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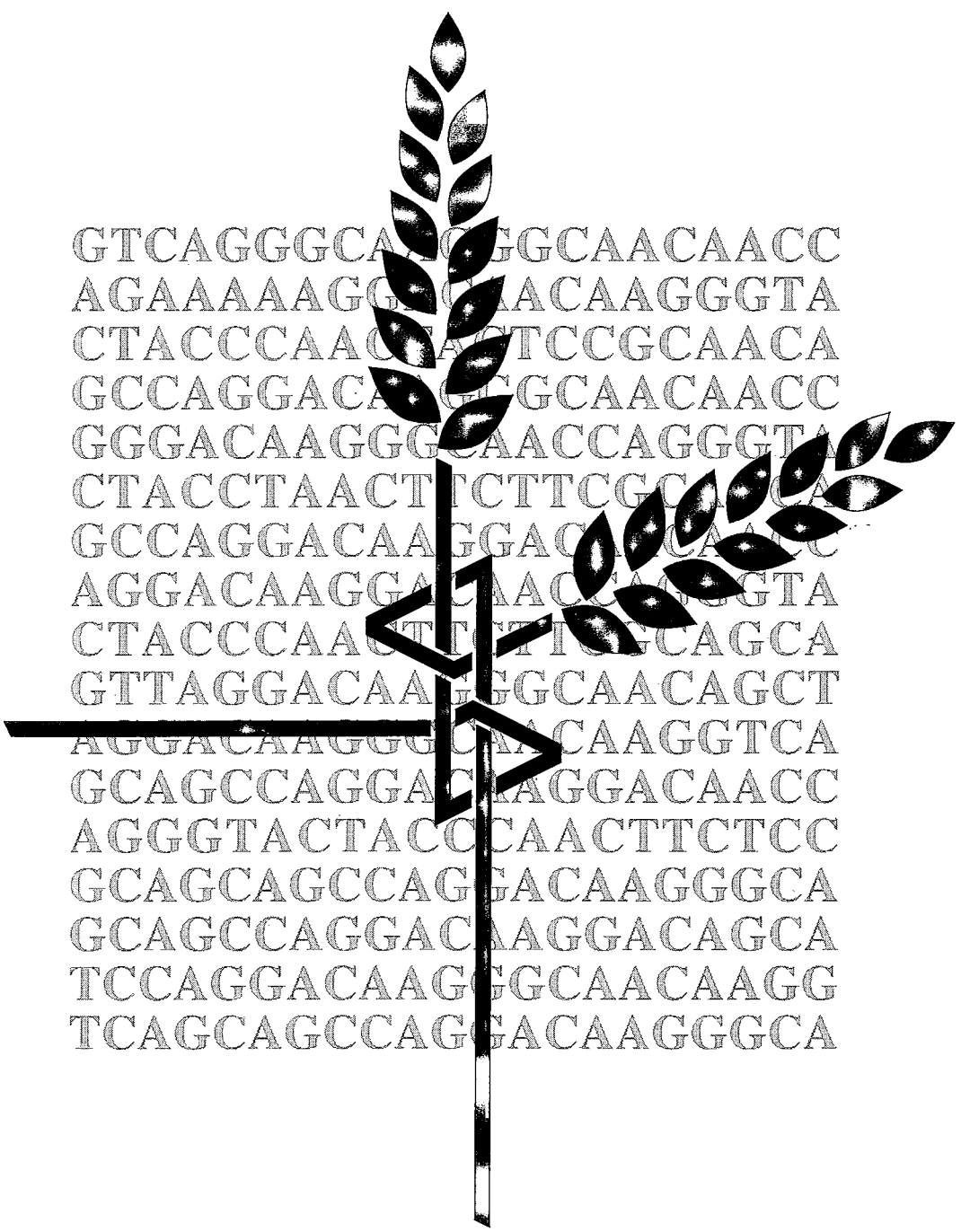

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Research article

Evidence of homoeologous introgression of the first and fourth groups of chromosomes of *Aegilops umbellulata* Zhuk. into wheat (*Triticum aestivum* L.)

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Summary

Homoeology between the chromosomes of the first and fourth groups in ten wheat lines derived from the population of F_4 BC_1 obtained after crossing between *T. aestivum* cv. Aurora and the wild species *Aegilops umbellulata* was investigated by analyzing the electrophoretic patterns of the gliadins, glutenins and albumins. Five lines expressed qualitative differences in two gliadin subunits GliB1 and GliA1 belonging to the ω- and γ-gliadins respectively and quantitative differences in the albumin subunits compared to cv. Aurora. The other five lines were indistinguishable from cv. Aurora based on the gliadins. The possession of *Ae. umbellulata*-type subunit composition of the gliadins and glutenins controlled by the chromosomes 1BS (locus *GliB1*), 1AS+1DS (locus *GliA1*) and 1AS (locus *GluB3*) and the quantitative differences in the albumin subunits controlled by the chromosomes 4DL and 4BL provide evidence of homoeologous introgression from the U genome of *Ae. umbellulata* into cv. Aurora.

Key words: wheat, *Aegilops umbellulata*, seed storage proteins, homoeologous relationships

Introduction

Besides cytological parameters (Maestra and Naranjo 1997), biochemical markers provide an effective means for determining the homoeologous relationships of alien chromosomes from the related species and chromosomes of hexaploid wheat. Protein and isoenzyme markers have been established for all homoeologous groups of chromosomes in tribe Triticeae (McIntosh 1988) and successfully used for determining the homoeology of the added or substituted alien chromosomes. William and Mujeeb-Kazi (1995, 1996) identified a high molecular weight (HMW) glutenin and beta-amylase markers for the presence of *Thinopyrum bessarabicum* and *Aegilops variabilis* chromosomes in amphiploids and disomic addition wheat lines. As the genes for HMW glutenins and beta-amylase isoenzymes are respectively located on the long arms of homoeologous group 1 chromosomes (Lawrence and Shepherd 1981) and 4A, 5A and 4D chromosomes (Ainsworth et al. 1983) in wheat, the observed markers could serve as evidence for determining chromosome homoeology between the two alien species with wheat. Similarly, by analyzing beta-amylase isoenzymes identification of alien chromosome and determination of chromosome homoeology of wheat were made in *T.aestivum-Aegilops markgrafii* amphiploid and addition lines of wheat (Schmidt et al. 1993). The study of the homoeology of the chromosomes by analyzing the protein patterns of interspecific hybrids thus provides effective approach as it enable to reveal the relationships between most of the wheat and alien chromosomes.

Aegilops umbellulata Zhuk. (syn. *Triticum umbellulatum*, 2n=14, UU) is a potential donor of a complex of genes for disease resistance (Sears 1954,

1956), early maturity and high grain protein content (Bochev 1988). With the objective of transferring genes of *Ae. umbellulata* chromosomes and establishing homoeology with the wheat chromosomes, a set of F_4 BC_1 lines have been selected from crosses between bread wheat cultivar Aurora and *Ae. umbellulata* (Ganeva et al. 2000). They were used to study the homoeologous relationships between the first and fourth chromosome groups of wheat and *Ae. umbellulata* by comparing endosperm protein markers.

Materials and methods

Ten hybrid wheat lines derived from the F_4 BC_1 population after crossing between *Triticum aestivum* cv. Aurora (2n=42, AABBDD) and the wild diploid species *Ae. umbellulata* Zhuk. (2n=14, UU) were investigated. As a bridge for the transfer of genes from *Ae. umbellulata*, the genome substituted line Aurolata was used in which the D genome of the cv. Aurora is substituted with the U genome of *Ae. umbellulata* (Zhirov 1989). Fig. 1 presents the breeding scheme. The wheat lines having 42 chromosomes were established in the Institute of Genetics 'D. Kostoff', Sofia, Bulgaria. The seed samples of *Ae. umbellulata* (accession No. C01) was obtained from the collection of the same Institute. The electrophoretic resolution of the gliadins was conducted according to Cooper (1987). The glutenins and albumins were extracted with buffers containing sodium dodecyl sulphate (SDS) and 2-mercaptoethanol according to Smith and Payne (1984) and Gupta et al. (1991). The glutenin and albumin subunits were fractionated in 12% polyacrylamide gel electrophoresis (PAGE) (Laemmli 1970) on the electrophoresis apparatus GE-2/4 (Pharmacia, Sweden) using power supply EPS 500/400. The gel size was 175/140/1.5 mm. The protein analysis was made individually using more than fifteen seeds from each of the wheat lines. For studies of *Ae. umbellulata*, mixed samples from fifteen individual seeds were used.

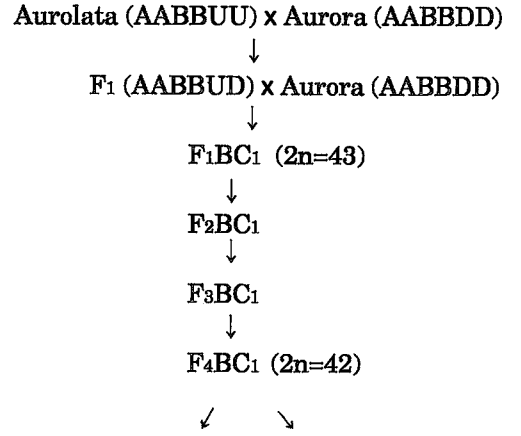

Fig. 1. Breeding scheme of the F_4 BC_1 wheat lines

Results and discussion

The wheat lines selected for this study showed considerable similarity in the overall composition of the three protein groups, i.e. gliadin, glutenin and albumin. However, some differences were observed in their gliadin patterns. Variants detected at the *GliB1*, *GliA1* and *GluB3* loci are shown in Table 1 together with their parental species. Two Aurora-specific gliadin subunits belonging to the ω- and γ-gliadins respectively controlled by chromosomes 1BS and 1AS+1DS (Shepherd 1973) were lacking in the five selected lines Nos. 10–14, similar to *Ae. umbellulata*, while they were present in the lines Nos. 15–19. A low molecular weight (LMW) glutenin subunit controlled by the *GluB3* locus on chromosome 1AS (Singh and Shepherd 1988) was present in the lines Nos. 10–14 similar to *Ae. umbellulata* and cv. Aurora but was absent in the lines Nos. 15–19. The probable reason for the absence of the mentioned LMW glutenin subunit is the repression of the locus in the chromosome 1AS, responsible for the same subunit in the lines Nos. 15–19.

Detectable variation occurred in several albumin subunits between cv. Aurora and *Ae. umbellulata* (Fig. 2). However, differences in the albumin patterns

Table 1. Gliadin and glutenin allelic variants in F_4 BC_1 wheat lines and the parental species

Line	Presence (+) and abcense (–) of loci†		
	GliA1	*GliB1*	*GluB3*
Nos. 10, 11, 12, 13, 14	–	–	+
Nos. 15, 16, 17, 18, 19	+	+	–
T. aestivum cv. Aurora	+	+	+
Ae. umbellulata	–	–	+

† *GliA1* and *GliB1* represent γ- and ω- gliadin loci, respectively (Payne et al. 1984) and *GluB3* represents a LMW glutenin locus (Singh and Shepherd 1988).

among the wheat lines were only quantitative. In the lines Nos. 15 and 16 (slots 5 and 7 in Fig. 2) the two subunits near the cathode (arrowed in the figure) resembled those of cv. Aurora by their higher intensity than those in the lines Nos. 10 and 11 (slots 4 and 6). It was reported that the above two albumin subunits in cv. Chinese Spring were controlled by chromosomes 4DL and 4BL (Gupta et al. 1991).

The data suggest that the introgression from *Ae. umbellulata* in the lines Nos. 10–14 involves the short arms of the chromosomes 1BS (locus *GliB1*), 1AS+1DS (locus *GliA1*) and 1AS (locus *GluB3*). This result is in agreement with the statement that at least two chromosomes controlling gliadins in *Ae. umbellulata* are homoeologous with two chromosomes in bread wheat (Shepherd 1973). Law and Payne (1983) located *GluU1* locus on the long arm of 1U chromosome of *Ae. umbellulata* and indicated the homoeology between this chromosome and the group 1 chromosomes of bread wheat. A possible explanation for the introgression of the gliadin and glutenin subunits controlled by the chromosomes 1BS and 1AS in the wheat lines Nos. 10–14 might be related to the position of their loci. The *GliB1* locus is located near the end of the short arm of the satellited region of the chromosome 1B (Payne et al. 1981) and the *GluB3* locus is also located on the short arm of the chromosome 1B (Payne and Lawrence 1983).

SDS-PAGE study of the protein bands controlled by 4DL and 4BL chromosomes indicated that they were albumins according to Gupta et al. (1991). The authors revealed by using immunoblotting that the bands correspond to beta-amylases of wheat grains. The participation of the same wheat chromosomes in the genetic control of β-amylase isoenzymes is established by Ainsworth et al. (1983). Considering the fact that the amount of qualitative variation in these proteins observed by SDS-PAGE is limited (Gupta et al. 1991), the quantitative variation in the albumins can serve as a potential marker for the presence of *Ae. umbellulata* chromosomes homoeologous to 4DL and 4BL of wheat.

The present results provide evidence of homoeologous introgression of the first and fourth groups of chromosomes of *Ae. umbellulata* into bread wheat. This information is useful for breeding purposes because it can be used in diagnostic detection of *Ae. umbellulata* chromosomes or chromosome segments in the wheat background.

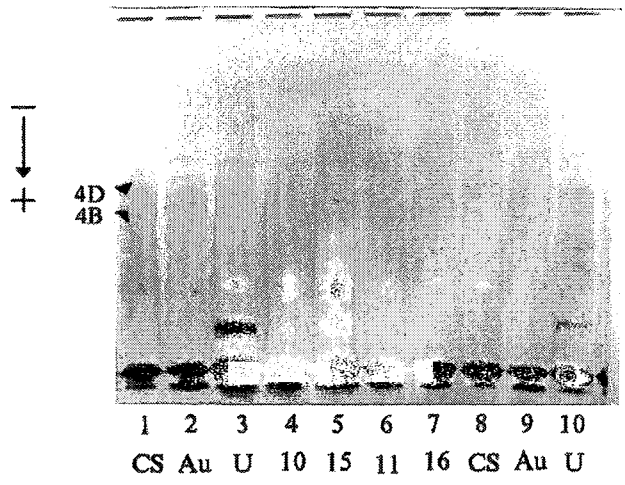

Fig. 2. SDS - PAGE patterns of albumins in F_4BC_1 wheat lines Nos. 10, 15, 11 and 16 (slots 4, 5, 6, 7), *T. aestivum* cv. Aurora (slots 2 and 9), *Ae. umbellulata* (slots 3 and 10) and the reference cv. Chinese Spring (slots 1 and 8). The albumins were obtained following the method of Gupta et al. (1991). The flour samples (10 mg) were treated in 1 ml bidistillated water for 1h at room temperature (21°C). The top 80% portion of the supernatant obtained after centrifugation at 15,000 g for 10 min was dried in evaporator at 40°C. The pellet was dissolved in 80 µl of 0.125 M Tris-HCl buffer containing 4% (w/v) SDS and 1.5% (v/v) 2-mercaptoethanol, and aliquots of 20 µl of this solution were loaded into each slot for elecrophoresis. The albumin subunits were fractionated in 12% PAGE (Laemmli 1970). The staining of gels was conducted by Coomassie Blue R-250-0.2 % in 12.5 % trichloroacetic acid.

Acknowledgments

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Research article

Genetic analysis of flag leaf area in durum wheat over environments

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Summary

Parental, F_1, F_2, BC_1, BC_2, BC_{11}, BC_{12}, BC_{21}, BC_{22}, BC_1 self and BC_2 self generations of three crosses involving six cultivars of durum wheat (*Triticum durum* Desf.) were studied for flag leaf area under normal and late sown environments to analyze the nature of gene effects. Various models i.e. 3-parameter model in the cross Cocorit-71 x A-9-30-1 and Raj 911 x DWL 5002 in late sown; 6-parameter model in the cross HI 8062 x JNK-4W-128 and Raj 911 x DWL 5002 and 10-parameter model in the cross Cocorit-71 x A-9-30-1 and HI 8062 x JNK-4W-128 were found adequate to account for the variability in generation means. Of the epistatic interactions, dominance x dominance (l) and dominance x dominance x dominance (z) played significantly greater role in controlling the inheritance of this trait. Absolute totals of non-fixable gene effects were much higher than the fixable gene effects in all the crosses in both the environments, indicating the greater role of non-additive effects in controlling the inheritance of flag leaf area in durum. Significant heterosis was attributed by the major combined effects of dominance (h) effect and dominance x dominance (l), additive x dominance x dominance (y) and dominance x dominance x dominance (z) epistatic interactions in the cross HI 8062 x JNK-4W-128 under late sown environment only. Restricted recurrent selection by the way of intermating the most desirable segregates followed by selection and diallel selective mating, which exploit both fixable and non-fixable components, have been suggested for the improvement of this trait.

Introduction

Grain yield is the ultimate aim for cereal breeders. The flag leaf makes a major contribution towards the grain yield of cereals. It contributes 41 to 43 percent to kernel weight and is the major photosynthetic site during the grain filling stage (Athwal 1968; Berdhal et al. 1972; Ibrahim and Abo Elenein 1977). In wheat grain yield is the end product of the interaction of a large number of physiological and biochemical processes in the plants and therefore, is genetically complex. Physiological studies in wheat have indicated that flag leaf contributes to the formation of about 60 percent of dry matter in the kernel at maturity. It is also the only leaf, together with a small contribution from the penultimate one, to carry out an activity essential to grain filling during the period from anthesis to maturity. Since the flag leaf plays a predominant role, its size likely to be important. The leaves being the major site of photosynthetic activity appears to have an obvious relation to the plant grain yield ability. Compared to other leaves the flag leaf contributes most photosynthetic assimilates in wheat and thus it assumes the greatest importance from the grain yield point of view (Lupton 1973). Monyo and Whittington (1973) have shown that leaf area is an indicator of potential grain yield in wheat. Vogele and Grossman (1985) found that flag leaf removal after ear emergence caused a 7 to 9% reduction in kernel weight. Similarly, grain yield and number of kernels /spike were reduced by 10.7 and 11.1%, respectively

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(Duwayri 1984), number of endosperm cells by 6 and 11%, single kernel weight by 10 to 29%, and grain yield by 15 to 25% (Natt and Hofner 1987). These results indicate the association of flag leaf with yield and its components in the positive sense. Many researchers (Briggs and Aytenfisu 1980; Mahmood et al. 1991; Adnan et al. 1994; Chowdhry et al. 1999) have reported a positive correlation of flag leaf with grain yield, number of grains/spike and kernel weight. These data justify the particular attention devoted by the breeder to this structure. Apart from the direct approach, the problem of yield increase may, in certain situations, be more effectively tackled on the basis of the performance of yield components and other closely associated characters. Therefore, this character would be of great importance as a criterion for selection to enhance the yield potential in durum.

Genetical studies for the inheritance of such an important trait was mostly based on diallel analysis (Ilyhchenko 1977; Hsu and Walton 1970; Jain and Singh 1976; Bariga 1980), which does not provide the estimates of different non-allelic interactions, could inflate the measure of additive and dominance components. To evolve a physiologically efficient and productive genotype in durum wheat, the knowledge of the different non-allelic gene actions, operating in the inheritance of the physiological traits like flag leaf area would be helpful. Information on the nature of the genetic control of flag leaf area is lacking in durum wheat (*Triticum durum* Desf.), which is the second important wheat species of India. However, scope of such studies is limited if these are not carried over environments. Keeping this in view, the present investigation was conducted to obtain information on the genetic control of flag leaf area under different environments through generation mean analysis.

Material and methods

The experimental material generated from six diverse parents, comprised three crosses namely, Cocorit 71 x A-9-30-1, HI 8062 x JNK-4W-128 and Raj 911 x DWL 5002. In each cross combination one of the parents (A-9-30-1, HI 8062 and Raj 911) had larger flag leaf area. Twelve basic generations, involved in these studies were two parents, F_1 and F_2, first backcross generations with both parents (BC_1 and BC_2), where BC_1 was the cross between F_1 x female parent and BC_2 was F_1 x male parent, their selfed progenies (BC_1F_2, BC_2F_2) and second backcross generations (BC_{11}, BC_{12}, BC_{21}, BC_{22}) i.e. the BC_1 and BC_2 plants again crossed with both original parents (BC_1 x female parent; BC_1 x male parent and BC_2 x female parent; BC_2 x male parent). These twelve populations of each of the three crosses were evaluated in randomized block design with three replications in two parallel experiments, one sown on 20th November (normal sown condition) and other sown on 20th December (late sown condition) in the same cropping season. Each replicate was divided into three compact blocks. The crosses, each consisting of twelve populations were randomly allotted to the blocks. All the twelve generations were then randomly allotted to twelve plots within a block. The plots of various generations contained different number of rows i.e. each parent and F_1 plots consisted of 2 rows, while each backcross generation in 4 rows and F_2 and the second cycle of backcrosses in 6 rows. Each row was 5 m long accommodating 33 plants spaced 15 cm apart, row to row distance being 30 cm. Border rows were provided at the beginning as well as at end of experimental rows in each block. The experiment was planted at Research Farm of Rajasthan Agricultural University, Agricultural Research Station, Durgapura, Jaipur, Rajasthan, India. The length and the maximum breadth of the flag leaf of the main spike of the each sampled plant was measured in centimeters and area was calculated following Simpson's (1968) formula as: Flag leaf area = (flag leaf length x flag leaf breadth) x 0.79. The data were recorded on 15 random plants in each parent and F_1, 30 plants in each backcross generation and 60 plants in each F_2 and second backcross generations in each replication under both the environments.

Standard statistical procedures were used to obtain means and variances for each generation and each environment separately, as suggested by Snedecor and Cochran (1968). While calculating variances, replicate effect was eliminated from total variances to obtain within replicate variance. These variances were used to compute standard error for each generation mean in each environment. Joint scaling test proposed by Cavalli (1952) were used to estimate genetic parameters by 3-parameter non-epistatic model [m, (d), (h)], 6-parameter model assuming digenic epistatic interaction [m, (d), (h), (i), (j), (l)], 10-parameter model, which allowed specification of digenic and trigenic non-allelic interactions [m, (d), (h), (i), (j), (l), (w), (x), (y), (z)].

The estimates of gene effects were obtained by weighted least square technique. Initially twelve equations were developed by equating observed generation means with their expectations in presence of digenic and trigenic interactions as proposed by Hill (1966). Generation means and their expectations were weighted, appropriate weights being the reciprocals

of the square standard errors. Twelve simultaneous equations so obtained were solved by way of matrix inversion as follows:

$M = Js^{-1}$

Where, M= the column vector of the estimates of the parameters; S= the matrix of score (right hand side); J= the information matrix; J^{-1} = the inverse of information matrix J and is a variance-covariance matrix.

The adequacy of a model was tested by predicting twelve generation means from the estimates of each of the 3, 6 and 10-parameter model by the comparison of the weighted deviations of the observed and expected generation means in the form of chi-square test with 'n–p' d.f., which provides a test of the goodness of fit of a model. In this situation 'n' is the number of statistics or generations and 'p' is the number of parameters. The estimates of χ^2 (n–p) is obtained as:

$\chi^2(n-p) = \sum (O_i - E_i)^2 W_i / E_i$

Where, O_i= is the observed mean of i^{th} generation; E_i = is the expected mean of i^{th} generation; W_i = is the weight of i^{th} generation, which is calculated as:

$W_i = 1/V\bar{x} = 1/SE^2x$

In the trigenic epistatic model the parameters estimated were: m = mean of all possible homozygous lines; (d) = additive gene effects pooled over all loci; (h) = dominance gene effects pooled over all loci; (i) = over all additive x additive epistatic gene effects; (j) = over all additive x dominance epistatic gene effects; (l) = over all dominance x dominance epistatic gene effects; (w) = additive x additive x additive gene interaction effects; (x) = additive x additive x dominance gene interaction effects; (y) = additive x dominance x dominance gene interaction effects; (z) = dominance x dominance x dominance gene interaction effects.

Relative magnitude of various gene effects in percent were also calculated by dividing the estimated value of each parameter by 'm' and multiplying it by hundred.

Components of heterosis were calculated as the difference between the mean value of F_1 generation and that of its better parent was taken as a measure of heterosis. From the weighted least square estimates of components of generation mean, components of heterosis in the presence of digenic interaction were calculated as follows (Jinks and Jones 1958):

$\overline{F_1} - \overline{BP} = [(h) - (i)] - [(d) - 1/2\,(j)]$ or $[(h) - (i) - (d) + 1/2\,(j)]$

The formula given by Hill (1966) was used to calculate the component of heterosis when trigenic interactions were also present:

$\overline{F_1} - \overline{BP} = [(h) + 1/4(l) + 1/8(z)] - [(d) + (i) - 1/2(j) + 1/4(l) + (w) - 1/2(x) + 1/4(y) - 1/8(z)]$

which equals

$[(h) - (i) + 1/2(x) + 1/4(z)] - [(d) - 1/2(j) + (w) + 1/4(y)]$

or $[(h) - (i) + 1/2\,(x) + 1/4(z) - (d) + 1/2(j) - (w) - 1/4(y)]$

Percent heterosis over better parent and inbreeding depression were calculated as follows:

Heterosis (over better parent) = $[(\overline{F_1} - \overline{BP})/\overline{BP}] \times 100$; S.E. $(\overline{F_1} - \overline{BP}) = (2EMS/r)^{1/2}$

Inbreeding depression = $[(\overline{F_1} - \overline{F_2})/\overline{F_1}] \times 100$; S.E. $(F_1 - F_2) = (2\,EMS/r)^{1/2}$

$\overline{BP}$ = Better parent; S.E. = Standard error; EMS = Error mean sum of squares.

Parameters (h), (l) and (z) were not affected by the degree of association 'r' therefore interpretation of the different interactions in this study was based on the basis of magnitude and relative signs of these parameters (Hill 1966).

Results and discussion

The results of joint scaling test indicated that the inheritance of flag leaf area could be explained on the basis of the 10-parameter model in the cross Cocorit 71 x A 9-30-1 and HI 8062 x JNK-4W-128 in normal and late planting condition, respectively (Table 1). Six-parameter model was observed adequate in the cross HI 8062 x JNK-4W-128 and Raj 911 x DWL 5002 in normal sown condition, whereas, 3-parameter model was adequate in the cross Cocorit 71 x A-9-30-1 and Raj 911 x DWL 5002 in late sown condition. Thus, it is clear that epistatic interactions had a greater role in controlling the inheritance of this trait in most of the cases. It may be noted that changes in planting dates somehow reduce/break the complex genetic behavior of different gene effects in some cases. Further, results indicated that the relative significance of main effects i.e. additive (d) and dominance (h) changed frequently with the cross as well as with the sowing environments.

Among digenic interactions, dominance x dominance (l) and among trigenic interactions, dominance x dominance x dominance (z) played significantly greater role in the inheritance of this trait than other interactions. This indicated the greatest significance of interactions purely involving the dominance effects in both the environments. However, their relative magnitude and signs changed frequently with the cross and the environments. In

the cross Cocorit 71 x A-9-30-1 and Raj 911 x DWL 5002 under late sown condition, where additive-dominance model was adequate, the additive (d) effect was either higher than or comparable to dominance (h) effect (Table 1). Earlier studies (Saini 1987; Kathiria 1991; Khedar 1998) are in agreement with the present findings.

The results of absolute totals of epistatic effects indicated that the second order interactions were more important than the first and both were higher than the main effects, where 10-parameter model was adequate (Table 2). Similarly the absolute totals of the first order interactions were higher than the main effects, where 6-parameter model was adequate. Thus, it is evident that epistatic interactions were more important than the main effects in all the cases where different epistatic models were appropriately fitted the data. Ilyhchenko (1977), Patel (1981) and Dhindsa (1982) also reported the role of epistasis in controlling the inheritance of this trait in bread wheat, whereas, in durum wheat Ghalib Al-Shalaldeh and Duwayri (1986) observed that this trait was controlled by polygenic gene effects. Duplicate epistasis at two-gene level and at three-gene level was observed only in the cross HI 8062 x JNK-4W-128 in normal and late planting condition, respectively. No conclusion regarding type of epistasis could be drawn in other crosses because (h) was found non-significant. Except in the late sown condition in the crosses Cocorit 71 x A-9-30-1 and Raj 911 x DWL 5002, where additive-dominance model was adequate, the non-fixable gene effects were higher than the fixable gene effects (Table 2). This indicated the requirement of complicated procedures of breeding for their further exploitation. However, if the crosses viz., Cocorit 71 x A-9-30-1 and Raj 911 x DWL 5002 are sown under late sown condition, simple breeding methods will be appropriate to exploit additive gene effects, which could be used successfully to improve this trait. Yadav et al. (1981) and Bariga (1989) also reported that additive effects were more important than non-additive effects in the inheritance of this trait while Hsu and Walton (1970), Jain and Singh (1976), and Prabu and Sharma (1984) reported that non-additive gene effects were predominant in the inheritance of this trait. Such varied results obtained in the present study may be due to difference in the materials used or even from the difference in the environments (sowing time).

Analysis of components of heterosis over better parent revealed that non-allelic interactions were the major source of heterosis. However, significant heterosis and inbreeding depression were not frequently observed in most of the cases (Table 3). The significant heterosis was only observed in the cross HI 8062 x JNK-4W-128 in late sown condition due to dominance (h), dominance x dominance (l), additive x dominance x dominance (y) and dominance x dominance x dominance (z) components of heterosis. Significant inbreeding depression in two cases was observed only due to the dissipation of non-additive dominance effects or epistatic effects involving dominance in F_2 generation. Absence of heterosis in the crosses could be explained on the basis of internal cancellation of heterosis components.

As a consequence of higher magnitude of

Table 1. Results of joint scaling test and gene effects for flag leaf area over environments

Effects	Cocorit 71 x A-9-30-1		HI 8062 x JNK-4W-128		Raj 911 x DWL 5002	
	Normal sown	Late sown	Normal sown	Late sown	Normal sown	Late sown
m	39.50±3.74**	40.59±0.91**	25.73± 0.93**	28.80± 1.53**	35.34± 1.17**	32.31± 0.25**
(d)	0.13±3.61	–4.50± 0.87**	0.78± 0.93	–0.42±1.06	4.86± 0.50**	5.96± 0.20**
(h)	7.32±7.24	–0.70± 1.67	12.91± 1.82**	6.63± 2.12**	–1.09±1.61	5.15± 0.94**
(i)	–3.42± 4.65		10.53± 1.82**	3.64± 4.48	–2.82±1.70	
(j)	–1.64±1.64		–0.29± 2.25	–3.61± 4.61	1.77±1.37	
(l)	–73.37± 30.72*		–18.66± 4.10**	–40.03± 15.63*	10.70± 2.50**	
(w)	–6.97± 13.61			5.76± 3.99		
(x)	–94.38± 33.28**			–36.02± 19.66		
(y)	4.92±26.32			–26.29± 7.16**		
(z)	172.99± 46.62**			76.39± 21.71**		
χ^2 for 10 parameter model	2.30 (2)†	6.30 (9)	14.37(6)	2.90 (2)	2.72 (6)	12.80 (9)

*, ** Significant at the 5 and 1 % level, respectively. †Degrees of freedom for χ^2

Table 2. Absolute totals of epistatic effects, fixable and non-fixable gene effects for flag leaf area over environments

Cross	Environment	Main effects		Epistatic effects†		Total gene effects‡	
		(d)	(h)	I order	II order	Fixable	Non-fixable
Cocorit x A-9-30-1	Normal	0.13	7.32	78.43	279.26	10.53	354.62
	Late	–4.50	–0.70	-	-	4.50	0.70
HI 8062 x JNK-4W-128	Normal	0.78	12.91	29.48	-	11.32	31.85
	Late	–0.42	6.63	47.33	144.45	9.81	189.02
Raj 911x DWL 5002	Normal	4.85	–1.09	15.29	-	7.67	13.56
	Late	5.96	5.15	-	-	5.96	5.15

†[(i), (j) ,(l), (w), (x), (y), (z)]. ‡[(d), (i), (w), (h), (j), (l), (x), (y). (z)]

Table 3. Components of heterosis for flag leaf area over environments

Effects	Cocorit 71 x A-9-30-1		HI 8062 x JNK-4W-128		Raj 911 x DWL 5002	
	Normal sown	Late sown	Normal sown	Late sown	Normal sown	Late sown
(h)	7.32	–0.70	12.91	6.63	–1.09	5.15
– (i)	3.42		–10.53	–3.64	2.82	
1/2(x)	–47.15			–18.01		
1/4(z)	43.25			19.10		
– (d)	–0.13	4.50	–0.78	0.42	–4.85	–5.96
1/2(j)	–0.82		–0.14	–1.18	0.89	
– (w)	6.97			–5.76		
–1/4(y)	–1.23			6.57		
Heterosis (%)	2.44	7.55	4.64	10.26**	–5.08	–4.20
Inbreeding depression(%)	12.24*	3.56	5.79	7.99*	7.95	1.04

*, ** Significant at the 0.05 and 0.01 level, respectively.

interactions (digenic/trigenic type) in most of the cases, the absolute totals of non-fixable gene effects were recorded higher than the fixable gene effects. Naturally, the successful breeding methods will be the ones, which will exploit the non-additive gene effects. The methods which mop-up the non-additive effects are restricted recurrent selection by the way of intermating the most desirable segregates followed the selection (Joshi 1979) and diallel selective mating (Jensen 1978). Furthermore, as the duplicate type of epistasis was observed in the cross HI 8062 x JNK-4W-128 under both the environments, so as the selection intensity should be mild in the earlier and intense in the later generations for tangible advancement of this trait. Furthermore, an appropriate choice of the environment should be made in such a way that character show relatively simple inheritance, so that flag leaf area could be improved in the crosses Cocorit 71 x A-9-30-1 and Raj 911 x DWL 5002 by simple breeding method (progeny selection) for tangible advancement of grain yield in durum.

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Research article

Breeding of bread wheat (*Triticum aestivum* L.) for semi-dwarf character and high yield

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Summary

Lines IN-06-92 and IN-14-92 had the highest grain yields. Line IN-14-92 was comparatively late in days to heading with having higher number of spikelets per spike, higher above-ground biomass production and also increased number of grains per main spike than the check varieties. Individual varietal correlation results suggest that number of grains per spike and spikelet were positively and significantly correlated with main spike grain yield in all the genotypes. Combined correlation results showed that grain weight was negatively and highly correlated with days to heading, number of spikelets and number of grains per spike, however, it exhibited positive correlation with plant height. The important character plot grain yield was not correlated with all the characters under this study except above-ground biomass production.

Key words: semi-dwarfism, agronomic characters, correlation analysis

Introduction

Semi-dwarf varieties of wheat (*Triticum aestivum* L.) have been cultivated in Asia (Japan and Korea) for more than four decades. Their introduction into Pakistan during 1965 has doubled the yield of wheat crop particularly with the release of Mexipak variety with the cooperation of CIMMYT Mexico. Universal acceptance of semi-dwarf wheats followed the pioneering work of Norman Borlaug whose CIMMYT wheat germplasm features in the pedigree of modern, high-yielding, semi-dwarf wheat varieties (Gale and Youssefian 1985). The semi-dwarf genotypes became particularly important with their concurred response to high doses of chemical fertilizers without lodging. Waddington et al. (1986) who studied more recent semi-dwarf cultivars (released after 1975), pointed out the importance of increased kernels per spike and indicated that most recent progress appeared to raise from increased biomass and not increased harvest index. Allan (1997) has reported from the studies of near-isogenic lines (NILS) of Nugaines variety that the semi-dwarf genotypes had mean yields of 22 to 36% greater than the non semi-dwarf (tall) genotypes. In another genetic backgrounds, yield increases associated with semi-dwarf genotypes averaged 2 to 16% greater than non semi-dwarf NILS (Allan 1989). The aim of the present study was to develop semi-dwarf germplasm with high yield and comparatively better agronomic characteristics for the release of new varieties.

Materials and methods

The $F_{4.5}$ lines were each derived from a range of fourteen different crosses during the year 1992 (CIMMYT International Nursery). Four varieties were used as check viz. Sarsabz, Soghat-90, Anmol-91 and Mehran-89. Genotypes were planted in six rows each with the row length of 4.5 meters. The genotypes were grown in a randomized complete block

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design with four replicates. The data of field experiments were subjected to analysis of variance (Steel and Torrie 1980). Association among the characters were examined by correlation analysis (n=20 for individual varietal correlation, n=72 for combined correlation).

Results and discussions

Lines IN-06-92 and IN-14-92 had the highest grain yields. Line IN-14-92 was comparatively late in days to heading with having higher number of spikelets per spike, higher above-ground biomass production and also increased number of grains per main spike than the check varieties. The same line (IN-14-92) had the lowest grain weight. Subsequent high yielding lines were IN-04-92, IN-08-92, IN-07-92, IN-05-92 and IN-03-92. Line IN-04-92 was not significantly different than the lines IN-06-92 and IN-14-92 for their plot grain yield. However, line IN-04-92 was also not significantly different than the check varieties Sarsabz and Soghat-90. Line IN-08-92 had a short plant height with a high harvest index value. Line IN-08-92 had a comparatively higher number of tillers/meter^2 (Jamali and Ahmad 1998). Line IN-07-92 had the highest above-ground biomass production than the lines and varieties.

Correlation studies of individual genotypes were conducted for main spike yield with other characteristics (Table 2). In this environmental condition only the number of grains per spike and spikelet showed positive and significant correlation in all the varieties and lines. While studying the adaptation of advanced CIMMYT wheat lines to water stress environments in Australia Cooper et al. (1994) have reported that grain number per fertile tiller/ spike was positively associated with grain yield. Sayre et al. (1997) also reported the strong relationships between grain yield and kernels per square meter. It suggests that number of grains per spike is a very important character and the selection should be based on this character for developing new high yielding wheat varieties. Plant height showed positive and significant correlation with main spike yield in lines IN-17-92, IN-54-92, Sarsabz, Anmol and Mehran. These results agree with the findings of Law et al. (1978) in which yield was positively related to height within major dwarfing gene group. However, Busch and Rauch (1993) reported the lack of a positive association between plant height and grain yield.

Table1. Comparative performance for agronomic characters of the selected F_5 genotypes.

Genotype	Days to heading	Plant height (cm)	No. of spikelets	No. of grains per spike	Main spike yield (g)	Grain weigh (mg)	Grains per spikelet	Plot yield (kg)	Harvest index	Biomass (kg)
IN-03-92	79.25g	102.60a	19.40ij	41.15e	2.07g	50.36a	2.12i	2.50abcd	35.70cdef	7.06bcd
IN-04-92	79.00g	97.00b	21.85def	56.30d	2.50de	44.60cde	2.59efgh	2.63ab	35.81cdef	7.38ab
IN-05-92	84.75e	86.95cde	26.05a	60.95bcd	2.72bcd	44.50cde	2.37ghi	2.55abcd	38.99abc	6.56bcdefg
IN-06-92	82.00f	90.20cd	22.90cde	61.25bcd	2.55cde	41.92ef	2.68cdef	2.68a	37.53abcde	7.15bc
IN-07-92	85.25e	88.80cde	24.15bc	58.90cd	2.59cde	44.77cde	2.43fgh	2.60abc	32.33fg	8.08a
IN-08-92	80.00g	73.90f	20.15hi	55.80d	2.17fg	39.09g	2.73cdef	2.63ab	39.77ab	6.60bcdefg
IN-14-92	90.25c	89.80cd	23.60bc	66.05bc	2.58cde	38.30g	2.79cde	2.68a	36.46abcde	7.35ab
IN-15-92	80.25fg	87.20cde	23.40bc	78.70a	3.18a	40.53fg	3.37a	2.28d	39.76ab	5.75gh
IN-17-92	96.25b	84.80e	26.35a	76.45a	3.03ab	39.89fg	2.90bcde	2.43abcd	37.79abcde	6.88bcde
IN-42-92	68.00i	90.40cd	20.75fgh	61.10bcd	2.95ab	48.25ab	2.95bcd	2.28d	39.89a	5.50h
IN-45-92	100.75a	90.75c	23.80bc	54.95d	2.30efg	42.12def	2.31hi	1.96e	31.87g	6.15efgh
IN-51-92	91.00c	101.00a	24.50b	68.20b	2.61cde	39.09g	2.79cde	2.30cd	34.54defg	6.65bcdef
IN-54-92	82.00f	94.95b	23.00cd	60.45cd	3.01ab	50.22a	2.65defg	2.24d	34.36efg	6.53bcdefg
IN-59-92	64.50j	86.30de	21.10fg	62.6bcd	2.87abc	45.81bc	2.95bcd	2.25d	37.17abcde	6.06efgh
Sarsabz	63.50j	85.45e	18.70j	55.75d	2.61cde	46.85bc	2.99bc	2.31bcd	37.11abcde	6.25defgh
Soghat-90	85.50e	88.10cde	20.55fghi	55.45d	2.44def	44.09cde	2.70cdef	2.31bcd	38.30abcd	6.05efgh
Anmol-91	73.25h	88.65cde	19.45hij	61.05bcd	2.75bcd	44.92cd	3.15ab	2.27d	39.52abc	5.93fgh
Mehran-89	87.75d	94.60b	21.65ef	57.60d	2.56cde	44.64cde	2.61efgh	2.28d	38.54abc	6.33cdefg

Means followed by the same letters do not differ significantly.

Villareal et al. (1992) reported negative correlation for plant height and grain yield within the groups of single gene dwarfs. The results contradict the earlier findings presented by Law et al. (1978) wherein yield was positively related to height. It suggests that the genetic background or environment may be playing a significant role for either positive or negative impact of plant height on grain yield. However, line IN-45-92 showed negative but significant correlation of plant height with main spike yield. Lines /varieties showed positive and significant correlation for number of spikelets per spike are IN-07-92, IN-45-92, IN-59-92 and Anmol. Line IN-05-92 showed negative and significant correlation for number of spikelets with main spike grain yield. Grain weight is an important character for improving wheat yields. In this study the lines which showed positive and significant correlation for grain weight with grain yield are IN-04-92, IN-08-92, IN-15-92, IN-42-92, IN-59-92, Soghat-90 and Anmol.

Combined correlation analysis studies are presented in Table 3. These studies show that days to heading was positively correlated (r = 0.627) with number of spikelets per spike, negatively associated (r = –0.439) with grain weight (mg), grains per spikelet (r = –0.297) and harvest index (r = –0.364). Flood and Halloran (1986) have reported that days to heading is positively correlated with number of spikelets. Negative correlation of days to heading with grain weight, grains per spikelet and harvest index suggests that high temperature affect negatively for grain development. Plant height is positively associated (r = 0.378) with grain weight and negatively correlated (r = –0.355) with harvest index. The main characteristic of semi-dwarf wheats is to transmit assimilate towards the developing grain. Number of spikelets are positively associated (r = 0.554) with number of grains per spike and main spike yield (r = 0.311), however, it is negatively correlated (r = –0.382) with grain weight and harvest index (r = –0.244). It is a physiological process that higher number of grains will increase competition for assimilate requirement and hence the grain weight may be reduced. Main spike yield is positively associated (r = 0.689) with grains per spikelet. It suggests that higher fertility may increase the main spike yield. Grain weight is negatively correlated (r = –0.256) with number of grains per spikelet. It is already stated that higher number of grains may reduce the grain weight. Number of grains per spikelet is positively correlated with harvest index. The higher harvest index may increase the spike fertility. Above-ground biomass was positively and significantly correlated with days to heading (r = 0.284) and plant height (r = 0.287).

Table 2. F_5 studies of main spike yield correlated with other characters

Genotypes	Plant height	No. of spikelets	No. of grains/spike	Grain weight	No.of grains / spikelet
IN-03-92	0.065	0.266	0.835 ***	0.198	0.708 ***
IN-04-92	0.172	0.095	0.536 **	0.583 **	0.446 *
IN-05-92	–0.420	–0.654**	0.860 ***	0.115	0.913 ***
IN-06-92	0.253	0.075	0.836 ***	0.109	0.857 ***
IN-07-92	–0.186	0.441 **	0.839 ***	–0.124	0.805 ***
IN-08-92	–0.117	–0.100	0.763 ***	0.437 *	0.690 ***
IN-14-92	0.326	0.252	0.878 ***	0.323	0.885 ***
IN-15-92	0.024	0.233	0.746 ***	0.479 *	0.634 **
IN-17-92	0.454 *	0.122	0.758 ***	0.047	0.817 ***
IN-42-92	–0.103	0.252	0.885 ***	0.537 *	0.816 ***
IN-45-92	–0.508 *	0.665 ***	0.875 ***	–0.016	0.599 **
IN-51-92	–0.007	0.422	0.656 ***	0.122	0.519 *
IN-54-92	0.557 **	0.278	0.910 ***	0.314	0.780 ***
IN-59-92	0.339	0.762 ***	0.939 ***	0.481 *	0.861 ***
Sarsabz	0.733 ***	0.361	0.930 ***	0.269	0.764 ***
Soghtat-90	0.353	0.029	0.890 ***	0.455 *	0.936 ***
Anmol	0.732 ***	0.511 *	0.933 ***	0.604 **	0.640 **
Mehran	0.501 *	0.356	0.957 ***	–0.095	0.783 ***

*, **, *** Significant at the 5%, 1% and 0.1% level, respectively

Table 3. Combined correlation analysis of F_5 genotypes

Genotype	Plant height (cm)	No. of spikelets	No. of grains per spike	Main spike yield (g)	Grain weight (mg)	Grains per spikelet	Plot grain yield (kg)	Harvest index	Biomass (kg)
Days to heading	0.154	0.627***	0.167	–0.073	–0.439***	–0.297**	0.016	–0.364***	0.284*
Plant height (cm)		0.078	–0.094	0.131	0.378***	–0.221	0.087	–0.355**	0.287*
No. of spikelets			0.554***	0.311**	–0.382***	–0.085	0.215	–0.244*	0.374***
No. of grains per spike				0.781***	–0.444***	0.774***	0.088	0.110	0.039
Main spike yield (g)					0.125	0.689***	0.008	0.033	0.016
Grain weight (mg)						–0.256*	–0.070	–0.125	0.025
Grains per spikelet							–0.063	0.330**	–0.254*
Plot grain yield (kg)								0.139	0.729***
Harvest index									–0.523***

Flood and Halloran (1986) reported that longer growing period habit is positively associated with higher dry matter accumulation at anthesis (Villareal et al. 1992). Biomass production was positively and highly significantly correlated with number of spikelets (r = 0.374) and plot grain yield (r = 0.729). A number of workers observed positive correlation between dry matter at anthesis and grain yield (Flood and Halloran 1986; Villareal et al. 1992). However, biomass production was negatively correlated with number of grains/spikelet (r = –0.254) and harvest index (r = –0.523). These results are in agreement with those reported by Villareal et al. (1992) that above-ground biomass production was negatively correlated with harvest index. The negative correlation between biomass production and number of grains/spikelet suggests that increased number of spikelets creates competition for assimilate supply to the developing grain during grain filling period. The very important character plot grain yield is non-significantly correlated with all the characters except above-ground biomass production. It is not known that some of the positive associations why do not affect final yield at this environment.

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Research article

Anther culture and long-term culture ability of androgenic calli in durum wheat (*Triticum durum* Desf.)

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Summary

To study the effects of culture media and genotypes on anther culture, anthers of sixteen durum wheat genotypes were cultured on N6 NB medium with BA and NAA and N6 DK medium with 2,4-D and kinetin. Overall, the calli and plants obtained were more than 1.7 times higher with N6 DK medium as compared to N6 NB. Among sixteen genotypes tested, nine genotypes responded for androgenesis in vitro. Genotype HI 8381 produced maximum plants (nearly 4 per 100 anthers) followed by A 9-30-1, which produced 2.25 plants on N6 DK medium. Formation of androgenic calli and regeneration of green plants were significantly higher with maltose and agarose as compared to media with sucrose and agar combination. For long-term maintenance androgenic calli were sub-cultured on three different combinations of MS media. Medium with 2,4-D and silver nitrate maintained embryogenic status of androgenic calli more affectively than other two media with 2,4-D alone.

Key words: *Triticum durum*, anther culture, genotypes, culture media, long-term culture.

Introduction

In vitro techniques and methods for genetic manipulation of higher plants have made considerable progress in recent years. The contribution of this area of research is increasing with a major role of haploids in crop improvement. The haploidization and subsequent chromosome doubling is particularly useful as an alternative to the numerous cycles of inbreeding or back crossing needs to obtain pure lines in conventional breeding program.

The production of doubled haploid lines of cereals from anther culture is limited by relatively low callus/ embryoid induction frequency, genotype dependent response and poor regeneration, a large number of which are albino plants. In durum wheat, Headwiger and Heberle-Bors (1986) reported very low frequency of haploid plants from anther culture. Foroughi-Wehr and Zeller (1990) observed embryogenesis in calli produced from durum wheat anther culture but only of non-regenerative type. Following the reports of considerable regeneration of androgenic plants by Ghaemi et al. (1995) and Tiwari (1997), durum wheat should no more be assigned recalcitrant status.

The use of anther culture as a tool in genetic improvement of most of the cereals has been hampered by the fact that plant regeneration from various cultures is of low frequency and of short duration. Maintenance of embryogenic source for prolonged duration is essential for conducting biochemical and physiological studies, mutation and in vitro selection for genetically modifying the material. However, so far in cereals we do not have any reports of efficient methods for long-term maintenance of androgenic cultures except in barley, where embryogenic cultures of androgenic source have been studied (Tiwari et al. 1990 a, b). Present study was conducted to determine the influence of several genotypes and medium components on the androgenic plant regeneration from durum wheat anther culture. Another objective of this study was to investigate the possibility of long-term culturing of androgenic calli of durum wheat.

Materials and methods

Main tillers were harvested from field grown plants soon after the emergence of flag-leaf ligule and stored at 7±1°C with their base dipped in plain water for 3–5

days. Before isolating anthers, the spikes enclosed in leaf sheath were surface sterilized with 70% ethyl alcohol. Anthers from only such spikes were selected for culturing which on microscopic examination revealed mid to late uninucleate stage of microspore development.

For all the experiments, media modified with various supplements were used for anther culture. For culture, 90 mm diameter glass petri dishes were used. In each dish, approximately 100 anthers were cultured. To avoid variations within ear head, anthers from each spike were evenly distributed among culture media tested in the respective experiments. All the cultured dishes sealed with Parafilm were incubated at 28°±1°C in absence of light for 7 days and then transferred under dim light conditions for 16 hr photoperiod at 22 ± 2°C.

Effect of culture media and genotypes for anther culture (Exp. 1): Experiment was conducted with 16 genotypes of durum wheat (2n=4x=28) (Table 1). Seeds were supplied by the Wheat Improvement Research Programme, JNKVV, Jabalpur, India. Cultivars were selected for their high yielding ability,

Table 1. Average number of calli and plants per 100 anthers as influenced by genotype and medium

Genotypes†	Calli/100 anthers		Plants/100 anthers	
	N_6 NB	N_6 DK	N_6 NB	N_6 DK
1.A 9-30-1	6.94d‡	11.78e	1.73b	2.25b
2.GW 1112	1.18a	3.85c	0.00	0.00
3.HI 8381	4.35c	10.00e	1.63b	3.89c
4.HI 8498	2.13b	5.06dc	0.53a	0.00
5.Jairaj	1.63a	0.53a	0.00	0.53a
6.JWJ 908	4.40c	7.39d	1.67b	0.57a
7.MPO 965	0.00	2.82b	0.00	1.13a
8.Raj 1555	1.70a	3.98c	0.00	1.14a
9.Tawa 215	6.63d	4.07c	0.00	0.00
Mean§	3.22	5.50	0.66	1.06
CD (0.05)				
Genotypes	0.67		0.68	
Media	0.32		0.32	
G x M	0.95		0.96	

†Total sixteen genotypes were tested. Seven genotypes namely A 206, JU 12, HD 4502, GW 2, OWR 137, Vijay and Gulab were excluded from the analysis, as they did not respond to either culture media.

‡Means followed by the same letter are not significantly different (p=0.05).

§t test significant (p=0.05) for paired observations of two culture media.

crop duration, drought tolerance and disease resistance properties due to which they are often included in the wheat breeding programs of Central India. Anthers from sixteen durum wheat genotypes were cultured on two modified N_6 (Chu 1978) media, (i) N_6NB supplemented with 1.0 mg/l NAA +1.0 mg/l BA and (ii) N_6 DK with 2.0 mg/l 2,4-D + 0.5 mg/l kinetin. Both the media were added with 326 mg/l L-glutamine + 100 mg/l m-inositol + 90 g/l sucrose + 7.5 g/l agar.

The experiment was conducted in completely randomized design in three replications. The statistical significance of treatment difference was evaluated by the t-test (Steel and Torrie 1980). For each experiment, percentages were transformed to arcsin $\sqrt{P}$.

The calli attaining 2–3 mm diameter size were transferred for plant regeneration to MS (Murashige and Skoog 1962) medium containing 0.4 mg/l IAA, 0.4 mg/l BA, 20 g/l sucrose and 7.5 g/l agar. The cultures were kept under light (fluorescent 3600 lx intensity) and dark cycle of 16/8 h at 22±2°C. In case, where no root formation took place, shoots were transferred to MS medium containing 0.5 mg/l IBA, 20g/l sucrose and 7.5 g/l agar.

All observations were based on initial culture media, irrespective of regeneration medium or rooting medium.

Effect of sugars and gelling agents on anther culture (Exp.2): Anthers from two high responding genotypes for in vitro androgenesis, A 9-30-1 and HI 8381 were cultured on three N_6 DK media (as described in Exp. 1) and as carbon source and gelling agent supplemented with (i) sucrose 90 g/l + agar 7.5 g/l, (ii) maltose 90 g/l + agar 7.5 g/l, and (iii) maltose 60 g/l + agarose 5 g/l. For plant regeneration, similar methodology as described in the Exp. 1 was used.

The experimental design used was split plot design, approximately 480 anthers were plated for each genotype and medium combination. Comparisons were made by Fisher's protected LSD test after transforming the data (Steel and Torrie 1980).

Long term maintenance of androgenic callus cultures (Exp. 3): Opaque and nodular embryogenic calli, generated after 45 days culture of anther of HI 8381 during previous experiments were sub-cultured on three different MS maintenance media supplemented with (i) 1.0 mg/l 2,4-D (ii) 2.0 mg/l 2,4-D and (iii) 2.0 mg/l 2,4-D + 5.0 mg/l silver nitrate. All the media were supplemented with 30 g/l sucrose and 8 g/l agar. Sub-culturing in fresh media was done at every 1-month interval. The culture conditions for callus maintenance were 22±2°C under 16/8h light/dark cycle

of 2000 lux illumination.

The experiment was conducted in completely randomizes design and the data were analyzed by Duncan's new multiple range test (Steel and Torrie 1980).

Results

Effect of culture media and genotypes on anther culture: Analysis of variance (Table 2) showed that genotype and culture medium had very highly significant effects (p=0.0001) on the capacity of androgenesis.

Calli and plants were regenerated from the anther culture of durum wheat on two media tested, N6 NB and N6 DK. The initiation of calli and plants varied significantly on the medium used (p<0.05). The N6 DK medium showed higher frequency of androgenesis in vitro, compared with N6 NB medium (Table.1). For eight genotypes N6 DK produced higher number of calli and plants, while on N6 NB medium only cv. Tawa 215 produced more calli than the other. Overall, the number of calli and plants per 100 anthers was more than 1.7 times in N6 DK as compared to N6 NB medium.

Among sixteen genotypes tested for their androgenic ability, nine genotypes produced calli and plants on both or either of the anther culture media used (Table 1). Genotype A 9-30-1 and HI 8381 produced significantly higher number of calli (>10 per 100 anther cultured) on N6 DK medium, whereas genotypes A 9-30-1 and Tawa 215 produced higher number of calli (>6) on N6 NB medium as compared to other genotypes.

Genotype HI 8381 produced maximum plants (nearly 4 per 100 anthers cultured) on N6 DK medium followed by A 9-30-1 (2.25 plants) on the same medium. On MS NB medium, A 9-30-1, JWJ 908 and HI 8381 regenerated only 1.63–1.73 plants per 100 anthers cultured. Despite producing considerable amount of calli, Tawa 215 and GW 112 could not regenerate plants on either of the culture media. Overall, for androgenic callus induction and plant regeneration from various genotypes medium N6 DK was more effective as compared to N6 NB.

Effect of sugars and gelling agents on anther culture: The supplements in culture medium significantly affected the number of calli and plants regenerated per 100 anthers (Table 3). The number of calli and plants on medium with maltose and agarose were significantly higher than the medium with sucrose and agar. For callus formation and plant regeneration, media with maltose and sucrose produced average results for callus formation (15.5 per 100 anthers) and plant regeneration (2.6 per 100 anthers); however, it was non-significantly different with other two media.

The green plant formation was effected by the initiation medium as well as genotypes. Genotype HI 8381 produced more number of green plants as compared to A 9-30-1 on all three media tested. Maximum response was on N6 DK medium with 90 g/l maltose and 5 g/l agarose where HI 8381 regenerated 7.2 plants and A 9-30-1 regenerated 2.9 plants per 100 anthers.

Long-term maintenance of androgenic callus cultures: In order to select appropriate culture medium for long-term maintenance of androgenic calli, the potential to produce embryogenic calli have been examined. For

Table 2. Analysis of variance for callus formation and plant regeneration

Source of variation	d.f.	Calli/100 anthers		Plants per 100 anthers	
		MS	F value	MS	F value
Replications	2	15.78	48.11**	1.10	3.24*
Genotypes	8	70.18	214.00**	6.86	20.17**
Media	1	5.92	18.06**	13.67	40.17**
Interactions (G X M)	8	59.50	181.44**	19.37	56.92**
Error	34	0.33		0.34	

Table 3. Comparison of media gelling agent and carbon source for anther culture response in durum wheat genotypes A 9-30-1 and HI 8381

N6 media supplemented with (g/l)	Genotypes	Calli/ 100 anthers	Plants/ 100 anthers	Green plants/100 anthers
Sucrose (90)	A 9-30-1	11.8	2.2	0.6
+agar (7.5)	HI 8381	10.0	3.9	1.1
	Mean	10.9a†	3.1a	0.8a
Maltose (90)	A 9-30-1	17.9	6.4	1.7
+agar (7.5)	HI 8381	13.1	5.7	3.4
	Mean	15.5ab	6.0ab	2.6ab
Maltose (90)	A 9-30-1	16.2	8.1	2.9
+agarose (5)	HI 8381	19.9	11.1	7.2
	Mean	18.6b	9.6b	5.1b
C.D. at 5%		6.81	3.87	2.6

†Means followed by the same letter are not significantly different (p=0.05).

this experiment, only one genotype HI 8381 was tested as it produced sufficient required quantity of embryogenic calli. Among the three media tested, medium with 2 mg/l 2,4-D and 5 mg/l silver nitrate maintained embryogenic calli more significantly than other two media for a period of six months (Table 4). The sub-culturing during the first month on various media reduced the embryogenic potential considerably being minimum on medium with 2 mg/l 2,4-D and maximum on 2,4-D and silver nitrate combination. During subsequent sub-culturing, embryogenic calli either degenerated or converted into non-embryogenic friable calli. Although no callus regenerated plants after four months of culture, few calli maintained their embryogenic status up to six months.

Discussion

Both genetic and environment factors effect callus formation and plant regeneration in cereal anther culture (Kasha et al. 1990; Rahimbaev et al. 1992; Raina 1997). Recent reports of increase in cereal anther culture results have been based mainly upon one or few responsive genotypes, while other genotypes show a wide range of response to culture conditions and media components. These strong genotypic differences are yet to be resolved for breeding purposes in most of the cereals. Anther culture ability in wheat can be divided into three independently inherited components: callus induction, plant regeneration and green plant formation, usually governed by more than one gene (Lazar et al. 1984; Deaton et al. 1987; Agache et al. 1989; Szakacs et al. 1988). Genotype seems to be one of the major determinants of callus and embryoid production in this work with durum wheat, since wide range of variations were observed among them for callus initiation (0–11.78%) and haploid plant formation (0–3.89%).

For many years, 2, 4-D has been considered as an essential growth hormone for callus induction in cereal tissue culture. However, for direct regeneration, i.e. without transferring calli or embryos to regeneration media, the replacement of 2,4-D by IAA or NAA has been important (Ouyang 1986; Liang et al. 1987). During present investigation, modified N_6 NB supplemented with NAA and BA was less effective for callus induction as compared to N_6 DK with 2,4-D and kinetin.

Most media used for wheat anther culture contain high sugar level between 6 and 10 percent. During present investigation as compared to sucrose, maltose increased the androgenesis considerably. Similar findings have been obtained for bread wheat (Last and Brettell 1990; Orshinsky et al. 1990; Zhou et al. 1991, 1992; Trottier et al. 1993; Novaro-Alvarez et al. 1994; Orshinsky and Sadasivaiah 1994). The mode of action of maltose is still unknown, however, it has been suggested that the slow hydrolysis of maltose could provide a carbon source for a longer period than sucrose, which is rapidly hydrolyzed (Orshinsky et al. 1990). Furthermore, a break down product of sucrose hydrolysis, fructose is also believed to inhibit androgenesis in vitro (Last and Brettell 1990). In addition, maltose could be more efficient than sucrose as its hydrolysis rate that is closer to the rate of utilization of glucose by embryos (Robert-Oehlschlager et al. 1990).

Both genetic and environmental factors effect callus formation and plant regeneration in cereal anther culture. Interactions among these factors are often significant. This usually makes comparison of work from various laboratories difficult as genotypes, media and culture conditions differ considerably. The results of current study indicate the importance of considering such interactions in the development of protocols with locally adopted genotypes and methodology.

For the maintenance of androgenic calli, it is desirable to have levels of hormones that provide good callus growth and do not hinder subsequent regeneration. Only few reports of successful maintenance of androgenic calli are available on barley with low levels of 2,4-D (Tiwari et al. 1990 a, b). During our preliminary experiments with durum

Table 4. Long-term culture effect on embryogenic callus formation in *Triticum durum* wheat cv. HI 8381 sub-cultured† on MS maintenance media

Maintenance MS media supplemented with	Number of embryogenic calli / 100 androgenic calli subcultured					
	Months					
	1	2	3	4	5	6
1.0 mg/l 2,4D	46.7b‡	40.0b	36.5b	30.0b	10.0c	6.7b
2.0 mg/l 2,4-D	36.4b	26.7c	23.3c	16.7c	16.7b	10.2b
2.0 mg/l 2,4-D + 5.0 mg/l $AgNO_3$	63.3a	63.3a	56.7a	43.4a	33.3a	20.0a
CD (0.05)	11.9	13.0	6.4	12.6	5.2	6.8

†Initial sub-culture of embryogenic calli was made after 45 days of anther culture.

‡Means within a column followed by the same letter are not significantly different (p=0.05).

wheat androgenic calli 2,4-D concentrations higher than 3 mg/l and other growth regulators such as NAA and IAA resulted in very low regeneration potential and therefore, were found to be unsuitable for long-term maintenance study. Silver nitrate, an ethylene inhibitor has shown to increase embryo production in cereals (Lentini et al. 1995; Ghaemi et al. 1994). During present investigation, silver nitrate was efficiently used to maintain cultures in combination with 2, 4-D as compared to auxin alone. This effect was probably due to low callus proliferation with increased embryogenic response.

In conclusion, it was shown that anther culture response of recalcitrant species *T. durum* can be improved by selecting responsive genotypes and suitable culture medium. However, if compared with many cereals, the present results are still very low and suggest that the medium currently used for anther culture of durum wheat may not be optimal for green plant production. Therefore, adjustments of medium components such as concentration and type of growth hormones, sugars, gelling agents etc. should be made prior to culture.

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Research article

Genetic divergence study in durum wheat based on seed vigor parameters

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Summary

Genetic divergence by using Mahalanobis D^2 statistics was studied for eight seed vigor parameters in 24 durum wheat genotypes. All the genotypes were grouped into six different clusters, which were not related to their geographical origin. It could be suggested that hybridization between parents included in clusters III and II followed by selection in segregating generations may help in isolating the desirable strains with early seedling vigor.

Introduction

India is a major durum wheat (*Triticum durum* Desf.) growing country in the world. It is traditionally grown under rainfed conditions in marginal soils. In such situation, the crop remains in stress right from the sowing to harvesting which results in poor and marginal yields, consequently low average productivity. It is also well known fact that highly vigorous seed performs better under wide range of environmental conditions, particularly stress conditions. For genetic amelioration of this crop, precise information on the nature and degree of genetic diversity helps in making of choice of desirable parents for a meaningful hybridization program. For identifying genetically divergent parents for hybridization, few worker adopt D^2 statistics based on yield and its components in tetraploid wheats (Lee and Kaltsiek 1973; Joshi and Singh 1979; Raut et al. 1985; Sethi et al. 1992; Singh 1994; Deshmukh et al. 1999). But no such information is available on the basis of seed vigor parameters, hence an attempt has been made to study the genetic diversity in twenty-four genotypes.

Materials and methods

Present study was carried out on twenty-four genotypes (including elite international germplasm nursery strains, advanced breeding line and cultivars) of durum wheat. Various seed vigor tests were employed in seed quality testing laboratory of Department of Seed Science and Technology, CCS Haryana Agricultural University, Hisar (India). The observations were recorded on the following parameters viz. seed density (mass/volume, g/cc), standard germination (ISTA 1996), vigor index-I (germination % x seedling length), vigor index-II (germination % x seedling dry weight), electrical conductivity of seed leachates (µmhos/cm/seed), accelerated ageing test (41°C, 100% rh, 72 hrs). Field emergence index was calculated following the procedure given by Maguire (1962). The total number of seedlings after 20 days of sowing was considered as seedling establishment potential. Following the ANOVA, the genetic diversity was assessed by using D^2 statistics of multivariate analysis given by Mahalanobis (1936) and the method suggested by Tocher (Rao 1952) was used to determine the group constellation.

Results and discussion

Highly significant mean sum of squares due to genotypes revealed the existence of substantial amount of variation and significant differences among the genotypes for all the seed vigor parameters except seed density. Block effects were non-significant in all the cases. D^2 values corresponding to 276 possible comparison among 24 genotypes, taking two genotypes at a time, were computed separately. Based on these estimates of genetic divergence, the genotypes were grouped into six clusters (Table 1). The cluster I comprised of maximum number of thirteen genotypes followed by cluster II and III with three genotypes each. Cluster IV and V consisted two genotypes each whereas cluster VI had single genotype. The clusters contained genotypes of heterogeneous origin coming from different regions. Even the exotic lines were grouped with indigenous materials. This indicated lack of parallelism between genetic and geographic diversity.

Average intra- and inter-cluster distance (D values) among six clusters are given in Table 2. The intra-cluster distance varied from 0.11 to 7.79 and did not transgress the limits of any of the inter-cluster distances. The zero value of intra-cluster distance for cluster VI was due to only one genotype in the cluster, whereas cluster V showed highest intra-cluster distance (7.79) having two genotypes. Moreover, cluster I and II also have high intra-cluster distance (6.83), which include thirteen and three genotypes, respectively. It indicated that the genotypes of these clusters were genetically less divergent and almost parallel divergent.

Minimum divergence at inter-cluster level was recorded between clusters II and VI (8.92) followed by between clusters I and VI (9.05) indicating that genotypes of these clusters were genetically close.

Maximum average inter-cluster distance was observed between clusters II and III (19.32) and between clusters II and IV (15.91). The genotypes belonging to these clusters were found genetically most divergent. Hence, it would be logical to incorporate such genotypes in the breeding program. The intra-cluster group means of the characters reveal the main fraction of the clusters (Table 3). An examination of the varietal composition of the clusters

Table 1. Clustering of durum wheat genotypes (seed vigour parameters)

Cluster	No. of genotypes	Genotypes
I	13	P5877, P5885, P5892, P5893, P5894, P5895, P5896, P5897, P5898, P5911, WH912, WH916, PDW233
II	3	P5875, P5876, P5888
III	3	P5915, WH922, WH896
IV	2	WH913, PDW215
V	2	P5899, WH899
VI	1	PBW34

Table 2. Average intra- and inter-cluster distances (D values)

Cluster	I	II	III	IV	V	VI
I	6.83	10.49	11.70	11.96	11.41	9.05
II		6.83	19.32	15.91	13.93	8.92
III			5.40	11.89	13.62	15.52
IV				3.88	11.49	9.51
V					7.79	9.52
VI						0.00

Table 3. Intra-cluster group means of seed vigor parameters

Character/Cluster	I	II	III	IV	V	VI
Standard germination (%)	94.10	92.22	96.00	95.67	90.00	90.67
Vigor index-I	20.76	20.51	23.83	21.43	21.95	18.64
Vigor index-II	5.85	5.16	6.57	7.20	2.50	4.82
Seed density (g/cc)	1.20	1.20	1.22	1.16	1.18	1.19
Electrical conductivity (μmhos/cm/seed)	4.71	4.04	5.97	6.67	5.58	5.31
Accelerated aging (%)	21.99	5.78	37.78	9.33	7.23	4.67
Field emergence index	7.60	5.42	8.37	8.10	7.23	9.40
Seedling establishment (%)	31.56	25.44	35.11	33.84	30.00	32.00

indicated the cluster III and composed of relatively high vigorous genotypes because of high mean values of most of vigor parameters viz. standard germination, vigor indices, seed density, germination after accelerated aging, field emergence index and seedling establishment. The cluster II was consisted of low vigor genotypes. Thus, the genotypes of these two clusters hold great promise as parents to obtain promising hybrid combination and create further variability for seedling vigor parameters.

The main criterion for selection of genotypes and parents for hybridization using D^2 analysis is the inter-cluster distances. The genotypes included in clusters with maximum inter-cluster distances are, obviously, genetically more divergent. It can, therefore, be suggested that hybridization between most divergent high vigor parents included in cluster III (P5915, WH992, WH896) and poor vigorous genotypes (P5875, P5876, P5888) followed by selection in segregating generations may be useful in isolating the desirable strains promising for growing under sub-optimal environmental conditions.

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Research article

DNA fingerprinting of wheat genotypes by RAPD markers

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Summary

Polymerase chain reaction (PCR) based randomly amplified polymorphic DNA (RAPD) markers were used to study the genetic relationship and genetic diversity among 27 Indian wheat accessions (17 hexaploids and 10 tetraploids). The size of PCR amplified products ranged between 0.03 and 3.0 kb. Out of the 103 amplified total RAPD bands, 82 (79.6%) were polymorphic. Within hexaploids, of the total 98 amplified bands, 64 (65.3%) were polymorphic whereas within tetraploids, of the total 103 bands, 78 (75.7%) were polymorphic. The similarity coefficient between hexaploids and tetraploids ranged from 0.630 to 0.952 and 0.400 to 0.966, respectively. Primers UBC-535, UBC-552, UBC-600, UBC-534 and UBC-386 showed maximum number of polymorphic bands. The primers UBC-18, UBC-535, UBC-337, UBC-600, UBC-572 and UBC-534 were found to give distinguishable number of unique RAPD markers. These six primers were able to distinguish some of the genotypes like, IC-82233, IC-99785, IC-35161-D, IC-35177-D and IC-35720-D. As the similarity matrix value showed, the tetraploid genotypes had more wider genetic distance value than hexaploids.

Key words: genetic diversity, RAPD, Indian wheat, PCR

Introduction

Wheat (*Triticum aestivum)* is the world's leading cereal grain and most important food crop. The genetic origin of wheat is a classic example of how closely related species combine in nature to form a polyploid series. The species of *Triticum* are grouped into diploids (2n=2x=14), tetraploids (2n=4x=28) and hexaploids (2n=6x=42). Only two species of *Triticum* are commercially important: the hexaploid species, *T. aestivum* (the bread wheat and the principal wheat in commerce), and the tetraploid species, *T.turgidum* (the durum wheat that is used for making pasta). The total numbers of accessions in international and local gene banks around the world are estimated to be in excess of 400,000, although many accessions may probably be duplicated in the different collections. FAO has developed a set of descriptor for wheat to facilitate uniformity in describing wheat accessions in different gene banks and to assist breeders in locating genes specific breeding programs (Poelham and Sleper 1995). Knowledge of genetic diversity and relationship among a set of germplasm and the potential merit of the genetic diversity would be beneficial to all phases of crop improvement. Assessments of genetic diversity of the elite germplasm have been sought and used by plant breeders for numerous reasons e.g. genetic relationships, parent selection, germplasm management and protection among others (Lee 1995).

Traditionally, the assessment of the genetic composition of crop germplasm has been conducted on the basis of morphological and phenotypic characters, which frequently lack the resolving power needed to identify individual genotypes. Estimation from biochemical markers viz. isozyme analysis may also be biased by the general consideration that only a minor portion of the genome is represented by these markers (Second 1982). In the last decade, molecular markers such as RFLP, RAPD, SCAR, AFLP etc. have been used to assess genetic variation at the DNA level, allowing an estimation of degree of relatedness between individuals without the influence of

environmental variation (Miller and Tanksley 1990). Among the various techniques available, RAPD analysis is a potentially simple, rapid, reliable and effective method for detecting polymorphism in wheat (Vierling and Nguyen 1992). To date, no information is available on variation in Indian tetraploid and hexaploid wheat genotypes at the molecular level. In view of the above, we have done this study to evaluate genetic relationship between and within tetraploid and hexaploid wheat genotypes.

Materials and methods

Seventeen accessions of bread wheat (*T. aestivum*) and ten of durum wheat (*T. turgidum*) were obtained from National Bureau of Plant Genetic Resources (NBPGR), New Delhi, used for the present study (Table 1). DNA was isolated from 8 days old seedlings using a method of Dellaporta et al. (1983) with the modification of replacing TE and $T_{50}E_{10}$ by 10 mM Tris-HCl.

PCR amplification was performed at 10 ng/µl, 20 ng/µl and 50 ng/µl with primer UBC-350 and UBC-386, and accessions IC-82198 and IC-82199 combinations to optimize the concentration of template DNA. PCR amplification was performed in a volume of 25 µl volume containing 200 µM dNTPs, 1.25 unit *Taq* DNA polymerase (Bangalore Genei Limited, India), 10 ng primers, 1.5 mM of $MgCl_2$ and 10x PCR assay buffer (supplied by the manufacturer of *Taq* polymerase) at a final concentration of 1x.

A set of ten decanucleotide RAPD primers was used for PCR amplification. The sequences of these primers were selected from literature (Table 2). The mixture was gently mixed and centrifuged for 10 seconds. The PCR amplification was conducted in Biometra thermal cycler programmed as initial denaturation at 94°C for 5 min; remaining 45 cycles with 94°C denaturation, 35°C annealing and 72°C

Table 1. List of plant material used in the present study

S. No.	Accession No.	Village	State	100 seed wt. (gram)	Plant height (cm)	Days to spike emergence	Class
1.	78754	Jhunjhunu	Rajasthan	-	116.3	106	*T. aestivum*
2.	78868	Pali	Rajasthan	2.78	138.0	95	*T. aestivum*
3.	78944	Jhunjhunu	Rajasthan	3.17	141.6	98	*T. aestivum*
4.	79037	Hamirpur	Himachal Pradesh	3.34	128.6	93	*T. aestivum*
5.	79105	Hamirpur	Himachal Pradesh	2.84	101.6	107	*T. aestivum*
6.	82198	Simla	Himachal Pradesh	2.88	146.3	112	*T. aestivum*
7.	82199	Simla	Himachal Pradesh	2.37	147.6	107	*T. aestivum*
8.	82202	Simla	Himachal Pradesh	2.79	126.6	107	*T. aestivum*
9.	82233	Simla	Himachal Pradesh	2.75	98.6	109	*T. aestivum*
10.	82234	Kinnaur	Himachal Pradesh	3.02	139.0	106	*T. aestivum*
11.	82272	Kinnaur	Himachal Pradesh	3.46	135.6	108	*T. aestivum*
12.	82280	Kullu	Himachal Pradesh	2.57	100.0	109	*T. aestivum*
13.	82449	Chittorgarh	Rajasthan	3.01	127.0	98	*T. aestivum*
14.	82526	Ahmedabad	Gujarat	3.17	139.3	94	*T. aestivum*
15.	99785	Chamoli	Uttaranchal	2.51	141.6	100	*T. aestivum*
16.	104522	-	-	2.76	110.6	107	*T. aestivum*
17.	104637	Alwar	Rajasthan	3.02	88.5	112	*T. aestivum*
18.	35102-D	Bijapur	Karnataka	3.41	130.3	100	*T. durum*
19.	35107-D	Bijapur	Karnataka	3.27	133.3	93	*T. durum*
20.	35144-D	Bellary	Karnataka	3.69	135.0	93	*T. durum*
21.	35148-D	Dharwar	Karnataka	-	111.3	93	*T. durum*
22.	35149-D	Dharwar	Karnataka	3.60	106.6	89	*T. durum*
23.	35140-D	Raichur	Karnataka	-	127.6	106	*T. durum*
24.	35141-D	Raichur	Karnataka	2.73	136.6	107	*T. durum*
25.	35161-D	Bijapur	Karnataka	3.77	139.3	98	*T. durum*
26.	35177-D	Bijapur	Karnataka	3.65	79.0	103	*T. durum*
27.	35720-D	Palampur	Himachal Pradesh	3.31	111.6	94	*T. durum*

polymerization temperature. Final extension was given at 72°C for 5 min.

Amplification products obtained were electrophoresed on 1.5% agarose gel (Sambrook et al. 1989). The gel image was viewed and stored in gel documentation system. Photographs from ethidium bromide stained gel were used to score the data manually and independently for RAPD analysis. Presence of amplified product was scored as 1 and its absence as 0. These data matrices were then entered into NTSYS-PC (numerical taxonomy and multivariate analysis system program) (Rohlf 1992). The data were analyzed by SIMQUAL (similarity for qualitative data) routine to generate Jaccard's similarity coefficients (Sokal and Sneath 1963). The similarity coefficients were then used to construct dendrogram using the UPGMA (unweighted pair-group method with arithmetic average).

Results and discussion

Optimization of template DNA concentration was done and 20 ng/µl concentration was found optimum with primer UBC-350 and UBC-386 and accessions IC-82198 and IC-82199 combination, respectively.

PCR amplification of DNA extracted from 27 genotypes was performed following the same protocol for all the ten primers. The number of RAPDs generated by the ten primers with 27 genotypes is presented in Table 3. The total number of bands amplified from the ten polymorphic primers was 103. This gave an average of 10.3 bands per primer. Out of the 103 bands, 82 (79.6%) were polymorphic. The size of the amplified products ranged between 0.3 to 3.0 kb. This gave an average of 8.2 polymorphic and 2.1 monomorphic bands per primer. The highest number of polymorphic bands was obtained with

Table 2. Arbitrary 10-mer nucleotide primers used for the RAPD analysis

Serial No.	Genei Lot No.	Primer code	Sequence 5' to 3'	GC content
1.	BG 80 GB 10 A1/201	UBC-18	GGGCCGTTTA	60
2.	BG 86 GB 10 G7/201	UBC-535	CCACCAACAG	60
3.	BG 81 GB 10 G2/200	UBC-337	TCCCGAACCG	70
4.	BG 87 GB 10 G8/201	UBC-552	CTAAATGGCG	50
5.	BG 89 GB 10 C10/201	UBC-600	GAAGAACCGC	60
6.	BG 84 GB 10 G5/201	UBC-532	TTGAGACAGG	50
7.	BG 88 GB 10 C9/201	UBC-572	TTCGACCATC	50
8.	BG 85 GB 10 C6/201	UBC-534	CACCCCCTGC	80
9.	BG 82 GB 10 C3/201	UBC-350	TGACGCGCTC	70
10.	BG 83 GB 10 G4/201	UBC-386	TGTAAGCTCG	50

Table 3. Approximately lower and higher molecular weight band size, number of unique bands amplified and the distinguished genotypes by its respective primers

Serial No.	Primer	Lower mol. wt. band size (bp)	Higher mol. wt. band size (bp)	No. of unique bands	Distinguished genotypes
1.	UBC-18	300	2100	1	IC-35177-D
2.	UBC-337	300	2800	1	IC-82233
3.	UBC-350	500	3000	-	-
4.	UBC-386	500	2000	-	-
5.	UBC-532	300	2500	-	-
6.	UBC-534	400	2500	2	IC-35161-D, 35177-D
7.	UBC-535	300	2000	4	IC-99785, 35161-D, 35177-D, 35720-D
8.	UBC-552	400	3000	-	-
9.	UBC-572	400	1800	1	IC-35720-D
10.	UBC-600	300	2500	1	IC-35161-D

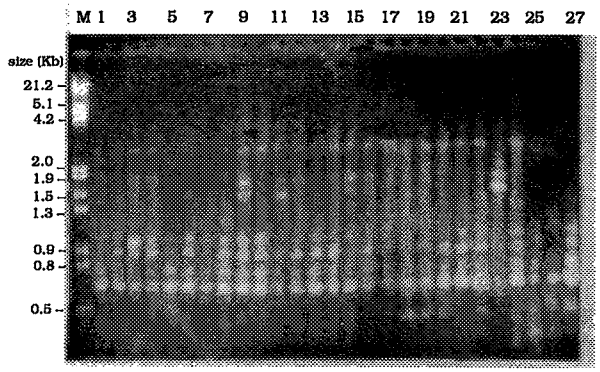

Fig. 1. The RAPD profile of 27 wheat genotypes generated by the primer UBC-552 on 1.5% agarose gel

primer UBC-535 and the lowest with UBC-532. The primers UBC-18, UBC-337, UBC-534, UBC-535, UBC-572 and UBC-600 were found to give distinguishable number of unique RAPD markers (1, 1, 2, 4, 1 and 1 bands, respectively).

The RAPD amplification within 17 hexaploid wheat genotypes resulted in 98 amplified products. Of the total amplified products, 64 (65.3%) were polymorphic. Of the polymorphic hexaploid bands, the highest number of bands was obtained by primers UBC-535 and UBC-552, while the lowest by UBC-532. The size of amplified hexaploid products ranged between 0.3 and 3.0 kb. This gave an average of 6.4 polymorphic and 3.4 monomorphic bands per primer.

RAPD amplification with 10 tetraploid wheat genotypes showed 103 amplified products, of which 78 (75.7%) were polymorphic. The highest number for polymorphic bands was obtained by primer UBC-535 and UBC-552 (Fig. 1), while the lowest by primer UBC-532. Ten primers gave an average of 7.8 polymorphic and 2.5 monomorphic bands per primer. Association among the 27 genotypes revealed by UPGMA cluster analysis is presented in Fig. 2. The dendrogram readily separated the 27 genotypes into two clusters (cluster I and cluster II). Cluster I comprised of 2 sub-clusters, of which sub-cluster I consisted of 6 hexaploid accessions with similarity coefficient of approximately 0.75. In this sub-cluster IC-82199 and IC-82202 showed higher genetic similarity followed by accessions IC-78754 and IC-78868. Sub-cluster II could be classified into 2 groups. Group I was further divided into 2 sub-groups of hexaploid wheat accessions having 3 and 8 accessions per sub-group. Group II included 7 tetraploid wheat accessions and in turn it could be divided into 2 sub-groups having 2 and 5 accessions per sub-group, respectively. Cluster II comprised of 3 unique tetraploid wheat accessions and could be divided into 2 sub-clusters. Accessions IC-35720-D is in one sub-cluster and the other two accessions IC-35177-D and IC-35161-D are in the other cluster.

Data of RAPD markers scanned from the 27 genotypes of wheat with 10 RAPD primers was used to generate similarity coefficients. The similarity coefficient among hexaploids and tetraploids ranged from 0.361 and 0.828. The two accessions of tetraploid wheat (IC-35107-D and IC-35144-D) were highly related as indicated by high value of similarity coefficient (0.966), followed by two other accessions of hexaploid wheat (IC-82280 and IC-82526) with similarity coefficient of 0.952. Accession IC-35177-D (tetraploid) and IC-78868 (hexaploid) were highly unrelated having a low value of similarity coefficient (0.361). The similarity coefficient among hexaploid wheat genotypes ranged from 0.630 to 0.952, whereas among tetraploids it ranged from 0.400 to 0.966. It

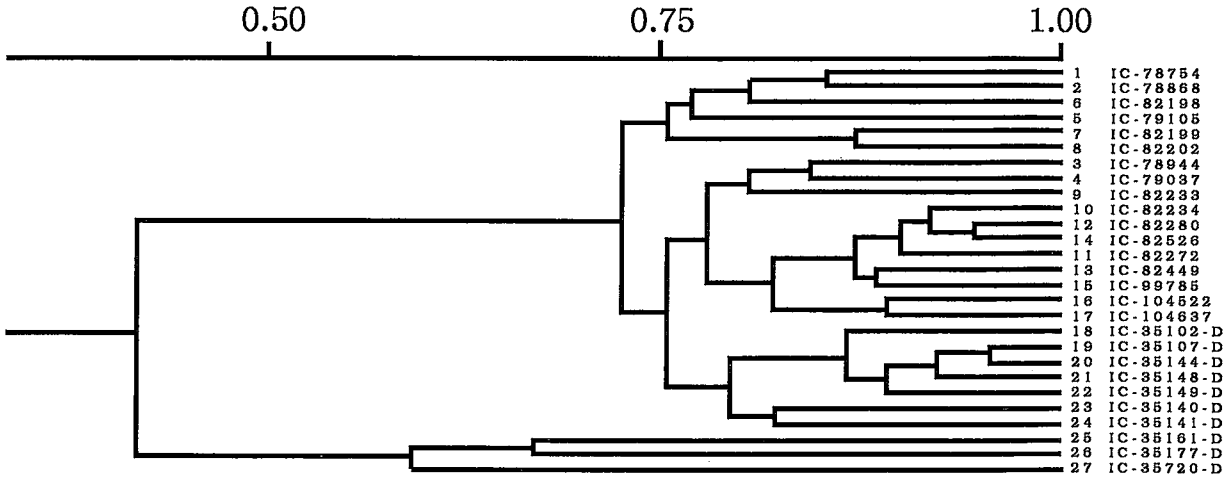

Fig. 2. Dendrogram of wheat genotypes constructed using UPGMA based on Jaccard's similarity coefficients. Scale on top is Jaccard's coefficient of similarity. Note: 1-17 are hexaploids and 18-27 are tetraploids)

clearly showed that tetraploid genotypes had more wider genetic distance value than hexaploid genotypes with relatively narrow genetic distance value.

The RAPD method has been in the past used successfully for the genetic diversity analysis in wheat (Pujar et al. 1999; Castagna et al. 1997; Nagaoka and Ogihara 1997). In this investigation genetic analysis of 17 hexaploid and 10 tetraploid Indian wheat accessions was done. Although investigations on diversity analysis of tetraploid and hexaploid wheat have been reported (Joshi and Nguyen 1993; Pujar et al. 1999; Sun et al. 1998), no one has analyzed above mentioned Indian tetraploid and hexaploid accessions separately or together. Therefore, this is the first report in which both are analyzed together at the same time.

Of the total amplification products scored in the RAPD analysis of this study, 79.6% were polymorphic and detected varied levels of polymorphism between hexaploid (65.3%) and tetraploid (75.7%) wheat accessions, which is consistent with the findings of Joshi and Nguyen (1993) and Pujar et al. (1999). The genetic distance (GD) values of tetraploid accessions were significantly higher than the GD values of hexaploid accessions. The narrowness of genetic base in the modern improved wheat cultivars is widely accepted and demonstrated by both pedigree (Cox et al. 1986) and molecular analysis (Sun et al. 1996).

The results of this study indicate that RAPD analysis can be used to study the genetic relationship of genotypes, and also to assess the genetic diversity of accessions.

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Research article

Genetic architecture of grain weight in durum wheat under normal and late sown environments

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Summary

Twelve basic generations, namely, P_1, P_2, F_1, F_2, BC_1, BC_2, BC_1self, BC_2self, BC_{11}, BC_{12}, BC_{21} and BC_{22} of three crosses involving six diverse cultivars of durum wheat were studied under normal and late sown conditions to understand the nature of gene effects on grain weight. The 10-parameter model was found adequate only in the two crosses to account for the variability in generation means under normal sown environments. However, in other cases even the 10-parameter model did not fit the data, confirming that more than trigenic interactions or linkages were involved in the inheritance of this trait. Of the epistatic interactions, trigenic interactions were invariably more important than other gene effects in the genetic control of grain weight in durum wheat. Additive x additive (i), additive x additive x dominance (x) and dominance x dominance x dominance (z) epistatic effects contributed maximum than other effects towards significant heterosis. Duplicate epistasis in sets of three genes was frequently observed. High magnitude of inter-allelic interactions at digenic and trigenic level and manifestation of high degree of heterosis and inbreeding depression, breeder should follow diallel selective mating and biparental mating which can mop-up the genes to form superior gene constellations interacting in a favorable manner to accelerate the pace of its genetic improvement.

Key words: durum wheat, gene effects, duplicate epistasis, heterosis, non-allelic interactions

Introduction

Durum or macaroni wheat, *Triticum durum* (2n = 4x = 28, genomes AABB) is grown on about 30 million hectares and accounts for almost 8 percent of total world wheat production. It is the second important cultivated species of the genus *Triticum* in India, occupying about 2.5 million hectare and has a lot of potential both for domestic consumption and for export market since durum wheat is used for making special products. Despite its importance for the human diet little progress has been made in improving the yield and nutritional qualities on durum wheat. Historically durum wheat has received insufficient attention from plant breeder. Therefore, any efforts to increase yield in durum will be directly supportive to boost up the over all wheat production of the country and it could be helpful to meet out the food requirements for the burgeoning population. Secondly, the surplus durum wheat could be exported in the international market to earn foreign currency.

Generally, the yield levels of durum are low as compared to bread wheat under irrigated as well as rainfed conditions. Systematic attempts for improvement in durum are needed through manipulations of various yield components. Grain weight is an important yield contributing component particularly under high temperature conditions (Chowdhery et al. 1996; Nayeem and Veer 2000). The exploitation of genetic information has not been very well demonstrated for this vital yield contributing trait

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in durum. The choice of plant breeding methodology for upgrading the yield potential largely depends on the availability of reliable information on the nature and magnitude of gene effects present in the population. Diallel analysis, although effective and most widely used, does not provide the estimates of non-allelic interactions. Epistasis, which is known to play a major role in the expression of heterotic potential, has been reported by Jinks (1955), Hayman (1958), Brim and Cockerham (1961), Gamble (1962), Hill (1966), Matinzinger (1968) and Stuber and Moll (1974), clearly indicated the role of epistatic gene actions besides additive and dominance type. Hence, it is essential to know precisely the genetic architecture of character(s) for further improvement of yield.

Generation mean analysis, which provides the estimates of the main gene actions (additive and dominance) and their digenic [(i), (j) and (l)] and trigenic [(w), (x), (y) and (z)] interactions, helps in understanding the performance of the parents used in the crosses and potential of the crosses to be used either for heterosis exploitation or pedigree selection. Since durum wheat is grown under varied environmental conditions, knowledge of gene action operating under different environments is essential, because in the absence of such information the breeding methods used may not result in appreciable improvement. The present investigation was therefore carried out to estimate the type of gene action under normal and late sown conditions for selection of most efficient breeding methodology for genetic improvement of grain weight in durum. Therefore, the three, six and ten-parameter models have been utilized to study and analyze the genetic control of grain weight involving six diverse cultivars of durum wheat.

Materials and methods

The experimental material generated from six diverse parents, comprised three crosses namely, Cocorit 71 x A-9-30-1, HI 8062 x JNK-4W-128 and Raj 911 x DWL 5002. In each cross combination one of the parents (Cocorit 71, JNK-4W-128 and Raj 911) had higher

Table 1. Results of joint scaling test and gene effects for grain weight under different environments

Effects	Cocorit 71 x A-9-30-1		HI 8062 x JNK-4W-128		Raj 911 x DWL 5002	
	Normal sown	Late sown	Normal sown	Late sown	Normal sown	Late sown
m	49.95**	51.24**	43.34**	49.04**	37.82**	51.17**
	±1.29	±0.47	±1.13	±0.66	±1.23	±0.42
(d)	5.51**	2.93**	2.74**	–0.85	8.99**	6.62**
	±1.14	±0.51	±0.93	±0.57	±0.99	±0.39
(h)	4.04	5.13**	6.92**	5.79**	11.97**	–3.50**
	±2.29	±1.04	±1.87	±1.17	±1.99	±0.78
(i)	–9.91**	–10.78**	11.63	–4.62**	45.13**	2.38*
	±2.74	±1.16	±2.91	±1.66	±3.19	±0.97
(j)	6.50	15.61**	–18.00**	–10.96**	30.03**	6.02*
	±5.12	±2.54	±4.41	±2.88	±4.68	±6.05
(l)	–60.65	–38.60**	–10.34	–20.50*	35.85**	38.57**
	±11.57	±5.07	±10.60	±6.64	±11.45	±3.15
(w)	-4.84	5.97**	–24.47**	–9.48**	6.36	–6.42**
	±4.33	±1.89	±3.44	±2.59	±3.59	±1.05
(x)	–56.95	–41.35**	0.56	–25.40**	42.87**	106.21**
	±13.46	±5.84	±12.90	±7.16	±14.09	±4.03
(y)	13.74	1.70	34.27**	7.50	32.43**	38.80**
	±9.20	±6.12	±8.89	±8.87	±8.32	±4.54
(z)	99.36	43.46**	63.82**	49.90**	–80.59**	–42.76**
	±17.05	±9.63	±16.88	±12.04	±17.00	±7.28
χ^2 for 10-parameter model	0.42 (2)†	18.21 (2)	14.02 (2)	25.33 (2)	3.35 (2)	26.95 (2)

*, ** Significant at the 0.05 and 0.01 level, respectively. †Degrees of freedom for χ^2

grain weight. Twelve basic generations, involved in these studies were two parents, F_1 and F_2, first backcross generations with both parents (BC_1 and BC_2), where BC_1 was the cross F_1 x female parent and BC_2 was F_1 x male parent, their selfed progenies (BC_1F_2, BC_2F_2) and second backcross generations (BC_{11}, BC_{12}, BC_{21}, BC_{22}) i.e. the BC_1 and BC_2 plants again crossed with both original parents (BC_1 x female parent, BC_1 x male parent and BC_2 x female parent, BC_2 x male parent). These twelve populations of each of the three crosses were evaluated in randomized block design with three replications in two parallel experiments, one sown on 20th November (normal sown condition) and other sown on 20th December (late sown condition) in the same cropping season. Each replicate was divided into three compact blocks. The crosses, each consisting of twelve populations were randomly allotted to the blocks. All the twelve generations were then randomly allotted to twelve plots within a block. The plots of various generations contained different number of rows i.e. each parent and F_1 plots consisted of 2 rows, while each backcross generation in 4 rows and F_2 and the second cycle of backcrosses in 6 rows. Each row was 5 m long accommodating 33 plants spaced 15 cm apart, row to row distance being 30 cm. Border rows were provided at the beginning as well as at end of experimental rows in each block. The experiment was planted at Research Farm of Rajasthan Agricultural University, Agricultural Research Station, Durgapura, Jaipur, Rajasthan, India. The weight of 100 seeds (g) counted at random from the single plant yield. The data were recorded on 15 random plants in each parent and F_1, 30 plants in each backcross generations and 60 plants in each F_2 and second backcross generations in each replication under both the environments.

Standard statistical procedures were used to obtain means and variances for each generation and each environment separately, as suggested by Snedecor and Cochran (1968). The data of each population in both environments were analyzed separately by joint scaling test of Cavalli (1952) to determine the nature of gene action. Components of heterosis in the presence of digenic (Jinks and Jones 1958) and trigenic interactions were calculated as suggested by Hill (1966).

Results and discussion

The results of generation mean analysis based on twelve generations (Table 1) revealed that even the 10-parameter model could not be fitted to explain the inheritance confirming the involvement of the more complex interaction or linkage in most of the cases. Both the epistatic effects, digenic as well as trigenic were frequently significant with the highest contribution of epistatic effects involving dominance (h). The absolute totals of the second order interactions (Table 2) were many times higher than the main effects and the first order interactions, indicating greater importance of trigenic interactions in controlling the inheritance of this trait. The greater significance of trigenic interaction is also obvious from the fact that the maximum contributing factors in all the crosses were trigenic effects such as parameter dominance x dominance x dominance (z) in the cases Cocorit 71 x A-90-30-1, HI 8062 x JNK-4W-128 and parameter additive x additive x dominance (x) in Raj 911 x DWL 5002. The first order interactions were also higher than the main effects and were quite important. Bhullar et al. (1980), Srivastava et al. (1980) and Singh (1981) also reported the role of epistasis in controlling of this trait in aestivum wheat.

Table 2. Absolute totals of epistatic effects, fixable and non-fixable gene effects for grain weight under different environments

Cross	Environment	Main effects		Epistatic effects†		Total gene effects‡	
		(d)	(h)	I order	II order	Fixable	Non-fixable
Cocorit 71 x	Normal	5.51	4.04	77.06	174.89	20.25	241.24
A-9-30-1	Late	2.93	5.13	64.99	92.50	19.70	145.85
HI 8062 x	Normal	2.74	6.92	39.98	123.12	38.83	133.92
JNK-4W-128	Late	–0.85	5.79	36.08	92.28	14.95	120.05
Raj 911 x	Normal	8.99	11.97	111.01	225.58	60.48	297.07
DWL 5002	Late	6.62	–3.50	46.99	130.84	15.42	172.54

† First order interactions: [(i), (j), (l)], Second order interactions: [(w), (x), (y), (z)]

‡ Fixable components: [(d), (i), (w)], Non-fixable components: [(h), (j), (l), (x), (y), (z)]

Table 3. Components of heterosis for grain weight under different environments

Effects	Cocorit 71 x A-9-30-1		HI 8062 x JNK-4W-128		Raj 911 x DWL 5002	
	Normal sown	Late sown	Normal sown	Late sown	Normal sown	Late sown
(h)	4.03	5.13	6.92	5.79	11.97	–3.50
– (i)	9.91	10.78	–11.63	4.62	–45.13	–2.37
1/2 (x)	–28.47	–20.68	0.28	–12.70	53.11	21.43
1/4 (z)	24.84	10.86	15.96	12.48	–20.15	–10.69
– (d)	–5.51	–2.93	–2.74	0.81	–8.99	–6.62
1/2 (j)	3.25	7.81	–9.00	–5.48	15.02	3.03
– (w)	4.84	–5.99	24.47	9.48	–6.36	6.42
–1/4(y)	–3.44	–0.43	–8.57	–1.88	–8.11	–9.70
Heterosis	22.66**	9.72**	15.12**	14.87**	–14.91**	3.68**
Inbreeding depression	–1.35	–3.16**	16.35**	7.16**	10.01**	4.88**

*, ** Significant at the 0.05 and 0.01 level, respectively.

While in durum wheat Gill et al. (1983) observed epistasis for this trait and he further observed that it is dependent upon the environments.

In the crosses Cocorit 71 x A-9-30-1, HI 8062 x JNK-4W-128 in late sown and in Raj 911 x DWL 5002 in both the environments, all the three (h), (l) and (z) parameters were significant and differed in signs, indicating duplicate epistasis in sets of three genes whereas in remaining cases conclusion regarding type of epistasis could not be drawn because either (h) or (l) parameter was non-significant. No information is available in durum wheat but in aestivum wheat Bhullar et al. (1980) and Srivastava et at. (1980) also observed duplicate type of epistasis for this character. The absolute totals of the non-fixable gene effects were many times higher than the fixables, indicating their greater importance in the inheritance of this trait (Table 2). Earlier also importance of non-additive gene effects in durum wheat was reported by Widner and Lebsock (1973), Gupta and Ahmad (1979), Srivastava et al. (1982), Gill et al. (1983) and Patil et al. (1997).

Highly significant heterosis over better parent was observed in all three crosses in both the environments (Table 3). Similarly significant inbreeding depression was also recorded except in the cross Cocorit 71 x A-9-30-1 under normal sown condition. Negative heterosis over better parent was observed in the cross Raj 911 x DWL 5002 in normal sown condition only. Yadav and Narsinghani (2000) reported negative heterobeltiosis and Chakraborty and Tiwari (1995) also reported significant positive heterobeltiosis for this attribute in bread wheat.

Components of heterosis revealed that additive x additive x dominance (x), dominance x dominance x dominance (z) along with additive x additive (i) in the cross Cocorit 71 x A-9-30-1, contributed significantly towards heterosis in both the sowing conditions. In the cross HI 8062 x JNK-4W-128 additive x additive x additive (w), dominance x dominance x dominance (z) along with additive x additive (i) in normal sown and additive x additive x dominance (x), dominance x dominance x dominance (z) along with additive x additive x additive (w) in late sown condition contributed maximum towards heterosis. In the cross Raj 911 x DWL 5002, components additive x additive x dominance (x), additive x additive (i) and dominance x dominance x dominance (z) in normal sown and additive x additive x dominance (x), dominance x dominance x dominance (z) and additive x dominance x dominance (y) in late sown condition, contributed towards heterosis. In the cross Cocorit 71 x A-9-30-1 under late sown environment inbreeding depression was significant and negative which indicated improvement of F_2 over F_1 mean for grain weight.

This study shows that the epistatic interactions particularly at trigenic level played a greater role in controlling the inheritance of grain weight in all three crosses under both the sowing conditions, the absolute totals of non-fixable effects were higher than the fixable effects. Breeding procedure that may utilize both additive and non-additive gene effects would prove beneficial. The diallel selective mating and biparental mating that may exploit both the types of gene effects could be worthwhile for the genetic improvement of grain weight in these crosses under normal sown environment to enhance grain yield in durum.

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Genetic stock

Utilization of genetic stock for quality attributes in Indian wheat breeding programs

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National Genetic Stock Nursery (NGSN), in its sixth year as "Suggested Crossing Block" was provided to nearly 36 centers in Indian peninsula during rabi 2001-02. It is an important source of confirmed genetic stocks for characters like biotic and abiotic stresses, yield components, superior agronomic bases and quality attributes. In last few years there is growing concern to improve the quality of the wheat to make it more competent in international market. Among various steps, identification of confirmed genetic stock for quality characters and providing them through NGSN is a step forward. This is to supplement the crossing block of wheat breeders of the country with genotypes equipped with characters like high protein, hectoliter weight, sedimentation value, β-carotene etc.

In the absence of reliable genetic sources for quality parameters in the past, the wheat breeders could not initiate a target-oriented quality-breeding program. In 1998, the wheat quality scientists at DWR, Karnal were able to select and categorize some genotypes from various national trials which were superior for quality attributes like protein (%), hectoliter weight, etc., which can be used as a genetic stocks for quality breeding programs.

Since 1998-99, 69 confirmed genetic sources superior for quality attributes were made available to wheat breeding programs for quality through NGSN CountryWide (Fig 1). Among them 31 were bread wheat and 38 were durum. These genetic stocks were superior for quality parameters in a superior agronomic base thereby making it more useful to a wheat breeder to emphasis for both grain yield as well as quality. The data so generated was published in the form of Progress Report, Vol II, Genetic Resources every year (Mahajan 1997, 1998, 1999, 2002; Mahajan and Ganga Rao 2000, 2001).

Twenty-six centers had utilized the quality lines

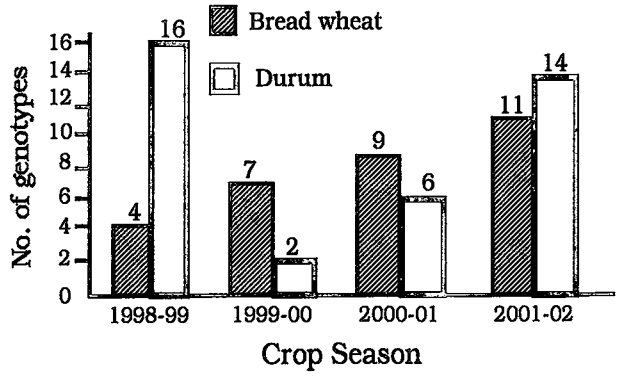

Fig. 1 Genotypes selected for quality attributes

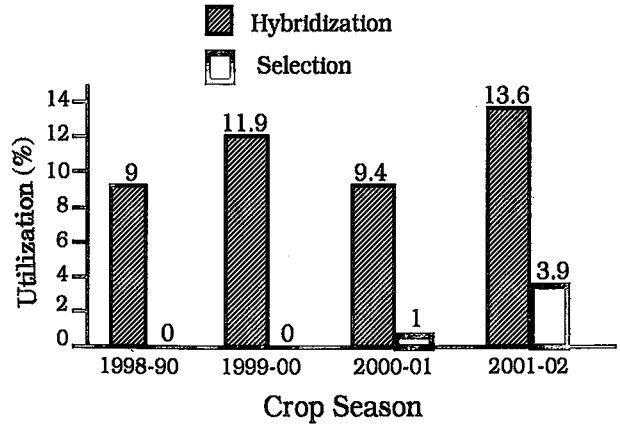

Fig. 2 Utilization of quality genetic stocks

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at least once in last four years. Among them Dharwar, Niphad, Pune, Kanpur and Burdwan had utilized the quality lines in all these years. Since 1999-2000 crop season, Akola, Hisar, Ranchi and Ludhiana had also initiated the selection among quality genetic stocks. The utilization of quality genetic stocks was either through hybridization or direct selection. The utilization of quality genetic stocks was primarily through hybridization during 1998-99, 1999-2000, 2000-01 and 2001-02 was 9.0, 11.9, 9.4 and 13.6 percent, respectively (Fig. 2). The direct selection of quality genetic stocks across the zones was reported during 2000-01 (1.0%) and 2001-02 (3.9%).

Due to growing awareness of quality parameters and their importance in breeding programs, the utilization of genetic stocks is constantly increasing from 14 centers (1998-99) to 19 centers (2001-02). This emphasizes the need to include more genetic stocks in NGSN thus focusing on future needs of wheat breeders.

The inclusion of the wheat genetic stocks has been an important step forward to support the wheat-breeding program of the country. In order to have a concentrated product oriented approach the group had decided to suggest the genetic stocks more suitable for different products like Chapati, bread, biscuits etc.

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Record

The 28th Japanese Wheat Genetics Symposium, August 23–24, 2002

Introductory remarks

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The 28th Japanese Wheat Genetics Symposium was held at Obihiro University of Agriculture and Veterinary Medicine, in August 23 and 24, 2002. The Local Organizing Committee consisting of H. Miura (Chairman) and K. Kato was in charge of its organization and management. Total number of the attendants were 104 belonging to 39 different institutions, including twelve researchers or students from foreign countries. As an address of welcome, Prof. S. Sawada presented the activity of late Prof. H. Kirara who stayed at Obihiro University in summer seasons during five years. The titles of the three oral sessions were : (1) wheat breeding in Hokkaido chaired by Drs. A. Yanagisawa and S. Ikeguchi (First symposium), (2) functional genomics of wheat organized by Drs. C. Nakamura and Y. Ogihara (Second symposium), and (3) general presentation including hot topics. In the poster session by young researchers or students, 14 papers were presented. The abstracts of all research papers are presented below. In the evening of August 23, the banquet was held in the University garden. More than 90 participants enjoyed a barbecue with home made vegetables, sweet corn, Irish potato, netted melon, and so on. In the business session, one agenda was discussed and agreement was made as follows; site of the 29th Japanese Wheat Genetics Symposium was decided to be Tottori University and Dr. Tsujimoto, a professor there, addressed willingness to become its host.

Abstracts of the first symposium

S-1-1 **Y. Amano** (Hokkaido Pref Tokachi Agric Exp Stn)

Wheat breeding and genetic research in Hokkaido

From the viewpoint of wheat breeding in Hokkaido, how to concern genetic research with breeding program was discussed. Agricultural technique of upland farming was introduced from United States to Hokkaido in early 20th century and wheat, potato, beans and sugar beets have been the main crops for the rotation system. European and American wheat varieties showed a high adaptability to Hokkaido environment in performance tests. Thereafter Hokkaido varieties were bred basically by combining European and American varieties with Japanese wheats. The winter wheat variety 'Chihokukomugi', released in 1981, has a good noodle tasting character. This variety was the first one that has a low amylose content and strong elasticity texture due to lack of the Wx-B1 protein synthesis. Introducing semi-dwarf genes, recent bred varieties had a high yielding potential and strong culm. In the cultivation of semi-dwarf varieties, high nitrogen application and high sowing density became possible, so average yield increased in twice. Rusts, winter killing and pre-harvest sprouting (PHS) had long been serious problems. For the protection from rust disease and winter killing, breeding program has been constantly carried out by introducing resistant genes of foreign materials. Biotype of pathogen of leaf rust in Hokkaido is more severe than that of Honshu islands and has always changed with lapse of cultivation of new varieties. Winter killing has been caused mainly by some snow molds and partly by cold injury. By recurrent selection, Hokkaido varieties were improved to have a comparatively high resistance to every factors of winter killing. More resistant materials to each factor were recently introduced and breeding program is now in progress. Snow mold resistance and cold resistance were quantitative characters but

heritability is high under restricted conditions. As combine harvest is widespread, PHS problem became serious. Wheat varieties in Hokkaido have been rather susceptible to PHS, so genetic sources must be introduced and breeding for PHS resistance has been a urgent program. Sprouting resistance is also a quantitative character and is connected to many parts of wheat grains. But embryo dormancy is the main factor, controlled by a small number of genes. Breeding program for sprouting resistance is now developing on.

S-1-2 **A. Yanagisawa[1] and S. Ikeguchi[2]** ([1]Hokkaido Pref Kitami Agric Exp Stn, [2]Hokuren Agric Res Inst)
Future prospects of wheat breeding in Hokkaido

The yields of wheat in Hokkaido have increased from 1.5t/ha in 1960 to 4.3t/ha in 1985. Those increases are due to the introduction of new varieties and the improvement of cultivation method, more efficient application of fertilizers and more efficient controls of diseases. Wheat production in Hokkaido is about 60% of domestic wheat in Japan. However the production has been unstable in recent years because of rain damage. Epidemics of scab also causes yield loss and degradation of quality. Several snow molds are another factors that affect winter wheat production. We have selected resistant lines to pre-harvest sprouting by the artificial rain treatment. Also α-amylase activities/falling number of late harvested samples are checked. In the sprinkled field, spikes are inoculated with *Fusarium graminearum* at flowering stage to promote disease to select resistant lines. We also select resistant lines to snow molds by the inoculation with *Typhus ishikariensis*. The domestic wheat are processed into mainly Japanese noodles. Good flour color and 3–5% lower amylose content than wild type wheat are desirable. Spring wheat in Hokkaido has enough protein content and quality to bread. Gluten quality, mixing propriety and HMW-glutenin, LMW-glutenin subunit patterns are investigated to improve bread-making qualities. DNA markers have been tried to identify resistant lines to pre-harvest sprouting and scab.

S-1-3 **S.I. Osanai**
Development of highly resistant wheat to pre-harvest sprouting

In Tokachi and Abashiri districts in eastern Hokkaido, Japan, the minimum temperature often drops to below 10°C and rain in common during the harvest month of August. As a consequence, the development of varieties that are tolerable to sprouting under low temperature and water absorption condition is necessary. The most tolerant spring semi-dwarf line, OS21-5, to germination at 10°C was selected under low temperature conditions from the first cross of Tordo (*Rht3*)/Zenkouji-komugi in 1982. It was widely used as gene source for breeding of pre-harvest sprouting resistance and year after year OS21-5 has contributed to produce transgressive lines as follows; spring wheats OS38 and OS39, selected from 8019R1/OS21-5//OS21-5, winter wheats OW77 from OS21-5/Lancer, OW104 from OS21-5/61199, OW93 from KK1551/OS21-5//W140-37/KK1646, and OW97, 98 and 99 from OW63/KK1647. Recent some winter wheat lines have been well improved on the agronomic characteristics except flour quality.

S-1-4 **K. Takata** (National Agric Res Center for Hokkaido Region)
Breeding for bread-making quality of winter wheat

More than decade has passed since the beginning of our breeding program for bread-making quality. Relationship between high molecular weight glutenin (HMWG) subunits and bread-making quality was studied for first step. HMWG subunits 5+10 coded by *Glu-D1* showed much larger effect on the physical properties of dough than subunits 2+12, 4+12 and 2.2+12. Subunit 20 was the weakest physical property of dough at the *Glu-B1* allele. Both subunit 20 and subunits 2.2+12 showed extremely poor functional properties. We have introduced subunits 5+10 to improve bread-making quality at first step. Now we are pursuing balance of strength and extensibility of dough for next step. Canada Western Extra-Strong Red Spring wheat, which has extremely strong dough properties, shows unique properties. For the improvement of flour quality in Japanese wheat, it would be useful to detect the protein components related to the unique properties and introduce them into Japanese wheat cultivars. We have noticed extra strong properties of Hard Red Winter wheat line KS 831957 and have tried to introduce its properties into Japanese wheat. It is considered that combination of a specific low molecular weight glutenin (LMWG) and HMWG subunits 5+10 give extra strong property. Bread-making quality is a complex character consists of HMWG, LMWG, gliadin, lipids, pentosan and starch. We have to elucidate each key factor and it will be very helpful tool for the breeding of bread-

making quality.

Abstracts of the second symposium

S-2-1 **N. Sakurai** (Kazusa DNA Research Institute)
Current macroarray analysis and its extent of utilization — implications from a study of *Arabidopsis* tetraploids.

We have prepared macroarrays which carry 13,536 clones of non-redundant ESTs from *Arabidopsis*, and have utilized them to profile gene expression in various states of tissues. By improving the spotting procedure, hybridization and detection methods, and introducing statistical processing for data analysis, we can now obtain expression data for each gene with annotations on the data reliability. Nonetheless, some problems remain to be resolved. A case is when we compare two individuals or tissues that are in different developing stages. As long as we apply mean, median, or other measures of central tendency for normalization of signal intensity, there is a postulate that most of genes are unchanged, or sum of all variance is constant between the both. In our laboratory, diploid and tetraploid *Arabidopsis* were applied to macroarray analysis as a model system to depict a difference between both nuclear phases. Some genes were significantly extracted as a feature of tetraploids. However, this is rather skeptical interpretation, because there is no certification that the total abundance of mRNAs is the same in diploid and tetraploid plants, even if they were grown under a same condition. How we can overcome this problem? Over discussing such topics, I'd like to introduce the utilization and application of array technology.

S-2-2 **Y. Ogihara** (Kihara Inst Biol Res & Grad Sch Integrated Sci, Yokohama City Univ)
Toward development of functional genomics in wheat

Recent developments of functional genomics in model plants such as *Arabidopsis* and rice, enable us to investigate overall gene constituent and expression patterns. In wheat, earnest research works such as mapping of DNA markers, especially Bin mapping of sequenced cDNA clones, and large scale analysis of ESTs in various tissues during wheat life cycle, can offer the tools for functional genomics. Recently, we have developed some of such basic tools for functional genomics: these are a large body of EST data base, cDNA microarray including microarray spotted full length cDNAs, and SNPs analysis of hexaploid wheat. These tools for functional genomics are now available to wheat researchers. Furthermore, we have a plan to set up data base for comparative and functional genomics in wheat.

S-2-3 **K. Mochida** (Kihara Inst Biol Res & Grad Sch Integrated Sci, Yokohama City Univ)
SNPs analysis of hexaploid wheat

Single nucleotide polymorphisms (SNPs) are frequently observed in the genes as well as intergenic regions among related strains. And SNPs are effectively used for genotyping of various strains. In wheat, however, the nature of polyploidy prevents, to some extents, SNPs analysis by the ordinary methods. Critical point to carry out SNPs analysis in wheat is to assign the individual SNPs into each of three genomes, namely A, B and D. We developed an EST assembling method that can distinguish the contigs transcribed from each loci located on different parts of chromosomes. By way the method 116,232 EST sequences of Chinese Spring (CS) were assembled into 25,971 contigs. Out of 25,971 contigs containing more than 5 members were selected and classified into related gene groups by combination of BLAST and phrap methods. Single copy gene groups per genome were supplied for further analysis. The grouped contigs were aligned, and the SNPs positions were determined. The frequency of SNPs examined was estimated to be once per 144.9 bp. Pyrosequencing™ method in combination with the nulli-tetrasomics series of CS was adopted to assign individual SNPs into each chromosome of hexaploid wheat. Most SNPs can be assigned into each chromosome, showing applicability of the Pyrosequencing method for SNPs analysis of hexaploid wheat.

S-2-4 **K. Murai** (Dept Biosci, Fukui Pref Univ; murai@fpu.ac.jp)
Functional genomics using EST data in wheat — A case of MADS box gene family —

To explore the gene expression underlying wheat life cycle, a large-scale analysis has been done on the cDNAs from various tissues at different growth stages in wheat. A set of about 70,000 expressed sequence tags (ESTs) was analyzed and grouped into about

26,000 independent clusters. Among these, 36 MADS box gene contigs were found. MADS box genes encode trascriptional regulators involved in diverse aspects of plant development. Based on the deduced amino acid sequences, the wheat MADS box genes were classified into seven groups. The largest group consists of 16 MADS box genes which show homology to *AGL2/SEP1*-like genes in *Arabidopsis*. It is well known that the sequence homology of MADS box genes is highly related to the functional homology. Four wheat MADS box gene clones, #3558, #4273, #5651 and #13055 of *AGL2/SEP1*-like group, were closely related to *OsMADS1* in rice, and *ZMM8* and *ZMM14* in maize, which play an important role in floral meristem determination in spikelets. This suggests that these wheat MADS box genes may also be involved in conferring determinacy of floret identity.

S-2-5 **K. Kato** (Obihiro Univ Agric & Vet Med)
Functional genomics combined with QTL analysis in wheat

To clarify the genic control of flowering time and seed dormancy in hexaploid wheat, we have applied comparative genetic mapping of QTLs and candidate genes across plant species including rice, maize and barley. We identified the genetic map positions of two candidate genes, *tck2a* on chromosome 5A and *taVp1* on 3A. Comparative mapping showed that *tck2a* was syntenous to the rice *CK2a* or the heading date QTL *Hd6*. On the other hand, *taVp1* was expected to be an orthologue of *Vp1*, the maize viviparous gene. However, our data did not support the direct relationship between candidate genes and QTLs. We will clarify the genic interaction between candidate genes and QTLs in future study. Alternatively, near isogenic lines carrying each QTL and/or chromosomal segment have been developed by marker-assisted selection using DNA markers. These plant materials will be profitable to characterize the functions of QTLs.

S-2-6 **C. Nakamura** (Grad Sch Sci & Technol, Kobe Univ)
Functional genomics approaches towards nucleus-cytoplasm interactions in wheat

The genetic system of the cytoplasmic genome (plasmon consisting of chloroplast and mitochondrial genomes) differs significantly from that of the nuclear genome in various aspects. The plasmon is semi-autonomous and yet can sustain its structural and functional integrity only through an intimate interaction with the resident nuclear genome. One important question directly related to the plasmon biology including nucleus-cytoplasm interaction and retrograde regulation of the nuclear gene expression is; if and how we can develop experimental systems that should enable us to carry out functional genomics research in wheat and its related species. The symposium talk is divided into three parts. First, several new trends in plant mitochondrial research are introduced, based on interesting reports in the 6th Int. Congress of Plant Mitochondria, July 9-14, 2002, Perth, Australia. Second, our recent research results and future perspectives of the cold responsive genes/proteins in wheat are reported. The genetic systems of low temperature and dehydration stress responses involving nuclear genes and proteins targeted to chloroplasts and mitochondria hopefully provide model systems for functional genomics study of the NC interaction. Third, a peculiar phenomenon of mitochondrial (mt) DNA heteroplasmy depicted as mixed presence of the maternal and paternal mtDNA copies in individual plants is addressed. Biological and evolutional significance of the observed mtDNA heteroplasmy remains largely unknown, but this system provides a new and unique tool for evolutional and functional genomics study in wheat and its related species.

Abstracts of the general presentation

Hot topics

Y. Matsuoka (Fukui Pref Univ)
Maize domestication — when, where, and how many times? (A recent view from plant genetics)

There exists extraordinary morphological and genetic diversity among the maize (*Zea mays* ssp. *mays*) landraces developed by pre-Columbian cultivators. To explain this high level of diversity in maize, several authors have proposed that maize landraces were the products of multiple independent domestications of a wild relative of maize, teosinte. Contrary to the view, a series of genetic studies by John Doebly and his colleagues strongly suggest that the high level of diversity of maize can be explained by a single domestication and subsequent diversification.

Recently, comprehensive microsatellite-based phylogenetic analyses of maize and its progenitor, teosinte, have been reported, which successfully distinguish between these two models regarding the origin of maize and indicate that all existent maize arose from a single domestication event in southern Mexico about 9,000 years ago. It is also indicated that the oldest surviving maize types appear to be those of the Mexican highlands with maize spreading from this region over the Americas along two major paths. I will discuss the implications of this work in the light of other genetic and archaeological evidence.

O-1 **K. Tsunewaki** (Fukui Pref Univ)
Aneuploid analysis of albino genes in tetraploid wheats

Although occurrence of albinos in hybrid offspring between cultivars or strains of polyploid wheat is not uncommon, no loci have been identified for albinism (McIntosh et al. 1998). It is due to lethality of albino, that makes maintenance of fixed albino stocks impossible. I succeeded to identify two types of homozygotes, *abn1/abn1* +/+ and +/+ *abn2/abn2*, for two complementary (or duplicated) recessive albino genes, *abn1* and *abn2*. To identify their chromosomal locations, I carried out aneuploid analysis of those genes, using a set of disomic D-genome chromosome substitution (abbrev. DS) lines of *T. durum* cv Langdon 16 (abbrev. Ldn) (Joppa and Williams 1988). Ldn and Line 183 were selected to represent the genotypes, *abn1/abn1* +/+ and +/+ *abn2/abn2*, respectively. Crosses were made between 14 DS lines and normal Ldn as female and Line 183. The F_1 hybrids were self-pollinated, and the F_2 seeds were sown to observe segregation of albinos. The F_2 population of the cross, DS 2D(2A) x Line 183, did not segregate any albinos, indicating that chromosome 2A of Ldn carries the *abn1* gene and chromosome 2D of DS 2D(2A) has its normal homoeoallele. All other F_2 population segregated albinos in the same frequencies as the control F_2 population. These results suggest that chromosome 2B of Line 183 carries the *abn2* gene, because, if not, the F_2 population of DS 2D(2B) should give much higher albino frequency than those of normal Ldn or other DS lines. The present conclusion explains well the results of Nishikawa (1986).

O-2 **Y. Mukai[1], G. Suzuki[1], A. Nakano[1] and M. Yamamoto[2]** ([1]Osaka Kyoiku Univ, [2]Kansai Women's Col)
Molecular breeding by genome fusion in rice. I. Introduction of wheat genome into rice

The most important task for plant breeding research is to introduce a set of agronomically useful genes of a certain crop into another distant crop beyond the barrier of reproductive isolation. For the revolutionary improvement of a crop, a new system which can transform a number of genes controlling many functions to plants is indispensable. Therefore, it is of urgent necessity for practical use of plant genomics to establish the transformation system that introduces large fragments of plant genome to other crops. In order to expand genetic variability in rice, we are trying to introduce the large genomic DNA fragments containing agronomically important genes of wheat into rice. We are doing research that breeds rice with wheat genome by successive introduction of the huge DNA fragments such as BAC clones. We call this method "genome fusion". In the first step of our research, we aim to develop the system which can introduce a mass wheat genome efficiently and the simple method of detecting the introduced genome or gene in transgenics. Our goal is to make bread and udon noodles from rice powder in the future by giving bread making nature and the noodle aptitude to rice. It is useful also to environmental preservation by not only contributing to consumption expansion of rice by aiming at conversion into the rice from wheat flour, but maintaining a paddy field by rice cultivation.

O-3 **M. Yamamoto[1], T. Ohta[2] and Y. Mukai[2]** ([1]Kansai Women's Col, [2]Osaka Kyoiku Univ)
Application of molecular combing to wheat research

Wheat has the large-sized genome including a large amount of repetitive DNA. The presence of such junk DNA stands in the way of plant genome analysis. When bacterial artificial chromosome (BAC) clones were used as probes in fluorescence in situ hybridization (FISH) experiments, hybridization signals were detected on the almost chromosomes and extended DNA fibers of wheat. Genome organization of agronomically important genes in wheat has been analyzed by southern blots and sequencing using lambda or BAC clones. Molecular combing is a new technique to directly map target DNA sequences onto individually stretched DNA molecules. This technique can give the visual information on the structure of BAC before its molecular biological analysis. This information results in saving time and labor in analysis and provides us with a useful finding for the construction of BAC contig. The orientation of the

seed storage protein genes and the grain-hardness genes was analyzed on wheat BAC DNA molecules (circular and linear types). Furthermore, application of molecular combing FISH includes determining the structure of the clone, the copy number of genes, and the order of genes and specific sequences.

O-4 **R. Ohno, F. Kobayashi, S. Takumi and C. Nakamura** (Fac Agr, Kobe Univ)

Characterization of wheat cold-responsive protein WCOR14

A wheat cold-responsive gene, *Wcor14*, encodes a chloroplast-targeted polypeptide of 14 kDa with a putative N-terminal transit peptide. The *Wcor14* gene expression was specifically regulated by low temperature and not induced by ABA, drought and salinity. Accumulation of the *Wcor14* transcript and the WCOR14 protein occurred in a coordinate fashion under cold acclimation; the protein accumulation precisely followed the transcript accumulation with a clear time lag. The time course of the transcript and protein accumulation also showed a good correlation with the developmental time course and the level of freezing tolerance in two wheat cultivars. Importantly, the winter-hardy cultivar M808 accumulated larger amounts of the transcript and protein more rapidly and for a longer period than the spring-type cultivar CS. A de-acclimation study clearly showed that the WCOR14 protein was more stable also under the normal temperature condition in M808 than in CS. Accumulation of the WCOR14 protein was maintained throughout at least two months under the low temperature condition. Studies using the near-isogenic lines of *Vrn* loci suggested that the transcript and protein accumulation was regulated either by the *Vrn* genes or by the *Fr* genes, which are linked with the *Vrn* genes. In vitro phosphorylation study showed that the recombinant WCOR14 protein was a substrate for a 50 kDa wheat kinase. The activity of this kinase was modulated by low temperature in a similar manner to the *Wcor14* gene expression.

O-5 **S. Takumi[1], R. Morimoto[1], T. Kosugi[1], H. Koga[2] and C. Nakamura[1]** ([1]Fac Agr, Kobe Univ, [2]Res Inst Agr Res, Ishikawa Agr Col)

Ectopic expression of *Kn1*-type homeobox genes in a wheat *Hooded* mutant

Hooded (*Hd*) is well known to be one of dominant inhibitors of awn development in common wheat (*Triticum aestivum* L.). The *Hd* near-isogenic lines of S-615, in which the *Hd* locus from CS is introgressed, show not only repressed awn growth but also knot-formation at the awn base. The knot-formation is associated with the altered cell morphology and abnormal cell division under the epidermal layer at the awn base and at the top of lemma. The map location of the *Hd* alleles showed the chromosomal synteny among the wheat *Hd*, barley *Hooded* (*K*) and maize *Knotted1* (*Kn1*). The *Kn1* and *K* phenotypes were respectively caused by structural mutations occurred in the long intron of the maize *Kn1* homeobox gene and its barley orthologue. Overexpression of the wheat class 1 *Kn1*-type homeobox gene exhibited abnormal leaf morphology with occasional ectopic leaves on the adaxial leaf surface in the transgenic tobacco, while no mutations possibly associated with the dominant phenotype were found in the wheat 4A loci of the *Kn1* orthologue *Wknox1*. To find a responsive gene, we compared sequences of RT-PCR products amplified from lemma of S-615 with those from the *Hd* near-isogenic line. We observed ectopic expression of one *Kn1*-type homeobox gene homologous to *Knox3*. These results suggest that the wheat *Hd* gene is an ectopically expressed dominant *Knox3* allele closely linked with the *Wknox1* locus on the wheat chromosome 4A.

O-6 **M. Matsumoto, I. Endo and N. Kawakami** (Fac Agric, Meiji Univ)

Effect of temperature on germination and phytohormone response in wheat seeds

Low ambient temperature is the main environmental factor for pre-harvest sprouting of wheat. The grains of cultivar Gifu-komugi have shown to have very strong dormancy. Imbibition of the dormant grains at low temperature (5°C for 4 days), however, eliminated dormancy fairly and reduced abscisic acid (ABA) sensitivity of the embryos. We also examined the effect of temperature on germination and post-germinative growth in a gibberrelic acid (GA) insensitive near isogenic line, *Rht-B1c*. Germination percentages of after-ripened grains were not clearly different between *Rht-B1a* (CS) and *Rht-B1c* after imbibition for 7 days at low temperature (5 to 20°C). At 30°C, however, 88% of *Rht-B1a* grains germinated but only 26% of *Rht-B1c* grains germinated. Lengths of roots and shoots of *Rht-B1c* seedlings were shorter than those of *Rht-B1a* at 25°C and 30°C, but the lengths were not different between the two genotypes at low temperature (15°C and 5°C) conditions. These results suggest that low temperature abolishes and

high temperature enhances the suppressive effect of *Rht-B1c* on GA response, including germination and post-germinative growth. Temperature might affect dormancy and germination of wheat grains through adjustment of ABA and GA signaling.

O-7 **T. Sasanuma[1,2], K. Chabane[3] and J. Valkoun[3]** ([1]Grad Sch Agri, Kyoto Univ, [2]Kihara Inst Biol Res, Yohokaha City Univ, [3]Genet Resour Unit, ICARDA)

AFLP analysis of genetic diversity and phylogenetic relationship of seven diploid *Aegilops* species

To evaluate the genetic diversity of genus *Aegilops* and to clarify its phylogeny, genetic variation of seven diploid *Aegilops* species (*Ae. umbellulata, Ae. caudata, Ae. tauschii, Ae. speltoides, Ae. bicornis, Ae. searsii, Ae. mutica*) were investigated with amplified fragment length polymorphism (AFLP) technique. Five accessions from each species, thus, a total of 35 accessions were used in this study. Four primer combinations were used for the analysis of intraspecific genetic diversity and 15 primer combinations were used for that of interspecific phylogenetic relationship. As for the genetic diversity within a species, *Ae. speltoides* and *Ae. mutica* had the highest level of variation, followed by *Ae. umbellulata, Ae. caudata, Ae. bicornis* and *Ae. searsii*. *Ae. tauschii* had the lowest level of variation. In general, geographical relationship among populations was detected for each species. In the phylogenetic analysis, three Sitopsis species formed a cluster. The C and U genome species formed another cluster. These results are more consistent with the result of the cytological genome analysis than with that of the molecular plasmon analysis, suggesting that the nuclear genome have evolved differently from the cytoplasmic genome in genus *Aegilops*.

Poster presentation

P-1 **G. Suzuki[1], T. Ohta [1], M. Yamamoto[2], M. Morell[3], S. Rahman[3], and Y Mukai.[1]** ([1]Osaka Kyoiku Univ, [2]Kansai Women's Col, [3]CSIRO)

Genomic organization of the *SBEI* multigene family in the D genome donor of wheat. II.

A wheat *SBEI* (starch branching enzyme I) multigene family is related to synthesis of starch. We isolated 120-kb and 100-kb clones containing *SBEI* genes from a large-inserts genomic library of *Aegilops squarrosa*, D genome donor of wheat. DNA gel blot of the clones with several different *SBEI* probes indicated that three *SBEI* genes were clustered within 100 kb. Sequence analysis of subclones and lambda clones demonstrated that *wSBEI-D2* was located 3-kb downstream of *wSBEI-D3*, and 3-kb upstream of *wSBEI-D4* gene; of these only *wSBEI-D4* encodes the SBEI purified from the endosperm. The possible existence of other *SBEI*-related sequences near the cluster was also suggested by genomic DNA gel blot analysis. We tried to visualize tandem organization of the *SBEI* multigene family by FISH on circular DNA fibers of the large insert clones.

P-2 **T. Shimizu[1], A. Meguro[1], S. Takumi[2], Y. Ogihara[3], and K. Murai[1]** ([1]Dep Biosci, Fukui Pref Univ, [2]Fac Agr, Kobe Univ, [3]Kihara Inst Biol Res, Yokohama City Univ)

Isolation of homoeologous genes of wheat *AGAMOUS* homologue *WAG*, which are located on 1A, 1B and 1D chromosomes

Homeotic transformation of stamens into pistil-like structures (pistillody) has been observed in alloplasmic lines of common wheat with the cytoplasm of a wild relative species, *Aegilops crassa*. To investigate the molecular mechanism of the induction of pistillody, we isolated a MADS box gene WAG (wheat *AGAMOUS*) from a cDNA library of young spikes, which shows high homology to *AGAMOUS*. *AGAMOUS* is class C MADS box gene specifying the identity of stamens and carpels in *Arabidopsis*. Northern blot analysis revealed that there are two *WAG* transcripts in the wheat floral organs, one of which is expressed specifically in pistil as well as in the pistillate stamens of the alloplasmic lines. Because Southern blot analysis showed that *WAG* presented three homoeologous genes, which located on homoeologous group 1 chromosomes (1A, 1B, 1D), we hypotheses that the pistil-specific *WAG* transcript is derived from one of three *WAG* homoeologous genes. To verify this hypothesis, we are now cloning and characterizing genomic clones of *WAG*.

P-3 **E. Hama[1], S. Takumi[2], Y. Ogihara[3] and K. Murai[1]** ([1]Dep Biosci, Fukui Pref Univ, [2]Fac Agr, Kobe Univ, [3]Kihara Inst Biol Res, Yokohama City Univ)

Class B MADS box genes are associated with the induction of pistillody in alloplasmic wheats

It is well known that class B MADS box genes are

involved in specifying the identity of petals and stamens in dicot species. To investigate molecular mechanism of pistillody, homeotic transformation of stamens into pistil-like structures, in alloplasmic wheats with *Aegilops crassa* cytoplasm, we isolated three cDNA clones of *PISTILLATA* type class B MADS box genes, *WPI-2* (wheat *PISTILLATA* #2), *WPI-6* and *WPI-7*. Among these clones, *WPI-2* was highly homologous to *OsMADS4*, rice ortholog of *PISTILLATA*, and preferentially expressed in young spikes, suggesting that *WPI-2* is wheat ortholog of *PISTILLATA*. In situ hybridization analysis revealed that *WPI-2* is expressed in stamen primordia of normal lines, but not in pistillate stamens of the alloplasmic line. These results indicate that pistillody in the alloplasmic wheats is caused by a change of *WPI-2* gene expression pattern.

P-4 **F. Kobayashi[1], S. Takumi[1], R. Ohno[1], T. Nakamura[2] and C. Nakamura[1]** ([1]Fac Agr, Kobe Univ, [2]Tohoku Nat Agr Cent)

Low-temperature inducibility of the RAB gene family in wheat seedlings

To investigate contribution of the wheat RAB (responsive to ABA) gene family to cold acclimation and freezing tolerance, expression patterns of the four RAB genes, *Wrab15, Wrab17, Wrab18* and *Wrab19*, were monitored under cold- and ABA-treated conditions in seedlings of two wheat cultivars Chinese Spring (CS) and Mironovskaya 808 (M808). *Wrab19* and *Wrab17* were isolated from cold-treated M808 cDNA library, and *Wrab15* and *Wrab18* were from an EST library of wheat developing embryos. *Wrab18* is likely a homoeologue of *Wrab19*, based on their nucleotide sequence similarity. mRNAs of *Wrab18* and *Wrab19* accumulated more in the roots than in the leaves under the low-temperature condition. After ABA-treatment the transcript appeared within 5 hours in M808 leaves but no transcript appeared in CS leaves. *Wrab17* mRNA appeared in the above-ground tissues within a day of cold treatment in both cultivars and reached a maximum level within 3 days in M808, while a maximum level reached at the 7th day in CS. The *Wrab17* mRNA accumulation was maintained throughout at least two months of cold treatment. In M808 seedlings treated with ABA, the *Wrab17* transcript accumulated to reach a maximum level within 1 hour. As to *Wrab15*, a barley HVA22 orthologue, the transcript accumulated more in the roots than in the leaves under the cold condition. The *Wrab15* expression was not induced by ABA-treatment in the leaves of both cultivars. A genomic clone of *Wrab17* was isolated.

P-5 **M. Nakata, R. Ohno, S. Takumi and C. Nakamura** (Fac Agr, Kobe Univ)

Characterization of a wheat cold-responsive gene *Wcor15* and its *E. coli*-synthesized protein

Low temperature induces expression of the *Cor/Ltr* gene family in wheat. *Wcor14* and *Wcor15* genes are responsive to cold and light, and they contain the same signal peptide for targeting into chloroplast. Their expression patterns such as cold-responsiveness and cultivar differences are also similar. These observations indicate that the 5' upstream sequences of these two *Cor* genes contain the same cis-regulatory elements. To analyze the 5' regulatory sequences, we isolated a *Wcor15* genomic clone from the wheat genomic library and determined its nucleotide sequences. The 5' upstream region of *Wcor15* contains four CRT/DRE motifs, which are known as responsive motifs to low temperature and light. The 5' upstream sequence of the *Wcor15* was fused to GUS reporter gene and introduced into tobacco genome. Both low temperature and light induced the GUS expression in the transgenic tobacco leaves. The expression pattern of the GUS gene in the tobacco leaves was well consistent with that of the *Wcor15* gene in the native wheat seedlings. To characterize WCOR15 protein, the *Wcor15* gene was expressed in *E. coli*. After centrifugation, the extracted histidine-tagged WCOR15 protein remained in the insoluble fraction. However, after solubilized with urea, the purified protein showed boiling-soluble feature. These results suggest that the WCOR15 protein interacts with membranes and plays a chaperon-like role to protect the membrane integrity against cold injury in the wheat chloroplasts.

P-6 **R. Morimoto[1], Y. Ogihara[2], C. Nakamura[1] and S. Takumi[1]** ([1]Fac Agr, Kobe Univ, [2]Kihara Inst Biol Res, Yokohama City Univ)

Mutations accumulated in the wheat *Kn1*-type homeobox gene *Wknox1*

The plant knotted 1 (*kn1*)-like homeobox (*knox*) genes are known to play important roles in the maintenance of shoot apical meristem (SAM), determination of cell fate and differentiation of vegetative tissues. Three homoeologous cDNAs encoding the kn1-like homeobox protein were previously isolated and designated as *Wknox1a, Wknox1b* and *Wknox1d*. These were assigned to the homoeologous group 4 chromosomes.

To investigate accumulation of mutations in the three homoeologous loci of wheat genome, we compared three homoeologous genomic sequences of the *Wknox1* region. The three *Wknox1* genes consisted of six exons and five introns. In the exon/intron regions, 29 mutations of insertion/deletion (more than 6bp in length) were found and most of them were located in the 4th intron. Four insertions, which were found in the 4th intron of *Wknox1a*, *Wknox1b* and *Wknox1d*, belonged to the *Stowaway* family of MITEs. The insertion events might have occurred in various times during wheat evolution, such as diversification of the ancestral diploid species, amphidiploidization and domestication of tetraploid wheat. The mutation rate in the *Wknox1b* locus was higher than that in the *Wknox1a* and *Wknox1d* loci. Moreover, the same expression level was observed of the three *Wknox1* loci by RT-PCR analysis, thus no mutations in the three homoeologous *Wknox1* loci affected the transcription level. The *Wknox1a* locus therefore can not be considered as a candidate locus for the Hooded phenotype in wheat.

P-7 **K. Mizumoto[1], K. Murai[2], C. Nakamura[1] and S. Takumi[1]** ([1]Fac Agr, Kobe Univ, [2]Fukui Pref Univ)

Cloning of genes involved in wheat ovule development and their expression in pistillate stamens

Homeotic transformation of stamens into pistil-like structures (pistillody) has been observed in the alloplasmic line of common wheat with *Aegilops crassa* cytoplasm. The induction of pistillody is suppressed by *Rfd1* gene located on the long arm of chromosome 7B in Chinese Spring (CS). Because of the absence of *Rfd1*, the alloplasmic line of CS ditelosomic 7BS((*cr*)-CSdt7BS) exhibits pistillody in all florets, whereas the euplasmic CS ditelosomic 7BS (CSdt7BS) with normal cytoplasm forms normal stamens. (*cr*)-CSdt7BS exhibit not only pistillody but also female sterility. In the (*cr*)-CSdt7BS pistils, abnormal ovules fail to form inner epidermis and integuments in the chalazal region. The pistillate stamens also show incomplete ovule-like structures either with inner integuments or with both inner and outer integuments. To study ovule development of wheat and the effect of *Ae. crassa* cytoplasm on the ovule formation, we cloned a wheat *BEL1* homologue *Wbel1* and an *ANT*(*AINTEGUMENA*) homologue *Want*. Both *BEL1* and *ANT* are essential for normal ovule development in *Arabidopsis thaliana*. According to RT-PCR analysis of the isolated genes, we detected the *Want* transcript specifically expressed in pistils and pistillate stamens of both CSdt7BS and (*cr*)-CSdt7BS. However, the expression level of *Wbel1* in the pistils and pistillate stamens was highly reduced in both (*cr*)-CS and (*cr*)-CSdt7BS. These results suggest that the expression of *Wbel1* and *Want* is related to the pistillody phenotype and that the *Ae. crassa* cytoplasm affects the *Wbel1* expression level.

P-8 **K. Uno[1], D. Ogawa[1], H. Tsujimoto[2], K. Noda[3] and N. Kawakami[1]** ([1]Fac Agric, Meiji Univ, [2]Fac Agric, Tottori Univ, [3]Res Inst Biol Res, Okayama Univ)

Analysis of seed dormancy and phytohormone crosstalk in *Rht-B1c* near isogenic line

Abscisic acid (ABA) and gibberrelic acid (GA) have been proved to be involved in grain dormancy and germination. However, the contribution of GA and its signaling on dormancy has been still obscure. We used a near isogenic line of wheat, *Rht-B1c*, which was GA insensitive in genetic background of CS, to see the effect of GA sensitivity on grain dormancy. At grain maturity, only 3 to 7% of *Rht-B1c* grains germinated, whereas more than 90% of CS grains germinated after imbibition at 22°C for 7 days. Germinability of *Rht-B1c* increased along with time of storage at room temperature (after-ripening), and almost all the grains germinated after 30 to 40 days. This clearly suggests that *Rht-B1c* allele invests the seeds with dormancy. Half grains with embryos of *Rht-B1c* showed higher sensitivity to ABA than CS during the after-ripening. *Rht-B1c* allele might affect on dormancy development and/or maintenance by enhancing ABA signaling via its GA insensitive character. We have amplified fragments of *Rht* gene, and identification of the sequence of *Rht-B1c* allele is now in progress. We are trying to clone and identify downstream genes, which expressions are affected by *Rht*, with PCR-aided subtraction method and microarray analysis.

P-9 **C. Guo[1], S. Takumi[2] and T. Terachi[1]** ([1]Dept Biotech, Fac Eng, Kyoto Sangyo Univ, [2]Lab Plant Genetics, Fac. Agr, Kobe Univ)

Preliminary report on the wheat plastid transformation by microprojectile bombardment

Plastid transformation technology is an attractive tool in plant biotechnology. The advantages of transplastomic plants over conventional nuclear transformants are as follows; high-level expression

of foreign proteins, absence of gene silencing and position effect, lack of transgene transmission to environment through pollens, etc. Although transplastomic plants are demanded, application of this technology is virtually limited to tobacco. In order to extend transplastomic technology to crops, we have tried to transform wheat plastid. In the present study, two wheat cultivars, Akadaruma and Fielder, were used as the material plants. Two plastid transformation vectors containing either *rbcL-psaI* or *trnV-rps12/7* sequence were constructed. Both vectors contain a spectinomycin/streptomycin-resistance gene (*aadA*) and a GFP gene (*gfp*) in the middle of chloroplast sequences. The homologous recombination at *rbcL-psaI* or *trnV-rps12/7* should integrate *aadA* and *gfp* into chloroplast genome. The calli were obtained from immature embryos, and they were bombarded with the vector DNAs. Two days after the bombardment, explants were transferred to selection medium containing streptomycin. Resistant calli were maintained on the selection medium for 2–4 months, and then transferred to shoot regeneration medium containing antibiotics. The 51 resistant calli were obtained from 7,837 bombarded calli. Further investigations are in progress to regenerate transplastomic wheat.

P-10 **N. Ohnishi, E. Himi and Kaz. Noda** (Res Inst Biores, Okayama Univ)

DFR (dihydroflavonol 4-reductase) gene structure of common wheat and it ancestor

Wheat (*Triticum aestivum* L. cv Chinese Spring) has three *DFR* genes on group 3 chromosomes (*TaDFR-A*, *TaDFR-B*, *TaDFR-D*). The nucleotide sequence of these *DFR* showed more than 92 % similarity and this amino acid sequence maintained more than 95 % similarity. *DFR* genes contained three introns: the first intron showed 79.3 % similarity on average, the second intron 69.1 % and the third intron 75.9 %. Similarity between the intron regions was lower than that between cDNAs, especially the second intron showed lower similarity than the other introns. cDNA of *T. monococcum* and CS *DFRs* showed 92.8 % similarity on average and *Ae. squarrosa* and CS was 87.9 % on average. Compared the intron sequences of *TaDFR-A* and *T. monococcum DFR*, the first intron showed 100 % similarity, the second intron 97.8 % and the third intron 98.8 %. While, the introns of *TaDFR-B* and *TaDFR-D* showed lower similarity to the introns of *T. monococcum DFR*. Also the introns of *Ae. squarrosa DFR* showed higher similarity to those of *TaDFR-D* than those of *TaDFR-A* and *-B*. Especially, the second intron of *Ae. squarrosa DFR* was less similar to those of *TaDFR-A* and *-B*.

P-11 **S.K. Ghimire[1]*, H. Tsujimoto[2] and K. Kato[1]** ([1]Fac Agr, Okayama Univ, [2]Fac Agr, Tottori Univ, *dns14715@cc.okayama-u.ac.jp)

Analysis of genetic diversity in wheat germplasm introduced from Nepal and Bhutan based on isozyme and RAPD polymorphism

Genetic diversity in wheat accessions collected from various parts of Nepal and Bhutan was analyzed by RAPD and isozyme analysis as well as field observation. Heading date showed large variation, ranging from 18.3 April to 13.5 May. Positive correlation was detected between heading date and the altitude of their collection sites ($r=0.424$, $P \leq 0.01$). Percentage of spring type varied among collection sites, and significant negative correlation with altitude was observed in east sites of Nepal ($r=-0.475$, $P \leq 0.01$). A total of 17 and 53 polymorphic bands were scored by RAPD and isozyme analysis, respectively. Gene diversity in newly introduced accessions was less than that in wheat germplasm introduced from Nepal and Bhutan a few decades ago, indicating rapid genetic erosion. By cluster analysis using isozyme data, a total of 48 populations were grouped into 5 distinct clusters. Most of South Asian countries like India, Nepal and Bhutan were clustered together, indicating their close genetic relationship. Among the Nepalese and Bhutanese wheat, new and old accessions were separated into two different sub-clusters, as also shown by RAPD analysis. Chinese accessions were clearly divided into two groups, and those from Xinjiang, Tibet and Yunnan were grouped with other Himalayan wheat, and independent of those in the Coast and Yangtze River basin of China.

P-12 **S. Nakayama[1]*, H. Tsuyuzaki[2], K. Takeda[3] and K. Kato[1]** ([1]Fac Agr, Okayama Univ, [2]Akita Pref Univ, [3]Res Inst Biores, Okayama Univ, *dns14717@cc.okayama-u.ac.jp)

Structural analysis of _Stowaway_-like transposable element and its intraspesific variation in _Ae. tauschii_ collected in Shaanxi Province, China

Insertion sequence of 113 bp was detected in 5' upstream region of *α-Amy* 3 in one accession of *Ae. tauschii* from Iran. This insertion was considered as a *Stowaway*-like transposable element because of its short length, conserved terminal inverted repeats, and

the presence of target site duplication (TA). Since *Stowaway*-like elements exist abundantly in *Ae. tauschii* genome, IMP (Inter-MITE Polymorphisms) analysis should be applicable to detect genetic variation. The analysis of *Ae. tauschii* introduced from Shaanxi Province showed no polymorphism by RAPD analysis. Then, IMP analysis was performed for 49 accessions of *Ae. tauschii* introduced from Shaanxi Province. Polymorphism was detected in two accessions (AT55 and AT56) from Yangling, in which a fragment of 220bp and 266bp was absent, respectively. The latter fragment was amplified in all accessions from Shaanxi Province excluding AT56, while it was amplified only in one accession (AT4, var. *typica*) from Afghanistan among 31 accessions from other areas. Nucleotide sequence of this fragment was almost same between AT4 and accessions from Shaanxi Province, suggesting that *Ae. tauschii* established in Shaanxi Province was principally derived from very limited population consisted of AT4 type.

P-13 **N. Kodama[1*], M. Ishii[2], K. Kato[1], K. Takeda[2]**
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PCR based marker for the selection of spring growth habit genes (*Sgh*) of barley

Most of the Japanese two-rowed barley are of spring type carrying *sgh* gene, and are considered to be inferior in cold tolerance and productivity. For the breeding of winter type cultivar, we tried to develop PCR marker for the selection of spring growth habit genes. The Japanese six-rowed winter barley, Hayakiso 2 and Dairokkaku 1, and their NILs for *Sgh* genes were used in this study. STS primers were designed to amplify 9 RFLP loci which linked to *sgh* or *Sgh2* genes, and nucleotide sequence of PCR products was compared among the NILs. Among 9 RFLP loci analyzed, polymorphism was detected in *XB-Amy1* whose primers were designed to amplify the third intron. The size of PCR products was different between Hayakiso 2 (*sgh*) (1200bp) and Hayakiso 2 (*Sgh*) (1350bp). The analysis of their F_2 population showed that recombination value between *XB-Amy1* and *sgh* was 4.4 cM which was comparable to the map distance reported so far. Most of the Japanese two-rowed cultivars with spring growth habit proved to be of 1200bp type, while foreign cultivars with winter growth habit, such as Igri and Proctor, showed a band of 1350bp. Therefore, a PCR based marker of *XB-Amy1* can be utilized for the introduction of *Sgh* gene into the Japanese two-rowed barley.

P-14 **I. Onishi, A. Hongo and H. Miura** (Obihiro Univ Agric & Vet Med)

Quantitative evaluation of shattering traits in hexaploid wheat

The speltoid-suppressing gene *Q* on chromosome 5A of common wheat (*Triticum aestivum* L.) is supposed to associate with free-threshing and square-headed spikes with non-fragile rachies. On the other hand, *T. spelta* and *T. macha* carrying the *q* allele are characterized by a narrow and long spike with brittle rachies. Rachies brittleness affects shattering tolerance that is important for cultivated wheat species. While genetic variation in shattering traits has been suggested, quantitative evaluation of the traits has not yet been established. So we developed a system measuring kinetic energy of shattering. The system can measure physical force and moving distance with 1 kg load-cell and displacement when rachies snap. Using this system, genetic variation for shattering traits within *T. aestivum* and *T. spelta*, as well as dosage effect of the *Q* allele, was investigated.

News

10th International Wheat Genetics Symposium (2nd circular)

Co-chairmen of Local Organization Committee (Norberto Pogna, Gian Tommaso Scarascia Mugnozza, Angelo Bianchi)

We are pleased to invite you to the 10th International Wheat Genetics Symposium (TIWGS) at the Ariston Conference Centre in Paestum, Italy, next 1– 6 September 2003.
The objective of the Symposium, patronizes by the Italian Ministry of Agriculture and Forestry, is to bring together researchers who deal with genetics of wheat, a crop widely grown in five continents providing over 20% of the calories of the world population of 6 billion persons.

In selecting the themes of the Symposium, we attempted to cover an extensive range of subjects including Evolution and Genetic diversity, Cytogenetics, Genomics, Technologies for genetic modification, Transgenic wheat, Classical and molecular breeding, Tolerance to abiotic stress, Resistance to pathogens and parasites, Nutritional and technological quality of wheat grain. The major focus of the Symposium will be on common (bread) wheat. However, durum (pasta) wheat, triticale, tritordeum and minor wheat species such as *Triticum dicoccum* (the ancient Roman "farrum") and *T. monococcum* will be considered as well because of their historical and economical relevance in the Mediterranean area. Short workshops will be devoted to other noteworthy aspects such as Taxonomy and Genome Symbols, Hybrid Wheat, Breeding in the Third Millennium, etc.

Moreover, we are delighted that the Symposium will once again host the International Triticeae Mapping Initiative (ITMI) workshop, with formal and poster presentations.

The poster and oral papers presented at the Symposium will be printed in a refereed proceedings. This will be a useful reference to people dealing with wheat genetics and breeding.

The old Magna Glacier City of Pestum, filled with Mediterranean hospitality, charm and culture, is the location of this exciting event, which is immediately followed the 8th Gluten Workshop scheduled for 8–10 September 2003 in Viterbo, Central Italy. We look forward to welcoming you to Paestum in September 2003.

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Web Symposium
www.cerealicoltura.it

Symposium in brief

Attendance requirements
At least one paper author and all speakers must attend the Symposium. Symposium registration fee must accompany submission of the final paper or poster submission.

Badges
All delegate will be provided with a name badge to be worn throughout the Symposium.

Certificate of attendance
A Certificate of attendance will be available on the request at the registration desk on the last day of the Symposium.

Exhibition
An exhibition will be arranged. For further information please contact the Organizing Secretariat.

Language
The official Symposium language will be English.

Proceedings
All accepted papers and poster presentations will be published in the Symposium Proceedings distributed to participants upon registration.

Symposium activities
- Symposium plenary sessions
- Poster sessions
- Workshops
- Social programme
- Social Programme for accompanying persons

Time table
April 30 Final deadline for submission of proposals
May 9 Notification of acceptance of scientific contribution
May 30 Deadline for early registration
June 16 Deadline for submission of papers
September 1 Opening of the 10th IWGS
September 6 Closing of the 10th IWGS

Editorial remarks

The 10th International Wheat Genetics Symposium is going to be held soon in coming September in Italy. The 2nd circular announcement of the Symposium is cited in this volume p46, and the details should be in Web-site www.cerealicoltura.it. Various important achievements will be presented, and serious but sincere discussion is expected there.

It is worthwhile recalling the brief history of IWGS and Wheat Information Service because the 10th anniversary of IWGS is in Italy and WIS is approaching to be issued for 50 years. It was in 1953 when 26 pioneers of wheat scientists from nine countries gathered in a room of Grand Hotel Bretagne at Bellagio, Italy, on the occasion of the IX International Congress of Genetics to discuss on promotion of wheat genetics research under international information exchange and cooperation. The discussion concluded three important issues; 1) organization of International Wheat Genetics Symposium, 2) establishment of Wheat Information Service, and 3) establishment of genetic stock centers of wheat and its wild species in Beltsville (USA) and Kyoto (Japan). Since then, WIS has been published to reach Vol. 96, and IWGS is increasing its attendance as listed below.

	Year	Place	Number of participants
1 st	1958	Winnipeg, Canada	125
2 nd	1963	Lund, Sweden	231
3 rd	1968	Canberra, Austraria	147
4 th	1973	Columbia, USA	327
5 th	1978	New Delhi, India	369
6 th	1983	Kyoto, Japan	222
7 th	1988	Cambridge, UK	432
8 th	1993	Peijing, China	ca.500
9 th	1997	Saskatoon, Canada	463

In this number of WIS, 7 research articles, 1 Genetic stock and Record (abstracts of papers read at the 28th Japanese Wheat Genetics Symposium) are included. We should say again that articles for Research information will be most welcome to be published in WIS. Also, please follow the instruction of manuscript preparation because some of contributed papers were over 5 pages in print (note about 810 words in a printed page), or figures are poorly prepared not suitable to direct photo-printing.

Dr. McIntosh had sent the latest supplement of gene catalogue. However, since it will be renewed and formally registered in the coming symposium, it was not included in this issue. On the other hand, the gene catalogue is now available on web in KOMUGI (http://www.shigen.nig.ac.jp/wheat/komugi/genes) for test version, and it is, also, a matter of issues for discussion in 10th IWGS.

We are looking forward to seeing many of you soon in Italy, and give us valuable suggestion for editing WIS.

June, 2003
Editors of WIS
K. Nishikawa (chief), T. Sasakuma, H. Tsujimoto and K. Furukawa (secretary)

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 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.

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