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Research article

Variability occurred in longterm-maintained monosomic lines of wheat

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Summary

Disomic lines were selected cytologically in the pedigree monosomic for 3A, 4A, 5A, 6A, 7A, 3B, 5B, 6B, 7B, 2D, 4D, 5D, and 6D of spring wheat cv. Milturum 553. The disomic lines differed significantly from each other and from original Milturum 553 in a number of quantitative traits. Monosomic state decreased the plant viability to some extent. The complex of genes compensating the decrease can be formed in these lines after some generations of maintaining. The relation of this phenomenon to the variability in longterm-maintained genetic stocks is discussed.

Key words: Aneuploids, Disomics, Genetic stocks, Instability, Wheat

Introduction

Monosomic lines are widely used to investigate in detail the genetic control of quantitative traits in wheat. In these experiments significant differences were found between monosomic lines and the euploid control in the expression of a large number of quantitative traits. In general, the absence of a critical chromosome accounts for these differences (Larson 1966; Maystrenko and Aliev 1986; Rigin and Barashkova 1984). Near-isogenic lines are used for revealing the effect of conventional markers on other traits (Koval and Koval 1997) and the linkage to other markers including molecular markers (Muehlbauer et al. 1988). However, the incorporation of some genes into the genome or a longtime absence of any chromosome, may in a way lead to a decreased plant viability. In this case, the formation of the so-called compensatory complex of genes (CCG) can be expected in these lines after some generations of maintaining. The CCG was described in silkworm as a result of selection for increased vitality in the inbred lines carrying semilethal genes (Strunnikov 1983). In these experiments, the individuals were selected with an increasing amount of minor alleles that compensated the harmful effect of the semilethal gene. These minor alleles

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acting additively form the CCG. In plants, the existence of CCG was shown in the experiments with diploid species *Pisum sativum* L., mutant chlorina. The CCG was transferred from the mutant line to the initial variety by several backcrosses. As a result, the line displaying the effect of stable heterosis was obtained (Sokolov 1990; Gostimsky et al. 1992). Thus, the CCG formation may bring about the variability in a number of traits including quantitative traits. This investigation reports the variability in genetic stocks of hexaploid wheat provoked by the aneuploid state of the genome. The results will be discussed in the light of possibility of CCG formation.

Material and methods

The monosomic series (BCs) of spring wheat variety Milturum 553 was produced in 1970, maintained for several generations with cytological identification, and which was kindly provided by Prof. R.A. Tsilke, Agricultural University, Novosibirsk, Russia (Tsilke and Zharkov 1981). Monosomics BCs of the cultivars Diamant and Saratovskaya 29 were kindly provided by Dr. O.I. Maystrenko, Institute of Cytology and Genetics, Russian Academy of Sciences, Novosibirsk, Russia (Arbuzova et al. 1996). Disomics were cytologically identified in the pedigree of each monosomic. Out of them, only the plants having regular meiosis were propagated for one generation under the same conditions with their heads bagged. These disomic families were planted in field; each line was grown in four separate replicates as row containing 20 plants per meter, 35 cm between the rows and 5 cm between the plants in a row. A sample of 10 plants from each row was scored at maturity. Each disomic family was designated as letter d followed by the initial monosomic family designation and number, for example, d5A-1, d5A-2. etc. The following traits were recorded: heading date, number of fertile spikes, main stem length, length of the upper internode, number of spikelets and grains in the main spike, the main spike grain yield, and plant yield (in gram). The main spike yield was divided by the number of grains in this spike to estimate the average grain weight. The spike density in disomics of Diamant and Saratovskaya 29 were calculated by dividing the spike length by the number of spikelets (cm/spikelet).

Results and discussion

The disomic families d4A, d5A, d3B, d5B, d6B and d7B had the same heading date as the euploid control variety Milturum 553 (52±1 days). The disomic families d3A, d6A, d7A, and d5D headed 5-6 days earlier than the control; the families d2D and d6D were 5 days later. The variability within the disomic families from the same monosomic plant was recorded only for d4D: d4D-1 and d4D-2 had 47±1 and 55±1 days to heading, respectively. Only the families d5A-2 and d4D-2 exhibited no significant differences from the variety Milturum 553 in the other traits studied (Table 1). The number of quantitative traits in which the rest d-families differed from the control varied from one to two (d3B-4, d5B-1, d5B-7, d7B-7, and d6D-2) to six to seven characters (d3A-7, d6A-4, d6A-9, d7A-1, d7A-5, and d5D-5). Accounting each trait separately or taking into consideration all the traits, the percentage of the families that showed significant differences from the control was highest in the families derived from monosomics for A genome chromosomes; intermediate and lowest, in D and B genome families, respectively (Table 2). The main stem

Table 1. Mean values of yield characteristics for disomic families from Milturum 553 monosomic lines

Disomic families	Stem length (cm)	Upper internode (cm)	Fertile spikes (number)	Spikelets in main spike	Grains in main spike (number)	Grain yield per spike(g)	Grain yield per plant(g)	One grain weight (mg)
Euploid	84.0	33.8	4.45	20.8	36.5	0.95	2.64	26.0
d3A-6	86.4	40.1*	6.33*	16.5*	35.4	1.15	5.04*	32.5
d3A-7	95.3*	46.1*	6.87*	16.3*	36.8	1.22*	5.90*	31.2
d4A-4	94.5*	38.8	5.62	19.7	42.0*	1.29*	4.84*	30.7
d5A-1	97.4*	36.8	6.82*	19.8	39.4	1.18*	5.35*	29.9
d5A-2	82.9	30.8	5.58	19.1	35.5	0.89	3.20	25.1
d6A-4	93.6*	46.1*	6.79*	16.5*	37.7	1.25*	5.12*	33.2
d6A-9	98.5*	48.9*	7.25*	16.7*	39.1	1.26*	6.03*	32.2
d7A-1	90.7*	43.5*	7.39*	17.6*	41.2*	1.19	5.93*	27.6
d7A-5	95.8*	42.5*	6.78*	18.9*	46.7*	1.29*	5.62*	28.9
d3B-2	93.4*	34.2	5.95	19.6	39.3	1.20*	4.58*	30.5
d3B-4	81.7	31.2	5.64	19.0*	32.5	0.80	3.13	24.6
d5B-1	88.4	32.5	5.75	21.0	40.3	1.16	4.37*	28.8
d5B-7	94.1*	35.6	5.45	20.7	40.6	1.33	4.63*	32.8
d6B-8	91.4*	30.5	5.64	21.3	41.0	1.26*	4.38*	30.7
d7B-5	89.9*	32.0	6.20*	20.5	42.6*	1.27*	5.18*	29.8
d7B-7	88.7	35.1	5.35	19.3*	40.6	1.22*	4.22	30.0
d2D-7	85.6	29.7*	4.08	22.0*	36.8	1.20*	3.50	32.6
d2D-9	96.0*	34.0	4.62	20.0	40.3	1.33*	4.16*	33.0
d4D-1	98.1*	41.6	6.08*	17.9*	38.5	1.13	4.34	29.4
d4D-2	91.0	33.4	5.59	20.6	36.2	1.10	3.51	30.4
d5D-5	91.2*	47.5*	6.20*	18.1*	37.9	1.32*	5.39*	34.8
d5D-6	86.4	40.9	5.03	18.3	46.9*	1.34*	4.64*	28.6
d6D-1	94.7*	35.7	6.13*	21.2	39.9	1.34*	4.98*	31.0
d6D-2	90.5	37.3	5.36	20.4	36.5	1.32*	4.91*	31.4

* Differences from the control euploid line are significant at the 0.05 level.

length (plant height) and the plant yield (grain weight per plant) are the only exceptions. The families derived from the monosomics for B genome displayed higher variation of these traits than the disomic pedigrees of the monosomics for D genome. The most variable traits were the following: the stem length, the main spike and plant yields. The length of the upper internode and the number of grains in the main spike were the least variable traits. Increased values of the parameters were recorded in the disomic families compared with the euploid control in the majority of observations. Only the number of spikelets in the main spike was less in ten of eleven disomic families compared with the control. The spikes with a decreased spikelet number had yet an

Table 2. Percentage of families significantly differed from the recurrent parent in quantitative traits

Traits	Genome			Total for all families
	A	B	D	
Number of fertile spikes	77.8	14.3	37.5	43.5
Spike length	77.8	57.1	50.0	62.0
Upper internode length	66.7	0.0	25.0	30.5
Main spike				
number of spikelets	66.7	28.6	37.5	44.2
number of grains	33.3	14.3	12.5	20.0
grain weight	66.7	57.1	75.0	66.2
Grain weight per plant	88.9	71.4	62.5	74.2
Total for all characters	68.6	34.7	42.8	48.7

increased grain number. It resulted from the increased number of fertile flowers in a spikelet, thereby increasing the grain number per spikelet and grain yield per plant (families d3A-7, d6A-4, d6A-9, d7A-1, d7A-5, and d5D-5). Thus, the increased main spike yield (measured in gram) resulted from both the increased grain weight due to intense grain filling and the increased number of fertile flowers (Table 1). Both the grain weight and the spike number contributed to the increased plant yield. The development of the extremely variable traits listed above depended on the efficiency of the plant metabolism. The less variable traits (the grain and spikelet numbers in a spike, the stem and upper internode lengths) depended rather on the rates of morphogenesis (Kuperman et al. 1982). Thus, the increase of the values in both these groups of traits can be achieved by the increase in the functioning efficiency of their physiological systems. The differences between the sibs that are progenies of the same monosomic plant suggest the heterogeneity of this plant. However, the entire series was bred basing on only one genotype of the recurrent parent. Consequently, the heterogeneity in certain monosomic lines appeared later in the process of reproduction under monosomic state maintenance, which may have contributed to the formation of CCG. Thus, there are grounds to suggest that longterm maintenance of the aneuploid state in certain cases can lead to increased yield. In other cases, the compensatory aneuploid state of the relevant alleles can disturb the genetic balance of the plant. In the latter situation, a decrease in the yield can be observed. In the similar experiment, Arbuzova and Maystrenko (1981) did not reveal any significant differences among the disomic families in the pedigrees of cv. Saratovskaya 29 monosomics (Maystrenko 1971). The monosomics used in this experiment were derived by selfing BC_9 plants from final crosses in the development of these monosomic lines. Any backcrosses in the course of monosomic line development disrupted the CCG by introducing the genome of the recurrent parent. In this experiment, only one selfing was performed after the last back-cross, but several generations of selfing are necessary to reveal the working compensatory alleles. In our experiment, 15 generations of selfing passed after the final crosses in the development of Milturum 553 monosomic line before we started to isolate the disomic families. We can suppose

Table 3. Mean values of yield characteristics for disomic families from Diamant and Saratovskaya 29 monosomic lines

Disomic families	Spikelets in main spike (number)	Spike density (cm/spikelet)	Grains in main spike (number)	One grain weight (mg)	Harvest index (%)
cv. Diamant					
Euploid	17.1	0.62	34.4	37.6	34.9
d1B	17.6	0.60	35.2	34.8*	33.9
d2B	17.9	0.58*	35.9	36.0	33.3
d5B	17.2	0.57	32.6	32.4*	29.7*
d1D	18.3*	0.56*	35.1	36.7	32.6
d4D	17.2	0.58	31.6	35.2	33.6
cv. Saratovskaya 29					
Euploid	14.4	0.50	32.5	39.7	43.2
d4D	1.4	0.46*	28.2*	35.0*	40.7

* Differences from the control euploid line are significant at the 0.05 level.

that, during all these 15 generations, the breeders unconsciously chose for further propagation the monosomics with higher yield parameters.

In other experiment carried out in 1998, we used several disomic progenies of the same monosomics of cultivars Saratovskaya 29 and Diamant (bred by Dr. O.I. Maystrenko) after over fifteen generations in monosomic state. Their comparison with the recurrent parents demonstrated significant differences in several quantitative traits (Table 3). However, unlike Milturum 553 disomics, the mean value deviations were mainly directed towards the decrease in grain production.

The CCG effects observed depend on the genome of the recurrent parent: differences between sibs from monosomic lines Saratovskaya 29 and Diamant occur rarer than in Milturum 553 monosomic progenies and the character of changes observed is different. The results obtained are not unambiguou, however, the authors tend to believe that such phenomenon may be found also in other monosomic series. Thus, both the maintenance under conditions of compulsory monosomic status during a large number of generations and unconscious selection for high yield parameters provided by breeders resulted in the formation of the compensation system in the sets of monosomic lines that enhanced significantly the plant metabolism. The increased values of most of the parameters recorded in the disomic families compared with the recurrent parent can be explained by this enhanced metabolism. The differences among sib disomic families from the same monosomic plant evidence heterogneity as one of the constituents of CCG and the variability of possible combinations of the genes involved in this complex. Our results provide additional arguments to suggestion (Goncharov 1992) that the results obtained with monosomic lines in investigation of quantitative traits should be considered carefully. In this paper, the compensation effects were almost not revealed earlier than in the tenth generation of selfing. Only some of the lines studied demonstrated these effects. Not all the lines that depress the growth and productivity cause the formation of the CCG. We attempted to obtain the CCG in near-isogenic lines of cv. Novosibirskaya 67 carrying dwarfing genes. Selection for the increased

height and yield was carried out in ANK-11 (*Rht3*) and ANK-12A (*Rht1* and *Rht2*) starting from BC_9S_9 and lasted without any results for six generations. Our data suggest that formation of the CCG may constitute a problem for stable maintenance of lines in genetic collections, especially if the line is deficient for chromosome number or carries a semilethal allele. The CCG formation can be also caused by other factors decreasing viability, for example, an alien cytoplasm or inbred depression in cross-pollinating species. An unconscious selection for more productive individuals cannot be excluded while maintaining genetic collections. Thus, we can expect the formation of the CCG changing a number of characters.

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Research article

Inheritance of flag leaf in bread wheat genotypes

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Summary

A 6x6 diallel analysis was conducted for the estimation of gene action, combining ability and heterosis for flag leaf parameters in wheat. Additive gene action and partial dominance was observed for flag leaf area and flag leaf weight, respectively. While dominance and overdominance were observed for specific flag leaf area and specific flag leaf weight. High heterosis for flag leaf traits was observed in the hybrids involving LU26S or 4072 as one of the parents. These parents were also good general combiners for these traits and thus, are suggested as useful to be incorporated in the future breeding program for improving flag leaf characteristics in wheat.

Key words: Flag leaf, Specific flag leaf, Additive, Dominance, Heterosis

Introduction

Flag leaf or the top most leaf in cereals is the most effective photosynthetic structure as compared to other green parts of the plant. Similarly in case of wheat, most of the photosynthates or assimilates accumulated in the grain in the form of starch and other carbohydrates are translocated mainly from the flag leaf. Ibrahim and Abo Elenein (1977) found that the flag leaf contributed 41-43% to the grain weight due to increase in kernel weight and number per spike since the flag leaf is photosynthetically the most active leaf during grain formation stage. Photosynthetic efficiency also relates to the amount of light interception and flag leaf is the one where maximum light interception can be obtained in cereals like wheat. Therefore, cultivars with greater flag leaf area generally have high grain weight. Monyo and Whittington (1971) obtained a significant positive correlation coefficient of 0.41 for the association between grain yield per tiller and flag leaf area in wheat. Similarly Briggs and Aytenfisu (1980) recorded a positive and significant association of flag leaf area with grain yield per plant and 1000-grain weight. Although genetic studies pertaining to flag leaf parameters (flag leaf and specific flag leaf area and weight) have been occasionally conducted by few scientists (e.g. Briggs and Aytenfisu 1980), this information is insufficient. Genetic information like gene action, combining ability and heterosis for these traits will be helpful for the breeders to select potential genotypes/combinations that may be

incorporated in productive breeding project.

Materials and methods

The experiment was conducted in the research area of the Department of Plant Breeding & Genetics, University of Agriculture, Faisalabad. The experimental material comprised six wheat genotypes viz., Pak.81, LU26S, Faisalabad 85 (Fsd.85), Pasban 90 (Psbn.90), 4943 and 4072. These genotypes were crossed in all possible combinations in a diallel fashion during the crop season 1995-96. All the F_1's along with their parents were planted in the next crop season in lines using a triplicated randomized complete block design. Plant to plant and row to row spacings were 15 and 25 cm, respectively. Seeds were sown in holes (made with the help of dibble) at the rate of 2 seeds per site which were later thinned to single healthy seedling per site after germination. Each treatment was a single line of 5 meter length comprising of approximately 30 plants. All the other cultural operations including hoeing, weeding, irrigation, fertilizers, etc. were carried out to reduce experimental error.

For the measurement of flag leaf traits, flag leaves from the main tillers of ten guarded plants from each treatment were collected when the plants attained their maximum vegetative growth and the leaves had fully expanded. Flag leaf area (FLA) was measured according to Muller (1991). Flag leaf weight was also recorded and specific flag leaf area and weight were calculated as under:

$$\text{Specific flag leaf area} = \frac{\text{Flag leaf area}}{\text{Flag leaf weight}}$$

$$\text{Specific flag leaf weight} = \frac{\text{Flag leaf weight}}{\text{Flag leaf area}}$$

Data collected were subjected to analysis of variance according to Steel and Torrie (1984). To determine the gene action, graphical analysis according to Hayman (1957) was carried out.

Results and discussion

Gene action studies

Analysis of variance revealed highly significant differences among genotypes for all the flag leaf traits studied. Graphical analysis conducted revealed that in case of flag leaf area positive intercept of regression line (Fig. 1a) indicated an additive gene action with partial dominance. These results are similar with those of Singh et al. (1988) and Alam et al. (1990) who also reported an additive gene action for the trait. On the basis of location of array points, Psbn. 90 possessed the maximum dominant genes while 4072 possessed the most recessive genes. To see whether the distribution of dominant alleles was correlated with the phenotype of the common parent, the values of Wr+Vr from each array were plotted against parental values. The graph (Fig. 1b) presented that parents with smaller flag leaf area had smaller Wr+Vr values and parents with larger flag leaf area had greater Wr+Vr values. The correlation coefficient was positive (0.49).

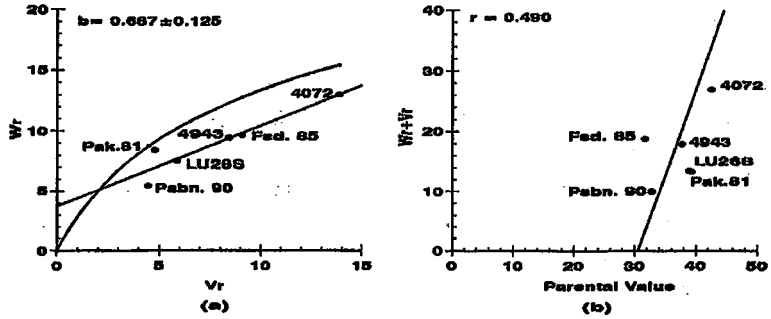

Fig. 1. Graphs for flag leaf area. a: Wr/Vr, b: Wr+Vr/P.

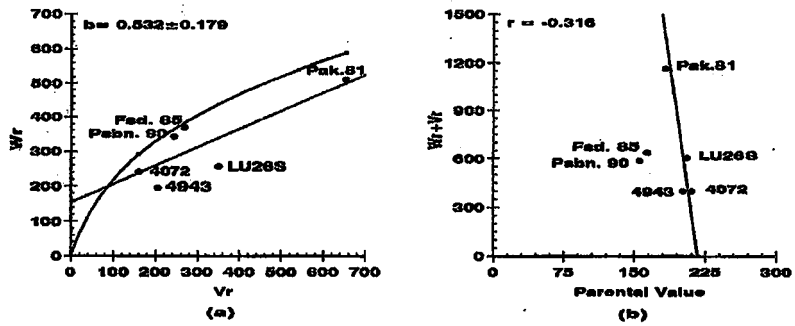

Fig. 2. Graphs for flag leaf weight. a: Wr/Vr, b: Wr+Vr/P.

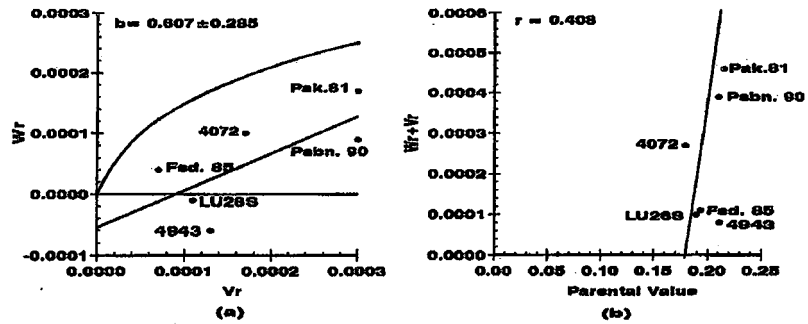

Fig. 3. Graphs for specific flag leaf area. a: Wr/Vr, b: Wr+Vr/P.

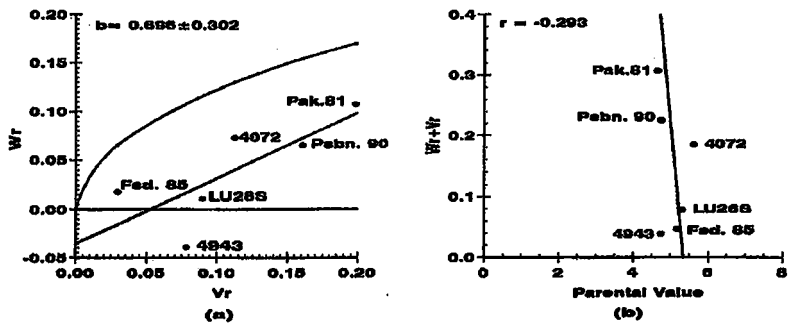

Fig. 4. Graphs for specific flag leaf weight. a: Wr/Vr, b: Wr+Vr/P.

Thus, it was clear that greater flag leaf area resulted due to more recessive genes. Dominant genes decreased the flag leaf area. Therefore, 4072 which possessed maximum recessive genes had largest flag leaf area and Psbn. 90 with minimum recessive genes or more dominant genes had the smallest flag leaf area. Lonc et al. (1993) has also reported recessive gene control for flag leaf area.

Positive intercept of the regression line in Wr/Vr graph (Fig. 2a) for flag leaf weight indicated the absence of complete dominance but partial dominance with additive gene action. Array points displayed that Pak. 81 contained the maximum recessive genes for flag leaf weight while 4943 and 4072 had the maximum dominant genes. Distribution of genes among the other parents was intermediary. The negative correlation (-0.32) revealed the involvement of dominant genes to increase the flag leaf weight (Fig. 2b). That is why, 4943 and 4072 which had the most dominant genes with lower Wr+Vr values were having greater flag leaf weight. Lesser dominant genes in Pak. 81 produced flag leaves of smaller weight. However, awkward position of Fsd. 85 and Psbn. 90 gave an idea that dominant gene control for flag leaf weight in these parents was not certain.

Wr/Vr graph (Fig. 3a) for specific flag leaf area showed that the intercept was negative; the regression line cut the Wr-axis below the origin, indicating an overdominant gene action. The position of array points in the graph throws light on the distribution of dominant and recessive genes in the parents. Fsd. 85, LU26S and 4943 were located closest to the origin thus, contained the maximum number of dominant genes. Pak. 81 was located farthest from the origin having lowest number of dominant genes. The correlated response (0.41) of dominance and parental phenotype (Fig. 3b) indicated that recessive genes controlled specific flag leaf area while the dominant alleles tended to reduce it. The position of the parental points along the graph line affirmed this fact.

For specific flag leaf weight Wr/Vr graph (Fig. 4a) showed the presence of over dominance. Figure further depicted that Fsd. 85 and LU26S being nearest to the origin contained the maximum number of dominant genes while Pak. 81 which occupied the farthest position from the origin contained the most recessive genes. Wr+Vr/P graph (Fig. 4b) depicted that specific flag leaf weight was under the control of dominant genes. Negative correlated response (r=-0.29) between Wr+Vr and P also agreed this point.

Heterosis studies

Flag leaf area is an effective yield related parameter. A larger FLA helps to synthesize photosynthates in greater quantities which are translocated to grains increasing their weight. Positive heterosis for FLA is thus, important. Heterotic studies for FLA (Table 1) revealed that 25 cross combinations manifested a positive increase over the mid-parent value. However, significant positive heterosis was indicated in 8 of the crosses. The considerable combinations in this respect included Fsd. 85 x LU26S, LU26S x Fsd. 85, 4943 x Fsd. 85 and LU26S x 4072, in descending order. Including these crosses, heterobeltiosis was positive in 14 crosses but none of them had significant values. Significant and negative heterobeltiosis was shown by 7 crosses.

An overview of the Table 1 indicated that 23 crosses showed positive increase over mid-parent value for flag leaf weight. Positive and significant heterosis was found in only 6 crosses. Maximum mid-parent heterosis (23.5%) was observed in Pak. 81 x LU26S while the same cross also exhibited maximum positive and significant increase (16.7%) over the better parent. Heterobeltiosis was positive and significant in only 4 of the crosses.

Out of 30 crosses 17 showed positive heterosis for specific flag leaf area while significant

Table 1. Percentage heterosis (H) and heterobeltiosis (HB) for flag leaf traits in all possible hybrids of six wheat genetypes.

Genotypes/Traits		Pak.81		LU26S		Fsd. 85		Psbn. 90		4072		4943	
		H	HB	H	HB	H	HB	H	HB	H	HB	H	HB
Pak.81	FLA			5.81	5.23	2.94	-7.07	3.36	-4.99	1.72	-2.16	3.66	1.55
	FLWT			23.54**	16.72**	4.43	-1.09	2.07	-5.47	2.63	-4.12	3.30	-1.49
	SpFLA			-13.86**	-19.07**	-0.98	-6.05	1.65	0.46	0.51	-7.91	-1.88	-2.79
	SpFLWT			17.54**	10.42**	1.24	-3.83	-0.91	-2.02	-1.09	-9.55**	1.98	0.95
LU26S	FLA	6.12	5.53			12.30**	1.87	4.84	-3.35	11.47**	6.65	8.09	6.46
	FLWT	19.41**	12.82**			3.25	-7.30	8.77	-4.38	5.69	4.44	9.02	7.95*
	SpFLA	-10.89*	-11.28**			8.90	7.77	-3.26	-8.09	2.17	-0.53	2.50	-2.84
	SpFLWT	12.86**	6.00			-7.91	-8.99	3.62	-1.62	-2.42	-5.18	-2.67	-7.68
Fsd.85	FLA	1.99	-7.93*	13.27**	2.76			4.30	2.49	0.38	-12.47**	9.55*	0.76
	FLWT	0.96	-4.38	10.49*	-0.81			-0.52	-2.86	2.59	-8.87*	6.58	-3.48
	SpFLA	1.47	-3.72	2.62	1.55			6.70	2.38	6.45	2.59	-5.45	-9.48*
	SpFLWT	-1.53	-6.46	-2.39	-3.35			-3.60	-7.44	-6.03	-9.99**	5.42	1.12
Psbn.90	FLA	4.18	-4.44	7.28	-1.10	4.10	2.28			-7.74	-18.08**	-3.71	-9.98*
	FLWT	-7.59	-14.42**	-2.31	-14.12**	-3.87	-6.12			-8.05	-19.67**	5.70	-6.29
	SpFLA	12.94**	11.63**	8.77	3.33	8.68	4.29			3.34	-4.29	-12.58**	-12.80**
	SpFLWT	-10.85**	-11.85**	-8.17	-12.82**	-7.41	-11.10**			-4.12	-11.42**	14.13**	14.01**
4072	FLA	3.01	-0.93	8.67*	3.97	0.76	-13.47**	-6.76	-17.45**			5.66	-0.36
	FLWT	5.51	-1.43	3.77	2.54	1.52	-9.52*	9.11 *	-5.07			-1.05	-3.17
	SpFLA	-1.01	-9.30*	4.35	1.59	9.68*	5.70	-11.57*	-18.09**			5.13	-2.84
	SpFLWT	-0.02	-8.57	-4.34	-7.05	-8.92**	-12.50**	12.20**	3.67			-5.59	-12.86**
4943	FLA	5.19	3.05	8.18*	6.55	11.58**	2.63	-2.40	-8.75*	4.23	-1.70		
	FLWT	12.33**	7.12	14.43**	13.31**	1.10	-8.44*	6.26	-5.79	-8.99*	-10.93**		
	SpFLA	-8.45*	-9.30*	-5.00	-9.95*	-1.48	-5.69	-12.59**	-12.80**	16.41**	7.58		
	SpFLWT	8.99	7.89	4.84	-0.57	1.31	-2.82	14.32**	14.20**	-14.70**	-21.26**		

*=P≤0.05, **=P≤0.01

FLA: flag leaf area, FLWT: flag leaf weight, SpFLA: specific flag leaf area, SpFLWT: specific flag leaf weight

positive heterosis (Table l) was recorded in 3 of the crosses (4943 x 4072, Psbn. 90 x Pak. 81 and 4072 x Fsd. 85). Maximum increase (16.4%) was recorded in 4943 x 4072 hybrid. The cross Psbn. 90 x Pak.81 also showed significant positive heterobeltiosis.

Twelve cross combinations depicted positive heterosis for specific flag leaf weight and only 5 crosses (Pak.81 x LU26S, 4943 x Psbn. 90, Psbn. 90 x 4943, LU26S x Pak.81 and 4072 x Psbn. 90, in descending order) reached the level of significance. Out of these five crosses, 4943 x Psbn. 90, Psbn. 90 x 4943 and Pak.81 x LU26S also showed significant positive heterobeltiosis.

These results indicated the possibility of exploiting the hybrid vigor for the improvement of wheat genotypes for flag leaf traits through selection of transgressive segregants in the later generations. The hybrid Pak.81 x LU26S indicated high heterosis as well as heterobeltiosis for flag leaf weight and specific flag leaf weight while heterosis for flag leaf weight was observed in hybrid LU26S x Pak.81, suggesting the possibility of useful selection for these traits in these crosses. Similarly, the hybrid 4072 x LU26S displayed hybrid vigor for flag leaf area. High heterosis for flag leaf area was also indicated in the cross 4943 x LU26S. These crosses, thus, can be utilized for the improvement in the traits for which they showed heterosis.

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Research article

Gliadin and HMW-glutenin variations in *Triticum turgidum* L. ssp. *turgidum* and *T. aestivum* L. landraces native to Sichuan, China

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Summary

Forty *Triticum turgidum* L. ssp. *turgidum* and 89 *T. aestivum* L. landraces native to Sichuan, China, were evaluated for the variability of gliadins and HMW-glutenins. Low variability was observed for both gliadins and HMW-glutenins in these landraces. No *Glu-D1* variation was observed and only two *Glu-A1* and *Glu-B1* variations were found in *T. aestivum* landraces. Eighty-seven out of 89 landraces (97.8%) had the identical HMW-glutenin pattern with bread wheat cultivar Chinese Spring. Landrace Chengdu-guangtou had the identical gliadin and HMW-glutenin patterns with Chinese Spring, supporting the proposal that Chinese Spring is a strain of Chengdu-guangtou. In *T. turgidum* landraces, 37 out of 40 landraces (92.5%) had identical HMW-glutenin pattern (i.e. subunits 2* and similar to 17+18). All HMW-glutenin patterns found in *T. turgidum* landraces differed from those in *T. aestivum* landraces. It suggested that the AABB genomes of *T. aestivum* landraces native to Sichuan were not closely related to those of *T. turgidum* landraces. The gliadin and HMW-glutenin loci of *T. turgidum* landrace can express in hexaploid wheat background. Thus, the *Gli* and *Glu-1* loci of *T. turgidum* landraces can be used to increase the genetic variability of Sichuan wheat cultivars.

Key words: Gliadin, HMW-glutenin, Landrace, *T. aestivum* L., *T. turgidum* L. ssp. *turgidum*

Introduction

In the endosperm of wheat (*T. aestivum* L.), the main storage protein classes are glutenin and gliadin. Glutenins were composed of high-molecular-weight (HMW) subunits and low-molecular-weight (LMW) subunits. HMW-glutenin subunits were controlled by three gene loci, located on the long arm of chromosomes 1A, 1B and 1D, and identified as *Glu-A1*, *Glu-B1* and *Glu-D1*, respectively (Payne et al. 1982). Specific HMW-glutenin allelic variants were associated with bread-making quality (Payne 1987). Gliadins were encoded by *Gli-1* and *Gli-2* loci located on the

distal part of the short arm of the homoeologous group 1 and group 6 chromosomes in tetraploid (Lafiandra et al. 1983) and hexaploid wheat (Lafiandra et al. 1984). Gliadins are inherited as blocks or linked groups (Mecham et al. 1978) and a vast multiple allelism has been established at each of these loci (Sozinov and Poperelya 1980). Although quality parameters associated with the presence of individual gliadins are not as well defined as they for glutenins, their impact on quality is well established and different gliadin blocks have been found to produce differential quality (Sozinov and Poperelya 1980).

A long period before 1980s, the bread-making quality of Sichuan wheat was very poor in China (Yen 1999). It is thought that the climatic condition in Sichuan is the limiting factor for high bread-making quality, but the genetic foundation for bread-making quality of Sichuan wheat is still unknown. One objectve of this paper is to interpret the genetic foundation for bread-making quality based on the variations of HMW-glutenin and gliadin in *T. aestivum* landraces from Sichuan, China.

At the same time, bread wheat cultivar Chinese Spring has been worldwide used in cytogenetic and molecular studies of wheat. It is indicated that Chinese Spring was originated from Sichuan, China (Sears and Miller 1985; Yen et al. 1988; Ward et al. 1998). Chinese Spring is one of the Sichuan white wheat (SWW). The SWW, widely distributed throughout Sichuan Province, is a group of Chinese endemic wheat. It is composed of cultivated common wheats characterized by thin leaves, light green color, square spike with multifloret spikelets, rounded glumes and high crossability with rye (Yen et al. 1988; Luo et al. 1992). There also have *T. turgidum* L. ssp. *turgidum* landraces in the distributed regions of *T. aestivum* landraces in Sichuan, China where Chinese Spring originated. Moreover, from the view of morphology, Sichuan *T. turgidum* and *T. aestivum* landraces have high similarities. The other objective of this paper is to describe the genetic relationship between *T. turgidum* and *T. aestivum* landraces by using the variations of HMW-glutenin and gliadin in these landraces.

Materials and methods

Plant materials

The gliadin and HMW-glutenin variations of 40 tetraploid (*T. turgidum* L. ssp. *turgidum*) and 89 hexaploid (*T. aestivum* L.) wheat landraces collected from different regions in Sichuan, China were analyzed. Synthetic hexaploid wheat RSP, obtained from the cross between *T. turgidum* landrace Ailanmai native to Sichuan and *Aegilops tauschii*, was included. Some bread wheat genotypes with different HMW-glutenin subunit combination as markers were also included in the HMW-glutenin analyses.

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-polyacrlamide-gel-electrophoresis (A-PAGE) at pH 3.1 according to the procedure of Cooke (1987).

According to the procedure of Ng and Bushuk (1987), HMW-glutenin subunits were separated by polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE). Using the bread wheat with different HMW-glutenin subunit combinations as references, HMW-glutenin subunits were identified according to Payne and Lawrence (1983) and Ciaffi et al. (1993).

Table 1. Gliadin patterns in 89 *T. aestivum* L. landraces

Pattern *	Landrace	Frequency (%)
1	Chimai, Hongtiaomai	2.2
2	Baihua, Dalaoke, Fanmai, Honghuamai, Pingshanheshangmai, Zhaoyaguangtou	6.7
3	Nanchongdalaoke, Wumang, Yangchengdiao, Youmang	4.5
4	Bailan	1.1
5	Nanchongheshangmai	1.1
6	Baimaizi	1.1
7	Chengdu-guangtou, Lezhihonghuamai	2.2
8	Bishanheshangmai, Doumai	2.2
9	Dahonghua	1.1
10	Luxia, Sanyuehuang, Xiaoyoutiao, Yuwei, Zhuweiba	5.6
11	Baihuamai, Baixiaomai	2.2
12	Zaohuang	1.1
13	Liulengmai	1.1
14	Bangchui, J-11, Heshaomai, Ludou, Siyuehuang	5.6
15	Luxianheshangmai	1.1
16	Baike, Dengtai, Fenglu, Gaoxianguangtou, Mianmai, Penganheshangmai, Pushan, Tuomai, Xuxumai	10.1
17	Paideng, Sihonghuaguangtou	2.2
18	Baituomai, Erlengxuxu, Fangmai, Luohan, Temai, Zhuermai, Wuyang, Luxian-guangtou, Yichengke, Huayang, Bijian, Lehan	13.5
19	Dahongpao	1.1
20	Baihuaguangtou, Baixumai	2.2
21	Guangtouhonghua	1.1
22	Chaoxieban	1.1
23	Dongmai	1.1
24	Baixiaomai, DayiBaihua, Hongpaideng, Maierzi, Tianlu, Wanyuanhonghuamai, Yizaixaomai, Youmang	9.0
25	Biantaer, Dahuangpao	2.2
26	Dahonghua, Hongxiaomai	2.2
27	Niuniumai	1.1
28	Zhangchumai	1.1
29	Luoxiamai	1.1
30	Yongchuanguangtou	1.1
31	Dalongxu, Huanghuamai, Youmanglanmai	3.4
32	Hefangxiaomai, Pengxiguangtou, Qinggangmai	3.4
33	Nuermai	1.1
34	Youyang-guangtou	1.1
35	Ermai	1.1

* The type of gliadin pattern was corresponding to that of the Fig. 1a-c.

Results

Gliadin variations

There were 35 different gliadin patterns among 89 *T. aestivum* landraces (Table 1; Fig. 1 a, b, c). Only 18 landraces did not have identical gliadin patterns with other landraces, while 17 gliadin patterns were found among 71 landraces. Gliadin patterns 16, 18 and 24 were most frequently appeared among the landraces (Fig. 1b lanes 16, 18; Fig. 1c lane 24). Two landraces, Chengdu-guangtou and Lezhihonghuamai, had identical gliadin patterns with Chinese Spring (Fig. 1a lane 7), and five landraces had identical gliadin patterns with J-11 (Fig. 1b lane 14), which is a representative of Sichuan white wheat with higher crossability with rye than Chinese Spring (Zheng et al. 1992). There had only one or two different bands among many gliadin patterns (Fig. 1a, b, c). These results indicated that low level of gliadin variations existed in these Sichuan landraces.

Only seven different gliadin patterns were obtained among 40 *T. turgidum* landraces (Table 2; Fig. 1d). Thirty-one out of 40 landraces (77.5%) had identical gliadin pattern 3 (Fig. 1d lane 3), and four landraces (10%) had identical gliadin pattern 4 (Fig 1d lane 4), while each of the five remainder gliadin patterns had only one landrace, respectively (Table 2; Fig. 1d). There has only one different band between gliadin patterns 3 and 4 (Fig. 1d lanes 3, 4). It indicated that there was only one different *Gli* locus among these landraces. These results suggested that very low level of gliadin variability existed in *T. turgidum*.

HMW-glutenin variations

In 89 *T. aestivum* landraces, only three HMW-glutenin phenotypes were observed, where 87 out of 89 landraces (97.8%) had identical phenotype (Fig. 1e; Table 3) with Chinese Spring. No variant on *Glu-D1* was found, while there were only two different loci on *Glu-A1* and *Glu-B1* respectively. All landraces had the same *Glu-D1a* allele (subunits 2+12). On *Glu-B1* locus, Ermai

Table 2. Gliadin patterns in 40 *T. turgidum* L. ssp. *turgidum* landraces

Pattern *	Landrace	Frequency (%)
1	Bangbanglanmai	2.5
2	Bazhonglanmai	2.5
3	Ailanmai, Baiqingke, Banglanmai, Beichuanlanmai, Changdiaoxu, Changxuxu, Dongmai, Gbailanmai, Goutoushan, Guinanmai, Jlanmai, Kangdinglanmai, Lanmai, Lezhilanmai, Ludoumai, Nvermai, Paonanmai, Renshoulanmai, Tbailanmai, Wugonglanmai, Yaanlanmai, Yanbianqinkemai, Youmang, Yunxianlanmai, Yuweilanmai, Yuechilanmai, Yvermai, Zaozuo1, Zhumao, Zm8046, Zm8455	77.5
4	Qingkemai, Luonanmai, Kaixianganmai, Jinchuanganmai	10.0
5	Zaozuo2	2.5
6	Fenzhilanmai	2.5
7	Sanlicun	2.5

* The type of gliadin pattern was corresponding to that of Fig. 1d.

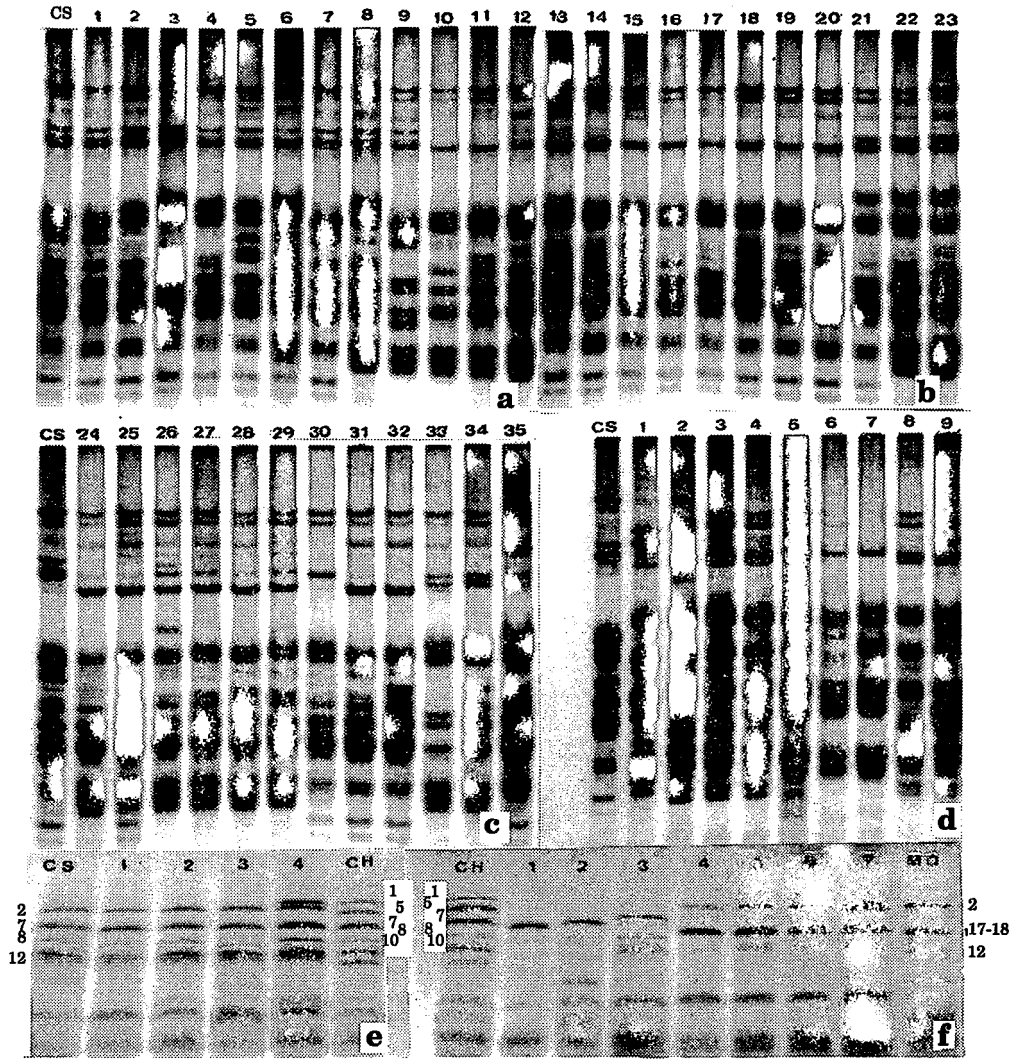

Fig. 1. Electrophoretic patterns of *T. aestivum* and *T. turgidum*

a, b and c: Gliadin patterns from 35 *T. aestvum* landraces representative of the different gliadin patterns observed in the 89 landraces. Chinese Spring (CS) was analyzed as control.

d: Gliadin patterns from seven *T. turgidum* landraces representative of the different gliadin patterns observed in the 40 landraces. Chinese Spring (CS), Ailanmai (lane 8) and synthetic hexaploid wheat RSP (lane 9) are shown.

e: HMW-glutenin patterns of *T. aestivum* landraces Chengdu-guangtou (lane 1), J-11 (lane 2), Ermai (lane 3) and Chaoxieban (lane 4). Chinese Spring (CS) (subunits: null, 7+8, 2+12) and Chuanyu 12 (CH) (subunits: 1, 7+8, 5+10) were used as references.

f: HMW-glutenin patterns of *T. turgidum* Zaozuo 2 (lane 1), Bangbanglanmai (lane 2), Sanlicun (lane 3), Banglanmai (lane 4), Changdiaoxu (lane 5), Ailanmai (lane 6) and synthetic hexaploid wheat RSP (lane 7). Chinese Spring (CS), Moulin (MO) (subunits: null, 2+12, 17+18) and Chuanyu12 (CH) were used for comparison.

Tale 3. HMW-glutenin subunits combinations in 89 *T. aestivum* L. landraces

Glu-A1	*Glu-B1*	*Glu-D1*	Landrace	Frequency (%)
null	7+9	2+12	Ermai	1.1
1	7+8	2+12	Chaoxieban	1.1
null	7+8	2+12	The remaining landraces, such as J-11	97.8

Tale 4. HMW-glutenin subunits combinations in 40 *T. turgidum* L. ssp. *turgidum* landraces

Glu-A1	*Glu-B1*	Landrace	Frequency (%)
null	20	Zaozuo 2	2.5
null	7	Bangbanglanmai	2.5
null	6+8	Sanlicun	2.5
2*	Similar to 17+18	The remaining landraces, such as Ailanmai	92.5

had the *Glu-B1c* allele (subunits 7+9), while other genotypes contained the *Glu-B1b* allele (subunits 7+8). On *Glu-A1* locus, Chaoxieban carried *Glu-A1a* allele (subunit 1), while other genotypes had *Glu-A1c* allele (null). The results indicated that there had very low level of HMW-glutenin variations in *T. aestivum* landraces from Sichuan, China.

A total of four different *Glu-1*-encoded allelic variants were identified among the 40 genotypes of *T. turgidum* landraces, resulting from the combination of two *Glu-A1* and 4 *Glu-B1* (Fig. 1f; Table 4). Three landraces had the *Glu-A1c* allele (null), while 37 out of 40 landraces (92.5%) had *Glu-A1b* allele (subunit 2*). More variations were observed in *Glu-B1*. It had four different allelic variations. Thirty-seven out of 40 landraces (92.5%) possess *Glu-B1*-encoded subunits with electrophoretic mobility similar to subunits 17+18 of the wheat cultivar Moulia, while only three landraces had unique *Glu-B1* loci. Zaozuo 2 had *Glu-B1e* allele (subunit 20) (Fig. 1f lane 1), Bangbanglanmai had *Glu-B1a* allele (subunit 7) (Fig. 1f lane 2), and Sanlicun had *Glu-B1d* allele (subunits 6+8) (Fig. 1f lane 3). Bangbanglanmai, Sanlicun and Zaozuo 2 had the same *Glu-A1c* allele (null), but different *Glu-B1* allele. The remainders had the same subunit combinations, subunit 2* encoded by *Glu-A1b* and subunits similar to 17+18 encoded by *Glu-B1*.

The expression of *Gli* and *Glu-1* loci of *T. turgidum* in hexaploid wheat background

In order to investigate the expression of *Gli* and *Glu-1* loci of *T. turgidum* in hexaploid wheat background, the synthetic hexaploid wheat RSP, which derived from the cross between Ailanmai and *Aegilops tauschii*, was employed in this study due to Ailanmai possess *Glu-1*-encoded subunits which has a frequency of 92.5% in *T. turgidum* landraces. All gliadin patterns and HMW-subunits of Ailanmai appeared in the synthetic hexaploid RSP (Fig. 1d lanes 8, 9 and Fig. 1f lanes 6, 7). It suggested that the *Gli* and *Glu-1* loci of *T. turgidum* cv. Ailanmai were totally expressed in the synthetic hexaploid wheat RSP.

Discussion

Based on the gliadins and HMW-glutenin subunit variations in *T. aesitvum* landraces native to Sichuan of China, we found that the genetic variations among these landraces were very low. It is agreement with the results obtained from isoenzyme (Yang et al. 1992) and RFLP markers (Ward et al. 1998).

From morphological (Yen et al. 1988) and RFLP analysis (Ward et al. 1998), it proposed that Chinese Spring is a strain of Sichuan landrace Chengdu-guangtou, a famous landrace of the Chengdu Plain, which is one of the Sichuan white wheat. In this study, only two out of 89 Sichuan white wheats (a frequency of 2.2%) showed identical gliadin patterns with Chinese Spring, but as many 87 out of 89 wheats (a frequency of 97.8%) showed identical HMW-glutenin subunit patterns with Chinese Spring. Chinese Spring and Chengdu-guangtou had the identical gliadins pattern and HMW-glutenin subunits, providing further support to the proposal that Chinese Spring is a strain of Chengdu-guangtou and a member of the Sichuan white wheat.

Allelic variation in HMW-glutenin subunits is largely responsible for bread-making quality in bread wheat, and quality scores had been assigned to each subunit (Payne 1987). From this study, the good 5+10 subunits encoded by *Glu-D1d* were not found in these *T. aestivum* landraces, while 88 out of 89 landraces (98.9%) had the *Glu-D1a* (subunits 2+12) and *Glu-A1c* allele (null) which was poor for bread-making. The mean quality score among these landraces was 6.01. It was much low and not suitable for bread-making quality. In Sichuan Province, these landraces had been widely cultivated or used as parents in wheat breeding, which was the reason of the poor bread-making quality in Sichuan wheats. For a long time, however, many agronomists in China have thought that they can not produce good bread-making quality in Sichuan due to the bad weather. In practice, by introgressing the subunits 5+10 encoded by *Glu-D1* and the subunit 1 or 2* encoded by *Glu-A1* from exotic wheat cultivars, wheat with good bread-making quality can be produced in Sichuan (Yen 1999).

Liu et al. (1999) reported that the *kr* genes in Sichuan *T. turgidum* landrace were different from that of Sichuan white wheats. In this study, we obtained the similar results. The gliadin patterns and the HMW-glutenin subunits in *T. turgidum* landraces from Sichuan were quite different from that of *T. aestivum* landraces, indicating that *Gli* and *Glu-1* loci among *T. turgidum* landraces from Sichuan were different from those of *T. aestivum* landraces. These results suggested that AABB genomes of *T. aestivum* landraces were not closely related to those of *T. turgidum* landraces. The variations of *Glu-A1* and *Glu-B1* in *T. turgidum* landraces were higher than that of *Glu-A1* and *Glu-B1* in *T. aestivum* landraces. From this study, we found that many *T. turgidum* landraces possessed *Glu-A1b* allele (subunit 2*), which was superior to *Glu-A1c* (null), and the *Gli-1* and *Glu-1* loci of *T. turgidum* landrace can express in hexaploid wheat background. Thus, these *Glu-1* loci can be used to improve the bread-making quality and increase the HMW-glutenin variations in Sichuan wheat breeding.

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Intraspecific genetic diversity for resistance to wheat rusts in wild *Triticum* and *Aegilops* species

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Summary

Intraspecific variability for resistance to leaf rust (*Puccinia recondita* f. sp. *tritici*) and stripe rust (*P. striiformis*) was studied in four wild *Triticum* and nine *Aegilops* species to assess their potential as reservoir of novel sources of resistance. Multipathotype seedling tests of wild *Triticum* and *Aegilops* species with individual *P. recondita* and *P. striiformis* pathotypes showed significantly large intraspecific diversity for rust resistance. A number of reaction patterns were observed among the limited number of accessions tested for each of the species. Segregation for rust reaction to individual pathotypes of rusts in F_2 generations of 31 intraspecific crosses in wild *Triticum* and *Aegilops* species further confirmed the genetic diversity for rust resistance within these species. Of nine crosses among five accessions of *Aegilops triuncialis*, eight crosses gave a 15:1 segregation ratio of resistant: susceptible F_2 plants indicating at least four different dominant resistance genes within the five accessions. Similar data were obtained from intraspecific crosses in other species, even when the accessions of a species studied were collected from the same site. These observations have important implications in designing strategies for utilizing wild species as donors of disease resistance in wheat.

Key words: *Aegilops*, *Triticum*, Diversity, Rust, Resistance

Introduction

Wild relatives of wheat provide a rich reservoir of genes for resistance to various wheat diseases (Sharma and Gill 1983; Jiang et al. 1994; Friebe et al. 1996; Harjit-Singh et al. 1998). Resistance to all the three wheat rusts, viz. leaf rust (*Puccinia recondita* f. sp. *tritici*), stripe rust (*P. striiformis*) and stem rust (*P. graminis tritici*) has been transferred from wild *Triticum* and *Aegilops* species (McIntosh 1998). Many of the alien rust resistance genes transferred into cultivated wheat and deployed have been overcome.

The transfer and exploitation of an alien gene for resistance to a particular disease from a donor species often precludes its utilization as a source for other genes. However, the same

species may have additional intraspecific diversity for resistance and could be a useful potential donor for more resistance genes for the same disease. Therefore, before looking for new sources for resistance genes in other species, it would be desirable to study intraspecific diversity for resistance in the same species for future use.

The present study was conducted to assess the extent of intraspecific diversity for rust resistance in four wild *Triticum* and nine *Aegilops* species using Indian leaf rust and stripe rust isolates with diverse avirulence/virulence on leaf rust (*Lr*) and stripe rust (*Yr*) resistance genes, respectively. All the species investigated revealed large intraspecific variability for rust resistance for their continuous exploitation for resistance breeding.

Materials and methods

Various accessions of four wild *Triticum* and nine *Aegilops* species from the germplasm collection maintained at the Punjab Agricultural University, Ludhiana were used for this study. Four to ten accessions each of four wild *Triticum* and six *Aegilops* species were tested for seedling reaction to five to six Indian isolates of leaf rust (*Puccinia recondita*). The eight Indian leaf rust isolates used in this study were selected on the basis of their avirulence/virulence on the leaf rust resistance (*Lr*) genes so as to have large diversity for pathogenicity among them (Nayar et al. 1997). The standard procedure for inoculation of seedlings (Nayar et al. 1997) was followed and the seedling reactions were recorded two weeks after inoculation according to the scale developed by Mains and Jackson (1926). The reactions 0; 0 and 2 were classified as resistant whereas reactions 3 and 4 were categorized as susceptible. Similarly, two to twelve accessions each of three wild *Triticum* and nine *Aegilops* species were tested with three diverse isolates of stripe rust (*P. striiformis*) by following the standard inoculation procedure (Nayar et al. 1997). The resistant and susceptible categories were made the same way as for the leaf rust.

Thirty one intraspecific crosses were made among different accessions of each of seven *Aegilops* and three wild *Triticum* species. Forty to ninety F_2 seedlings of each of 30 intraspecific crosses were tested with an individual isolate of leaf rust. In three of these crosses, the tests were made with two or three individual rust isolates. The chi-square test was used to assess the goodness of fit to the expected ratios of resistant and susceptible F_2 segregants. In the case of intraspecific crosses of *T. urartu*, where one parent exhibited an intermediate reaction (i.e. 2+ to 3- or X) and the other parent was resistant, three categories (resistant, intermediate and susceptible) were made to test the goodness of fit to the expected ratio. For genetic analysis of resistance to stripe rust, F_2 seedlings of another intraspecific cross between two accessions of *T. dicoccoides*, exhibiting intermediate (Acc 4667) and resistant (Acc 13985) reactions to isolate N of stripe rust, were tested with this isolate.

Results and discussion

Seedling tests of different accessions of wild *Triticum* and *Aegilops* species with individual *P. recondita* pathotypes showed large intraspecific diversity for rust resistance. Seven accessions of *T. boeoticum* (A^b) showed seven different reaction patterns to six pathotypes (Table 1). Similarly, there was large variability for reaction patterns among small number of accessions of *Ae. longissima*

Table 1. Seedling reactions of diploid *Aegilops* and wild *Triticum* species to individual pathotypes of leaf rust

Species (genome)	Accession no.	Reaction to pathotype@						Reaction pattern
T.boeoticum (A^b)		77	77A-1	77-1	77-2	77-3	77A	
	4638	S	R	S	S	—	—	I
	4668	R	S	—	S	S	R	II
	4671	R	R	R	S	R*	—	III
	4672	R	S	R	R	—	—	IV
	4796	R	S	R	S	R*	R	V
	4856	R	X	S	R	R	—	VI
	4945	—	R	R	R*	R	R	VII
T.urartu (A^u)		77	77-1	77-2	77-3	77-A		
	5319	S	R	S	R	R		I
	5340	X	R*	R	R	R		II
	5343	R	—	R	R	R		III
	5357	S	—	—	S	R		IV
	5360	R	S	S	S	—		V
Ae. speltoides (S)		77	77A-1	77-1	77-2	77-3	104-1	
	3574	R	R	R	R	—	R	I
	3577	R	R	S	R	—	R	II
	3593	R	R	R	R	—	—	I
	3594	R	R	R	R	R	—	I
	3596	R	R	R	R	R	—	I
	3601	R	R	R	R	R	—	I
	3602	R	R	S	R	R	R	II
	3604	R	R	R	R	R	—	I
	3608	R	R	R	R	R	—	I
	3808	R	R	R	R	—	—	I
Ae. longissima (S^l)		77	77A-1	77-1	77-2	104-1		
	3506	S	S	—	R*	R		I
	3770	S	R	R	R	R		II
	3819	S	S	—	R*	—		I
	3821	R	R	—		—		III
Ae. squarrosa (D)		77A-1	77-1	77-2	77-3	104-1		
	3733	S	S	R#	S	—		I
	3737	S	R(S)#	S	S	X		II
	3738	S	S	S	S	S		III
	3742	S	S	S	S	S		III
	3748	—	R	R	R	R		IV
	3749	—	R	R	R	R		IV
	3767	S	X	S	—	R*		V

@ R: Resistant (0-2), S: Susceptible (3-4), X: Mesothetic, R*: Reaction varies from 1-3, —: not tested, # Different reactions obtained in tests conducted at different times.

Table 2. Seedling reaction of polyploid wild *Triticum* and *Aegilops* species to individual pathotypes of leaf rust

Species (genome)	Accession no.	Reaction to pathotype@					Reaction pattern
T. dicoccoides		77	77A-1	77-1	77-2	104-1	
(AB)	4627	S	S	S(R)#	R	S*	I
	4637	R	R	R	R	R	II
	4654	S	S	S(R)	R	S	III
	4656	S	R*	R*	—	—	IV
	4667	R	S*	S	—	—	V
	13985	—	R	S	S	S	VI
T. araraticum		77	77A-1	77-1	77-2	104-1	
(AG)	4679	S	S	S	S	S	I
	4741	–	R	R	R	—	II
	4747	X	R	R(S)	R	R	II
	4749	R(X)	S	S	S*	X	III
	4756	S	S	X	S*	—	IV
Ae. triuncialis		77	77-1	77-2	77-3	104-1	
(UC)	3549	R	R	R	R	R	I
	3621	—	R	R	R	R	I
	3622	R	R	R	R	R	I
	3630	R	R	R	R	—	I
	3649	R	R	R	R	—	I
	3662	R	R	X	R	R	II
	3665	R	R	R	R	—	I
	3669	S	R	S(R)	S*	R	III
Ae. ovata (UM°)		77	77A-1	77-1	77-2	104-1	
	3514	R	R	R	R	R	I
	3547	R	R	R	R	R	I
	3548	R	R	R	R	R	I
	3559	S	R	S	—	—	II
	3563	S	R	S	X	—	II
	3565	R	R	R	R	R	I
	3798	S	S	—	R	R	III
	5507	R	S	R	—	—	IV
Ae. triaristata		77	77A-1	77-1	77-2	77-3	
(UM^t)	3492	S	—	S	S	—	I
	3526	S	X	S	—	—	II
	3545	R	R	—	R	—	III
	3689	S	—	S	—	R	II
	3693	S	R	S	—	R	I

@ R: Resistant (0-2), S: Susceptible (3-4), X: Mesothetic, R* and S*: reaction varies from 1-3, —: not tested, #: Different reactions obtained in tests conducted at different times.

Table 3. Seedling reactions of diploid *Aegilops* and wild *Triticum* species to individual pathotypes of stripe rust

Species (genome)	Accession no.	Reaction to pathotype@			Reaction pattern
		K	38A	M	
T. urartu (A^u)	5319	R	S	R	I
	5341	R	S	R	I
	5343	S	R	S	II
	5349	R	S	R	I
	5357	R	S	R	I
	5360	S	R	R	III
Ae. speltoides (S)	45	R	S	S	I
	3475	R	R	R	II
	3566	R	S	S	I
	3567	R	S	R	III
	3569	R	S	R	III
	3570	R	S	R	III
	3571	R	R	R	II
	3573	R	R	R	II
	3574	S	S	R	IV
	3576	R	S	S	I
	3577	R	R	R	II
	3580	R	R	R	II
Ae. longissima (S^l)	3507	R	R	S	I
	3770	R	S	R	II
	3819	S	S	R	III
	3821	R	R	S	I
Ae. bicornis (S^b)	3782	S	R	S	I
	3799	S	S	R	II
	3804	S	S	S	III
Ae. squarrosa (D)	3727	S	S	R	I
	3737	R	S	R	II
	3743	R	R	R	III
	3748	R	R	R	III
	3749	R	R	R	III
	3754	S	R	R	IV
	3757	R	S	R	II
	3806	R	R	R	III
	3760	R	R/S	R	II

@R: resistant (0-2), S: susceptible (3-4)

(S^l) and *Ae. squarrosa* (D). However, only two distinct patterns were observed among ten accessions of *Ae. speltoides* (S). Variability in reaction pattern was also high among tetraploid species (Table 2). Six accessions of *T. dicoccoides* (AB) showed six different reaction patterns. Five accessions of *T. araraticum* (AG) had at least four different reaction patterns. Eight lines of *Ae. ovata* (UM^o) had four reaction patterns. Testing of diploid (Table 3) and tetraploid (Table 4) wild wheats and *Aegilops* species with individual pathotypes of *P. striiformis* also revealed significant intraspecific diversity for seedling response.

The study of segregation for reaction to individual pathotypes of *P. recondita* in the F_2 generations of 30 intraspecific crosses further supported the existence of significant intraspecific diversity in the diploid and tetraploid species (Table 5). Segregation for rust resistance was observed in the F_2 of all the crosses among five accessions of *T. urartu* (A) tested with *P. recondita* pathotype 77A. Similarly, of nine crosses among five accessions of *Ae. triuncialis* (UC) having resistant reactions to pathotype 77-2, eight crosses segregated in 15 resistant : 1 susceptible ratio. This suggested at least four different dominant genes for resistance to leaf rust among the five accessions. Therefore, this species could be a large reservoir of leaf rust resistance genes. Furthermore, segregation in limited number of crosses between different resistant accessions of *Ae. longissima* (S^l), *Ae. triaristata* (UM^t) and *Ae. ovata* (UM^o) supported the prevalence of considerable intraspecific diversity within wild *Aegilops* species. However, no susceptible plant was observed in F_2 generation of crosses among different accessions of *Ae. speltoides* (S). This is in agreement with the observation that there were only two reaction patterns among the accessions of *Ae. speltoides* (S). Since there are two major groups of *P. recondita* ('Group I' and 'Group II') based on aecial and telial host range (Anikster 1997) and one of the types ('Type C') belonging to one group ('Group II') is specific to *Aegilops* species having S genome, the larger resistance of *Ae. speltoides* (S) accessions to a number of leaf rust pathotypes from wheat may be a case of non-host resistance (Niks and Dekens 1991).

Study of the F_2 generation of cross of resistant Acc 3749 of *Ae. squarrosa* with susceptible accession (Acc 3754) showed that the resistant parent possesses one dominant and one recessive gene for resistance to pathotype 77-1. Both of these genes were individually effective against this pathotype. However, testing of the F_2 generation of Acc 3754 (S) x Acc 3749 (R) with pathotype 77-4 indicated that Acc 3749 possesses two dominant genes for resistance where both genes are individually completely effective against pathotype 77-4. It has been observed that dominance or recessiveness of resistance genes is not absolute and that the dominance relationship can change with pathogen isolate. The stem rust resistance gene *Sr6*, which in most cases is dominant, displays recessive inheritance with some pathogen cultures (Roelfs 1988). Therefore, it is possible that the two genes of Acc 3749 providing resistance to 77-1 are the same as those that provide resistance to 77-4, and that only the dominance relationship of one of the genes in Acc 3749 changed with change in rust pathotype. However, it is difficult to prove or disprove this assumption with the limited data available and, therefore, the presence of more than two leaf rust resistance genes in Acc 3749 cannot be ruled out.

Study of the intraspecific cross of *T. dicoccoides* between Acc 4667 exhibiting intermediate reaction (; to 0N on first leaf and 0N to 3-N reaction on second leaf of seedling) and Acc 13985 exhibiting resistant reaction (; on both the leaves) to pathotype N of stripe rust showed that the former accession possesses a dominant gene for intermediate reaction and the latter possesses another dominant gene conferring complete resistance (12 resistant : 3 intermediate : 1 susceptible ratio; $\chi^2 = 1.02$)

Table 4. Seedling reactions of polyploid wild *Triticum* and *Aegilops* species to individual pathotypes of stripe rust

Species (genome)	Accession no.	Reaction to pathotype@			Reaction pattern
		K	38A	M	
T. dicoccoides (AB)	4632	S	R	R	I
	4660	S	S	S	II
	4665	S	S	S	II
	7081	S	S	S	II
T. araraticum (AG)	4679	S	S	R	I
	4689	S	S	S	II
	4692	S	—	R	I
	4697	R	S	S	III
	4747	R	R	R	IV
Ae. cylindrica (CD)	3511	S	S	R	I
	3717	S	R	S	II
	3724	S	S	S	III
	3725	S	S	R	I
Ae. triuncialis (UC)	3541	R	R	R	I
	3549	R	R	S	II
	3621	R	R	R	I
	3661	S	R	R	III
	3669	S	R	R	III
Ae. ovata (UM^o)	3514	S	R	S	I
	3547	R	R	S	II
	3565	S	R	S	I
	5507	R	S	R	III
Ae. triaristata (UM^t)	3492	R	R	R	I
	3693	R	R	R	I
	3797	R	R	R	I
Ae. peregrina (US)	3477	S	R	S	I
	3791	R	R	R	II

@ R: resistant (0-2), S: susceptible (3-4), —: not tested.

In the present study, examination of variability for resistance to leaf rust and stripe rust by testing of different accessions of wild *Triticum* and *Aegilops* species with individual isolates possessing diverse pathogenicity showed that there is large intraspecific variability for rust resistance within each of these species. Existence of a number of rust reaction patterns among small samples of accessions of each species showed that each species possesses a number of rust resistance genes. If these accessions were tested with even more number of pathotypes, further variability for rust resistance genes among different accessions may be revealed. These observations with multipathotype seedling tests were highly supported by testing of F_2 generation of the intraspecific crosses with individual rust pathotypes. Inspite of the fact that all accessions of *T. urartu* used in the present study were collected from Turkey, F_2 of all crosses between different accessions of this species segregated for rust reaction (Table 5). This observation has an important implication in the utilization of wild relatives of wheat as donors of rust resistance. It suggests that when one or more genes transferred from a wild donor species are overcome by new pathotypes due to directional selection, the same donor species could still be a reservoir of a number of new resistance genes that can be transferred and deployed in the future. At least six leaf rust resistance genes (*Lr21*, *Lr22a*, *Lr*32, *Lr*41, *Lr*42 and *Lr*43) have been transferred from *Ae. squarrosa* (Cox et al. 1993; McIntosh 1998) and transfer of other *Lr* genes is in progress. This suggests that, as source of resistance, although *Ae. squarrosa* did not appear to be as good as other *Aegilops* species with C, U and M genomes (Dhaliwal et al. 1991, 1993; Harjit-Singh et al. 1998), still it possesses impressive intraspecific genetic diversity for leaf rust resistance.

Based on the higher proportion of accessions exhibiting resistance to prevalent isolates, it is often concluded that a particular wild related species is a better source of resistance than another with lower proportion of resistant accessions. However, the latter may still possess a number of genes for resistance that are not useful against the present pathotypes but may be useful against emerging pathotypes in future. Our observations at the Punjab Agricultural University, Ludhiana showed that *T. dicoccoides* (AB) is highly susceptible to leaf rust and stripe rust (Dhaliwal et al., 1993; Harjit-Singh et al. 1998) but still we could find different useful stripe rust resistance genes among the two accessions studied in the present study. Similarly, van-Silfhout (1989) found that 850 samples of *T.dicoccoides* collected from Israel possess at least eleven stripe rust resistance genes. Also, he found that several entries found to be susceptible to one or more isolates from Israel proved resistant to eight of the main stripe rust pathotypes from Netherland.

The significantly large intraspecific diversity revealed in the wild *Triticum* and *Aegilops* species in the present study suggests that these species shall continue to offer a number of novel resistance genes, in spite of the fact that many of the resistance genes contributed by these species have been overcome by new pathotypes. The progenitor species like *Ae.squarrosa* (Cox et al. 1993; McIntosh 1998), *T. dicoccoides* (van Silfhout 1989; van Silfhont et al. 1989) and *T. boeoticum* (Gill et al. 1995) continue to be promising sources of rust resistance for transfer and deployment. Thus, the wild progenitor species should be preferred as source of new genes for resistance against the prevalent pathotypes over the non-progenitor and distantly related species due to ease of transfer through recombination and reduced linkage drag from the former.

Table 5. F_2 segregation for seedling reaction to individual pathotypes of leaf rust in 30 intraspecific crosses.

Species and genome	Cross*	Patho-type	F_2 segregstion of R:I:S **		χ^2	P	Genetic control#
			observed	expected			
T. urartu (A^u)	1. 5357 x 5328(I)	77A	58:8:2	12:3:1[$]	3.92	0.10-0.20	Two Dom
	2. 5340 x 5343(I)	77A	52:8:3	12:3:1	2.59	0.20-0.30	Two Dom
	3. 5319(I) x 5357	77A	40:10:4	12:3:1	0.12	0.90-0.95	Two Dom
	4. 5340 x 5319(I)	77A	59:9:2	12:3:1	3.39	0.10-0.20	Two Dom
Ae. speltoides (S)	5. 3577 x 3604	77-1	87:0:0	No seg.[¶]	–	–	–
	6. 3593 x 3808	77-1	58:0:0	No seg.	–	–	–
	7. 3602 x 3601	77-1	48:0:0	No seg.	–	–	–
	8. 3601 x 3596	77-1	52:0:0	No seg.	–	–	–
	9. 3584 x 3577	77-1	43:0:0	No seg.	–	–	–
	10. 3577 x 3574	77-2	64:0:0	No seg.	–	–	–
Ae. squarrosa (D)	11. 13(S) x 3733	77-1	49:0:11	3:0:1	1.42	0.20-0.30	One Dom
	12. 3749 x 3748	77-1	91:0:0	No seg.	–	–	–
	13. 3749 x 3754(S)	77-1	72:0:14	13:0:3	0.34	0.50-0.70	One Dom & One Rec
	14. 3754 (S) x 3749	77-4	81:0:6	15:0:1	0.06	0.75-0.90	Two Dom
	15. 3761 x 9822(S)	77A-1	32:0:24	9:0:7	0.01	0.90-0.95	Two Dom
Ae. longissima(S^l)	16. 3818 x 3770	77-1	30:20:3	9:6:1	0.03	0.95-0.99	Two Dom
		77-2	51:0:3	15:0:1	0.04	0.90-0.95	Two Dom
T. araraticum (AG)	17. 4679 x 4747(S)	77-1	57:0:22	3:0:1	0.34	0.50-0.70	One Dom
		77-2	15:0:45	1:0:3	0.00	1.00	One Rec
		77A-1	18:0:54	1:0:3	0.07	0.70-0.80	One Rec
Ae. triuncialis (UC)	18. 3621 x 3549	77-2	63:0:4	15:0:1	0.004	0.90-0.95	Two Dom
	19. 3621 x 3622	77-1	45:0:0	No seg.	–	–	–
		77-2	66:0:0	No seg.	–	–	–
	20. 3622 x 3549	77-2	46:0:2	15:0:1	0.36	0.50-0.70	Two Dom
	21. 3622 x 3669	77-2	50:0:1	15:0:1	1.60	0.10-0.20	Two Dom
	22. 3669 x 3549	77-2	54:0:2	15:0:1	0.69	0.30-0.50	Two Dom
	23. 3669 x 3621	77-2	66:0:4	15:0:1	0.03	0.80-0.90	Two Dom
	24. 3541 x 3549	77-2	81:0:9	15:0:1	2.16	0.10-0.20	Two Dom
	25. 3541 x 3621	77-2	54:0:2	15:0:1	0.69	0.25-0.50	Two Dom
	26. 3541 x 3622	77-2	52:0:1	15:0:1	1.72	0.10-0.25	Two Dom
Ae. triaristata (UM^t)	27. 3492 x 3542	77-2	38:0:25	9:0:7	0.42	0.50-0.70	Two Dom
Ae. ovata (UM^o)	28. 3547 x 3565	77-2	51:0:3	15:0:1	0.05	0.80-0.90	Two Dom
Ae. cylindrica (CD)	29. 3724 x 3717(S)	77A-1	45:0:6	13:0:3	1.63	0.20-0.30	One Dom & One Rec.
	30. 3724 x 3695(S)	77-1	39:0:16	3:0:1	0.49	0.30-0.50	One Dom
		77-2	7:0:36	1:0:3	1.74	0.10-0.20	One Rec.

*(I): Accessions giving intermediate reaction to the given pathotype; (S): Susceptible accession, **R:I:S Resitant:Intermediate:Susceptible, #Dom: Dominant; Rec: Recessive, [$]One gene giving complete resistant and the other giving intermediate reaction (2+, X or 3-), [¶]No segregation observed in F_2.

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Research article

Detection of plasmon-specific RAPD markers using the alloplasmic hybrids of common wheat (*Triticum aestivum* L.) cv. Chinese Spring

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Summary

Plasmon-specific polymorphisms were detected by RAPD-PCR analysis of alloplasmic hybrids of common wheat (*Triticum aestivum* L.) cv. Chinese Spring (CS) with cytoplasms from various *Triticum* and *Aegilops* species. The analysis of total DNAs from 47 hybrid lines using 31 random primers revealed seven polymorphic RAPD markers, among which six were assigned to the plasmon variations. The polymorphic markers were either unique to single plasmons or common to groups of phylogenetically related plasmons. By combining the markers, at least T (*Ae. mutica*), T^2 (*Ae. mutica*), S^b (*Ae. bicornis*) and A^2 (*T. monococcum*) plasmons were distinguished. They can be effectively used for the plasmon identification of the alloplasmic hybrids.

Key words: Plasmon-specific RAPD markers, Cytoplasm identification, Alloplasmic hybrids, *Triticum, Aegilops*

Introduction

A large number of alloplasmic or nucleus-cytoplasm (NC) hybrids have been produced through substitution backcrosses in Triticeae, especially in *Triticum* and *Aegilops* species (Kihara 1951; Fukasawa 1953, 1959; Maan 1975, 1991; Tsunewaki 1980, 1996; Panayotov 1983; Ohtsuka 1981, 1991). These hybrids have provided useful experimental materials for studying diversity of the cytoplasmic genomes (plasmons) and interactions between nuclear genomes and plasmons based on the classical analysis of physiological and morphological characteristics manifested in the hybrids (Tsunewaki 1980, 1996) and molecular analysis of chloroplast and mitochondrial DNA variations in the hybrids (Ogihara and Tsunewaki 1988; Terachi and Tsunewaki 1992; Tsunewaki 1996).

One problem which did and possibly could occur during the course of the backcross program

for the production of NC hybrids is mis-identification of lines due to mistagging. If it occurred, its recognition and confirmation requires much time and labor as pointed out by Tsunewaki et al. (1996). To avoid such serious problem, molecular markers which can be easily and effectively used for the cytoplasm identification are required. RFLP analysis of total DNA using chloroplast and mitochondrial DNA-specific probes can be useful for this purpose. PCR-based methodology using plasmon-specific primers could also be useful. SSCP (single strand conformational polymorphism) analysis in fact has been proven to be a powerful method for the identification of both inter- and intraspecific plasmon polymorphisms in *Triticum* and *Aegilops* species (Ohsako et al. 1996; Wang et al. 1997).

In the present study we applied RAPD-PCR analysis which is generally simpler and more convenient to detect DNA polymorphisms than the above two methods. For the detection of plasmon-specific polymorphisms we used 47 lines of NC hybrids in which one nuclear genome of common wheat cv. Chinese Spring is combined with cytoplasms from various *Triticum* and *Aegilops* species. Although the scale of the present work was limited, the method was shown to be effective in identifying polymorphisms either unique to single particular plasmons or often common to phylogenetically related groups of plasmons.

Materials and methods

Plant materials

Forty-seven lines of alloplasmic or NC hybrids which have plasmons derived from various *Triticum* and *Aegilops* species combined with a single nuclear genome from *Triticum aestivum* L. em Thell. cv. Chinese Spring (CS) were used in this study. For some NC hybrids, their cytoplasms were replaced by that of CS after reverse crosses of the hybrids as male parents with CS as a female parent (see Results and discussion). The original hybrids were provided by Prof. K. Tsunewaki, Kyoto University (now Fukui Prefectural University). CS served as a euplasmic control.

RAPD-PCR detection of DNA polymorphisms

Total DNAs were extracted according to Liu et al. (1990) from one to five plants obtained after self-fertilization of fertile NC hybrids and from single progeny plants of male-sterile NC hybrids after backcrosses with the paternal CS. DNAs from the reverse F_1 hybrids were also extracted in the same way. RAPD-PCR was performed in a reaction mixture (10μl) containing 10 ng of total DNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2 mM $MgCl_2$, 0.001% gelatin and 0.32 μM random decamer primer (Operon Technologies). PCR program was as follows: a pre-denaturation step for 30 s at 93 °C; and 40 cycles of 1 min at 93 °C, 1 min at 36 °C, 1.5 min at 72 °C; followed by post-extension for 2 min at 72 °C. Amplified fragments were resolved by electrophoresis through 1.4 % agarose gels and visualized by staining with ethidium bromide.

Results and discussion

Polymorphic DNA fragments were detected by RAPD-PCR analysis using total DNAs extracted from 47 NC hybrid lines. Only 31 random primers were used in this study and seven were found to give polymorphic fragments among them. The polymorphisms detected were either amplification or non-amplification of DNA fragments unique in single lines or in several lines mostly with

phylogenetically related cytoplasms.

Six among seven polymorphic fragments were assigned to the cytoplasmic genomes (Table 1). Three types of cytoplasmic polymorphisms were detected as amplification of single fragments. A primer OPA02 amplified a 1.6 kbp fragment ($A02_{1,600}$) in three NC hybrids with M plasmon of *Ae. comosa thessalica* (Kyoto University code number C05), M^h plasmon of *Ae. herdreichii* (C06) and N plasmon of *Ae. uniaristata* (C07) (Fig. 1A). The polymorphism in the male-sterile M and M^h plasmons was detected in the progeny plants obtained after backcrosses of the NC hybrids with the paternal CS and thus considered to be derived from the plasmons. The cytoplasmic origin of this polymorphism was confirmed by RAPD-PCR analysis using DNAs from the reverse hybrids in which their cytoplasms were replaced by that of CS. As expected the fragment $A02_{1,600}$ could not be amplified in the reverse hybrids (Fig. 2A). Another primer OPB19 amplified a 1.3 kbp fragments ($B19_{1,300}$) in the NC hybrids with S^b (C12, *Ae. bicornis*), T (C13, *Ae. mutica*) and T^2 (C14, *Ae. mutica*) plasmons (Fig. 1B). OPE07 amplified a 3.2 kbp fragment ($E07_{3,200}$) in three NC hybrids with S^l (C10, *Ae. sharonensis*), S^b (C12, *Ae. bicornis*) and S^v (C33, *Ae. kotschyi*) but did not in NC hybrids with S (C08, C17, *Ae. speltoides*) and other S-derivative plasmons. They were assigned to the cytoplasmic variations in the same way using the reverse hybrids. The polymorphic fragment $A02_{1,600}$ detected in M and two M-related plasmons was absent in three NC hybrids with T and T^2 plasmons of *Ae. mutica* and M^o plasmon of *Ae. ovata*. M^o plasmon of *Ae. ovata* is thought to have evolved from T plasmon of *Ae. mutica* after hybridization with *Ae. umbellulata* as a pollen parent followed by amphidiploidization (Tsunewaki 1995). Therefore, as far as this polymorphism is concerned, T plasmon of *Ae. mutica* can be considered to have remained unchanged

Table 1. Plasmon-specific RAPD marklers detected in the NC hybrid lines of common wheat (*Triticum aestivum* L.) cv. Chinese Spring

Polymorphic fragment	Type of polymorphism[a]	Polymorphic line (plasmon code, species)[b]
$A02_{1,600}$	+	C05 (M, *Ae. comosa thessalica* 2x KU17-2), C06 (M^h, *Ae. heldreichii* 2x), C07 (N, *Ae. uniaristata* 2x)
$A02_{2,200}$	–	C04 (D, *Ae. squarrosa typica* 2x KU20-2), C05 (M, *Ae. comosa thessalica* 2x KU17-2), C06 (M^h, *Ae. heldreichii* 2x), C07 (N, *Ae. uniaristata* 2x), C19 (D, *Ae. squarrosa anathera* 2x KU2009), C28 (D, *Ae. cylindrica* 4x)
$A13_{1,100}$	–	C14 (T^2, *Ae. mutica* 2x)
$D05_{1,700}$	–	C16 (A^2, *T. monococcum flavescens* 2x)
$B19_{1,300}$	+	C12 (S^b, *Ae. bicornis* 2x), C13 (T, *Ae. mutica* 2x), C14 (T^2, *Ae. mutica* 2x)
$E07_{3,200}$	+	C10 (S^l, *Ae. sharonensis* 2x KU5-1), C12 (S^b, *Ae. bicornis* 2x), C33 (S^v, *Ae. kotschyi* 4x KU14-2)

[a] + and – represent amplification and non-amplification polymorphisms, respectively, relative to the nuclear parent CS and all other NC hybrids.

[b] According to Tsunewaki et al. (1996).

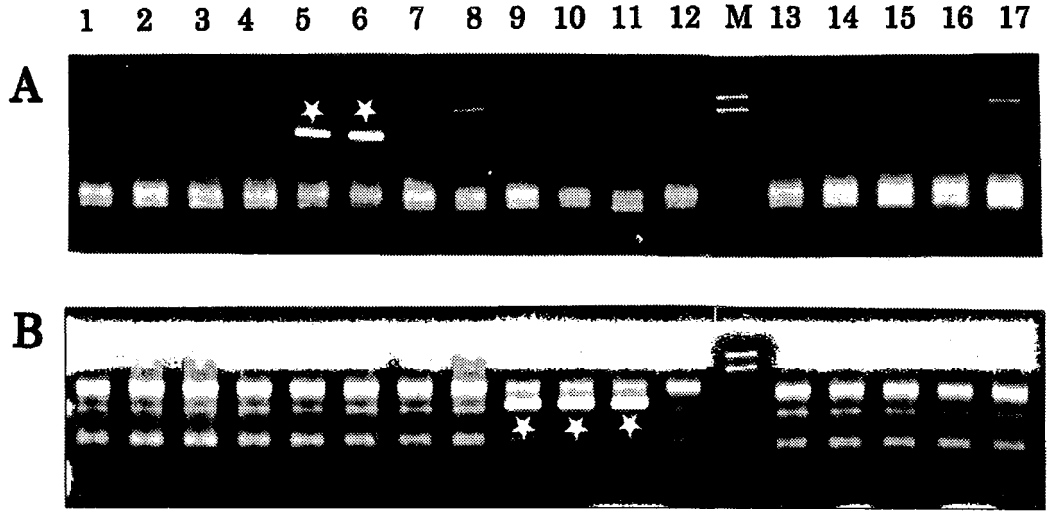

Fig. 1. RAPD markers detected by OPA02 and OPB19.
A: $OPA02_{1,600}$ amplified in C06 (M^h, *Ae. heldreichii*) and C07 (N, *Ae. uniaristata*); the same polymorphism was observed in C05 (M, *Ae. comosa*), B: $OPB19_{1,300}$ amplified in C12 (S^b, *Ae. bicornis*), C13 (T, *Ae. mutica*) and C14 (T, *Ae. mutica*). Asterisks indicate the polymorphic marker fragments. lane 1: C16 (*T. monococcum flavescens*), 2: C02 (*Ae. caudata polyathera* KU6-1), 3: C03 (*Ae. umbellulata*), 4: C04 (*Ae. squarrosa typica* KU20-2), 5: C06 (*Ae. heldreichii*), 6: C07 (*Ae. uniaristata*), 7: C08 (*Ae. speltoides ligustica*), 8: C10 (*Ae. sharonensis* KU5-1), 9: C12 (*Ae. bicornis*), 10: C13 (*Ae. mutica*), 11: C14 (*Ae. mutica*), 12: C25 (*T. timopheevi*), M: λ *Hin*dIII markers, 13: C30 (*Ae. columnaris* KU11-2), 14: C31 (*Ae. ovata*), 15: C33 (*Ae. koyschyi* KU14-2), 16: C35 (*Ae. crassa* 4x) and 17: C52 (*T. aestivum* cv. CS).

in M^o plasmon of *Ae. ovata* after its evolution. On the other hand, $B19_{1,300}$ was absent in a NC hybrid with M^o plasmon of *Ae. ovata*. This polymorphism thus can be considered to have evolved after amphidiploidization of *Ae. ovata*. A reason why this same polymorphism was present in S^b plasmon of *Ae. bicornis* remains unknown.

Three other types of cytoplasmic polymorphisms were detected as non-amplification of single fragments. One was recognized by non-amplification of a 2.2 kbp fragment ($A02_{2,200}$) by a primer OPA02. This polymorphism was detected in three NC hybrids with D plasmon of *Ae. squarrosa typica* (C04), *Ae. squarrosa anathera* (C19) and *Ae. cylindrica* (C28). The same polymorphism was detected also in NC hybrids with M plasmon (C05) and its related M^h (C06) and N (C07) plasmons. The localization of $A02_{2,200}$ in the cytoplasmic genomes was confirmed by RAPD-PCR analysis of the reverse hybrids, in which the fragment $A02_{2,200}$ appeared after replacing their cytoplasms by that of CS (Fig. 2B). $A02_{2,200}$ was amplified in four NC hybrids with D^2 plasmon of *Ae. crassa* 4x (C35), *Ae. juvenalis* (C53), *Ae. crassa* 6x (C55) and *Ae. vavilovii* (C56). A NC hybrid with the cytoplasm of *Ae. ventricosa* (C36) also showed the amplification of this fragment, although its cytoplasm is classified in D plasmon type (Tsunewaki et al. 1996). It has been suggested that *Ae. juvenalis*, *Ae. crassa* 6x and *Ae. vavilovii* were derived from *Ae. crassa* 4x, and that D^2 plasmon of *Ae. crassa* 4x and D plasmon of *Ae. ventricosa* evolved from D plasmon of *Ae. squarrosa* after

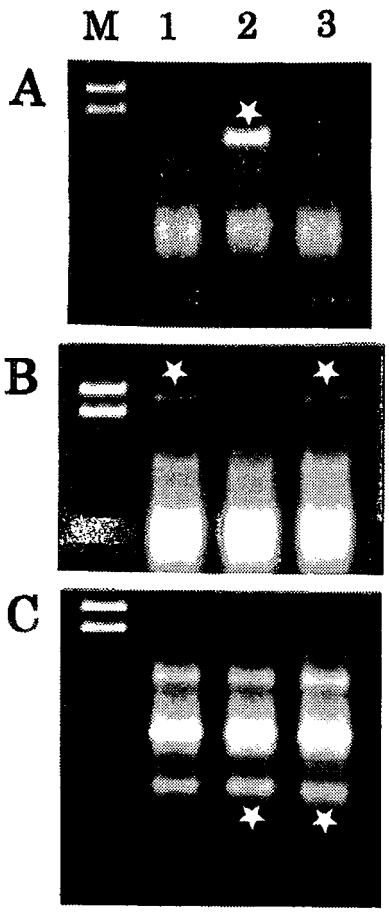

Fig. 2. Confirmation of the cytoplasmic and nuclear origin of the polymorphic RAPD markers using DNAs from the reverse F_1 hybrids in which cytoplasms of the NC hybrids were replaced by that of CS.
A: a cytoplasmic marker $OPA02_{2,200}$ in C07 (*Ae. uniaristata*), B: a cytoplasmic marker $OPA02_{1,600}$ in C28 (*Ae. cylindrica*) and C: a nuclear marker $OPA01_{600}$ in C20 (*Ae. longissima* TL05). Lane 1: CS, 2: NC hybrid, 3: reverse hybrid. Asterisks indicate the polymorphic marker fragments.

hybridization with *Ae. comosa* or *Ae. heldreichii* followed by amphidiploidization (Tsunewaki 1995). It was therefore suggested that nucleotide substitution(s) leading to the amplification of $A02_{2,200}$ occurred in the cytoplasms of *Ae. ventricosa* and *Ae. crassa* 4x after their evolution. A second non-amplification, cytoplasm-specific polymorphism was detected as a 1.7 kbp fragment by OPD05 ($D05_{1,700}$) in C16 with the A^2 plasmon of *T. monococcum*. A third non-amplification, cytoplasm-specific polymorphism of a 1.1 kbp fragment by OPA13 ($A13_{1,100}$) was detected in male-sterile C14 with the T^2 plasmon of *Ae. mutica*. By contrast, $A13_{1,100}$ was present in C13 with male-fertile T plasmon of *Ae. mutica*, confirming the differentiation of these 2 plasmons (Terachi et al. 1990).

The last polymorphism detected was amplification of ca. 0.6 kb fragment by primer OPA01 (designated as $A01_{600}$) unique in C20 with the cytoplasm of *Ae. longissima* TL05. This polymorphism was assigned to the nuclear genome because $A01_{600}$ was amplified in the reverse hybrid obtained after replacing the cytoplasm with that of CS (Fig. 2C). Tsujimoto (1994) reported that one or a pair of the maternal, gametocidal 5SL chromosomes are present in C20 and that they are fully transmitted to their selfed and backcrossed progenies. Root-tip chromosome counting confirmed the presence of this chromosome, thus suggested that $A01_{600}$ was derived from the gametocidal chromosome present in this NC hybrid.

In conclusion, we could effectively detect plasmon-specific polymorphisms using the NC hybrids of common wheat cv. CS having cytoplasms from *Triticum* and *Aegilops* species. Plasmon-specific polymorphisms detected were either specific to single plasmons or mostly common to groups of phylogenetically related plasmons. In combinations of these markers, D, T, T^2, S^b and A^2 plasmons were identified, although D plasmon of *Ae. ventricosa* was exceptional. For the convenient identification of the cytoplasms in the series of NC hybrids, a complete set of plasmon-specific RAPD markers have to be selected by further study.

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Research article

Phylogenetic study of five morphological groups of hexaploid wheat (*Triticum aestivum* L. em Thell.) based on cytological analysis

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Summary

The genetic relationships among the five groups of hexaploid wheat: common, *spelta*, *macha*, *vavilovii* and semi-wild wheat (SWW) are not clear although wheat taxonomic and phylogenetic studies have been conducted for several decades based on morphological and cytogenetic analyses. A cytological study of a half-diallel cross involving common, *spelta*, *macha*, *vavilovii* and semi-wild wheat was conducted to assess phylogenetic relationships among these five morphological groups of hexaploid wheat. C-value coefficients of genetic similarity in this study were calculated based on the number of chiasmata in hybrids. The dendrogram based on the C-value coefficients suggests that common wheat is most closely related to *vavilovii* followed by *spelta* and SWW, and least related to *macha*.

Key words: *Macha*, *Spelta*, *Vavilovii*, Semi-wild wheat, Phylogenetic relationships.

Introduction

T. aestivum was divided into six subspecies based on morphological characters: *vulgare*, *sphaerococcum*, *compactum*, *spelta*, *macha* and *vavilovii* (MacKey 1966). However these subspecies have more recently been recognized as groups within *T. aestivum* with distinct morphological characteristics (Barnes and Beard 1992). Among these groups, *spelta*, *vavilovii* and *macha* have a fragile rachis and are not free-threshing (Singh et al. 1957).

Recently, another hexaploid wheat, semi-wild wheat (SWW), was found in Tibet (Shao et al. 1983). This wheat has a spring habit and grows as a weed in barley and wheat fields. Cytogenetic

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analysis suggested that the genomic constitution of SWW was AABBDD because it exhibited full chromosome pairing and high fertility when crossed with common wheat (Shao et al. 1983; Chen et al. 1988). Based on meiotic analysis of F_1 hybrids with euploid or double ditelosomics of *T. aestivum* cv. Chinese Spring, and also N-banding, Chen et al. (1988) concluded that the chromosome constitution of SWW was similar to that of the cultivar Chinese Spring. Morphologically, SWW has a rachis fragility and a non-free threshing characters which distinguish it from common wheat. When SWW matures, its spikelets separate naturally and fall to the ground. Although wheat taxonomic and phylogenetic studies have been conducted for several decades based on morphological and cytogenetic analyses, the relationships among the five groups of hexaploid wheat: common wheat, *spelta*, *macha*, *vavilovii* and semi-wild wheat (SWW) are not clear. The objective of this study was to determine the phylogenetic relationships among these five morphological groups of hexaploid wheat based on cytological analysis.

Materials and methods

The Canadian cultivar Columbus, PI 355512, PI 348636, PI 428343 and semi-wild wheat (SWW), representing the groups common wheat, *macha*, *spelta*, *vavilovii* and SWW, respectively, were used in the cytological study. Crosses for a half-diallel among these wheats were made in the greenhouse. F_1's and their parents were planted in the field and young spikes were collected between 10:00 am and 1:00 pm and fixed in Carnoy's solution (6:3:1, alcohol:chloroform:acetic acid) for meiotic studies. The number of chiasmata was estimated for each F_1 and its parents by observing approximately 100 pollen mother cells and calculated by the following formula: estimated number of chiasmata = 2(number of ring bivalents) + 1(number of rod bivalents) + 2(number of trivalents) + 4(number of ring quadrivalents). The C-value (Driscoll 1979) is the observed number of paired chromosome arms per cell as a proportion of the theoretical maximum (42). Thus, the C-value was calculated by dividing the estimated number of chiasmata by 42. The C-value of an F_1 is a measure of the chromosome homology between two parents. The greater the C-value of the F_1, the more homologous the chromosomes of the two parents. Thus, the C-values were used as genetic similarity coefficients in this study.

Results

Chromosome pairing data are presented in Table 1. All parents had over 20 ring bivalents per pollen mother cell (PMC) except SWW with 19.81. Trivalents and quadrivalents were not found and the univalent frequency was very low in the parents. The number of ring bivalents ranged from 13.22 to 19.27. Most F_1's involving *macha* had poor chromosome pairing. In these F_1's, the number of univalents ranged from 0.75 to 4.64 per PMC. In addition, F_1's involving SWW also had a relatively high number of univalents with the exception of the *vavilovii*/SWW F_1. The F_1 of common wheat with *vavilovii*, however, had good chromosome pairing as did the parents, exhibiting only 0.04 univalents. The F_1's of *spelta* with common wheat and *vavilovii* had higher chiasma frequency compared to the F_1's of *spelta* with *macha* and SWW. The C-values of F_1's in Table 1 were used as genetic similarity coefficients. Based on these genetic similarity coefficients (Table 2), a dendrogram (Fig. 1) was constructed for the five groups of hexaploid wheat. Common wheat

Table 1. Chromosome pairing in five hexaploid wheats and their F_1's

Cross or parent	No. of cells observed	Mean chromosome configuration					X	C
		I	IIa	IIb	III	IV		
macha	97	0.02	20.01	0.98	0.00	0.00	41.00	0.976
vavilovii	104	0.00	20.73	0.27	0.00	0.00	41.73	0.994
SWW	243	0.06	19.81	1.16	0.00	0.00	40.78	0.971
spelta	208	0.02	20.23	0.76	0.00	0.00	41.22	0.981
CW	212	0.03	20.43	0.56	0.00	0.00	41.41	0.986
CW/*macha*	211	0.97	17.37	3.08	0.02	0.02	37.92	0.903
CW/SWW	230	1.06	17.04	3.41	0.01	0.01	37.49	0.893
CW/*spelta*	165	0.67	17.60	3.04	0.01	0.01	38.27	0.911
spelta/*macha*	211	2.68	14.81	4.83	0.01	0.01	34.50	0.821
spelta/*vavilovii*	136	0.19	18.33	2.55	0.01	0.00	39.24	0.934
spelta/SWW	200	2.58	14.50	5.20	0.02	0.00	34.22	0.815
vavilovii/CW	99	0.04	19.27	1.65	0.00	0.00	40.19	0.957
vavilovii/*macha*	105	0.75	17.08	3.53	0.01	0.00	37.69	0.897
vavilovii/SWW	191	0.46	17.75	3.02	0.00	0.00	38.52	0.917
macha/SWW	170	4.64	13.22	5.46	0.00	0.00	31.91	0.760

I: Univalent, IIa: Ring bivalent, IIb: Rod bivalent, III: Trivalent, IV: Quadrivalent, X: Estimated number of chiasmata, C: C-value, SWW: Semi-wild wheat, CW: Common wheat

Table 2. Genetic similarity coefficients among five hexaploid wheats based on C-values of F_1 hybrids

	CW	SWW	*spelta*	*macha*	*vavilovii*
CW	–				
SWW	0.89 (0.05)[a]	–			
spelta	0.91 (0.05)	0.81 (0.06)	–		
macha	0.90 (0.06)	0.75 (0.06)	0.82 (0.06)	–	
vavilovii	0.96 (0.05)	0.92 (0.05)	0.93 (0.07)	0.89 (0.07)	–

CW: Common wheat, SWW: Semi-wild wheat, ( )[a]: Standard deviation

was clustered with *vavilovii*. The genetic similarity coefficient for the two wheats was 0.957. *Spelta* was not included in the common wheat and *vavilovii* cluster but diverged only slightly as evidenced by a genetic similarity coefficient of 0.921, followed by SWW (0.875) and *macha* (0.843).

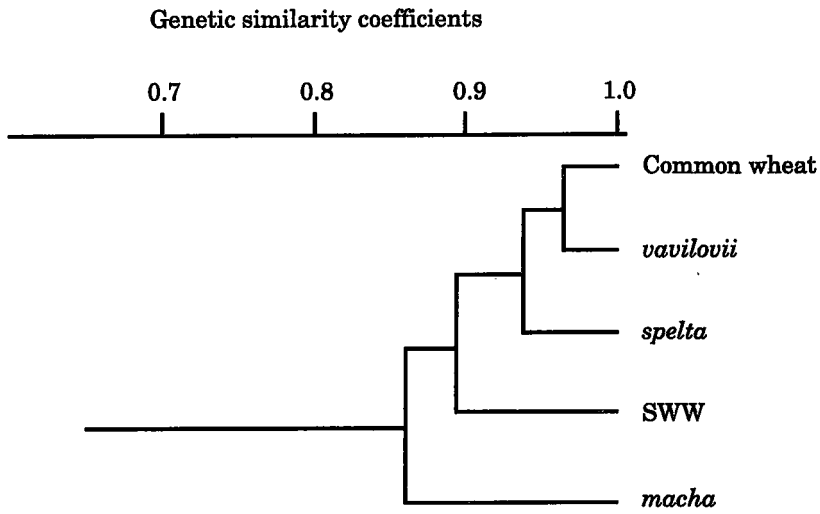

Fig. 1. Dendrogram of five hexaploid wheats based on chromosome pairing in F_1's.

Discussion

Chromosome pairing information can be used to infer phylogenetic relationships between species (Jauhar 1988). A good measure of chromosome homology and the effectiveness of pairing is chiasma frequency. In general, a higher chiasma frequency indicates better pairing and greater homology among the parental chromosomes (Jauhar and Joppa 1996). In the current study, chiasma frequency was investigated in the hybrids from a half-diallel of five accessions representing the five groups of wheat: common, *spelta*, *vavilovii*, *macha* and semi-wild wheat. Chiasma frequency varied from 31.91 to 40.12 among the 10 hybrids. Theoretically, two chiasmata can occur in a very long chromosome arm, however, the actual frequency of two chiasmata on the same chromosome arm, is extremely low because of the interference effect of chiasmata (Sybenga 1972). Instances of more than one chiasma in a pair of chromosome arms were not considered in this study.

The reported high levels of chromosome pairing in hybrids of common wheat (Chinese Spring) with *spelta* wheat suggested that common wheat and *spelta* have a common origin (Riley et al. 1967). They found that the F_1's between common wheat and *spelta* or *vavilovii* showed only a single chromosome translocation, however, F_1's between common wheat and *macha* showed two translocations in metaphase I of meiosis. In the current study, translocations were not found between common wheat and *spelta*, *vavilovii* or *macha*. However, most F_1's involving *macha* had relatively poor chromosome pairing. This would suggest that *macha* is distantly related to common wheat, *spelta* and *vavilovii*. Sachs (1953) found an average of 20.60 bivalents/cell in the F_1 of *spelta* with *vavilovii*, 18.75 in the F_1 of *vavilovii* with *macha* and 18.66 in the F_1 of *spelta* with *macha*, indicating that *spelta* and *vavilovii* are more closely related to each other than they are to *macha* wheat. In the present study, the mean number of bivalents/cell was 20.92 in the F_1 of common wheat with *vavilovii*, 20.61 in the F_1 of *vavilovii* with *macha* and 20.45 in the F_1 of common wheat with *macha*. The dendrogram (Fig. 1) based on C-value coefficients suggests that

common wheat is most closely related to *vavilovii* followed by *spelta* and SWW, and least related to *macha*. However, *Ph* gene might affect chromosome pairing. The genetic relationships among these five groups of wheat need to be conformed by modern tools, such as random amplified polymorphic DNA, amplified fragment length polymorphism etc.

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Research information

Genotypic variation for chlorophyll content and leaf area in wheat and their relation to grain yield

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Wheat (*Triticum aestivum* L.) is an important cereal crop of world and a staple food of the people of Pakistan. In order to feed the ever growing population increase in the cultivated land area has a limitation due to the problems waterlogging, and salinity, drought and unavailability of new lands. Methods to increase yield per unit area have therefore, to be explored. Although with the advent of high yield varieties, the increase in wheat grain production has been commendable during the past few years, there is scope yet for improvement. Since yield is the result of genotype by environment interaction, it has been suggested that by increasing photosynthetic efficiency, productivity could be increased (Rosenow et al. 1983). It is known that photosynthetic efficiency depends on leaf area, chlorophyll content and the stomatal response/gas exchange. Therefore, it is worth determining these parameters and analyzing the correlation if any, in various locally available genotypes.

A preliminary experiment was conducted in the field at AEARC, Tando Jam, Pakistan, using 16 wheat genotypes with five replicates. Fertilization was done @ 172 kg N, 115 kg P_2O_5 and 5.6 kg K ha^{-1} with normal cultural practices. Ninety days after sowing, six upper leaves of each tiller of each plant were excised and their area was measured with a Leaf Area Meter (LI-3100; LI-COR, Inc, USA) and average/leaf was calculated. Chlorophyll content (a, b and total), of these leaves was determined according to Arnon (1949) and the yield per plant was determined at maturity.

The results showed variation for the chlorophyll content of the genotypes tested (Table 1). As the chlorophyll content was calculated on per gram fresh weight however, if same was done on per leaf, wide variations may be recorded as the case with Vu et al. (1987) and Ashraf et al. (1994) who also indicated that the plants with higher chlorophyll content may have higher photosynthetic efficiency. On the basis of these facts, the authors seem to be largely equivocal in suggesting a direct relationship between chlorophyll content and rate of photosynthesis (Vu et al. 1987; Ashraf and Khan 1993; Ashraf et al. 1994). By contrast, wide variation for chlorophyll content, have also been reported (Wright et al. 1983; Sinha and Patil 1986; Ashraf and Khan 1990; Estill et al. 1991). Leaf area and grain yield per plant also varied significantly in these genotypes. There are many reports indicating that genotypes with higher leaf area may have higher grain yield (Duncan et al. 1981; Rosenow et al. 1983; Ludlow and Muchow 1990; Ashraf et al. 1992). The highest leaf

Table 1. Chlorophyll content (a, b and total), leaf area and yield per plant of different wheat genotypes.

Genotype	Chl*-a	Chl*-b	Total-Chl*	Leaf area (cm^2)	Yield per plant (g)
	(mg /g fresh weigh)				
SI-8927	1.119a	0.409ab	1.528ab	22.03fg	6.09cdef
SH-8918	1.141a	0.409ab	1.550ab	24.91ef	8.52a
SH-8921	1.126a	0.409ab	1.535ab	39.12a	5.90def
SP-89126	1.047a	0.345b	1.393b	25.22ef	5.52ef
SP-89128	1.099a	0.406ab	1.505ab	21.62fg	6.54bcde
SI-9077	1.114a	0.340b	1.455ab	31.70bc	7.18bc
SH-90157	1.021a	0.371ab	1.392b	27.93cde	6.23cdef
PN-9044	1.165a	0.385ab	1.551ab	20.21g	5.20fg
PN-9005	1.098a	0.379ab	1.477ab	22.58fg	6.56bcde
PN-9041	1.133a	0.408ab	1.541ab	25.70ef	7.10bc
PN-9083	1.027a	0.349b	1.376b	18.97g	5.42fg
PN-9086	1.100a	0.444a	1.544ab	26.90de	7.12bc
PN-90111	1.030a	0.367ab	1.397b	33.02b	7.37b
Sarsabz	1.148a	0.448a	1.596a	30.36bcd	6.72bcd
Soghat-90	1.056a	0.397ab	1.453ab	29.85bcd	4.46g
Mehran-89	1.770a	0.441a	1.618a	28.70cde	7.54b

Means in the same column sharing the same letters did not differ significantly according to Duncan's multiple range test ($P \leq 0.05$). * Chlorophyll.

area was observed in SH-8921 and the highest yield per plant in SH-8918, but the non significant positive correlations were recorded among total chlorophyll content (r=0.37), leaf area (r=0.16) and grain yield per plant. However in many cases in present study, it was observed that varieties with higher chlorophyll content or leaf area may have higher grain yield per plant, which is also confirmed by the correlation values which are positive but non significant. Therefore, these results show that leaf area and chlorophyll content cannot be correlated with higher grain yield in the genotypes tested.

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Research information

Comparative performance of semi-dwarf wheat (*Triticum aestivum* L.) genotypes.

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Plant height in wheat (*Triticum aestivum* L.) and its relationship to grain yield has long been of interest to plant breeders. In wheat the GA-insensitive semi-dwarfing genes *Rht-B1b* (*Rht1*) and *Rht-D1b* (*Rht2*) have been successfully utilized by plant breeders worldwide for more than three decades. The yield advantage of wheats carrying the GA-insensitive semi-dwarfing genes is only partly due to the direct effect of the genes on plant height and increased lodging resistance (Gale and Youssefian 1985). Field experiments analyzing near-isogenic lines for GA-insensitive dwarfing genes clearly demonstrate that positive pleiotropic effects on increased number of grains per spike result in higher yields under most environmental condition (Borner et al. 1993; Flintham et al. 1997; Jamali and Ahmad 1998).

To feed the increasing population of Pakistan there is continuous and perpetual need to evolve new high yielding wheat varieties. The aim of this study was to compare the semi-dwarf genotypes for their yield and other agronomic characteristics. The trial was consisted of five F_6 semi-dwarf genotypes with four commercial varieties viz. Sarsabz, Soghat-90, Anmol and Mehran-89. The genotypes were planted in six rows, with row length of 4 meters in a randomized complete block design with three replicates. The pedigree/parentage of genotypes is presented in Table 1.

Table 1. List of pedigree of genotypes under study

Genotypes	Pedigree
9-6	Cham4//Bow'S'/Dove'S'
25-1	Vee#7/High Protein
29-2	Tsi/Vee#5'S'//Nkt'S'
30-2	Tsi/Vee#5'S'//Nkt
37-1	Kauz'S'//Prl'S'/Vee#6
Sarsabz	(Pit. Frond)(Pit. Mazoe)/Mexipak
Soghat-90	Mutant of Pavon
Anmol	Kvz/Trm/Ptm/Ana (parent variety Lira)
Mehran-89	Kvz/Buho'S'//Kal/BB

Table 2. Comparative performance of wheat genotypes.

Genotypes	Days to heading	Plant height (cm)	No. of tillers	No. of spike-lets	No.of grains per spike	Grain yield per spike	Grain weight (mg)	No.of grains per spikelet	Plot ($4.8m^2$) yield (kg)
9-6	98.3b	76.2cd	483.7a	20.8b	50.0b	1.6c	33.2d	2.4b	1.1a
25-1	99.7a	93.5a	283.3cd	25.3a	65.0a	2.3a	35.4cd	2.6b	1.0a
29-2	94c	77.5bc	254.7d	21.2b	66.7a	2.5a	38.2ab	3.1a	0.9a
30-2	83f	72.9d	260.0d	21.1b	62.7a	2.5a	40.8a	3.0a	1.1a
37-1	88.7d	72.7d	328.0bc	20.5b	64.5a	2.4a	37.0bc	3.2a	1.2a
Sarsabz	85.3e	78.7bc	287.7bcd	20.8b	63.1a	2.5a	38.9ab	3.0a	1.1a
Soghat-90	93.7c	75.4cd	350.7b	21.3b	52.1b	1.9b	37.0bc	2.5b	1.2a
Anmol	89.3d	80.7b	315.7bcd	20.9b	63.3a	2.5a	39.5ab	3.0a	1.1a
Mehran-89	97.7b	81.0b	280.7cd	21.5b	63.9a	2.4a	38.2ab	3.0a	1.1a

Means followed by the same letters do not differ significantly at 5% level.

The genotypic comparison results are presented in Table 2. In this comparison all the genotypes were not significantly different from each other for grain yield per plot. The reasons for the non-significant differences may be due to saline patches in the experimental area, as we could not conduct the soil analysis. However, the line 37-1 and Soghat-90 had the highest yield and the line 29-2 the lowest yield. The possible reason for the lowest yield in line 29-2 may be due to reduced number of tillers per unit area. Number of tillers is one of the important yield components which affects the final yield. In this comparison, line 25-1 was late in heading, tallest in plant height and possessed the highest number of spikelets. Line 9-6 had the highest number of tillers with decreased main spike yield, grain weight (mg) and number of grains per spikelet. Final yield is a complex character and depends on its various components. Genotype, environment and its interaction may also significantly affect the yield. Each genotype has its own strategy to produce more yield.

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Long anther trait of rye (*Secale cereale* L.) – its chromosomal location and expression in bread wheat (*Triticum aestivum* L.)

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Rye (*Secale cereale* L.), a relative of bread wheat (*Triticum aestivum* L.), has contributed substantially in the genetic improvement of the latter. In most of the cases, rye chromatin conferring resistance to biotic and abiotic stresses has been transferred in bread wheat. At the same time, there are certain other traits, which can also be exploited in wheat improvement. One such trait is anther length, which is markedly more in rye than wheat. This trait can act as good morphological marker and has potential to be exploited in hybrid wheat breeding program if incorporated into the wheat genome. However, the chromosomal location of this trait and expression in the genomic background of bread wheat has not been reported.

The material for the present study comprised Chinese Spring wheat, Imperial rye, and a set of addition lines of the former with individual added chromosome of the latter, obtained from the Wheat Genetics Resource Center, Kansas State University, Manhattan, USA. Each genotype was raised in pots under similar conditions. Length of anthers from the florets of the central spikelet of 5 plants was recorded just before dehiscence under a stereoscopic microscope. Mean anther length of the genotypes under study was compared using t-test.

Data on mean anther length of the test genotypes (Table 1) depicted that rye had the longest anthers. Of the 7 addition lines, the one with 4R chromosome of rye had significantly longer anthers than the remaining 6 addition lines as well as Chinese Spring wheat, which were statistically at par with one another. These results support that the rye chromosome 4R is responsible for increased anther length. However, anther length of this addition line was statistically shorter than that of the rye parent. It is evident that the expression of anther length of rye gets reduced to an intermediate level in the genetic background of bread wheat. It is also a common observation that the anther length of rye gets reduced in its amphiploid with durum wheat, the triticale (x *Triticosecale*). Thus, the expression of the trait gets diluted in the genomic background of both durum and bread wheat, may be due to intergenomic interactions.

In the recent past, considerable progress has been made in the development of hybrid wheat. One of the bottlenecks in the successful production of commercial hybrid wheat is the poor seed set on the male sterile line by natural cross-pollination. The extent of hybrid seed set largely depends upon the amount of pollen shed by the pollinator/restorer (Wilson 1968), which is directly correlated with anther size (Beri and Anand 1971). Jost and Milohnic (1976) found significant positive correlation of restoration ability with anther length. It has been estimated that pollen

Table 1. Mean anther length (mm) of Chinese Spring wheat, Imperial rye and 7 addition lines

Genotypes	Anther length (Mean ± SE)
Chinese Spring (CS)	2.75 ± 0.012
Imperial (Imp)	7.13 ± 0.112
CS/Imp 1R	3.00 ± 0.049
CS/Imp 2R	2.55 ± 0.124
CS/Imp 3R	3.22 ± 0.070
CS/Imp 4R	4.24 ± 0.124
CS/Imp 5R	2.90 ± 0.058
CS/Imp 6R	2.62 ± 0.058
CS/Imp 7R	2.73 ± 0.095

production per inflorescence in wheat is only about 10% of that in rye. It is a common observation that triticales have longer anthers than wheat and also produce more pollen. So far, no emphasis has been laid to exploit the alien sources for transfer of long anthers in wheat, which can otherwise be useful in hybrid wheat production program.

The chromosome 4R of rye, which is involved in the expression of long anthers, also contains gene(s) for purple coleoptile and culm, *Pc* (Miller 1984), aluminum tolerance, *Alt* 3 (Aniol and Gustafson 1984), powdery mildew resistance, *Pm 6* (Lind 1982), and waxy endosperm, *Wx* (Korzun et al. 1997). Moreover, this chromosome contains a gene for male fertility restoration, *Rfc* 2 (Hossain and Driscoll 1983), which if linked to long anthers can have added advantage to exploit rye-introgressed wheats having these traits to be used as restorers/pollinators in hybrid wheat production program. Also, the trait under study can act as a good morphological marker in the basic and applied aspects of crop improvement. Using the available genetic manipulations, it is possible to introgress the rye chromatin conferring long anthers into bread wheat, which will be a boon in hybrid wheat production technology.

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Wheat Information Service
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Research information

Evaluation of cereal cyst nematode (*Heterodera avenae*) resistant wheat variety in Rajasthan, India

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The cereal cyst nematode CCN (*Heterodera avenae*) causing 'molya' disease in wheat crop is a severe problem in light sandy areas of India, like state of Hariana, Punjab, Himachal pradesh, Western part of U.P. and Rajasthan. The wheat grown in thirteen districts of Rajasthan state is being suffered by the infection of molya disease incited by *H. avenae* every year. The total area of wheat cultivation in Rajasthan is about 22.41 lac hectare (1998). Out of that about 1.5 lac hectare is mapped suffering with this nematode. This disease is used to cause about 40-50 % yield losses, which may attain up to 60-65% at its severity (Mathur 1969; Mathur et al. 1980). Looking towards the state wheat production (67.8 lac ton), the CCN suffered area (1.5 lac. ha) instead of producing 4.5 lac ton, only yielding 2.25 lac ton (1998-1999) and grain loss in terms of money amounts up to about Rs. 11.25 crores. Earlier certain efforts using cultural practices and chemicals were also exercised, but to achieve easy and economic goal, the breeding for the disease resistant program be initiated since 1991 for development of CCN resistant variety for tangible advancement in wheat production in molya infected areas of the country.

About 5000 wheat entries (genotypes / strains) received from exotic (ICARDA, CYMMIT,

Table 1. Cross combinations for CCN resistant in wheat

Cross combination	Resistant genotype
J-24 x AUS-15854	*CCNRV$_1$
Raj. 2184 x AUS -15854	CCNRV$_2$
Raj. 3077 x AUS - 15854	*CCNRV$_3$
Raj. 2329 x AUS -15854	CCNRV$_4$
Raj. 2535 x AUS -15854	CCNRV$_5$
H.D.2009 x AUS -15854	CCNRV$_6$
Kalyan sona x AUS -15854	*CCNRV$_7$

*Cereal cyst nematode resistant variety

Australia) and indigenous (NPGR & DWR) resources were screened in naturally and artificially CCN infested field conditions. Of the evaluated germplasm lines, one strain namely AUS-15854 was observed resistant to CCN in field condition. Further this strain was again vigorously tested for CCN resistant in subsequent years. Simultaneously, to find out the genetic behavior of this CCN resistance, an experiment was also planned out for three years. The results revealed that a single dominant gene controlled the CCN resistance in AUS-15854 wheat genotype.

Hybridization program that used AUS-15854 as donor parent was undertaken using high agronomic traits bearing varieties to involve CCN resistant gene. The promising and popular seven wheat varieties were developed through hybridization followed by pedigree method in naturally infested field conditions. The cross selected combination for this program is as shown in Table 1.

The selection of CCN resistant plants under CCN infested field conditions was made in different subsequent generations (F_2 - F_5) in each cross. Promising and desirable CCN resistant progenies were selected in each cross in F_6 generations. The yield trial was carried out to evaluate yield performance of evolved lines against most popular and widely cultivated wheat variety Raj.3077. Out of 7 cross progenies only three best yielders and CCN resistant genotypes ($CCNRV_1$, $CCNRV_3$, $CCNRV_7$) were selected for further testing program in large CCN infested areas of the state.

After generating sufficient seeds of above said three genotypes, trials were conducted at about five dozens of CCN infested cultivators field (at 6-10 larvae/g soil). During experiment a CCN susceptible but widely grown variety (Raj. 3077) was kept as compared check. The result of trials (Table 2) exhibited that all the three CCN resistant lines were recorded significantly higher grain yield over the check variety Raj. 3077. However, all the three lines were observed to be at par in grain yield. Of these three lines, $CCNRV_1$ possess better grain quality and more straw yield than others ($CCNRV_3$, $CCNRV_7$). Thus $CCNRV_1$ line could be used for planting in CCN infested areas as well as for better straw yield.

Execution of large scale field trials are also going on under mega environments of the state to confirm higher yield potential along with other desirable traits. The release of such types of

Table 2. Performance of selected cereal cyst nematode resistant lines under field conditions

Wheat lines	Plant height (cm)	Tillers per plant	Ear length (cm)	Grain yield (q/ha)	Straw yield (q/ha)	Nematode reaction		Root growth
						No. of Cyst/plant	Reaction*	
$CCNRV_1$	84.68	6.00	12.05	40.77	72.49	1.76	Resistant	Normal
$CCNRV_3$	77.26	7.10	12.91	39.85	70.77	2.31	Resistant	Normal
$CCRNV_7$	81.40	6.01	11.56	38.58	67.15	3.28	Resistant	Normal
Raj.3077 (Local check)	55.78	2.48	7.50	24.18	44.00	13.31	Susceptible	Deformed & twiggy
L.S.D.(5%)	2.08	0.19	0.60	3.05	1.24	1.90		

Data are the averages of 6 CCN infested locations

*Scale of nematode (CCN): 0–4.0 cysts/plant=Resistant, 4.1–9.0 cysts/plant=Moderately resistant, 9.0–20.0 cysts/plant=Susceptible, 20.0 and above cysts/plant=Highly susceptible.

varieties shall certainly be revolutionized the wheat production in the CCN infested areas of different states of India. This would also be helpful to produce extra grain for rapidly growing population of the country.

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Wheat Information Service
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Proposal

Call to support an English translation of the 1979 Russian taxonomic monograph of *Triticum* by Dorofeev et al.

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While wheat researchers are familiar with the existence of the Russian monograph of *Triticum* L. (Dorofeev et al. 1979), this important taxonomic work is unavailable to the majority of them because of the language barrier. Acceptance also has been problematic because the monograph follows a traditional treatment concept that recognizes all morphological forms of wild and domesticated wheats and excludes the wild species of *Aegilops* L. This taxonomic approach had already fallen out of favor by the time of its publication in 1979. Thus, Dorofeev et al. has remained in relative obscurity under the dominating influence of the genetic concept of the wheat complex as embodied in the treatments of Morris and Sears (1967), Kimber and Sears (1987) and Kimber and Feldman (1987).

Taxonomy is usually a minor concern for wheat geneticists. However, it promises to play a significant role in the protection of germplasm diversity and intellectual property rights, issues of growing importance in the developing research arena and commercial markets of biotechnology. By virtue of its detailed morphological classification, Dorofeev et al. has direct application to all aspects of biodiversity research – *i.e.*, preservation, cataloguing, and utilization. This monograph provides the only comprehensive worldwide catalogue of all known infraspecific taxa of domesticated and wild wheat species. It is the culmination of a significant scientific effort that dates back to the time of Vavilov's leadership of the systematic wheat research in Russia. Dorofeev

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et al. can serve as an authoritative reference for both identifying distinct forms of wild and domesticated wheat and challenging the validity of proprietary claims on wheat genes and genetic lines that rightfully belong within the public domain.

The scientific value of Dorofeev et al. should not be underestimated. To produce such a taxonomic monograph *de novo* would be extremely difficult in the current research funding climate. Additionally, the combined knowledge and expertise of its authors cannot now be reproduced. An English version would open a wealth of information to botanists, plant breeders, geneticists, genebank managers, and others in the wheat research community. The 25 species and 1,242 infraspecific taxa described in Dorofeev et al. are fully catalogued with botanical descriptions, taxonomic keys, geographic distribution, disease traits, origin, and history. In nomenclature, Dorofeev et al. is unique among the modern taxonomic treatments of *Triticum* for its detailed synonymy and comprehensive compilation of names – over 3000 names for the wheats are listed in the index.

This project evolved from informal discussions that took place in July 1999 during the Percival Symposium (*Wheat – Yesterday, Today and Tomorrow*; University of Reading, UK). Our goal is to finance a quality translation that will be published at an affordable price. Any profits made from an English version of Dorofeev et al. will go into a fund for translation of other significant Russian scientific publications dealing with wheat. In February 2000, permission to proceed with the English translation was obtained from the Vavilov Institute (VIR, St. Petersburg, Russia), the holder of the copyright to the Russian edition of Dorofeev et al. A translator has been identified and various options for publication are currently being explored.

CIMMYT recently pledged $ 5000 to the project fund established to finance the translation and publication. We are posting this announcement to alert the research community of the need for this English translation and to request donations to the project fund. A minimum of $ 5000 in matching funds will be required to support the costs of translation and publication. Individuals and research entities wishing to help with meeting this funding goal should contact Laura Morrison at the above address for instructions on submitting donations. All contributions will be acknowledged in the published translation.

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Wheat Information Service
Number 90:54 (2000)
Report

GrainTax Synonymy Tables Project: June 2000 Progress Report

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Classification Tables for 28 taxonomic treatments of *Triticum* and *Aegilops* are now posted on the *GrainTax* web site (http://wheat.pw.usda.gov/ggpages/GrainTax/). All of these taxonomies were produced during the 20th century, and as a whole they present a confusing array of inconsistent names and competing claims of accurate phylogenetic concepts. While the Classifications Tables do not alleviate this problem, they do offer a reliable reference for treatments that are encountered in the literature and in communications among members of the wheat research community. Researchers are encouraged to make use of them for checking the correct citation of species names and for verifying the handling of taxa within a particular treatment.

Also available on the *GrainTax* site are the following supplementary tables: Table of Authorities with correct spelling and abbreviations for the publishing author of valid taxonomic names; Synonym Table with a list of the most commonly encountered synonyms; Rejected Name Table with a list of illegitimate, invalid, and ambiguous names. In the next stage of the project, cross-referencing links will be built to join names to classifications and to relate valid names to their synonyms.

The goals of the *GrainTax* project will best be realized with the cooperation of the wheat research community (Morrison and Raupp 1999). The Tables are now easily accessible and, when followed, ensure consistency in the use of taxonomic names. To avoid confusion in referencing wheat taxa (e.g., in research articles or in germplasm requests), it is advisable to cite the classification that is being followed and to follow the classification without change to the names of taxa, their ranking, and their authority citation.

References

Morrison LA and Raupp WJ (1999) *GrainTax* Synonymy Tables Project: June 1999 Progress Report. Wheat Inf Serv 88: 52-56.

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Wheat Information Service
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Information

PRELIMINARY INFORMATION

Genetic Collections, Isogenic and Alloplasmic Lines (International Conference)

Tentative date: July–August 2001
Location: Institute of Cytology and Genetics SB RAS, Novosibirsk, 630090, Russia
Working languages: English and Russian
Address for correspondence: Dr. Sergey F. KOVAL, Institute of Cytology and Genetics SB RAS, 630090, Novosibirsk, RUSSIA. Tel: (383-2) 33-34-62; Fax: (383-2) 33-12-78; E-mail: kovalvs@bionet.nsc.ru

Any genetic collections (testers on individual genes, substitution, near-isogenic, or alloplasmic lines) are of great value not only with respect to gene pool preservation, but also as model objects for studying gene-genotype or plasmon-genome interactions. Near-isogenic lines are used for genome molecular mapping and as testers in genetic analysis. Substitution and alloplasmic lines have become an indispensable tool in studies of phylogeny and systematics. CMS effects have found a wide application in practical breeding. Abundant information on alteration of nuclear gene manifestations under the plasmon effect has been accumulated.

However, numerous methodological questions on forming, maintaining, and using genetic collections (including isogenic and alloplasmic lines) require further discussing. There are no agreement on the similarity extent of the genotypes of recurrent parent and backcrossed lines. The remains of donor genetic material hinder the assessment of the phenotypic effect of either marker genes or cytoplasm. Worldwide banks of both data continuously maintained genotypes of tester, substitution, isogenic and alloplasmic lines are waiting to be formed. The existing material is dissolved in international and national gene pools, which are mainly of practical breeding importance. These and many other unsolved questions require a discussion by interested researchers.

The Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences, is involved in developing and investigating of substitution and near-isogenic lines. Twice, it organized the All-Union (USSR) Conferences on Genetic Collections, Isogenic and Alloplasmic Lines. These Conferences aided the information and material exchanges between the participants. Most interesting works presented were published in a volume on isogenic lines and three volumes on genetic collections.

Now, the Institute of Cytology and Genetics is planning to organize an international conference to discuss all these topics.

Would you, please, inform us on whether you are interested in participation in this Conference and publication in its proceedings.

We need to determine the number of possible participants of the Conference and would be very grateful for your advice on other scientists to be addressed with this Preliminary Information. Any comments or additions to the tentative program are welcome.

We propose to discuss the following topics:

1. Maintenance of genetic collections (as plants and tissue cultures; seed storage), their reproduction, nomenclature, and principles of their forming.
2. Databases (data storage, processing, and exchange).
3. Isogenic and substitution lines: use in gene molecular mapping, studies of marker phenotypic manifestations, and utility for genetic analysis of multiple markers.
4. Alloplasmic lines: plasmon-genome interactions, CMS, and use in phylogenetic and systematic studies.

Title/Name:_____
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Editorial remarks

We are glad to send you the 90th issue of Wheat Information Service in millennium year, in which author and subject index of last 10 numbers are included. From starting this year, a new editorial board has been organized; some of members of the board are new, and many have been re-elected (see the name of members on the back page). Thanks for their voluntary efforts for wheat science.

WIS has been developing gradually in recent years, that is revealed by the increased contributions of original research articles and research information. The editorial office has decided to change the printing block to A4 size from the next issue, which will allow more printing spaces. However, please remind the page limitation; five printing pages for research article, and two for research information.

Because the system for the scientific information service in Japan has recently changed, we are not able to publish "Recent Publications" from this issue. We appreciate continuous kindness of Cambridge Science Abstracts who allowed us to publish their information. Now era of Internet has been opened. We can obtain lots information through telephone line even from marginal regions. The "Recent Publication" in WIS may have performed its mission. Now, back issues of this journal can be read through Internet (http://www.shigen.nig.ac.jp/wheat/wis.html).

We thank continuous contribution of donation from many subscribers. This donation system had been established because of mutual helps for promoting exchange of research information. However, the present situation of financial affairs indicates that the contributed money covers only mailing costs. Please attention the spirit of donation system. Also, please use Credit Card (Visa or Master Card) or International Postal Money Order. Personal check costs more amounts of money for bank charge than the content.

The editorial office hopes good harvest of wheat crop to open the 21st century.

K. Nishikawa, T. Sasakuma, H. Tsujimoto, and K. Furukawa
(Editorial office)

May, 2000.

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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.

Business Office

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