterility to wheat under any condition of photoperiod, and hypothetical suppressor gene which suppresses the action of *Rfd1* gene under long-day conditions should be involved in PCMS induction in alloplasmic N26.

Y. Ogihara (Kihara Inst Biol Res, Yokohama City Univ; ogihara@yokohama.cu.ac.jp)
Functional plastomics in wheat: Monitoring of whole plastome gene expressions in the wheat life cycle as revealed by the plastid DNA microarray method

Plastids develop from proplastids to differentiated organella such as chloroplasts, etioplasts, chromoplasts, leucoplasts and amyloplasts. All plastids contain circular DNA with long inverted repeats that separate the rest of the molecule into small and large single copy regions. Recently, the complete nucleotide sequence of chloroplast DNA for Chinese Spring wheat (134,540 bp) has been determined. The 108 plastid genes, so far deduced, as well as 16 light-responsive and ten cold-inducible genes were spotted onto the microarray in order to investigate profiling of gene expressions of whole plastid genes in the wheat life cycle. RNAs were extracted from 13 different tissues: seedlings grown under light and in the dark, roots, fourth leaves at the heading stage, flag leaves at the heading stage, young spikelets, spikes at the heading stage, spikes at the flowering stage, seeds at 5, 20, 40, 50 days after pollination (DAP), and embryo. The cDNAs were labelled by incorporation of fluorolink Cy5-dUTP, and hybridized to the spotted genes. The hybridization signals of each gene in each tissue were measured, and compared. Each tissue showed representative hybridization patterns, displaying different gene expression patterns among tissues except the patterns between young seedling grown under light and fourth leaves at the heading stage.

K. Tsunewaki*, K. Ichikawa, I. Oda and K. Morinaga (Dep Bioscience, Fukui Pref Univ; *tunewaki@fpu. ac. jp)
Genetic analysis of three kinds of chlorophyll abnormality appeared in tetraploid offspring of a pentaploid wheat hybrid

Three chlorophyll abnormalities, striato-virescence, delayed virescence and albino, appeared in tetraploid offspring of the crosses, *T. durum* cv. Langdon x [(*T. aestivum* cv. Jones Fife x *T. dicoccum* cv. Vernal) x *T. dicoccum* cv. Vernal^3], where Vernal^3 means three backcrosses with Vernal as the recurrent parent. Striato-virescence is the appearance of albinotic stripes in leaves of the tillers formed only in winter. Delayed virescence is change of normally green seedlings to albinotic ones in mid-winter to early spring, with slow recovery of green color later. Albino is chlorophyll-deficiency of germinating seedlings. These three abnormalities were subjected to the aneuploid analysis using the disomic substitution series of Langdon durum. The analyses suggested that all three abnormalities are controlled by two recessive alleles; striato-virescence by *sv1* on chromosome 3A and *sv2* on 2A, delayed virescence by *dv1* on 2A and *dv2* on 2B, and albino by *abn1* on 2A and *abn2* on 2B. *T. aestivum* cv. Chinese Spring carries their normal homoeoalleles, *Sv3*, *Dv3* and *Abn3* all on 2D chromosome.

4. Poster session

T. Ibuki, J. Suzuki, Y. Kowyama and K. Kakeda* (Fac Bioresour, Mie Univ; *kakeda@bio.mie-u.ac.jp)

Search for self-incompatibility related gene transcripts in *Hordeum bulbosum*

A. Meguro[1], S. Takumi[2], Y. Ogihara[3] and K. Murai[1]* ([1]Dept Biosci, Fukui Pref Univ, [2]Fac Agri, Kobe Univ, [3]Kihara Inst Biol Res, Yokohama City Univ; *murai@fpu.ac.jp)

Cloning and characterization of wheat AGAMOU homologous gene, *WAG*

T. Kawahara[1], S. Taketa[2] and K. Murai[1]* ([1]Dept Biosci, Fukui Pref Univ, [2]Fac Agri, Kagawa Univ; *murai@fpu.ac.jp)

Development of RAPD markers useful for identifying wheat-barley 5H chromosome recombinant lines

H. Kato and K. Murai* (Dept Biosci, Fukui Pref Univ; *murai@fpu.ac.jp)

Detection of DNA markers linked to *Vrn-A1* gene by bulked segregant analysis.

T. Yoshii and K. Murai* (Dept Biosci, Fukui Pref Univ ; *murai@fpu.ac.jp)

Cloning of novel genes expressed in wheat young spike

S. Hirosawa[1], N. Mori[1]*, S. Takumi[1], T. Kawahara[2] and C. Nakamura[1] ([1]Fac Agr, Kobe Univ, [2]Fac Agr, Kyoto Univ)

AFLP analysis for the phylogeny and evolution of common wheat

H. Tanaka[1], N. Mori[1]*, S. Takumi[1], T. Kawahara[2] and C. Nakamura[1] ([1]Fac Agr, Kobe Univ, [2]Fac Agr, Kyoto Univ)

Allelic diversity at chloroplast microsatellite loci among diploid species of *Aegilops* and *Triticum*

Editorial remarks

Even number of WIS is regularly edited in the season of wheat flowering in the northern hemisphere, and planting in the southern. No. 92 contains articles of ideal balance. The editorial office has been receiving a constant number of manuscript for Research Article last year, and the proportion of acceptance after reviewing is about 60%. Rejection is mostly due to just agronomic performance for regional interest without analysis. Also, we would like to receive more articles for Research Information, which could be tentative data, experimental plan or even idea and proposal for research. Useful information of molecular markers or mapping information should be in this item.

Together with this volume, you may find a receipt of money contribution, or a form of money order sheet. Thanks for the formers, and please remind a spirit of mutual help for the latters.

We have received sad news of three wheat scientists recently; Dr. Masatake Tanaka (Kyoto University), Dr. Mutuo Sasaki (Tottori University), and Dr. A. J. Worland (John Innes Centre). Dr. M. Tanaka had worked on genome analysis of wheat and *Aegilops* under the late Dr. H. Kihara, and contributed for conservation of *Aegilops* germplasms, all of which are conserved in Germplasm Institute of Kyoto Univ. He was in charge of the chief editor of Wheat Information Service during 1986-1993. Dr. M. Sasaki had studied on Triticale and always helpful for WIS. Dr. A. J. Worland had worked on genetics of physiological characters in wheats. Their contributions will be succeeded and utilized in the wheat researches of the 21st century.

Editorial Office:
K. Nishikawa, T. Sasakuma, H. Tsujimoto and K. Furukawa

Kihara Memorial
Yokohama Foundation for the Advancement of Life Sciences

The Kihara Memorial Foundation (KMF) was established in 1985 in memory of the late Dr. Hitoshi Kihara, a world famous geneticist and evolutionary scientist. The activities of the KMF are promotion of life science by supporting symposia, workshops, and technical courses for researchers, enlightenment of scientific information to citizens, awarding of 'KMF Prize' and 'Child Scientist Prize', and publication of journals such as 'Wheat Information Service'.

The coming 21st century will be one of life sciences. KMF intends to continue contribution for a better future of the earth to solve many problems facing us such about health, food, resources and environment.

The recent economic condition in Japan is limiting our support of these KMF activities. KMF is, therefore, taking up subscriptions from colleagues who approve of the activities of KMF. We would appreciate receiving from you inquiries about this matter, thank you.

Kihara Memorial Foundation

Business Office

Wheat Information Service
c/o Kihara Memorial Yokohama Foundation for the Advancement of Life Sciences
641-12 Maioka-cho, Totsuka-ku, Yokohama 244-0813, Japan.
Phone: +81-45-825-3487. Fax: +81-45-825-3307, E-mail: yamabosi@yokohama-cu.ac.jp
Mr. K. Hasegawa (Managing director), Mr. K. Sugizaki (Chief officer), Ms. K. Furukawa (Publication secretary)

WIS No. 92

編　集　　西　川　浩　三

発行所　木原記念横浜生命科学振興財団

〒244-0813 横浜市戸塚区舞岡町641-12
Tel : (045)825-3487
Fax: (045)825-3307
E-mail: yamabosi@yokohama-cu.ac.jp

発行日　2001年6月28日

Wheat Information Service No. 92

Contents

I. Research articles

Koval SF, Baiborodin SI and Fedotova VD : Interlocus interaction of chlorina mutant genes *cn-A1* and *cn-D1* in near-isogenic lines of spring wheat Novosibirskaya 67 (*T. aestivum*). 1

Zheng YL, Chen YQ, Wei YM, Zhou YH, Zhang ZQ, Liu DC, Lan XJ and Yan ZH : Esterase, gliadins and RAPD variations among Sichuan wheat cultivars. 5

Iwaki K, Nakagawa K and Kato K : The possible candidate of *Vrn-B1* in wheat, as revealed by monosomic analysis of *Vrn* gene carried by Triple Dirk (B), the former *Vrn2*. 9

Aghaee-Sarbarzeh M, Dhaliwal HS, Chhuneja P and Harjit-Singh : Suppression of rust resistance genes from distantly related species in *Triticum durum-Aegilops* amphiploids. 12

Alam SM, Ala SA, Ansari R and Khan MA : Influence of leaf extract of bermuda grass (*Cynodon dactylon* L.) on the germination and seedling growth of wheat. 17

Menon MK and Tomar SMS : Transfer of *Agropyron elongatum*-derived rust resistance genes *Sr24* and *Lr24* into some Indian bread wheat cultivars. 20

II. Research information

Sharma RK, Chowdhury S and Sethi AP : Identification of hybrid necrosis genes among some bread wheat accessions. 25

Singh DP, Sharma AK and Grewal AS : Loose smut resistant lines in wheat and triticale with combined resistance to Karnal bunt, rusts, powdery mildew and leaf blight. 27

III. Genetic stocks

Ozkan H and Feldman M : Seeds of newly-synthesized allopolyploids of *Aegilops* and *Triticum* and of their parental plants. 30

IV. Record

The joint meeting of the 5th Japanese Society of Molecular Biology of Triticeae and the 27th Japanese Wheat Genetics Symposium, October 7–9, 2000. 32

V. Editorial remarks

..... 39