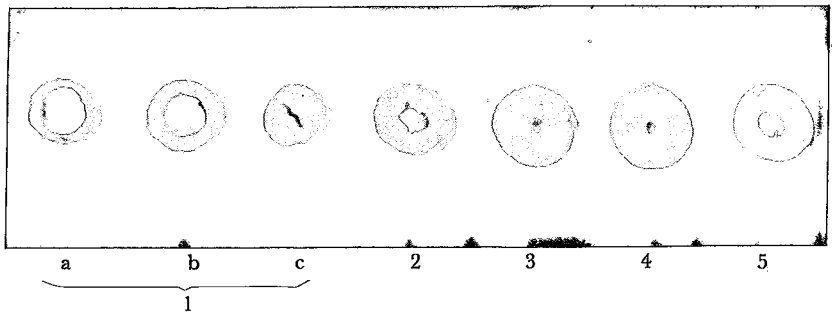
K. Yamashita

WHEAT INFORMATION SERVICE



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No. 38 March, 1974

I. Research Notes

The relationships of the S-genome diploids to polyploid wheats[1)]

G. KIMBER
Department of Agronomy, University of Missouri, Columbia, Missouri, U.S.A.

In assigning the genome symbols to the diploids in the Triticinae, KIHARA (1949) ascribed the letter S to the species which then constituted the Sitopsis section of the genus *Aegilops*. This section, the species of which have been given various taxonomic rankings, consists of, at least for the purposes of this paper, three forms: *Triticum speltoides* (*Ae. speltoides*), *T. longissimum* (*Ae. longissima* and *Ae. sharonensis*) and *T. bicorne* (*Ae. bicornis*).

Hybrids between these species had meiotic pairing patterns indicating that they each possessed essentially the same genome. The pairing averaged 6.70 bivalents per cell in the hybrid *T. speltoides* × *T. longissimum* (KIMBER, 1961), approximately 7 bivalents per cell in *T. speltoides* × *T. bicorne* (KIHARA, 1949) and the equivalent of 6.86 bivalents per cell in *T. bicorne* × *T. longissimum* (KIMBER, 1961). A quadrivalent was observed at meiosis in the hybrids involving *T. longissimum*, and it was ascribed to the presence of a reciprocal translocation. Therefore, in the classical concept of the genome relationships, each of these species should be considered to carry the same basic genome.

JENKINS (1929) reported the occurrence of a mode of seven bivalents in hybrids between *T. turgidum* and *T. speltoides*; however, some considerable time was to pass before any species of the Sitopsis section came to be accepted as the donor of a genome to the polyploid wheats. In 1940 PATHAK observed similarity between the satellites of the chromosomes of *T. speltoides* and *T. turgidum*. SEARS (1956) suggested *T. bicorne*, and also in 1956, on the basis of morphological evidence, SARKAR and STEBBINS suggested *T. speltoides* as the donor of the B genome to wheat. RILEY *et al.* (1958) concluded, on the basis of karyotypic, geographical and synaptic evidence, that *T. speltoides* had donated the B genome to the polyploid wheats.

1) Contribution from the Missouri Agricultural Experiment Station, Journal Series Number 67215. Copied from the Proceedings of the 4th Wheat Genetics Symosium by the kind permission of the Editors and Organizing Committee.

A conclusive demonstration of the homology of the genome of *T. speltoides* (or either *T. longissimum* or *T. bicorne*) to the B genome of the polyploid wheats was lacking. In the case of the hybrids of *T. speltoides* to polyploid wheats, considerable homoeologous chromosome pairing was observed (RILEY *et al.*, 1958), and this obscured the homologous-chromosome pairing pattern. In the cases of *T. longissimum* and *T. bicorne* little, if any, chromosome pairing, homologous or homoeologous takes place in hybrids with polyploid wheat (KIMBER, 1961; RILEY *et al.*, 1958), yet this did not seem to alert workers to the possibility that the Sitopsis section had not contributed the B genome of the polyploid wheats.

Recently (KIMBER and ATHWAL, 1972) the recognition of variation in the ability of *T. speltoides* to affect homoeologous chromosome pairing has led to a reconsideration of the evolution of the polyploid wheats. KIMBER and ATHWAL demonstrated three levels of chromosome pairing in hybrids between *T. aestivum* and different accessions of *T. speltoides*. The presence of heteromorphic bivalents in even the low-pairing hybrids was taken as evidence that homoeologous chromosome pairing was occurring and thus homologous chromosome pairing should also be possible. The very low pairing observed in these lines indicates the absence of homologous chromosomes. Confirmation that the low-pairing forms of *T. speltoides* do not interfere with homologous chromosome pairing was also obtained from the regular bivalent formation observed in a amphiploid derived by colchicine treatment from a low-pairing hybrid (KIMBER and ATHWAL, 1972; KIMBER, unpublished). That the low-pairing form of *T. speltoides* does not affect homologous chromosome pairing was also supported by a comparison of the meiosis of autotetraploids of the low- and high-pairing *T. speltoides* lines (LARSEN and KIMBER, 1973).

This recognition of the non-homology of the chromosomes of *T. speltoides* to those of the B genome of polyploid wheats points to a need for a re-examination of other pertinent evidence that may help to elucidate the situation.

The karyotypic evidence gathered by PATHAK (1940) and RILEY *et al.* (1958) was, in fact, based on a single accession in each case and thus is open to the possibility of undetected variation in the satellite size existing in these species. WAINES and KIMBER (1973) have shown such variation in the satellites of *T. monococcum*, and this type of variation may exist in other species of the Triticinae also.

In an attempt to demonstrate the nature of the genetic regulation of pairing of *T. speltoides*, KIMBER (1966) made the hybrid between a form of *T. speltoides* known to allow a high frequency of multivalent formation in hybrids with polyploid wheat and *T. longissimum* (*Ae. sharonensis*), which was characterised by very low-pairing in hybrids with wheat. The F_1 was back-crossed to *T. speltoides* twice, and a segregant, heterozygous for the pairing control, was crossed with *T. aestivum*. The six F_1 plants of this cross segregated four high-pairing to two low-pairing. SEARS (1969) pointed out that the average of 2.9 biva-

lents per cell in the low-pairing segregants was much lower than that expected if *T. speltoides* were the donor of the B genome.

DVOŘÁK (1972) recorded the pairing frequencies of wheat and *Agropyron* telocentrics in the presence of *T. speltoides* with differing abilities to affect homoeologous chromosome pairing. Surprisingly, telocentric 7B of wheat showed a reduced pairing affinity when the frequency of homoeologous pairing was at an intermediate level. DVOŘÁK (*loc. cit.*) also pointed out that the data of JOHNSON and KIMBER (1967) could not support a closer relationship between the B and S genomes than between the A and S or the D and S. The data of ATHWAL and KIMBER (1972) show a similar lack of homology between the B and the S genomes. RILEY and CHAPMAN (1966) calculated the affinity of the long arms of the chromosomes of homoeologous group 5 of *T. aestivum* and chromosome 5S of *T. speltoides*, and they concluded that chromosome 5B showed little affinity to chromosome 5S. Thus, when the pairing behavior of individual chromosomes is considered, there is little evidence that the S genome is homologous to the B genome.

The lack of pairing in hybrids between *T. longissimum* and *T. aestivum* (KIMBER, 1961) and the obvious genomic similarity between *T. longissimum* and *T. speltoides* indicates that the B genome of polyploid wheat could not have been derived from a species so closely related to *T. longissimum*.

In addition to the cytological evidence, recent studies of the electrophoretic banding patterns of seed proteins do not support the concept of *T. speltoides* as the donor of the B genome to polyploid wheats (JOHNSON and HALL, 1966).

At this symposium new evidence has been presented on the relationships of the B and S genomes. RUBENSTEIN and SALLEE (1973) have described the meiotic pairing configurations in various hybrids of *T. kotschyi* (*Ae. variabilis*) and *T. speltoides*, and it is apparent that there is a genome in common between these species. However, when *T. kotschyi* is crossed with *T. aestivum* (DRISCOLL and QUINN, 1968), there is virtually no meiotic chromosome pairing, clearly showing that there is little or no chromosome homology between these species. SHANDS and KIMBER (1973) have produced a series of hybrids between *T. speltoides*, and also between *T. turgidum* (*T. durum*) and low-, intermediate- and high-pairing *T. speltoides*. The *T. turgidum* hybrids show the same range in pairing behaviors seen in the *T. aestivum* × *T. speltoides* crosses described by KIMBER and ATHWAL (1972). The *T. timopheevii* hybrids, however, are quite different. Even in the hybrid with the low-pairing *T. speltoides*, where homoeologous chromosome pairing should be minimal, there is the equivalent of approximately seven bivalents. This pairing is taken to represent the homology of the S genome of *T. speltoides* to the G genome of *T. timopheevii*. In hybrids between *T. timopheevii* and *T. durum* there is apparently one genome in common (SHANDS and KIMBER, 1973), and this is assumed to be the A genome. Consequently, it is improbable that the S genome of *T. speltoides* is equivalent to the B genome of *T.*

turgidum.

Clearly some reconsideration of the criteria to be used in the establishment of genomic relationships is in order. Cytologists have always been faced with the problem of what is sufficient pairing in hybrids to establish homology on a scale large enough to be considered genomic. The problem is now further compounded by the recognition of genetic systems influencing the degree of chromosome pairing in hybrids. In the Triticinae, for example, variation has been recorded in several species. DOVER and RILEY (1972) have recognized four classes of pairing in *T. tripsacoides* (*Ae. mutica*) hybrids; DVOŘÁK (1972) and KIMBER and ATHWAL (1972) report three classes in *T. speltoides*, while DOVER and RILEY (*loc. cit.*) record four, but in the presence of B chromosomes. KIMBER (unpublished) has recovered a "super-high" pairing type of *T. speltoides* which differs from the super-high described by DOVER and RILEY (*loc. cit.*) in that its presence results in super-high pairing even in the presence of chromosome 5B. MELLO-SAMPAYO (1972) describes an intermediate-pairing type in *T. longissimum*, and recently KIMBER and SALLEE (1973) have found a high-pairing type in this species also.

In attempts to recognize genomic relationships, it would seem that the pairing pattern most likely to give a correct indication of similarity is that in which homoeologous chromosome pairing is reduced to a minimum but homologous chromosome pairing is not affected. Thus the low-pairing classes of DOVER and RILEY (1972) and KIMBER and ATHWAL (1972) and the class I of DVOŘÁK (1972) could be the pairing patterns best indicative of genomic similarity. It is unfortunate that the only type of *T. speltoides* pairing behavior in hybrids with polyploid wheat recognized prior to 1972 was the high-pairing. Had the accessions of *T. speltoides* been only of the low-pairing type, it is improbable that it would have been seriously considered as the donor of the B genome of polyploid wheat.

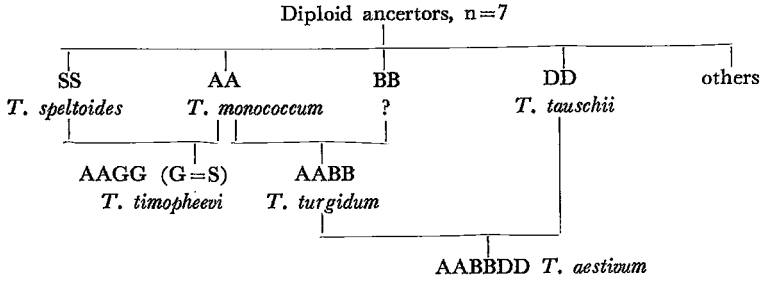

Fig. 1. A representation of the evolutionary pathway of some of the polyploid wheats.

In view of these considerations it is perhaps appropriate to present a diagram which may represent our current understanding of the evolutionary pathway of wheat (Fig. 1).

This diagram must, in some ways, be considered tentative. It does not, for example, recognize the genomic situation of *T. zhukovskii*. Similarly, it places the origin of the

B genome in some unidentified diploid species. KIMBER and ATHWAL (1972) considered various possibilities for the origin of the B genome. After eliminating such alternatives as an as-yet-undiscovered form of *T. speltoides*, autopolyploids of the A genome diploids and a hybrid B genome donor, they concluded that the polyphyletic origin of wheat was an alternative that must be considered seriously. Such an origin would most likely have involved two or more independently produced amphiploids involving diploid wheats and unspecified, but diverse diploids. Intercrossing of these amphiploids would cause little change in the A genome component, but might repattern the other genomes so that recognition of the diploid species involved would be impossible. However, there is another possibility: simply that the B genome donor species has become extinct.

Inheritance of leaf area in vulgare wheat

M. KRALJEVIĆ-BALALIĆ
Department of Genetics and Plant Breeding, University of Novi Sad, Novi Sad, Yugoslavia

In recent years, the role of the green parts of plants in determining the yield of different genotypes has been intensively studied. In order to recognize more completely the components of grain yield, studies on the role of different leaves, internodes, spikes, and awns have been conducted (WATSON *et al.*, 1963; DAVIDSON, 1965; KUMAKOV, 1968; SIMPSON, 1968).

No strong correlation between the leaf area and yield was found (WATSON, 1952; ASANA, 1955; STOY, 1965; BOROJEVIĆ and ĆUPINA, 1969). A particular significance in organic matter formation is attributed to the flag leaf and the green area above it.

The intention of this paper is to examine certain characteristics of the leaves which could be found useful in the selection for high yield. The objective of the research reported was to examine the mode of inheritance of leaf area, its heritability and combining ability.

Material and Method

In diallel crossings of four wheat varieties (short-straw Mara and Bezostaia-1, semi-dwarf Sava, and dwarf NS-732), the mode of inheritance, heritability, and combining ability of the leaf area were studied in the F_1 and F_2 generations.

The size of the leaf-lamina area per plant in cm^2 was determined by the weight method (ALEKSEENKO, 1959).

The analyses of variance of the data on the combining ability and the estimation of

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various effects were made by the Griffing technique, method 4 (1956). The heritability estimation was made according to MATHER (1949).

Results and Discussion

The Mara variety had the largest leaf area, then Sava and Bezostaia-1, while NS-732 had the smallest (Table 1). In the F_1 generation the combinations Bezostaia-1 × Mara and Mara × NS-732 had the largest leaf area. The hybrid Bezostaia-1 × Sava had the smallest (Table 1).

The total leaf area in wheat showed a continuous variability, which is typical for the majority of quantitative characters. In the F_1 hybrids examined the leaf area was largely intermediate in Mara × NS-732 and Sava × Mara and incompletely dominant in Bezostaia-1 × Mara (Table 1). This can be explained by the presence of a number of genes which had additive effects.

Table 1. Mean value, standard deviation, variance, coefficient of variation and heritability of the leaf area

Parents and crosses	Gen.	n	X±S.E.	δ	δ^2	V	h^2
Sava	P_1	45	118.82±3.60	24.17	584.04	20.34	
NS-732 × Sava	F_1	45	120.43±3.78	25.08	628.75	20.83	
〃	F_2	130	110.19±2.48	28.43	808.33	25.80	23.68
Sava × Mara	F_1	45	136.83±4.31	28.91	835.78	21.13	
〃	F_2	130	134.42±2.23	25.45	647.58	18.93	6.42
Bez.-1 × Sava	F_1	45	118.86±4.27	27.98	782.95	23.54	
〃	F_2	130	107.07±2.17	24.74	612.25	23.11	0
NS-732	P_1	45	105.52±3.77	25.26	637.94	23.94	
Mara × NS-732	F_1	45	141.77±3.49	23.43	549.08	16.53	
〃	F_2	130	139.80±2.97	33.91	1148.63	24.25	47.35
NS-732 × Bez.-1	F_1	45	128.38±3.09	20.72	429.25	16.14	
〃	F_2	130	112.21±2.21	25.19	634.41	22.45	13.97
Mara	P_1	45	159.03±3.74	25.08	628.82	15.77	
Bez.-1 × Mara	F_1	45	152.01±4.53	30.41	924.86	20.01	
〃	F_2	130	130.47±2.47	28.22	796.14	21.63	11.08
Bezostaia-1	P_1	45	116.45±3.59	23.87	570.19	20.50	

The F_1 combinations NS-732 × Sava, Bezostaia-1 × Sava, and NS-732 × Bezostaia-1 showed full dominance (Table 1). The differences between the parents of these hybrids were not significant.

In all the F_2 combinations segregation occurred, the mean value was lower than in the F_1 generation, and the total leaf area was intermediate in relation to the parents (Table 1).

The flag leaf comprised 44% to 50% of the entire leaf area. In their inheritance, the flag leaf and other leaves behave similarly to the total leaf area.

The genotypic variability was high in the morphological and physiological characteristics, which confirmed the results of research on barley conducted by YAP and HARVEY (1972). The heritability of the entire leaf area in barley ranged between 24% and 73% (FOWLER and RASMUSSON, 1969). In our tests the heritability ranged between 6% and 47%. The negative value of heritability in Bezostaia-1 × Sava indicated that probably no genetic difference in relation to the examined characters exists.

Combining Ability

The variance of GCA (general combining ability) was highly significant for the total leaf area, while the variance of SCA (specific combining ability) was not significant (Table 2). SINGH and GUPTA (1969) arrived at the same conclusion. BROWN *et al.* (1966) maintain that the SCA in wheat is absent.

The best combiner for the total leaf area was Mara, then Bezostaia-1 and NS-732,

Table 2. Analysis of variance of the general (GCA) and specific combining ability (SCA) and the ratio GCA/SCA of the leaf area

Source	DF	SS	MS	F exp.	F	
					0.05	0.01
GCA	3	796.76	265.59	26.56**	3.71	6.55
SCA	2	34.83	17.42	1.74	4.10	7.56
GCA/SCA			15.25			

** Significant for 1%

Table 3. GCA and SCA estimates of the total leaf area

Parents	SCA			GCA (gi)
	NS-732	Mara	Bez.-1	
Sava	3.15*	−0.50	−2.70	−11.53
NS-742		1.80	−5.45	− 4.28
Mara			3.15*	15.73
Bezostaia-1				0.08

LSD for 5%=2.56 *Significant for 5%
1%=3.64

and the worst was Sava (Table 3). The SCA was significant only in the combinations NS-732 × Sava and Bezostaia-1 × Mara (Table 3).

A separation into the SCA and GCA indicated that the additive genetic variance comprised the main part of the total variance of the six examined F_1 hybrids. This concurs with the results of HSU and WALTON (1970) and BHATT (1971).

Tissue culture of wheat, rye, and their hybrid[1)]

W.F. SHERIDAN
Division of Biological Sciences, University of Missouri, Columbia, Missouri, U.S.A.

Prior to 1967 there were few reports of success in inducing growth of monocotyledonous plants as callus in sterile culture. Since that time considerable progress has been made, with *Allium* (FRIDBORG, 1971), *Asparagus* (WILMAR and HELLENDORAN, 1968), and *Lilium* (SHERIDAN, 1968) in the Liliaceae and *Gladiolus* (ZIV *et al.*, 1970; SIMONSEN and HILDEBRANDT, 1971) in the Iridaceae having been induced to form callus on the media of LINSMAIER and SKOOG (1965) or the similar medium of MURASHIGE and SKOOG (1962), except for the *Allium* callus, which was induced on the 5B medium of GAMBORG *et al.* (1968), a medium originally developed for soybean culture.

Among the grasses, CAREW and SCHWARTING (1958) reported induction of callus from rye embryos on a 2,4-D-supplemented, modified Heller's medium; NICKELL in 1964 reported the successful production of sugarcane callus on a supplemented White's medium; and MASCARENHAS *et al.* (1965) reported callus induction with maize sporophyte tissue on a supplemented White's medium.

More recently callus induction and growth was reported for oats on 2,4-D-supplemented Linsmaier and Skoog's medium by CARTER *et al.* (1967) and for rice, *Oryza sativa,* on 2,4-D-supplemented Murashige and Skoog medium by the same laboratory (YAMADA *et al.*, 1967). Callus induction with rice on a modified 2,4-D-supplemented White's medium was reported by TAMURA (1968) and on Murashige and Skoog's medium supplemented with casein hydrolysate and 2,4-D by KAWATA and ISHIHARA (1968).

The successful culturing of sorghum callus in liquid and on solidified Murashige and Skoog's medium supplemented with 2,4-D was reported by MASTELLER and HOLDEN (1970). The successful culturing of tissue of barley, timothy, brome grass, oats and sorghum was achieved on a new, completely defined medium by SCHENK and HILDEBRANDT (1972). They noted, however, that other monocots, including rice, wheat, and maize, grew less well on this medium.

Barley and wheat (*Triticum monococcum* and *T. aestivum*) callus was induced by GAMBORG and EVELEIGH (1968) on their completely defined solidified PRL-4 medium. The callus was subsequently subcultured in suspension culture using their completely defined B-5 medium. Both of their media were supplemented with 2,4-D. The B-5 medium was subsequently used by GAMBORG (1970) and GAMBORG and SHYLUK (1970) to study the

1) This work was supported by NSF Grant GB 36809. I am grateful to Dr. E.R. SEARS for supplying the seeds used for this work and for his helpful criticism of the manuscript. Copied from the Proceedings of the 4th Wheat Genetics Symposium by the kind permission of the Editors and Organizing Committee.

effect of ammonium as the sole nitrogen source on the growth of callus of einkorn wheat and several dicots. TRIONE *et al.* (1968) grew wheat (*T. aestivum*) callus tissue on Hildebrandt's "D" medium (HILDEBRANT *et al.*, 1946), a modified White's medium, supplemented with 2,4-D. They were successful in growing the callus both in liquid and on solid media. For callus induction, they removed a section from the root-stem axis just below the cotyledonary node.

Callus induction from seedling roots of einkorn wheat (*Triticum monococcum*, 2n=14), emmer wheat (*T. dicoccum*, 2n=28), and common wheat (*T. aestivum*, 2n=42), and from stem pieces of common wheat were reported by SHIMADA *et al.* (1969). They used White's basal medium or a modification of it (RISSER and WHITE, 1964), both of which were supplemented with 2,4-D or IAA. They noted that the best callus growth occurred when casein hydrolysate or coconut milk was added to the media.

This study was initiated to determine: (1) conditions suitable for callus initiation and subculturing of wheat, rye, and a wheat-rye hybrid; and (2) the response of such callus lines to various culture media and conditions.

Wheat (*Triticum aestivum* cv. Chinese Spring), rye (*Secale cereale* cv. Gator), and a wheat-rye hybrid were used in this study. The wheat-rye hybrid was produced by pollinating a Chinese Spring wheat with pollen from the Gator rye plant which also served as a source of rye seed.

Culture media were those of Linsmaier and Skoog, modified White's according to SHARP *et al.* (1972), Gamborg's B-5, and Schenk and Hildebrandt. These media were at times supplemented with 2,4-D or NAA, casamino acids or casein hydrolysate, yeast extract, and sucrose in various combinations and concentrations as described in the results. The basal medium is that of Linsmaier and Skoog containing 4% sucrose, and supplemented with 1 g/l casamino acids and 4 mg/l of 2,4-D, solidified with 8 g/l of Difco purified agar. All culture tubes and flasks were covered with 0.0015-inch-thick polyetheline film cut into four-inch squares. All cultures were exposed to 16 hours of light followed by 8 hours of darkness unless otherwise noted.

Callus Induction

Callus induction of wheat, rye, and the hybrid was consistently obtained by the following procedure. Seeds were placed in a mixture consisting of one part bleach (5.25% of sodium hypochlorite) and five parts distilled water and shaken; after 10 minutes the seeds were rinsed three times with sterile, distilled water and placed on a shaker in sterile, 125ml Erlenmeyer flasks containing 25 ml of sterile distilled water. After 24 hours, the seeds were removed, treated again with bleach and rinsed as before. The seeds were then individually placed in sterile culture tubes containing the basal medium but lacking 2,4-D.

After two to four weeks or when the seeds had sprouted and plants were about four inches tall, they were removed under sterile conditions and the basal part of the stem was cut into thin cross sections about two millimeters thick. Those four or five sections closest to the cotyledon were placed in a one-by-six-inch culture tube containing 20 ml of basal medium. Within a few weeks, abundant callus formed on the upper surface of the stem slices.

When IAA at 10 or 50 mg/l was substituted for 2,4-D in the basal medium, little or no callus was formed on wheat sections. No callus was formed when IAA at 10 mg/l or 50 mg/l and NAA at the same concentration were substituted for the 2,4-D. However, good callus was produced when NAA at 10 mg/l was substituted alone for 2,4-D in the basal medium.

Among 360 wheat anthers placed on various modifications of the basal medium, callus was produced in 11 cultures, all of which originated from the filament tissue. Four calluses appeared on the basal medium containing 2 mg/l of 2,4-D, six calluses appeared on one-half strength basal medium (except for the iron stock, which was full strength) containing 2 mg/l of 2,4-D, and one callus formed on this same medium but containing 1 mg/l of NAA and no 2,4-D. In this last case, roots rapidly appeared and callus growth cased.

Subculture of Wheat Callus

Wheat callus derived from filament tissue was grown on 27 different combinations culture media to test for differences in growth rate. The media tested were: I, Linsmaier and Skoog; II, Linsmaier and Skoog, with the basal salts modified to contain 5312 mg/l KNO_3, 330 mg/l NH_4NO_3, and 167.5 mg/l $CaCl_2$ (personal communication, C.E. Green, Univ. of Minn.); and III, White's modified according to SHARP *et al.* (1972). Each of the media was supplemented with either 1 g/l casamino acids, 0.5 g/l of yeast extract or both 1 g/l casamino acids and 0.5 g/l of yeast extract. Sucrose was included at 2, 4, or 6%, and all of the media contained 4 mg/l of 2,4-D.

In most cultures, a cream-colored callus with a raspberry-like appearance was formed on the medium. Although callus survived and proliferated on all of the media, there was considerable difference in growth rate. The greatest increase in size occurred on the modified Linsmaier and Skoog's medium containing casamino acids only (IIa). Only a slight difference was apparent in the effect of sucrose concentration in this medium, with 4% sucrose appearing to be superior. The substitution of yeast extract for casamino acids (IIb) or its addition to the medium containing casamino acids (IIc) resulted in a marked inhibition in growth and the callus being yellow to tan in color.

The second-best culture medium was the regular Linsmaier and Skoog's medium containing casamino acids only (Ia). Little effect of sucrose concentration was evident,

but the inhibitory effect of yeast extract was clearly evident and affected callus growth and coloration in the same way as described above.

Wheat callus growing on the modified White's medium grew more slowly than on the other media, but showed slowest growth when only casamino acids were present. Inclusion of yeast extract alone (IIIb) was superior to casamino acids alone (IIIa), and the inclusion of both supplements (IIIC) gave the best growth response. There was little effect of sucrose concentration on growth.

Three conclusions regarding growth rate of wheat callus could be clearly drawn from this experiment. First, Linsmaier and Skoog's medium was superior to the modified White's medium; second, the inclusion of yeast extract in Linsmaier and Skoog's medium was clearly inhibitory alone or in combination with casamino acids, while its inclusion in the modified White's medium alone or in combination with casamino acids was clearly stimulatory; and third, the inclusion of sucrose at 2, 4, or 6% concentration in these media had only slight effects on growth rate.

Wheat callus, originally derived from filament, has been repeatedly subcultured in liquid basal medium containing 2% sucrose. Under these conditions the callus grows as white- to cream-colored clumps which continually fragment and thus remain a few millimeters or less in diameter. The callus remains undifferentiated in this 2,4-D-containing medium, but upon transfer to basal medium containing 2% sucrose and 4 mg/l of NAA and lacking 2,4-D, the callus differentiates into hundreds of roots, which rapidly elongate and form a filamentous mass radiating from the central callus clump. These roots remain white except for some green pigmentation appearing in the vascular region which occupies the core of the roots. No stem or leaf differentiation has been observed.

Subculture of Rye Callus

Rye callus has been repeatedly subcultured on the basal medium. This callus has a morphology different from that of wheat or the hybrid. It is white, often with a greenish core, and is distinctive in often producing an extensive, loose surface layer which is fluted or layered. There has been no tendency for organ differentiation on the basal medium.

Subculture of Wheat-Rye Callus

The hybrid callus has been repeatedly subcultured on the basal medium, where it grows as a white to yellowish- or cream-colored callus with a smooth to slightly rough surface.

This callus was subcultured onto the same 27 different media described in the subculturing of wheat callus. The best growth was clearly shown on the Linsmaier and Skoog medium supplemented with casamino acids only. On this medium 2% sucrose appeared to be superior. The next best medium for callus growth was the same medium supplemented with both casamino acids and yeast extract. In this case 4% sucrose was

superior. The other media gave fair to poor results, with the least growth occurring on the modified White's medium. On this medium the callus remained whitish where casamino acids only were added to the medium; however, when yeast extract was added in combination with casamino acids or alone, the callus became brown- or tan-colored and little or no growth was observed. Generally, except as noted above, sucrose concentration had little effect on callus growth.

The hybrid callus resembled the wheat callus only to a limited degree in its growth response on the 27 different media. Best growth was on the Linsmaier and Skoog standard medium rather than the same medium containing a modified amount of basal salts. Although poorest growth was on the modified White's medium; as was the case with wheat callus, the hybrid callus was even poorer on the modified White medium containing yeast extract, both in combination with casamino acids or alone. This is opposite from the response of wheat callus to the addition of yeast extract to this medium.

Comparative Growth Responses

Since wheat callus was reported previously to grow on the B-5 medium (GAMBORG and EVELEIGH, 1968; GAMBORG, 1970; and GAMBORG and SHYLUK, 1970) and by SCHENK and HILDEBRANDT (1972) on their medium, a series of cultures was established to compare the growth of the wheat, rye, and wheat-rye callus on these two media to their growth rate on Linsmaier and Skoog's medium.

Ten subcultures of each kind of callus were established on 20 ml of each of the three kinds of media. All three media contained 2% sucrose, 1 g/l of casamino acids, and 4 mg/l of 2,4-D. Explant sizes were 25 mg of rye callus, 25 mg of the wheat, and 20 mg of the hybrid. The ninety tubes were divided into two groups of 45 each. One group was kept in continuous dark while the other group was kept in the usual alternating light-dark cycle. After 40 days at 25°C the cultures were harvested and fresh weights were obtained.

The results of the experiment may be summarized as follows. All three kinds of callus grew to some extent on the three kinds of media. The wheat callus grew the most and the rye callus the least. For each kind of callus, the growth response was similar for the three kinds of media, and little difference was noted between growth rate of callus grown in a light-dark cycle and that grown in continuous dark. The growth response was quite variable, with the standard deviations exceeding the mean increase in fresh weight in most cases. This variability as well as the generally slow rate of growth was probably a result of the small size of the explants, since it has been observed that larger explants (100 mg or larger) commence growth more rapidly and fail to grow less often than the small explants used in this experiment.

Sources of earliness and winter habit in durum wheat

C.O. QUALSET and Y.P. PURI[1)]
Department of Agronomy and Range Science, University of California,
Davis, California 95616, U.S.A.

We have made a systematic survey of the U.S. Department of Agriculture World Collection of Wheat (durum section) for many characters to provide new sources of variability for use in an applied breeding program. Of major interest for the California breeding program was new sources of photoperiod- and thermal-insensitive genotypes. Many of the improved durum wheats with spring habit from the International Maize and Wheat

Table 1. Selected list of durum wheats with very early heading time in U.S.D.A. World Collection

PI or CI	Origin	PI or CI	Origin	PI or CI	Origin	PI or CI	Origin	PI or CI	Origin	PI or CI	Origin
306656	France	195079	Ethiopia	226573	Ethiopia	60608	Ethiopia	70719	Iraq	140959	Australia
192103	Portugal	195091	〃	273974	〃	60387	〃	70729	〃	165445	Mexico
192129	〃	195092	〃	273975	〃	59284	〃	70730	〃	CI 13719	U.S.A.
192130	〃	195094	〃	273989	〃	CI 8639	〃	70732	〃	CI 14046	〃
192179	〃	195096	〃	297832	〃	CI 8651	〃	225328	Iran	CI 12726	〃
192207	〃	195101	〃	297834	〃	145720	Arabia	268298	〃		
192487	〃	195103	〃	297835	〃	152567	〃	268300	〃		
192620	〃	195697	〃	297836	〃	155315	〃	127098	Afghanistan		
192741	〃	195711	〃	297837	〃	152445	Morocco	135048	〃		
204014	〃	195715	〃	297839	〃	220425	Egypt	135049	〃		
191235	Spain	195717	〃	297840	〃	60707	〃	135059	〃		
271895	Italy	195718	〃	297841	〃	60727	〃	94708	USSR		
271898	〃	195719	〃	297843	〃	60731	〃	115514	India		
290478	Hungary	195720	〃	297844	〃	60735	〃	143782	〃		
CI 13160	Ethiopia	196081	〃	297846	〃	60736	〃	164512	〃		
193868	〃	196082	〃	297847	〃	60737	〃	164546	〃		
193884	〃	196093	〃	297848	〃	237630	Cyprus	164754	〃		
193889	〃	196095	〃	297849	〃	226578	Israel	164759	〃		
193890	〃	196097	〃	297850	〃	249822	〃	176285	〃		
194028	〃	196098	〃	297853	〃	292038	〃	176293	〃		
194029	〃	196907	〃	297854	〃	295968	〃	183535	〃		
194030	〃	197478	〃	297857	〃	109597	Turkey	212925	〃		
194031	〃	197482	〃	58787	〃	119326	〃	214348	〃		
194035	〃	199993	〃	58793	〃	166938	〃	40940	〃		
194043	〃	199995	〃	60601	〃	211697	〃	42008	〃		

1) Tulelake Field Station, Tulelake, California 96134.

Improvement Center in Mexico are photo-insensitive, but many apparently have a vernalization requirement (thermal-sensitivity) as evidenced by failure to head when planted in July at Davis, California (minimum temperatures 50°F). Thus, sources not having a cold requirement for heading were desired in the breeding program.

The durum collection (about 3,500 entries) was grown at Davis (38°32'N; 16 m elevation) with July planting in 1969 and Tulelake, California (41°58'N; 1240 m elevation) with April planting. The methods used and the variation in heading time observed at the two locations were discussed in more detail elsewhere (QUALSET and PURI, 1973). By examining the heading responses jointly for the two locations it was possible to identify very early heading types that are very likely both photo- and thermal-insensitive. There were about 350 entries that fell in this category, of which 130 are listed in Table 1. About 1,000 of the entries failed to head at Davis with summer planting. Of this group, only 85 failed to head at Tulekake and these are believed to have strong vernalization re-

Table 2. Durum wheats having winter growth habit in U.S.D.A. World Collection

PI or CI	Origin	PI or CI	Origin	PI or CI	Origin	PI or CI	Origin
185188	Portugal	290496	Hungary	CI 3225	Tunisia	68268	U.S.S.R.
185189	〃	184172	Yugoslavia	CI 3229	〃	68270	〃
185190	〃	184174	〃	166473	Turkey	68271	〃
192760	〃	264959	〃	166639	〃	68283	〃
190978	Spain	264979	〃	166642	〃	68284	〃
190979	〃	265005	〃	167543	〃	73313	〃
190980	〃	265007	〃	178094	〃	78811	〃
191021	〃	265009	〃	178140	〃	181265	〃
191249	〃	265010	〃	178184	〃	262656	〃
191251	〃	265011	〃	245750	〃	181265	India
263562	Switzerland	CI 11245	〃	210855	Iran		
191375	Italy	CI 11246	〃	225237	〃		
191380	〃	277126	Bulgaria	243691	〃		
231363	〃	294909	〃	134116	Afghanistan		
243638	〃	295011	〃	134133	〃		
272536	Hungary	295012	〃	181258	〃		
272537	〃	295030	〃	220135	〃		
272549	〃	295039	〃	220689	〃		
272596	〃	295043	〃	221488	〃		
290481	〃	295070	〃	221489	〃		
290482	〃	278260	Greece	221494	〃		
290484	〃	278261	〃	68245	U.S.S.R.		
290487	〃	278262	〃	68264	〃		
290493	〃	278353	Sicily	68265	〃		
290495	〃	CI 3206	Tunisia	68267	〃		

quirements and would be useful in breeding durum varieties with winter habit (Table 2).

The group that failed to head at Davis in the summer, but did head at Tulelake and thus having spring growth habit, probably have weak photoperiod sensitivity and/or vernalization requirement. This group (not listed here) should be examined under conditions of better control of temperature and light intensity to determine the vernalization and photoperiod requirements in these spring wheats.

Requests for seed stocks of these entries should be made to Dr. Joseph C. CRADDOCK, Germplasm Resources Laboratory, Building No. 046, Beltsville, Agricultural Research Center, Beltsville, Maryland 20705, U.S.A.

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Possibility of 5B-like effect in diploid species

OKAMOTO, M. and N. INOMATA
Abteilung für Pflanzengenetik für Strahlen und Umweltforschung
8059 Grünbach 1, West Germany, and
Laboratory of Genetics and Plant Breeding, College of Agriculture,
University of Osaka Prefecture, Osaka, Japan

At meiosis of haploid plants from diploid species such as einkorn wheat, barley, oat and rye certain numbers of bivalents are usually observed although frequencies may vary according to plants. But in diploid species of the above plants no quardivalents have been found so far unless these diploid species were subjected to radiation treatment.

Nobody has suggested possibility of presence of 5B-like effect in the above species.

If chromosome pairing in pollen mother cells of haploids of the above plants is due to chromosome pairing between homologous segments of chromosomes, which seems to be more or less naturally accepted at the present moment, it is quite natural to expect that quardivalents might also be found in diploid species at meiosis, because four homologous segments are or must be present in diploid species. No quardivalent formation has so far been observed as has been mentioned above.

It is very interesting to suggest that a gene or genes which supress quadrivalent formation at meiosis of the above plants may be present in these diploid species.

In hexaploid species of wheat a gene or genes which suppress homoeologous pairing have already been found on chromosome 5B by OKAMOTO (1957) and RILEY (1958), and a gene or genes have recently been found on chromosome 3D by MELLO-SAMPAYO (1971) to have similar but less effect. MELLO-SAMPAYO also reminded OKAMOTO at the 4th International Wheat Genetics Symposium in Columbia (1973) of such effect in A genomes discovered by DRISCOL (1972).

We might then be able to say more strongly that such a gene or genes which suppress homoeologous pairing may also be effective in diploid species as well.

The things remain to be done is to find out whether such a gene or genes may be found in the original diploid species from which the A, B and D genomes come from.

The first approach to such a problem will be test whether the chromosomes of the *Aegilops squarrosa* have such effect or not.

The method may be outlined as follows.

(1) Cross synthesized hexaploid species on monosomics of Chinese Spring wheat.
(2) Pick up the monosomic plants.
(3) Cross the monosomic plants by *Secale cereale, Ae. sharonensis* or whatever species suitable for the test.
(4) Compare pairing configulations between the plants with or without the chromosome concerned.

If one of the seven chromosomes of the *Ae. squarrosa* is found to have the effect of suppressing homoeologous chromosome pairing, it may safely be asserted that *Ae. squarrosa* carries a gene or genes for suppression of homoeologous chromosome pairing.

The test for presence or absence of such a gene or genes for suppression of homoeologous chromosome pairing in the original species from which the A and B genomes come from may not be so simple as that in *Ae. squarrosa*. But trials are now being to find out such methods.

It may be worth while to mention that the method similar to that with the chromosomes of *Ae. squarrosa* may be employed with any addition or substitution lines of common wheat.

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Genome analysis in the triticinae using isoenzymes of phosphodiesterase[1)]

G. Wolf and B. Lerch
Institut für Pflanzenpathologie der Universität Göttingen, and Institut für Biochemie der Biologischen Bundesanstalt, Brauschweig, Deutschland

Introduction

The hitherto accepted concept of the origin of the three different genomes of hexaploid wheat has been questioned in recent times because the origin of the B genome is not clear (see the review by Sears, 1969). This concept was based mainly on cytological and morphological investigations. In recent years electrophoretical investigation have been used increasingly to elucidate the relationships in the Triticinae. In particular the spectra of the seed storage proteins (Johnson, 1972) and of several enzymes of different species (Jaaska, 1971; Mitra and Bhatia, 1971) have been compared by electrophoresis. However, efforts so far to elucidate the relationships in the Triticinae by comparing the isoenzymes of twelve different enzymes have been unsuccessful (Brewer *et al.*, 1969). While working on nucleic-acid-degrading enzymes in wheat, we found three isoenzymes of phosphodiesterase (PDE) in *Triticum aestivum* ssp. *vulgare* using disc electrophoresis in polyacrylamide gels (Wolf, 1968). We presumed that the three enzymes could be traced back to the three genomes of the hexaploid wheat. In the work presented here we compared the patterns of the isoenzymes of phosphodiesterase of 18 species or subspecies of *Triticum* and *Aegilops*, hoping that by this means we might obtain information about the donors of the three genomes of the hexaploid wheat.

Materials and Methods

Extraction

Leaves (about 1~8 g fresh weight) were ground at 4°C with sand in a mortar with 3x the amount (v,w) of 0.05 M Tris-HCl buffer (pH 7.5), containing 0.5 M KCl. The extracts were centrifuged at 20,000 g for 10 minutes and concentrated to about 1/3 of the original volume by polyethylene glycol (Aquacide, Calbiochem).

For some experiments the enzyme was partially purified by slowly heating the crude extract to 60°C in a water bath. Under these conditions about 50% of the proteins were precipitated without loss of PDE activity or changes of electrophoretic mobility (Lerch and Wolf, 1972).

1) We are grateful to Mrs. U. Oulevey for technical assistance and Dr. Ch. Lehmann, Gatersleben, Dr. E.R. Sears, Columbia, Dr. E. Fuchs, Braunschweig, and Dr. G. Röbbelen, Göttingen, for supplying seeds. We thanks Dr. E. R. Sears, Columbia, for helpful discussions. Copied from the Proceedings of the 4th Wheat Genetics Symposium by the kind permission of the Editors and Organizing Committee.

Electrophoresis

Disc electrophoresis was performed in 7% polyacrylamide gels (0.6×9 cm) at 4°C according to DAVIS (1964). Two hundred V were applied until the proteins had entered the running gel (after about 30 min) and 300 V until the bromphenolblue band had reached the bottom of the gel. Under these standard conditions the electrophoresis was completed after about 3 1/2 hours.

Detection of Enzyme

Each gel was stained for PDE (orthophosphoric diester phosphohydrolase, EC 3.1. 4.1) according to LERCH (1968) by incubation in a solution of 2.5 mg 2'-deoxythymidine-5'-naphthylphosphate (Merck, Darmstadt) and 15 mg fast red RC (Serva, Heidelberg) in 25 ml 0.05 M Tris-HCl (pH 7.5) for 15 minutes to one hour at room temperature. After staining, the gels were washed in a solution of 7% acetic acid and scanned in a Joyce-Loeble densitometer at 460 nm.

Results

All tested hexaploid species and subspecies have 3 PDE isoenzymes with the same electrophoretic mobility. Each of the two faster moving bands in always about twice as active as the slowest one.

The tetraploids contain only one band, which is identical with the fastest band of the hexaploids.

The band of three diploid species and one subspecies was fast moving, and under standard conditions it was difficult to decide whether this band and that of the tetraploids moved electrophoretically as fast or at a different rate as the fastest of the hexaploids.

Unambiguous results were obtained only by coelectrophoresis at a prolonged separation time (5 1/2 hours). Thus, by coelectrophoresis of partially purified extracts of *T. aestivum* ssp. *vulgare* var. *wernerianum* and extracts of the four diploid species or subspecies, it was shown that the diploid band was not identical with the fastest band of the hexaploids. Coelectrophoresis also demonstrated that the slow-moving band of *Ae. squarrosa* is identical with the slowest band of the hexapoids.

These results imply that the PDE isoenzymes of the four diploid species or subspecies are also not identical with the PDE of the tetraploids. Coelectrophoresis of extracts of *T. aestivum* ssp. *vulgare* var. *wernerianum* with extracts of the other hexaploid species and with those of the tetraploids also confirmed the results obtained under standard conditions; that is, all the bands of the hexaploids are identical and the fastest of these is identical with the band of the tetraploids.

Discussion

The use of seed proteins and of many of the enzymes employed in genetic investiga-

tions of wheat presents several problems. The seed proteins are very numerous and only characterized by their electrophoretic mobility. Some enzymes, e.g., the phosphatases and esterases, also give many bands, and their substrate specifity is not well defined. With other enzymes, such as esterase, alcohol dehydrogenase, catalase or aminopeptidase, the picture may be complicated by polymorphism (MacDonald and Brewbaker, 1972).

Phosphodiesterase, however, has a well-defined substrate specifity and occurs in the Triticinae only in the form of at most three isozymes, which are not composed of dissociable subunits (Wolf, unpublished). Therefore, in our opinion, it is particularly suited for genome analysis.

It is striking that the pattern of the isoenzymes of phosphodiesterase is the same within the di-, tetra-, and hexaploid group. Assuming the validity of the one-gene, one-protein hypothesis and considering the PDE as a genome marker, we draw the following conclusions from a comparison of the isoenzyme pattern:

1. The identity of the slowest moving band of the hexaploids and the band of *Aegilops squarrosa* confirms *Ae. squarrosa* as the donor of the D genome.

2. The identity of the band of the tetraploids and the fastest migrating band of the hexaploids confirms that the hexaploids derive from a tetraploid species.

3. No band corresponding to the middle band of the hexaploids could be found in the di- and tetraploids. However, the synthetic hexaploid *T. durum* × *Ae. squarosa* displayed three isoenzymes of phosphodiesterase. As the parents possess only one band each, we conclude that the middle band in the hexaploids is a hybrid enzyme (Wolf, unpublished).

4. The occurrence of only one band in the tetraploids can be explained best by the hypothesis of autotetraphloidy (Camara, 1935). The hypothesis is supported further by the observation that the fastest band of the hexaploids (originating from the tetraploids) contains about twice the activity of the slowest one (originating from *Ae. squarrosa*).

5. *T. monococcum, T. aegilopoides,* and *Ae. speltoides,* which are said to possess the genomes A or B, have an identical band which is different, however, from that of *Ae. squarrosa* and does not correspond to any band of the tetra- or hexaploids. We therefore conclude that the tetraploids have originated from a diploid species as yet unknown. Studies on occurrence and properties of hybrid enzymes of PDE in *Triticum* are nearly completed and will be published elsewhere.

Phylogenetic Relationships among DNA's of wheat, rye and *Agropyron*

J. Dvořák and E. Scheltgen
Departments of Crop Science and Bacteriology, University of Saskatchewan, Saskatoon, Canada

There is much evidence to indicate that the genera *Triticum*, *Secale* and *Agropyron* are closely related. Compensation for replaced wheat chromosomes in alien disomic substitutions has been demonstrated for a number of rye and *Agropyron* chromosomes, which indicates that homoeologous chromosomes of these species must carry similar genes. On the other hand, the fact that little or no pairing occurs between wheat and *Agropyron* or rye homoeologues suggests considerable dissimilarity among these chromosomes. Numerous structural rearrangements of chromosomes may have occurred in these genomes during their evolution. Indeed, it has been shown that the *S. cereale* genome differs from the more primitive genome of *S. montanum* by a minimum of three reciprocal translocations (Riley, 1955) and diploid *A. elongatum* differs from *Ae. squarrosa* by a minimum of one reciprocal translocation (Dvořák, 1971). However, the well-expressed homoelogy among *Triticum*, *Aegilops*, *Agropyron* and *Secale* chromosomes suggests that the fixation of translocations has been relatively rare.

Studies of pairing between specific wheat and *Aegilops* or *Agropyron* telocentrics in polyhaploids involving *Ae. speltoides* have revealed that chromosome pairing is usually restricted to one homoeologous group (Johnson and Kimber, 1967; Kimber, 1968; Athwal and Kimber, 1972; Dvořák, 1972a), which makes the presence of numerous translocations in these chromosomes highly improbable. However, as Kimber (1968) and Athwal and Kimber (1972) were able to demonstrate for *Ae. umbellulata* chromosomes, inversions would preserve the integrity of homoeologous groups and yet structurally differentiate homoeologues. The high incidence of inversions constituting intra- and inter-specific chromosome polymorphism within the genus *Drosophila* (Stone *et al.*, 1960; Wasserman, 1960; Bock, 1971) indicates that inversions may be the most significant type of chromosome rearrangement during evolution. The question of whether the low pairing affinity among homoeologous chromosomes is due to the accumulation of inversions in genomes of Triticinae or to some other cause cannot be resolved for lack of evidence.

While the mechanism of chromosome pairing is still obscure, there is some justification for a hypothesis that pairing is initiated in specific sites along chromosomes. It was shown in corn that segments either close to a telomere or proximal to a centromere have minor importance in the initiation of homologous pairing (Burnham *et al.*, 1972). Comings and Riggs (1971) proposed that nucleotide sequences in specific sites along

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chromosomes may play an essential role in the recognition of homologous chromosomes. DVOŘÁK (1972b) suggested that the diversification of such sequences during evolution may be responsible for low pairing affinity between homoeologues. From this point of view, it appears worthwhile to obtain information on the extent of similarity in DNA's of representative species in the Triticinae.

Results and Discussion

Unlabelled and ^{3}H-labelled DNA's were isolated from sterile embryo cultures of common wheat cv. Chinese Spring, rye cv. Prolific and diploid *A. elongatum* according to a method described by DVOŘÁK (1972a).

DNA Base Composition of Wheat, Rye and Agropyron

The mole percent of guanine plus cytosine (GC) of wheat, rye and *Agropyron* DNA's was determined from the thermal dissociation profiles in 0.1 SSC buffer (1 SSC is 0.15 M NaCl and 0.015 M Na citrate pH 7.0). The relative GC content was calculated from the midpoints of thermal dissociation profiles (Tm) according to a relationship proposed by SCHILDKRAUT *et al.* (1962).

The base composition of *A. elongatum* DNA was also determined by equilibrium centrifugation in a CsCl density gradient. Its buoyant density was found to be 1.699 g/cm^3 which, according to the reltionship of density to mole percent GC proposed by MANDEL *et al.* (1970) and SCHILDKRAUT *et al.* (1962), corresponds to a GC content of 40.4%.

The absence of significant differences among the T_m's of wheat, rye and *Agropyron* DNA's (Table 1) indicates that the relative base composition of the DNA of wheat, rye and *Agropyron* has been stable during evolution.

Table 1. Base composition of wheat, rye and *Agropyron* DNA's estimated from thermal dissociation profiles

Source of DNA	Ave. T_m in ℃	Relat. GC cont. in mole %
Wheat	70.5	41.4
Rye	71.7	43.8
A. elongatum	71.2	42.8

Hybridization among Wheat, Rye and Agropyron DNA's

The homology of nucleotide sequences among wheat, rye and *Agropyron* DNA's was studied by DNA-DNA hybridization which was carried out according to a procedure described by GILLESPIE and SPIEGELMAN (1964). Twenty-seven μg of unlabelled, single-stranded wheat or rye or *Agropyron* DNA was immobilized on nitrocellulose filters (Schleicher and Schuell Co., B-6, 25 mm in diameter). The filters with immobilized DNA were incubated with 0.7 ml of solution containing 5 μg/ml of either homologous

(same species) or heterologous (different species), single-stranded, sheared, ^{3}H-labelled DNA in 1 SSC and 50% formamide at 43°C.

The selectivity of the reassociation of DNA from solution with filter-immobilized DNA decreases with an increase in the concentration of Na^{+} in the solution. However, it increases with an increase in temperature above the point at which the maximum rate of reassociation occurs. The use of a formamide solution makes it possible to carry out DNA-DNA hybridization at lower temperatures (BONNER *et al.*, 1967; MCCONAUGHY *et al.*, 1969) with increased selectivity.

In a preliminary experiment it was found that the maximum rate of reassociation of wheat single-stranded DNA with filter-immobilized *Triticale* DNA occurred in 1 SSC and 50% formamide at 38°C. The temperature chosen (43°C) for the following DNA-DNA hybridization experiments makes the DNA reassociation highly selective, yielding only well matched DNA/DNA duplexes.

One estimation of the relatedness of nucleotide sequences among the DNA's studied was based on the amount of reassociation between heterologous DNA's as compared to that between homologous DNA's. The less related the nucleotide sequences of heterologous DNA's are, the less reassociation is expected to occur between ^{3}H-labelled DNA in solution and filter-immobilized DNA compared to the control in which both DNA's are from the same source.

Table 2. The relative amount of binding of radioactive DNA to filter-immobilized DNA

Filter-bound DNA	^{3}H-DNA in solution		
	Wheat	*Agropyron*	Rye
Wheat	100	60	—
Agropyron	73	100	51
Rye	—	61	100

The relative amounts of reassociation of *Agropyron* and wheat as well as *Agropyron* and rye DNA's compared to reassociation of homologous DNA's are shown in Table 2. Invariably, the amount of reassociation between heterologous DNA's was lower than that between homologous DNA's. From the DNA-DNA hybridization experiment in which *Agropyron* DNA was filter-immobilized and wheat or rye DNA was in solution, it appears that rye DNA nucleotide sequences differ more from those of *Agropyron* DNA than from those of wheat. However, in the reciprocal hybridization in which *Agropyron* was in solution and either rye or wheat DNA was filter-immobilized, rye and wheat nucleotide sequences differ equally from *Agropyron* DNA.

The comparison of wheat with rye DNA was not included because it has been carried out by BENDICH and MCCARTHY (1970a). They observed that when wheat DNA was

filter-immobilized and rye DNA was in solution, the reassociation between the DNA's of wheat and rye was only 60% of that of the homologous control. However, in the reciprocal hybridization the amount of binding of wheat DNA to filter-immobilized rye DNA was equal to that of the homologous control. The lack of reciprocity in the amount of reassociation between DNA of wheat and rye was also noticed in a DNA-DNA hybridization experiment employing a DNA-agar technique (BENDICH and BOLTON, 1967).

Thermal Stability of Reassociated DNA/DNA Duplexes

Another estimate of nucleotide sequence homology among the DNAs mentioned above was based on differences in the thermal dissociation profiles of homologous and heterologous reassociated DNA/DNA duplexes. The thermal dissociation of double-stranded DNA is proportional to the number of hydrogen bonds per unit of the DNA double helix (MARMUR and DOTY, 1962). Imperfect matching of strands in reassociated DNA due to the presence of noncomplementary bases results in a lower number of hydrogen bonds per unit of DNA/DNA duplexes and, consequently, in a decrease in thermal stability as measured by the T_m (BAUTZ and BAUTZ, 1964). The decrease in the T_m of heterologous DNA duplexes compared to the T_m of homologous DNA duplexes will be referred to as the ΔT_m.

Table 3. T_m's reassociated DNA/DNA duplexes and $\triangle$ T_m's between heterologous and homologous DNA/DNA duplexes

Filter-bound DNA	^{3}H-DNA in solution		
	Wheat	*Agropyron*	Rye
Wheat	78.5*	—	—
	ΔT_m=2.2		
Agropyron	76.3	76.8	76.2
		ΔT_m=0.8	ΔT_m=4.6
Rye	—	76.0	80.8

*T_m in ℃.

LAIRD *et al.* (1968) estimated that a ΔT_m of 1°C is approximately equal to the mismatching of 1.5% of the nucleotides in the reassociated DNA.

The thermal dissociation of homologous or heterologous DNA/DNA duplexes was investigated according to a procedure previously described by BENDICH and MCCARTHY (1970a). The ΔT_m's of heterologous DNA/DNA duplexes involving the DNA's of *Agropyron* and wheat or *Agropyron* and rye (Table 3) follow a pattern similar to the relative amounts of reassociation between these DNAs (Table 2). The ΔT_m of wheat-*Agropyron* heterologous DNA/DNA duplexes was 2.4°C smaller than the ΔT_m of rye-*Agropyron* heterologous DNA/DNA duplexes. Again the DNA nucleotide sequences of *Agropyron* appear to be more

like wheat than those of rye. The reciprocal hybridizations involving rye and *Agropyron* DNAs did not give identical results. While the ΔT_m was 4.6°C for heterologous DNA/DNA duplexes from hybridization in which *Agropyron* DNA was immobilized and rye DNA was in solution, the ΔT_m of DNA/DNA heterologous duplexes from the reciprocal hybridization was only 0.8°C.

BENDICH and MCCARTHY (1970a) suggested that the lack of reciprocity of DNA-DNA hybridization may be attributed to the different degree to which individual families of nucleotide sequences have been diversified within a genome. However, this explanation may be oversimplified. A certain portion of eukaryotic DNA is usually represented by short nucleotide sequences repeated many times, and because of this they readily reassociate (BRITTEN and KOHNE, 1968). It has been shown that in many species repeated sequences differ considerably in their GC content from the rest of the DNA (e.g. YASMINEH and YUNIS, 1971; MACGREGOR and KEZER, 1971; TRAVAGLINI *et al.*, 1972; GRAHAM and SKINNER, 1973). Evolutionary diversification of GC-rich and conservation of GC-poor repeated sequences or *vice versa*, followed by unequal reiteration of newly evolved sequences in different genomes, can easily account for the lack of reciprocity in DNA-DNA hybridization as measured by the relative binding of DNA's to each other as well as by the ΔT_m. The families of repeated sequences rich in GC will hybridize more readily under stringent conditions, and also the thermal stability of GC-rich duplexes will be higher than that of GC-poor duplexes. Since the T_m of rye homologous DNA/DNA duplexes was higher than the T_m's of wheat or *Agropyron* homologous DNA/DNA duplexes, it seems that there is a larger portion of GC-rich repeated sequences in rye DNA than in wheat or *Agropyron* DNA, although the GC content of native DNA's appeared to be the same (Table 1).

Conclusions

The results presented in this paper and those reported by BENDICH and MCCARTHY indicate that rye DNA is as distantly related to *Agropyron* DNA as to wheat DNA, while the two latter DNA's appear more closely related. A comparison of the thermal stability of *T. monococcum-Ae. squarrosa* heterologous DNA/DNA duplexes with homologous *T. monococcum* DNA/DNA duplexes revealed that these DNA's differ slightly from each other (BENDICH and MCCARTHY, 1970b). The ΔT_m of 1.0°C is smaller than that found for wheat-*Aygropron* heterologous DNA/DNA duplexes (ΔT_m of 2.2°C).

The data obtained from the present experiments and from those of BENDICH and MCCARTHY indicated that the DNA nucleotide sequence homology among *Aegilops, Triticum, Agropyron,* and *Secale* is in close agreement with the suggested phylogenetic relationships among these genera. The order of homology with wheat DNA nucleotide sequence is: *Aegilops, Agropyron* and *Secale*. The progressive decrease in homoeologous

pairing of *Aegilops, Agropyron* and *Secale* chromosomes with wheat chromosomes is thus paralleled by the diversification of nucleotide sequences of their DNA's. However, although this parallelism is consistent with the hypothesis that the level of pairing between homoeologous chromosomes is determined by the similarity of nucleotide sequences in their DNA's, it does not provide direct evidence for it. This hypothesis must be tested by other means.

Ble Tom Pouce Blanc (*T. aestivum* L.), a source of genes of solid stem*

S. TSVETKOV
The Institute for Wheat and Sanflower, General Toshevo, Bulgaria

For the period 1963~1972, an interspecific hybridization was carried out at the Institute with the aim of transferring stem solidity of *T. durum* DESF. on to *T. aestivum* L. In spite of the newly developed forms of common winter wheat with solid stem and resistant to *Cephus pygmaeus* L. (TSVETKOV, 1971), researches entailed great difficulties and time consumption. Our inferences turned analogous to those of YAMASHITA (1937), PLATT and LARSON (1944) MCNEAL (1956, 1961), LARSON (1959, 1959a), LARSON and MCDONALD (1963), WALLACE and MCNEAL (1966) and MCKENZIE (1965).

We thus were bound, in our later studies, to seek out another solving of the problem, namely, developing such common winter wheat varieties which to possess dominant and semidominant genes for solid stem, and be, afterwards, utilized for the selection of new productive forms of common winter wheat with solid stem and resistant to *Cephus pygmaeus* L.

By analysing stems of a number of common winter wheat varieties in 1970, we came across an interesting fact that individual plants of Ble Tom Pouce Blanc characterize for their various degree of stem solidity in the internodes. As a result of selection, a line was developed out of Ble Tom Pouce Blanc, with highly solid stem in the internodes. In fact there are reports about Ble Tom Pouce Blanc (JACUBCINER, 1959; ZEVEN, 1969; RUDENCO and UDACHIN, 1969), but there are no such about its solid stem as a source of genes for breeding. Stem solidity was determined by three crossesections in the first top (ear-next) internode and by a cross section in the middle of every next (2nd, 3rd, 4th, 5th) internode. The cross sections of the first top internode were made 2.5 cm. below ear, in the middle of the internode and 2.5 cm. above the base. Degree of stem solidity was determined by the scale of SAPEGIN (1938) and LARSON (1959). For conveniencies, recordings

*A newly selected line of Ble Tom Pouce Blanc with solid stem in the internodes.

were accomplished consecutively from top to bottom of every internode (five in the whole).

Data given in Table 1 show, that in the cross section of the first top internode 2.5 cm. below ear, the newly selected line of Ble Tom Pouce Blanc coincides by the degree of stem arrangement with the hollow stem of the standard Bezostaya 1 common wheat variety as well as the hard No. 1522 wheat (*T. durum* DESF.) with a hollow stem. However, the two lower cross sections show, that stem solidity of Ble Tom Pouce Blanc in the first top internode sharply increases from the middle of the internode thus reaching a degree of 4.6

Table 1. Degrees of stem solidity of the parental varieties and their F_1 interspecific hybrids.

Parents/hybrids	No. of plants studied.	Degree of stem solidity from top to bottom, in points (1～5*).						
		first top internode		lower internode				
		2,5cm below ear	middle	2,5cm. above base	2nd	3rd	4th	5th
Bezostaya 1 (*T. aestivum*)-Stand.	32	1.0	1.0	1.0	1.0	1.0	1.0	2.0
Ble Tom Pouce Blanc (*T. aestivum*)	60	1.0	2.1	4.6	3.4	3.9	4.1	4.2
No. 1522 (*T. durum* DESF.)	20	1.0	1.7	1.9	1.5	1.5	1.7	2.0
Ble Tom Pouce Blanc × No. 1522 F_1	16	2.0	4.7	4.9	4.7	4.3	4.3	4.3
No. 1522 × Ble Tom Pouce Blanc F_1	16	2.0	3.8	4.6	4.0	4.0	4.8	4.3

*1 : hollow stem ; 5 : solid stem ; from 1.1 to 4.9 : medial degrees from hollow to solid stem.

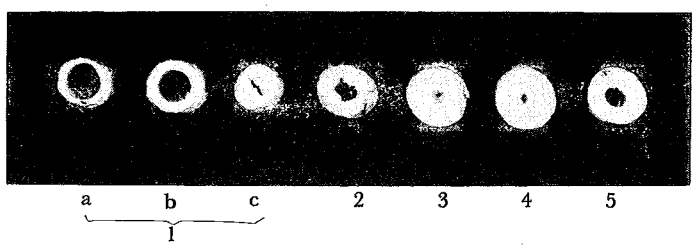

Fig. 1. Pieces demonstrating stem solidity in the newly selected line of Ble Tom Pouce Blanc by internodes : 1a. 2.5 cm. below ear ; 1b. in the middle of the topmost internode ; 1c. 2.5 cm. above the base of the topmost internode ; 2, 3, 4, 5. pieces in the middle of the next internodes from top to bottom.

which, according to the scale at use, proves almost solid with only a tiny opening. Stem solidity in the next four internodes (from top to bottom) also preserves the same (Fig. 1) with insignificant variations mainly in the second and third internodes; while Bezostaya 1 winter wheat variety and the hard No. 1522 wheat possess fully hollow internodes.

With the aim of transferring stem solidity of the newly selected line of Ble Tom Pouce Blanc to the progenies we made, in 1970, some reciprocal crosses between the solid-stem line of Ble Tom Pouce Blanc and the hard hollow-stem wheat No. 1522 (*T. durum* DESF.). From the cross sections it was established that stem solidity of the aforesaid line of Ble Tom Pouce Blanc transfers to F_1 hybrids as a dominant character. Hybrid plants characterize for the nearly solid stem, identical to that of Ble Tom Pouce Blanc (Table 1).

We believe that data obtained are a contribution to the genetics of this wheat variety, which apart from its possessing genes of dwarfness (ZEVEN, 1969) appears also as a source of genes of solid stem.

At present we are in possession of F_2 dwarf forms of common winter wheat with solid stem. Analyses of F_2 hybrid with respect to the factors causing stem solidity in Ble Tom Pouce Blanc are now in progress. Crosses were carried out between Ble Tom Pouce Blanc and other common wheats with the aim of finding out differences in the manner of inheritance of stem solidity in the intervarietal hybrids. Namely, some crosses were made between Ble Tom Pouce Blanc and the Bulgarian common winter wheat variety, named "Dobrich", which is known for its hollow stem and high resistance to diseases (No. 11–32-1140).

Pollen dispersal in wheat (*T. aestivum* L.)

S.C. ANAND and S.C. SHARMA
Punjab Agricultural University, Ludhiana, India

With the discovery of cytoplasmic male sterility and fertility resotration mechanism in wheat (WILSON & ROSS, 1962; SCHMIDT, JOHNSON & MANN, 1962) great deal of interest was created for the exploitation of hybrid vigour by the production of 'hybrid wheat'. Wheat being a self-pollinated crop, the problems associated with the production of hybrid seed on commercial scale was due to poor pollen shedding and dispersal (WILSON, 1968). MENGE *et al.*, (1965), ANAND and BERI (1971) showed that cross pollination of wheat was affected by pollen load and wind direction. ZEFEN (1968) reported that the number of pollen grains above a wheat field was an important factor affecting seed set. To measure pollen load, Bitzer and Patterson (1967) exposed vaseline coated slides in the field and the pollen grains trapped on the slides were counted. The present study was undertaken to measure pollen availability in wheat at various distances and in all the four directions of the pollen source under field condition.

Materials and Methods

Wheat variety C273 was used to study pollen dispersal C273 is a tall cormeroial cultiver and it was planted in a three square meter plot. During period of anthesis, microscopic slides fitted with adhesive tape were fixed to trap the pollen grains. They were placed towards east, west, north and south at 22, 44, 66 and 83 cm from the first row of the pollen source. Three slides were fitted just below spike level and three just below spike level and three were placed at the ground level at each distance and direction. The slides were mounted in the morning and removed in the evening for three successive days. The

exposed area of the adhesive tape was 2.5×2.0 cm (5 cm^2). The tape was removed, dipped in a solution of iron acetocarmine, and the pollen grains were counted under the microscope. The figures reported in this paper are the mean number of pollen grains collected in an area of 5 cm^2 each day. The experiment was replicated twice and the data were statistically analyzed for split plot design.

Result and Discussion

The number of pollen grains trapped at various levels and distances was checked in the four directions. Maximum pollen load was observed towards east and minimum towards south of the pollen source. Pollen count was higher in southern direction compared with that of northern and western direction. This was due to western and north western winds which prevailed during the days the experiment was conducted.

The pollen grains were greater near the pollen source. As the distance of pollen source increased, the pollen load decreased. The number of pollen grain at spike level towards east was 9.99, whereas, at the ground level, they were 25.55. On an average, the pollen grain which fell on the ground were almost double then the pollen grains available at spike level. This was true at all the directions and distances. It indicates that wheat pollen is quite heavy and it does not travel to any great distance. JENSEN (1968) reported that 90% of the wheat pollen remained within 20 feet of its source and much of it dropped to the ground within 5 ft. The results ontained in the present study support the findings of JENSEN (1968). Thus, in the crossing block of hybrid seed production of wheat, fewer male sterile rows would be desirable for adequate seed setting.

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II. Editorial Remarks

Announcement for future issues

WIS No. 39 will be planned for publication in October 1974. Manuscripts for this issue are accepted any time, not later than July 31, 1974.

WIS is open to all contributions regarding methods, materials and stocks, ideas and research results related to genetics and cytology of *Triticum, Aegiolops, Secale, Haynaldia* and related genera. Manuscripts should be typewriten in English, and submitted with duplicates. One article should not exceed five printed pages, including one textfigure (smaller than 7×7 cm^2). Lists of stocks are exempted from this page limit. Authors receive 50 reprints of their contributions free of charge. Extra copies are printed by order at cost price. Communications regarding editorial matters should be addressed to:

Kosuke YAMASHITA
Wheat Information Service
Kihara Institute for Biological Research
Misima 411, Japan

Raise of Membership Fee

Due to the economic situations, the yearly Membership Fee has been raised up to ¥700 for foreign member and ¥500 for Japanese member from the fiscal year beginning April 1973. The money should be paid by the Foreign Postal Money Order, othersiwe considerable loss is caused due to the bank charges. Back numbers are available.

Acknowledgement

The cost of the present publication has been defrayed partly by the Grant in Aid for Publishing Research Results from the Ministry of Education, Government of Japan, and partly by contributions from the Flour Millers Association, Tokyo, Japan, and the Jenkins Foundation for Research, Sallinas, California, U S A. We wish to express our sincere thanks to those organizations. We should also like to express our sincere gratitude for favorable comments regarding WIS Nos. 1~37, and valuable contributions for the present issue. Increased support would be appreciated

The Managing Editor

Explanation of the Figure on the Cover

Pieces demonstrating stem solidity in the newly selected line of Ble Tom Pouce Blanc by internodes: 1a. 2.5 cm. below ear; 1b. in the middle of the topmost internode; 1c. 2.5 cm, above the base of the topmost internode; 2, 3, 4, 5. pieces in the middle of the next internodes from top to bottom. (S. TSVETKOV, Fig. 1, p. 26, present issue WIS 38).