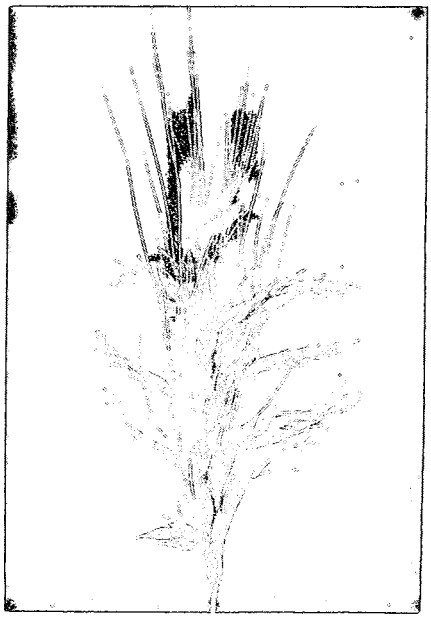
WHEAT INFORMATION SERVICE



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I. Research Notes

Cytoplasmic variability in Triticinae[1)]

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In higher plants cytoplasmic inheritance is essentially maternal and cytoplasmic genes are seldom transmitted through the male gametes. Therefore, phenotypic differences in F_1 hybrids from reciprocal crosses (JONES 1912), and differential segregation in F_2 or backcross progenies from reciprocal crosses generally indicate cytoplasmic differences between the parents (BATESON and GAIRDNER 1921, CHITTENDEN 1927, CHITTENDEN and PELLEW 1927, GAIRDNER 1929). The study of allelism or linkage relationships cannot be done among the non-nuclear genes from different cytoplasmic sources in higher plants, because these genes predominantly show maternal inheritance. Therefore, research on cytoplasmic inheritance is limited to the study of cytoplasmic differences and inheritance of nuclear genes controlling cytoplasmic effects. The relative similarities and differences between the hereditary constituents of cytoplasms from different sources are inferred from their interactions with certain nuclear genes or genotypes (MICHAELIS 1954, KIHARA 1951, FUKASAWA 1959).

In the Triticinae, F_1 hybrids from reciprocal crosses involving the species of *Triticum* have not been reported to differ phenotypically, even when the parental species had different cytoplasms. Hybrids between these species are usually completely male sterile and have a high degree of female sterility. Therefore, differential segregation in the F_2 cannot be studied. Complete elimination of the nuclear genes or chromosomes of the cytoplasm-donor species by extensive backcrossing with the recurrent male parent is usually necessary to reveal cytoplasmic effects on the expression of the substituted genome, because of the aneuploidy and associated sterility in the early generations.

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Therefore, cytoplasmic differences among the species of *Triticum* and *Aegilops* have been studied by the reciprocal-nuclear-substitution method (KIHARA 1951, FUKASAWA 1953). KIHARA and FUKASAWA selected *Triticum* and *Aegilops* species with no pairing homology between their chromosomes during meiosis in F_1 hybrids to prevent crossing over between the alien genomes. They reintroduced the *Aegilops* genome into *Aegilops* cytoplasm by backcrossing, to prove that *Aegilops* cytoplasm remained unaltered in male-sterile wheats *Aegilops* cytoplasm. The cytoplasms of *Ae. caudata* and *Ae. ovata* affected the expression of the substituted wheat genome differently. The *T. aestivum* plants with *Ae. caudata* cytoplasm had male sterility and pistillody (KIHARA 1951), and *T. durum* plants with *Ae. ovata* cytoplasm had male sterility and delayed maturity (FUKASAWA 1953). WILSON and ROSS (1962) reported that *T. aestivum* with *T. timopheevi* cytoplasm had male sterility.

T. durum or *T. aestivum* with the cytoplasms of *T. boeoticum, T. monococcum, Secale sereale* (MAAN and LUCKEN 1967, 1968, 1971a, 1971b), or *Ae. umbellulata* (MAAN 1972) were shown to be male sterile and have reduced plant vigor. MURAMATSU (1965) reported that *T. aestivum* 'Chinese Spring' with *Ae. umbellulata* cytoplasm had male sterility and normal plant vigor. MAAN and LUCKEN (1969) and MAAN (1973) reported that *T. aestivum* with the cytoplasm of *T. timopheevi, T. Zhukovskyi, T. araraticum, T. dicoccoides* var. *nudiglumis,* or *Ae. speltoides* had male sterility. The cytoplasms of these five species had no apparent differential phenotypic effects on durum or common-wheat plants. However, cytoplasmic differences among some of these species became apparent during subsequent research (MAAN and LUCKEN 1972). FUKASAWA (1959), KIHARA and TSUNEWAKI (1967), and MAAN and LUCKEN (1968b) reported that *T. dicoccoides* var. *Kotschyanum, T. spelta* var. *Duhamelianum,* and *T. aestivum* 'Chinese Spring' restored partial fertility to wheat with the cytoplasms of *Ae. ovata* or *T. timopheevi;* but *Kotschyanum, Duhamelianum,* and Chinese Spring did not restore fertility to wheat with *Ae. caudata* cytoplasm. However, *T. compactum* 44 did restore partial fertility to wheat with *Ae. caudata* cytoplasm (KIHARA 1967). Two common wheat lines R5 and R6 (with male-fertility-restoring genes from *T. Zhukovskyi* and *T. boeoticum,* respectively) restored complete fertility to wheat with *T. timopheevi* cytoplasm, restored partial fertility to wheat with *Ae. caudata* cytoplasm, and produced F_1 hybrids with a bushy and stunted growth habit from crosses with wheat having *Ae. ovata* cytoplasm (MAAN and LUCKEN 1972). These interatcions clearly differentiated the cytoplasm of *Ae. caudata, Ae. ovata,* and *T. timopheevi.*

This paper reports substitution of *Triticum* genomes into the cytoplasms of eight *Aegilops* species. Also, differential nucleo-cytoplasmic interactions are reported between certain FR-lines (common wheat with male-fertility-restoring genes) and common wheat with the cytoplasms of *T. boeoticum, S. cereale,* or *Ae. umbellulata.*

The genomes of *T. aestivum* and/or *T. durum* were substituted into the cytoplasms of

Table 1. Phenotypic effects in *T. durum* and *T. aestivum* plants with alien cytoplasms

Cytoplasm donors	Genome donors[1]			*T. aestivum* × FR-line interactions[2]		
	T. durum	*T. aestivum*				
	56-1	Chris	Selkirk	R3	R4	R5 or R6
Ae. bicornis	S-PFW[15]	PFW[18]	PFW[11]			
Ae. umbellulata	S-PFW[6]	SW[5]	PFW[9]	SN	SN	PFN
Ae. sharonensis	S-PFW[6]	PFN[5]	PFN*			
Ae. longissima	S-PFW[8]	PFN[5]	PFN[5]			
Ae. heldreichii	SW[3]		SW[3]			
Ae. squarrosa	SW[7]	FN[7]	FN[7]			
Ae. variabilis	S-PFW[6]	PFW[10]	PFW[10]			
Ae. cylindrica	SN[2]	SN[6]	SN[6]			
T. boeoticum	SW	SW[7]	SW[14]	SW	SW	FN
S. cereale	SW	SW[5]	SW[7]	SW	FN	SN

1) S=sterile, PF=partially fertile, F=fertile, W=weak or reduced plant vigor, N=normal or near-normal plant vigor and superscripts indicate number of crosses with the genome donors.

2) *T. aestivum* × FR-line interactions indicate phenotype of F_1 plants from *T. aestivum* 'Chris" or 'Selkirk' with alien cytoplasms/R3, R4, R5 or R6 with male-fertility-restoring genes. R3=*T. timopheevi*/[8] *T. aestivum* 'Marquis', R4=*T. timopheevi-Ae. squarrosa*/[8] *T. aestivum* 'Dirk', R5=*T. Zhukovskyi*/[8] *T. aestivum* 'Justin', and R6=*T. boeoticum-Ae. squarrosa*/*T. durum*/'Chinese Spring'. R5 and R6 did not completely restore plant vigor to common wheat with the cytoplasm of *T. boeoticum*, *S. cereale* or *Ae. umbellulata*.

eight *Aegilops* species (Table 1), by the backcross method (KIHARA 1951). *T. aestivum* lines with the cytoplasm of *T. boeoticum*, *S. cereale*, or *Ae. umbellulata* were crossed with FR-lines to study interactions between the nuclear genes derived from certain *Triticum* species and the *Aegilops* cytoplasms.

Reduced Male Fertility and Reduced Plant Vigor

T. aestivum and/or *T. durum* plants with the cytoplasm of *Ae. bicornis*, *Ae. umbellulata*, or *Ae. cylindrica* had complete or partial male sterility and reduced plant vigor (Table 1). In general, the *T. durum* genome was more sensitive to the alien cytoplasm than the *T. aestivum* genome. Fewer backcrosses were necessary to eliminate nuclear genes or chromosomes from the cytoplasm-donor species in the substitution-backcross series of *T. durum* than in the substitution-backcross series of *T. aestivum*. Therefore, the reduction in vigor of *T. durum* plants with alien cytoplasms was usually observed in early backcross progenies, and the reduction in vigor of *T. aestivum* plants with the alien cytoplasms became apparent only in advanced backcross progenies. The reduction in plant vigor was influenced by environmental factors. In the greenhouse, *T. durum* and *T. aestivum* in some alien cytoplasms had noticeably poorer growth during the fall growing season than during the spring season. Apparently the photoperiod and the light intensity during the growing season influenced plant vigor. However, under field conditions photoperiod and

light intensity were not limiting factors, and reduced vigor under field conditions may have been due to cool nights and low soil temperatures during the spring season.

T. durum and *T. aestivum* plants with *T. boeoticum* cytoplasm (MAAN and LUCKEN 1967, 1970) had stable male sterility under all environments tested. *T. durum* and *T. aestivum* plants with *Ae. umbellulata* (MAAN 1972) or *S. cereale* (MAAN and LUCKEN 1971b) cytoplasm had complete male sterility during the fall and partial fertility during the spring season in the greenhouse. The plants with *Ae. umbellulata* cytoplasm had normal anther extrusion and normal-appearing anthers; however, they did not dehisce, and seed set on bagged heads was poor. The manual bursting of anthers with forceps and hand pollination increased seed set on these comon-wheat plants. Similarly, *T. aestivum* plants with *S. cereale* cytoplasm set a few selfed seeds during the spring season, but anther extrusion was less noticeable in plants with *S. cereale* cytoplasm than in plants with *Ae. umbellulata* cytoplasm.

T. durum and *T. aestivum* plants with *Ae. bicornis* cytoplasm (from amphidiploid *Ae. bicornis-T. boeoticum*) were reported to have normal fertility and plant vigor (MAAN and LUCKEN 1972). Now after 14 back-crosses, *T. durum* plants with *Ae. biocrnis* cytoplasm have complete male sterility and reduced plant vigor during the fall and partial fertility and near-normal vigor during the spring season in the greenhouse. *T. aestivum* plants with *Ae. bicornis* cytoplasm (after 10 backcrosses) have relatively less reduction in plant vigor and fertility than *T. durum* plants with *Ae. bicornis* cytoplasm. Also. *T. aestivum* with *Ae. variabilis* cytoplasm was reported to have normal fertility and plant vigor (MAAN and LUCKEN 1972). However, in subsequent backcross progenies a progressive reduction in plant vigor became apparent.

T. durum and *T. aestivum* plants with the cytoplasms of *Ae. cylindrica, Ae. longissima, Ae. sharonensis,* and *Ae. heldreichii* had male sterility (Table 1), and *T. durum* plants with these cytoplasms also had reduced vigor. Additional backcrosses are necessary (in the substitution backcross series with *T. aestivum*) before valid conclusions can be made about the reduction in plant vigor in common wheat.

MAAN and LUCKEN (1971a, 1972) reported that *T. durum* and *T. aestivum* with *Ae. squarrosa* cytoplasm had normal fertility and plant vigor. Additional backcrosses have now been made, and *T. aestivum* plants with *Ae. squarrosa* cytoplasm (*Ae. squarrosa*/^{12} *T. aestivum*) still have normal fertility and plant vigor. However, results from additional backcrosses with the *T. durum* recurrent parent indicated that complete substitution of the *T. durum* genome into *Ae. squarrosa* cytoplasm resulted in non-germinating seeds. All seedlings from the fourth and fifth backcrosses with *T. durum* pollen had 29 chromosomes ($2n=14_{II}+a$ complete or telocentric D-genome chromosome). The sixth backcross with *T. durum* pollen again resulted in plants with the maternal chromosome number ($2n+29$; $14_{II}+1_I$ or t_I). However, seedlings were obtained by transferring the em-

broys from the mature non-germinating seeds to an artificial medium for embryo culture as described by SCHOOLER (1960). Apparently a growth factor in the embryo culture medium or an addition of a D-genome chromosome to the *T. durum* genome improved interactions between the *T. durum* genome and the *Ae. squarrosa* cytoplasum.

Interacting Nucleo-Cytoplasmic Systems

Common wheat with the cytoplasm of *T. boeoticum, Ae. umbellulata,* or *S. cereale* had reduced fertility and plant vigor. R5 and R6 restored male fertility and plant vigor to wheat with *T. boeoticum* cytoplasm (MAAN and LUCKEN 1970). R5 and R6 restored partial fertility and plant vigor to wheat with the cytoplasm of *Ae. umbellulata* or *cereale.* R4 (a common wheat with male-fertility-restoring genes from amphidiploid *T. timopheevi-Ae. squarrosa*) restored fertility and plant vigor to wheat with *S. cereale* cytoplasm. But R4 did not restore fertility or plant vigor to wheat with *T. boeoticum* cytoplasm. Also, one *S. cereale* chromosome added to the wheat genome restored fertility to common wheat with rye cytoplasm. This rye chromosome did not restore fertility to common wheat with the cytoplasm of *Ae. umbellulata* or *T. boeoticum.* These nucleo-cytoplasmic interactions clearly indicate cytoplasmic differences among *T. boeoticum, Ae. umbellulata,* and *S. cereale* (Table 1).

Similarly, differential fertility-sterility interactions of the F_1 hybrids from crosses with R5 and R6 indicated that *T. timopheevi* and *T. Zhukovskyi* differed cytoplasmically from *T. araraticum, T. dicoccoides* var. *nudiglumis,* and *Ae. speltoides. T. timopheevi* with the cytoplasm of *Ae. speltoides* had complete male sterility and reduced plant vigor. But *T. timopheevi* with the cytoplasm of *T. araraticum* or *T. dicoccoides* var. *nudiglumis* had normal fertility and plant vigor, indicating that there are cytoplasmic differences between *Ae. speltoides* and *T. araraticum* or *T. dicoccoides* var. *nudiglumis* (MAAN and LUCKEN 1972).

The review of literature and the results presented in this paper show that in the Triticinae cytoplasmic differences among species can be detected by the substitution of the genome of one species into the cytoplasms of other related species. The cytoplasms of the newly established nucleo-cytoplasmic combinations may show differential effects on the expression of the substituted genome. The cytoplasmic effects become apparent due to the absence of certain nuclear genes in the substituted genome. Certain cytoplasms with similar effects on the expression of the substituted genome may differ in their interactions with certain nuclear genes from other related cytoplasm-donor species.

T. durum or *T. aestivum* genomes have now been substituted into cytoplasms of the following species: *T. monococcum, T. boeoticum, T. timopheevi, T. Zhukovskyi, T. araraticum, T. dicoccoides* var. *nudiglumis, Ae. speltoides, Ae. squarrosa, Ae. bicornis, Ae. longissima, Ae. sharonensis, Ae. umbellulata, Ae. caudata, Ae. heldreichii, Ae. variabilis, Ae. ovata, Ae. cylindrica,* and *S. cereale.* Among these species, 16 distinct cytoplasms have been demonstrated. Apparently, *Triticum* species with genomes AABB or AABBDD have the same or similar

cytoplasms. The cytoplasms of the species including *Ae. uniaristata, Ae. biuncialis, Ae. triuncialis, Ae. triaristata, Ae comosa, Ae. kotschyi, Agropyron elongatum,* and *Haynaldia villosa* are currently under study. Apparently, cytoplasmic differences among species of Triticinae are common, and cultivated wheats with different cytoplasms are being developed to study possible economic use of cytoplasmic variability in wheat improvement.

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Genetic control of factors regulating the phenol reaction of wheat and rye grain

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The phenol test provides a ready means of checking the purity and identity of cereal grain samples (ANON 1966, WALLS 1965, WRIGLEY and SHEPHERD 1974). Its simplicity makes it attractive: coloration of the bran is assessed after incubating soaked grain on paper wetted with dilute phenol solution. The test is reported to be indicative of genotype and independent of growth conditions, provided grain ripens to less than about 30% moisture. As it is important in these applications of the test that it should be unequivocally characteristic of genotype, further investigation of its genetic regulation was warranted. Furthermore, the reaction should serve as a marker in genetic studies.

JOSHI and BANERJEE (1969) have reported that in emmer wheats, the phenol reaction is monogenically controlled and that a multiple allelic series is involved. BHOWAL *et al.* (1969) concluded that the A genome is the source of the gene in the emmers and in bread wheat.

ZEVEN (1972), using substitution lines of Chinese Spring, suggested that genes for phenol reaction of grain were located on chromosome 2A in Hope, and on 2A and 2D in Timstein. He suggested *Tc* (tyrosinase in caryopsis) as a gene symbol to replace earlier suggestions of *Pk, F* and *B.*

The phenol reaction of the outer glumes is also useful in varietal identification. The extent of glume coloration appeared to be phenotypically independent of grain reaction for about fifty Australian wheats (WRIGLEY and SHEPHERD 1974), although some genetic linkage between the loci has been suggested (FRAZER and GFELLER 1936). ZEVEN (1972) proposed the symbol *Tg* for the gene for the phenol reaction of glumes.

Chemically, the test is generally explained as involving the enzymic oxidation of

phenol, through diphenols to quinones and finally to dark brown melanins. The enzyme system involved has variously been called monophenoloxidase or tyrosinase (ZEVEN 1972), DOPA oxidase (ABROL *et al.* 1971), polyphenol oxidase (TIKOO *et al.* 1973), or the phenolase complex (MASON 1955).

Our studies indicate that the phenol reaction is mainly regulated by genes on chromosomes $2A^L$, $2D\alpha$ and 2R in the lines examined. The following results are reported in the present form because most aspects of the work are being discontinued.

Materials and Methods

Plant materials used were derived from various research programmes: E. R. SEARS, University of Missouri (Hope, Timstein, Red Egyptian and Thatcher substitution lines, Chinese Spring aneuploids and the Imperial Rye addition and substitution lines); L. A. SNYDER, University of Minnesota (Marquis and Kenya Farmer substitution lines); Rosalind MORRIS, University of Nebraska (Cheyenne substitution lines).

For phenol testing, grains or glumes were soaked overnight in water. After blotting off excess moisture, they were spread out on filter paper (Whatman No. 1) wet with 1% aqueous phenol (10 to 20 μl per cm^2), covered to prevent loss of moisture, and incubated at 40° for 2.5 hours.

Results and Discussion

Figure 1 shows the phenol reactions for grains and glumes of several cultivars. Grain reactions ranged in coloration as follows:

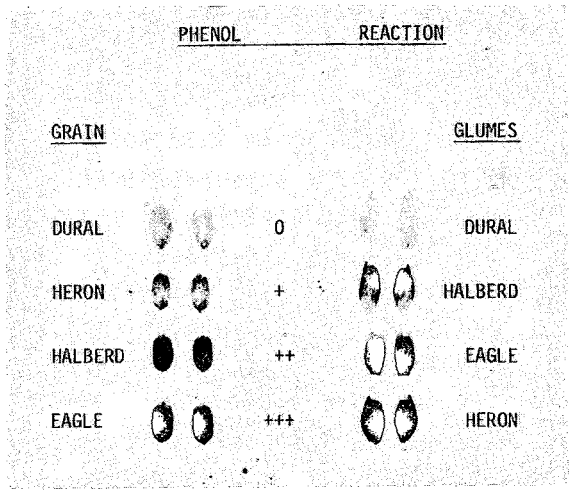

Figure 1. Coloration of grains and glumes of four Australian wheat cultivars after incubation with dilute phenol solution.

Reaction 0: no change in colour, e.g. Dural.
〃 1: light brown, e.g. Chinese Spring, Heron.
〃 2: medium brown, e.g. Halberd, Red Egyptian.
〃 3: dark brown, e.g. Cheyenne, Eagle, Hope, Holdfast, Kenya Farmer, Marquis, Thatcher, Timgalen, Timstein.

Chromosome substitution lines

Table 1 shows the phenol reactions for grain of chromosome substitution lines of homoeolgous group 2, in which nearly all reactions darker than Chinese Spring were observed. Samples for each series were obtained from several sources. Of the Thatcher series, only the 2A, 2B and 2D lines were examined. Lines 1D and 2B of the Cheyenne series were not available. All lines involving chromosomes of groups 1, 3, 4, 5 and 6 gave reactions indistinguishable from that of Chinese Spring. Darkening was obtained for a few lines involving group 7 chromosomes of the Cheyenne and Red Egyptian series, but usually only for some grains of the samples. Unequivocal grain colorations were obtained for chromosomes 2A and 2D as shown.

Table 1. Phenol reactions of grain of chromosome substitution lines

Substitution Line	Kenya Farmer	Timstein	Cheyenne	Hope	Marquis	Thatcher	Red Egyptian
2A	2, 3*	3	3	3	3	3	1
2B	1	1	–	1	1	1	1
2D	2	3, 2*	1	1	1	1	2

* Reactions relate to different samples

Rye

Further involvement of group 2 chromosomes was found by examining Chinese Spring addition and substitution lines involving Imperial Rye chromosomes. Grain of SEARS' Chinese Spring–Imperial Rye triticale gave a phenol reaction of 2. (The original Imperial Rye was not available). The 2R (2A) substitution line (probably a translocation 2R-$2A^L$ (SEARS 1968)) and the 2R addition line both gave grain reactions of 3. Other rye substitution lines were not tested, but the remaining six addition lines reacted the same as Chinese Spring. Rye addition lines based on Holdfast would not be suited to this approach, since the wheat parent itself reacts strongly with phenol.

The grain reaction of Chinese Spring

Of 34 available nullisomic-tetrasomic lines of Chinese Spring, only those nullisomic for 2D gave a 0 reaction. Nulli-2B tetra-2D produced a 2 reaction, considerably darker than grain of Chinese Spring. (Nulli-2A tetra-2D was not available). All other lines reacted similarly to Chinese Spring. Further evidence that chromosome 2D is involved

was provided by the 2 reaction of tetrasomic 2D. Chinese Spring ditelo-2Dα reacted identically to Chinese Spring. (Ditelo-2Dβ is not fertile and hence cannot be tested by this method). Thus the gene regulating the pale brown phenol reaction of Chinese Spring grain appears to be located on the α arm of chromosome 2D.

Genetic studies on the grain reaction of Timstein

The Timstein 2A substitution line was crossed to the respective ditelomonotelosomic 2A lines of Chinese Spring. The appropriate cytologically selected plants with 2n=42t (42 chromosomes including one telocentric) in each cross were test-crossed as female with Chinese Spring. Chromosome numbers of progenies were determined by root-tip mitotic analyses. These plants were grown to maturity and the grains harvested from them were tested for phenol colour reaction.

In the case of the $2A^L$ telosomic test-cross, 68 individuals were analysed as being positive (+ve) (significantly darker than Chinese Spring) or negative (−ve):

2n=42	25+ve(P)	17−ve(R)	42
2n=42t	8+ve(R)	18−ve(P)	26
	33	35	68

(P=parental, R=recombinant)

The approximate 1:1 ratio of positive: negative phenol colour reactions suggests that a single gene for reaction was involved, and based on that interpretation a linkage value of 36.8±5.8% between the locus involved and the 2A centromere was estimated.

In the test-cross involving the $2A^S$ telosome, only 18 plants were examined: 15 with 2n=42 all reacted positively whereas 3 with 2n=42t reacted negatively. Although the number of plants is small, the results are as expected if the locus under test is located in $2A^L$.

The observations that substitution lines Timstein 2A and Timstein 2D both react positively suggest that Timstein probably carried contrasting alleles to Chinese Spring at loci (possibly homoeoalleles) on these chromosomes. It can be hypothesized that the gene on Timstein 2D is allelic with the weaker allele observed on Chinese Spring 2D, and if so, then it would appear that the chromosome arms $2A^L$ and 2Dα are homoeologous.

To test the above hypothesis that Timstein carries two alleles relative to Chinese Spring involved in phenol colour reaction, we tested 51 F_2 plants from the cross Chinese Spring × Timstein. Thirty three plants reacted positively and 18 plants reacted negatively. This segregation is not significantly different from that expected for a single factor difference ($P>0.05$), but the result is difficult to reconcile in relation to the observations on the chromosome substitution lines.

Phenol reaction of glumes

A similar series of experiments to those listed above might be expected to elucidate the genetics of the glume phenol reaction, since exploratory studies have shown that

glumes of Chinese Spring give much paler coloration with phenol, than glumes of at least some of the donor varieties of the substitution lines. However insufficient glume samples were available in the present study for any definite conclusions to be established.

Biochemistry of the phenol reaction

Some evidence that a diphenol is an intermediate in the phenol reaction comes from the observation that the rate of oxidation of DOPA (dihydroxyphenyl alanine) by bran extracts corresponds directly to grain phenol reaction (ABROL *et al.* 1971). This observation, confirmed in the present studies, has been the basis for using DOPA as substrate when staining electrophoretic gels for "tyrosinase" activity (TIKOO *et al.* 1973). The methods of these workers were used to examine the possibility that different isoenzymes are involved in the various intensities of grain coloration, or are associated with the two different chromosomes involved. Up to five isoenzymes were observed for bran extracts, but their number and intensities were not related to the grain phenol reactions of a wide range of samples. Furthermore, isoenzymes were obtained for extracts of Duramba bran, and even of flour, neither of which shows a phenol reaction. The presence of such isoenzymes in flour has also been reported by KOBREHEL *et al.* (1972).

A possible explanation of the inconsistency between the results of gel electrophoresis and of photometric assay of the extracts is that the various colours of the phenol reaction are due to the presence of inhibitors which suppress coloration in Duramba bran and in flour, for example, but which are separated from the enzymes during electrophoresis. However, JOSHI *et al.* (1969) have advanced evidence against such a possibility.

There is also the further possibility that the phenol test is non-enzymic (CSALA 1972), since a reasonably normal phenol reaction can be obtained for grains that have been heated at 100° for a number of hours, without added water. However, we found that the phenol reaction is quite sensitive to moist heat, suggesting at least partial involvement of enzymic activity.

Clearly the biochemistry of the phenol reaction is not as simple as has sometimes been supposed, and caution is needed in interpreting the results of DOPA oxidation in relation to the phenol reaction. Until the steps involved are further elucidated, the term phenolase complex (MASON 1955) should be used, and specific names such as tyrosinase should be avoided as synonyms for the phenol reaction.

Radiation induced mutations in bread wheat (*Triticum aestivum* L. em THELL.) var. Hira

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In the last two decades, it has regularly been emphasized that in wheat it would be increasingly difficult to obtain further increase in yield through conventional breeding methods, utilizing existing germplasm. It is also difficult to locate additional natural sources for several useful characters, and if these useful attributes are found in alien species, their incorporation into wheat through cytogenetic techniques is very laborious. Under such circumstances, additional genetic variability will have to be created through induced mutations.

The research work on induced mutagenesis in wheat has received renewed attention due to the success achieved in this crop in recent years. At least eleven wheat varieties, utilizing induced mutations have so far been released (IAEA report 1972). Variability in floral morphology for outbreeding devices will be of some significance in hybrid wheat production, if artificially induced. Keeping these views in mind, radiation induced mutations in wheat var. Hira were studied in some detail in M_3 generation and are being reported in this communication.

The seed of wheat var. Hira was obtained from Genetics Division, I.A.R.I., New Delhi, and was irradiated in the winter of 1970~1971 with four different doses of gamma rays (10 Kr, 20 Kr, 30 Kr and 40 Kr). The M_2 generation was raised as single plant progenies in 1971~1972. The M_3 generation was similarly raised, but only from the suspected mutant lines of M_2 generation. In M_3 generation, 336 lines, which could be traced back to 42 plants of the M_1 generation, were analysed. Since whole population derived from M_1 plants could not be studied, it was not possible to work out the mutation frequencies and only qualitative study of the different kinds of mutations could be undertaken. The following mutants were recorded.

1. *Mutants for plant height:*

Considerable variation in plant height was observed. The plant height varied from 70~90 cms in control. In treated lines, the taller ones ranged from 90~120 cms and the dwarf ones ranged from 35~70 cms. There were other lines which segregated for tallness or dwarfness. The variation in plant height could be due to variation in the number and/or size of internodes.

2. *Erect leaf mutants:*

The leaves in the control were drooping, while in the treated popullation, 146 lines

out of 336 lines analysed, showed erect leaves without any segregation. There were other 50 lines which showed segregation for this character. It may be concluded that several genes must be controlling this character. However, the length and width of erect leaves were reduced. This morophological character is important because such erect leaves would receive maximum light without causing any shade to the leaves situated below. These leaves were sometimes found in combination with mutants for plant height.

3. Mutants for ear length:

Variation for ear length was also observed. Ear length in control ranged from 10～13 cms, while in treated population it ranged from 6～14 cms. Therefore, in general was a decrease in ear length in treated lines.

4. Mutants for awn length:

The M_3 population was also analysed for awn length. Both fully awned and awnless types were recovered. The control showed no variation and was fully awned. There were only six lines which were almost awnless. Another 70 lines showed segregation for variable awn length. Chimeric plants having variable awn length were also avaialable.

5. Mutants for ear morphology:

The ear morphology in treated lines was compared with control and a number of mutants were recorded:

(a) *Compactoids:* These were of three types: (i) squarehead, (ii) subcompactoid and (iii) compactoid. Several mutants showing variation in the density of ear were detected. The variation in ear density was often associated with a variation in plant height. For instance, a compactoid ear was associated with shorter plant height and subcompactoid with an intermediate plant height.

(b) *Speltoids:* Speltoid mutants were also isolated. In these mutants, the ears were lax and the glumes tightly enclose the kernels. The mutants were also late in maturity. These were hard in threshing and had sterile basal and terminal spikelets.

(c) *Sphaerococcoids:* Sphaerococcoid mutants were less frequent and were usually associated with mutations for erect leaves and short culm.

(d) *Vavilovoids:* These mutants were also infrequent and had short and lax ears with some sterile spikelets.

6. Mutants for heading time and maturity:

Mutants for heading time and maturity time were also isolated. In the control, the heading took 100～101 days for flowering. In the early flowering mutants, the heading time ranged from 95～99 days, while in the late flowering ones, it ranged from 102～110 days.

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Frequency of vavilovoid mutants induced by radiation and chemical mutagen treatments in *durum* wheat

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Occurrence of vavilovoid mutants in mutagenised populations of tetraploid and hexaploid wheat have been reported. These mutants phenotypically resemble *Triticum aestivum* ssp. *vavilovi* (TUM) Sears first described by Jakubziner. The vavilovoid mutants are slow growing with slender weak culms and narrow leaves. Their spikes show pseudobranching which is due to increased length and number of rachillae nodes, non-free-threshing habit, basal sterility and reduced development of awns in the speltoid background. The seed set is usually very poor.

In our mutation experiments with *durum* wheat cultivar Vijay, a number of M_2 families were found to segregate for the vavilovoid mutants (Fig. 1). A significant finding, however, was that all these segregating families were derived from seeds treated with chemical mutagens—ethylmethane sulfonate (EMS), N-methyl-N-nitrosourea (MNU) and N-ethyl-N-nitrosourea (ENU) (Table 1). None of the families originating from gamma-ray or neutron irradiated seeds segregated for vavilovoid mutants. As is evident from

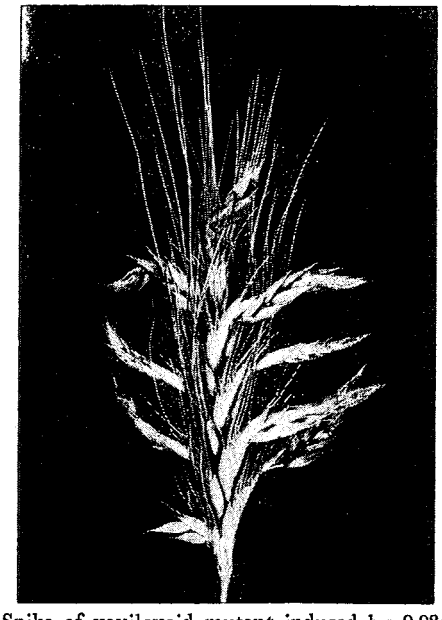

Fig. 1. Spike of vavilovoid mutant induced by 0.03% ENU in *durum* wheat cultivar Vijay.

Table 1. Frequency of vavilovoid mutants in *durum* wheat cultivar Vijay

Treatment	Number of families scored	Number of families segregating for vavilovoids	% families segregating for vavilovoids	Number of plants scored	Number of vavilovoid plants	% vavilovoid plants
Gamma-rays	506	0	0	46,245	0	0
Neutrons (thermal)	190	0	0	19,190	0	0
EMS (2%/3h)	259	7	2.702	19,617	10	0.0509
MNU (0.03%/3h)	160	3	1.875	5,160	5	0.0968
ENU (0.03%/3h)	207	2	0.966	19,030	2	0.0105

Table 1, a large number of families and plant populations have been scored both in radiation and chemical mutagen treatments. Though vavilovoid mutants have been obtained in durum wheat and in *T. carthlicum* following X-irradiation of seeds, the observed differences in the frequency of vavilovoid mutants in the radiation and chemical mutagen treatments in the present study could not be due to the chance alone. Further, data of PRASAD show that both in *durum* and *carthlicum* such mutants occurred in chemical treatments or in combination treatments of gamma-rays and chemicals. Vavilovoid mutants did not occur in gamma-ray treatments.

The vavilovoid phenotype is believed to result from mutation at a locus situated on the long arm of chromosome 5A between the *q* gene and the awn inhibitor B_1 and its expression is suppressed by the free-threshing gene Q. It is, therefore, inferred that in a free threshing *durum* cultivar only simultaneous mutation for both speltoidy and vavilovoidy can lead to vavilovoid phenotype.

Chemical mutagens are more effective than radiations in inducing chlorophyll mutations in hexaploid wheat and such mutants are presumed to result from intragenic changes. Further, numerous morphological mutations not observed following radiation treatments were induced by chemical mutagens like EMS. From the present study also it is evident that the vavilovoid mutants are readily induced by chemicals as compared to radiations in *durum* wheat. It implies that chemical mutagens may be more efficient than radiations in inducing the type of intragenic changes which will result in vavilovoid mutations.

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Nuclear DNA content of Einkorn wheat

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Information on DNA content of nucleus has been become indispensable for consideration of phylogeny of wheat. With regard to DNA content of nucleus each species of *Triticum* and *Aegilops* had been represented with a few varieties or strains in most of the earlier works. Our recent extensive study of DNA content of nucleus including 33 strains of Dinkel wheat, 33 of *Ae. squarrosa* and 38 of three species of Sitopsis (*Ae. speltoides;* 18, *Ae. longissima;* 10 and *Ae. bicornis*; 10) has revealed, however, two remarkable facts as descrived below; (1) there is no difference among subspecies or cultivars of Dinkel or hexaploid wheat and it is equal to the sum of its parental species, (2) on the contrary, there are considerable intraspecific as well as interspecific variations in diploid ancestral species of polyploid wheat. So it is of great significance to evaluate not only interspecific but also intraspecific variation in DNA content of nucleus.

Forty one strains of Einkorn or diploid wheat were subjected to Feulgen-microspectrophotometrical measurements. Of these, 39 strains were kindly provided for the present study from The Plant Germ-Plasm Institute, Kyoto University. Measurement was performed by Mendelsohn's two-wave length method (FURUTA *et al.* 1974). Ten nuclei of pollen tetrads were measured in each of four replicated preparations.

Mean value, standard error and relative value to *T. monococcum* cultivar "Early" are shown in Table 1, together with their sources. Significant differences were found among strains within three varieties, var. *monococcum,* var. *aegilopoides* and var. *thaoudar,* while no difference was found among three varieties. It is summarized that Einkorn wheat resembles to two other diploid ancestors of hexaploid wheat in variability of nuclear DNA content. Variability and its significance in wheat evolution will be discussed elsewhere in detail.

Literature Cited

FURUTA, Y., K. NISHIKAWA, and T. TANINO 1974. Stability in DNA content of AB genome component of common wheat during the past seven thousand years. Japan. J. Genetics 49: 179~187.

Table 1. Nuclear DNA content in forty one strains of *Triticum monococcum*

Strain number	Collection	Variety	DNA content	
			$\bar{X}\pm$S.E.	Ratio*
1501	BMUK	*aegilopoides*	208±3	1.01
1502	〃	〃	209±3	1.02
1503	〃	*thaoudar*	215±4	1.05
1504	〃	〃	211±3	1.03
1505	〃	〃	218±4	1.06
1507	〃	*aegilopoides*	199±4	0.97
1510	〃	〃	196±3	0.96
1516	〃	*thaoudar*	224±4	1.09
1517	〃	*aegilopoides*	207±3	1.00
1518	〃	〃	204±6	1.00
3601	BEC	*thaoudar*	210±8	1.02
3605	〃	〃	207±4	1.00
3614	〃	〃	216±5	1.05
3619	〃	〃	214±5	1.04
3620	〃	〃	200±4	0.98
3625	〃	〃	216±4	1.05
3630	〃	*aegilopoides*	215±4	1.05
3632	〃	〃	200±6	0.98
3636	〃	*monococcum*	203±4	0.99
3637	〃	〃	206±3	1.00
3640	〃	〃	200±3	0.98
3641 A	〃	〃	218±7	1.06
3641 B	〃	〃	220±5	1.07
8095	BEM	*thaoudar*	188±3	0.92
8097	〃	〃	212±3	0.92
8103	〃	〃	199±3	0.97
8120	〃	〃	200±4	0.98
8231	〃	〃	209±3	1.02
8270	〃	〃	213±3	1.04
8308	〃	〃	213±3	1.04
8326	〃	〃	211±3	1.03
8330	〃	〃	207±3	1.00
8345	〃	〃	204±6	1.00
8349	〃	〃	213±4	1.04
8382	〃	〃	214±4	1.04
8388	〃	〃	197±5	0.96
8404	〃	〃	194±3	0.95
8407	〃	〃	208±4	1.01
8410	〃	〃	203±3	0.99
1	Lab. strain	*monococcum*	205±2	1.00
2	〃	〃	204±4	1.00

* The ratio of each strain to *Triticum monococcum* cultivar "Early" (Laboratory strain #2).

Inheritance of protein content in backcross hybrids of *Triticum aestivum* L.

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Protein content is characterized by a relatively limited genetical variation and is rather susceptible to environmental conditions (Hehn and Barmore 1965). With regard to this many difficulties hindered the genetic study and selection of varieties on this trait.

The first works to notify the studies on protein inheritance were those of GERIKE 1925; CLARK and HOOKER 1926.

More systemic investigation on protein inheritance in simple intervarietal hybrids of *Triticum aestivum* L. appeared much later (DAVIS 1961, HAUNOLD *et al.* 1962, JOHNSON *et al.* 1963, LEBSOCK *et al.* 1964, KAUL and SOSULSKI 1965, KOTOVA 1966, TANDON 1967; RACHINSKI and RACHINSKA 1968, BOYADJIEVA 1972).

The object of the present investigation is protein inheritance in backcross hybrids of *Triticum aestivum* L. The studies in this aspect will directly contribute to the right choice of accurate methods for breeding on quality.

Materials and Methods

The studies were conducted on crosses of San Pastore × Bezostaya 1, Panonja × Dardo and Valdichiana × San Pastore.

P_1, P_2, F_1 and BCP_2 of the cross San Pastore × Bezostaya 1 urged forward the new investigations on the crosses Panonja × Dardo and Valdichiana × San Pastore. The last two ones were tested under field conditions. The study was carried out in 1969, 1970 and 1971.

The parental forms, F_1, BCP_1, BCP_2 and F_2 were comparatively tested in 1971. Thirty plants of the parental forms, F_1, BCP_1 and BCP_2 as well as 100 F_2 plants were tested.

Protein content was determined by Keldal's method. All three crosses were contrasting in protein content. The difference in the protein of the parental forms of the cross Panonja × Dardo was 1.7%, the one of San Pastore × Bezostaya 1 was 1.1% and that of the cross Valdichiana × San Pastore was 0.8%.

Results and Discussion

As it is presented in Table 1, the cross San Pastore × Bezostaya 1 showed intermediate inheritance in F_1, the lower protein content partially prevailing.

The average value of protein in BCP_2 of the cross San Pastore × Bezostaya 1 was higher than that of F_1 and occupied the place between F_1 and P_2.

Table 1. Grain protein content in F_1, BCP_1, BCP_2, F_2 and parental varieties of the crosses San Pastore × Bezostaya 1, Panonja × Dardo and Valdichiana × San Pastore

Hibrids	Protein content % $\bar{x}$	±m	VC %
♀ San Pastore	17.42	0.43	6.54
♂ Bezostaya 1	18.57	0.59	8.92
F_1	17.68	0.35	6.87
BCP_2	18.02	0.54	10.71
♀ Panonja	14.33	0.20	7.26
♂ Dardo	12.67	0.13	5.84
F_1	13.24	0.15	6.15
BCP_1	14.30	0.17	6.43
BCP_2	13.67	0.19	5.27
F_2	14.88	0.12	7.73
♀ Valdichiana	14.45	0.17	6.23
♂ San Pastore	13.64	0.13	5.28
F_1	13.70	0.22	6.58
BCP_1	13.55	0.29	6.91
BCP_2	13.59	0.21	6.18
F_2	14.13	0.12	7.05

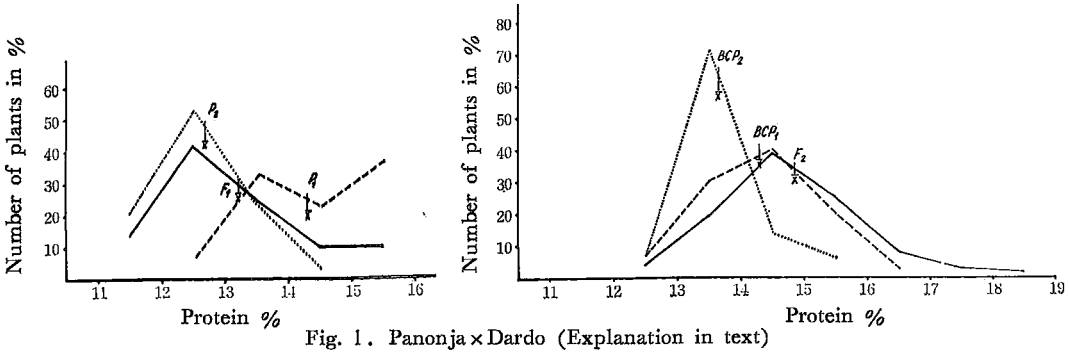

Fig. 1. Panonja × Dardo (Explanation in text)

When backcrossing with the high protein containing parent Panonja in the cross Panonja × Dardo, BCP_1 population was at the level of Panonja.

When backcrossing with the low protein containing parent Dardo, however, BCP_2 population keeps almost up to the level of F_1.

Fig. 1 presents the exact distribution of the values for the grain protein content of the parental varieties, F_1, BCP_1, BCP_2 and F_2 of the cross Panonja × Dardo.

It is evident that F_1 hybrid of the cross Panonja × Dardo encloses the limits between the two parental forms.

When backcrossed with the high protein containing parent Panonja, the protein in BCP_1 population reached 17%, its level being considerably above the levels of F_1 and

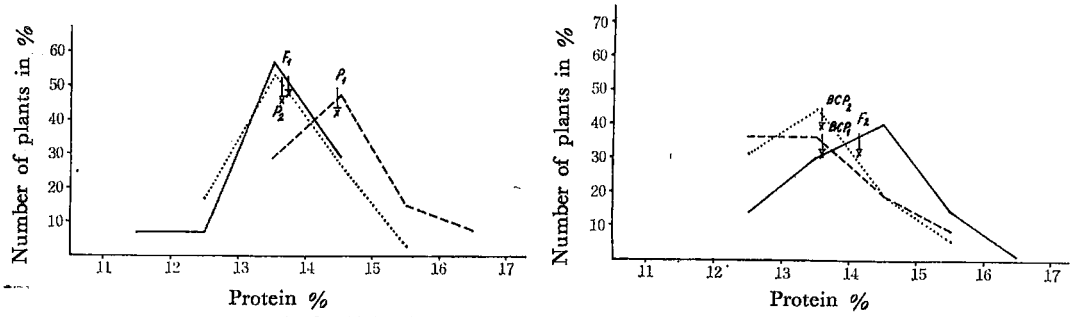

Fig. 2. Valdichiana × San Pastore (Explanation in text)

the high protein containing parent Panonja. The average value for the protein in F_2 surpassed the capacities of F_1 and Pannoja variety, which was obviously due to the transgressive form. As it is presented in the Figure, F_2 population was distinguished for its greater variability. Its limits were from 12% up to 19% protein, 14% of the individuals having higher protein content than Panonja variety.

The results obtained from the backcross with the high protein containing parents of the two crosses tested — San Pastore × Bezostaya 1 and Panonja × Dardo — and F_2 population of the cross Panonja × Dardo showed greater inheritance intensity of the genes conditioning the higher protein content of Bezostaya 1 and Panonja, and also the suppressive effect on the genes determining the lower protein content in San Pastore and Dardo varieties. This was also confirmed by the backcrossing with the low protein containing parent Dardo in the cross Panonja × Dardo, in which the level of BCP_2 population did not go below the level of F_1.

In all two crosses contrasting in grain protein content, the level of the population became higher than F_1, when backcrossing with the high protein containing parent. It set up at the level between F_1 and the level of the high protein containing parent or at its own level.

As it is shown in Table 1, the cross Valdichiana × San Pastore was a cross between varities, differing in grain protein content too, though at a smaller degree.

In F_1 of the cross Valdichiana × San Pastore the low protein content was dominant.

When backcrossing with the high protein containing variety Valdichiana, the level of BCP_1 population did not become higher than the level of F_1 but it remained almost at the level of the low protein containing San Pastore.

The limits of variability for BCP_1 and BCP_2 populations were the same as for F_1 (Fig. 2).

The variability of F_2 hybrids of the cross Valdichiana × San Pastore was relatively greater than F_1, BCP_1 and BCP_2 but it did not go beyond the limits of the parental varieties.

The results obtained for the protein in the cross Valdichiana × San Pastore indicate the smaller inheritance intensity of the genes, conditioning the high protein content of Valdichiana variety.

Through the method of backcrossing with the high protein containing parent, better conditions are created for the successful selection of high protein containing varieties. To a great extent, however, this depends on the inheritance intensity of the genes conditioning the high protein content in the varieties used for hybridizatoin.

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Results of nucleus substitution in *Aegilops* and *Triticum* species by means of successive backcrosses with common wheat

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KIHARA (1951) and FUKASAWA (1953) were the first to make studies on nucleo-cytoplasmic interactions in subtribe Triticinae. Substitution backcrosses were held among many species from *Triticum, Aegilops* and *Secale* later, connected with the new found sources of cytoplasmic male sterility and selection of hybrid wheat (WILSON and ROSS 1962, NETTEVICH and FEDOROVA 1966, MAAN and LUCKEN 1972, PANAYOTOV and GOTSOV 1973a, 1973b, POPOV, PANAYOTOV and GOTSOV 1974a). This was the base on which a voluminous amount of studies were made in the last years not only of theoretical, but of extreme practical significance too.

In 1970a special program was assumed in our country for investigation of nucleo-cytoplasmic interactions in subtribe Triticinae with the following main tasks:

1. Discovering a new genetic system of cytoplasmic male sterility for selection of hybrid wheat. Fertility restorers in this system should be of high stability under various condition and male sterile and restoring lines to be controlled by one gene for the easier selection of R lines.

2. Finding new sources for pollen fertility—restoring forms with *T. timopheevi* cytoplasm, which is basically used for selection of hybrid wheat. *T. timopheevi* cytoplasm is a fairly reliable source or male sterility, but fertility restoration is unstable and the selection of R lines is very difficult.

3. Studying the effect of foreign cytoplasms (sterile and fertile) on the phenotype reactions of wheat including the morphological characters, productivity, cold and disease resistance, vegetation period, grain quality etc.

Table 1. Obtained lines of common wheat with cytoplasms of *Triticum* and influence of the cytoplasms on fertility and growth

Cytoplasm donors	Genome type	Progeny	Fertility	Growth
T. monococcum L. ssp. *hornemannii* CLEM. cult. Greifswold	A	BC_7	F	normal
T. abyssinicum STEND.	AB	BC_7	F	〃
T. aethiopicum JAKUBZ. ssp. *harlani-unicum*	AB	BC_6	F	〃
T. dicoccum (SCHRANK.) SCHUBL. ssp. *farrum* BAYLE	AB	BC_7	F	〃
T. dicoccoides (KOERN.) AAR. ssp. *namuricum*	AB	BC_7		
line 1			F	〃
line 2			FPF	almost normal
T. paleocolchicum MEN. ssp. *schwamlicum*	AB	BC_6	F	normal
T. polonicum L. ssp. *villosum* DESF.	AB	BC_6	F	〃
T. turanicum JAKUBZ. ssp. *insigne* PERS.	AB	BC_6	F	〃
T. Haynaltricum ssp. *hungaricum*	VAB	BC_6	F	〃
ssp. *unicum* ZHUKOVSKYI	VAB	BC_6	F	〃
from *Debrecen*–HUNGARY	VAB	BC_6	F	〃
T. spelta L.	ABD	BC_5	F	〃
T. vavilovii TUM. ssp. *vaneum* JAKUBZ.	ABD	BC_5	F	〃
T. Zhukovskyi MEN. et ER.	AAG	BC_6	MS	〃
T. fungicidum VAV. et ZHUK. ssp. *nigrescens* ZHUK.	AABG	BC_6	F	〃

Table 2. Obtained lines of common wheat with cytoplasms of *Aegilops* and influence of the cytoplasms on fertility and growth

Cytoplasm donors[1)]	Genome type	Progeny	Fertility	Growth
Aegilops ovata L.	C^uM^0	BC_7	MS	normal, delay
Ae. triaristata WILLD. ssp. *contorta* ZHUK. 6x	$C^uM^tM^{t2}$	BC_7	MS	weak
Ae. recta ZHUK. 6x	$C^uM^{t1}M^{t2}$	BC_4	MS	〃
Ae. columnaris ZHUK.	C^uM^c	BC_6	MS	〃
Ae. biuncialis VIS.	C^uM^b	BC_6	MS	〃
Ae. machrochaeta (SHUTT. et HUET ex DUV. JOUVE) RICH.	C^uM^b	BC_4	MS	〃
Ae. variabilis EIG	C^uS^v	BC_6	FPF	normal
Ae. kotschyi BOISS.	C^uS^v	BC_5	FPF	〃
Ae. triuncialis L. ssp. *typica* ZHUK.	C^uC	BC_7	MS	〃
Ae. caudata L.	C	BC_6	MS	〃
Ae. cylindrica HOST	CD	BC_4	F	〃
Ae. comosa SIBTH. et SM.	M	BC_2	MS	weak
Ae. Heldreichii HOLZM.	M^1	BC_2	MS	〃
Ae. aucheri BOISS.	S	BC_7	MS	normal
Ae. longissima (SCHW. et MUSCHL.) EIG	S^1	BC_5	FPF	almost normal
Ae. sharonensis EIG	S^1	BC_5	FPF	〃
Ae. crassa BOISS. 6x	DD^2M^{cr}	BC_7	F	normal
Ae. ventricosa TAUSCH.	DM^v	BC_3	F	〃
Ae. juvenalis (THELL.) EIG	DC^uM^J	BC_6	F	〃

1) Classification and genome formulas are cited by KIHARA (1963).

Searching the solution of the problems we are making a number of substitution backcrosses using different *Aegilops* and *Triticum* species and common wheat (Table 1, 2). As a result of the hybridization 9 new sources of cytoplasmic male sterility for common wheat were found and these are: *Ae. triaristata, Ae. recta, Ae. columnaris, Ae. biuncialis, Ae. machrochaeta, Ae. triuncialis, Ae. comosa Ae. veldreichii* and *Ae. aucheri.* Some new restoring sources of *T. timopheevi* cytoplasm were discovered (POPOV, PANAYOTOV, GOTSOV, 1974b). The rest of the *Aegilops* and *Triticum* species and the amphidiploid *T. Haynaltricum* (*H. villosa* × *T. dicoccum*-ZHUKOVSKYI, 1964) do not influence the generative organs and pollen fertility. Some of them influence partialy on the fertility (Table 2).

One of the main idea in our investigations is finding a cytoplasm of favourable influence on the productivity of wheat as well as other characters which are subject of breeding work. For that reason we are making substitution backcrosses with 5 or 6 varieties of wheat in order to obtain several alloplasmic lines of the same cytoplasm. Comparative study of these lines with their analogues posessing their own cytoplasm under field and laboratory conditions will be held in the coming 1～2 years. Except yields, disease and cold resistance, protein content, baking qualities etc. will be studied.

Our aim is to gradually obtain lines of common wheat with cytoplasms of all *Aegilops* and *Triticum* species and later with *Agropyron, Secale* and other relatives to these species.

Preliminary studies show that wheat is highly influenced by foreign cytoplasms. Side by side with the negative effect in some cases a positive influence is to be observed as growth stimulation and a shorter vegetation period. According to us, the cytoplasms which do not influence on wheat fertility are of particular importance.

Studies in the vast field of interactions and nucleo-cytoplasmic combinations are required intensive scientific work. In order to include the whole subtribe Triticinae (enlisting more than 200 species) in the research works a close coordination between the Institutes from differenct internationally cooperated countries should be established.

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Significance of wheat-*Aegilops* crosses for the improvement of cultivated wheat

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Wheat-*Aegilops* crossings are of great importance to the studies of evolution as well as to the breedings of hybrids. Difficulties emerge in the production of amphidiploids

from tetraploid wheat (AABB) and diploid *Aegilops* species, because of difficulties in crossing. Crossing experiments as well as cytogenetic analysis showed the following results:

1. The best results were shown with *Aegilops longissima* crossings.

2. The resulting triploids $A_{Em.}$ $B_{Em.}$ $B_{Slt.}$ from AABB and $B_{Slt.}$ $B_{Slt.}$ showed in Meiosis a closer affinity between the B-genome from *Ae. speltoides* and the B-genome from AABB wheat. *Ae. longissima* and *Ae. bicornis* did not show such a close affinity.

3. The effectiveness of the 3 colchicine-methods (from SEARS, BELL, and SCHUMANN) were compared. The BELL- and SCHUMANN-methods were the best for obtaining hybrids.

4. The doubling of chromosomes in the triploid by colchicine treatment demostrated that the "Autoalloploids" forth the *Aegilops longissima* genome were more stable and fertile than those with the *Aegilops speltoides* genome.

5. The author suggests the following terminology for the new "Amphidiploids"

a) *T. dicoidspeltoides*=(*T. dicoccoides*×*Ae. speltoides*) colch.

b) *T. carthlispeltoides*=(*T. carthlicum*×*Ae. speltoides*) colch.

c) *T. dicospeltoides*=(*T. dicoccum*×*Ae. speltoides*) colch.

d) *T. dicoidlongissima*=(*T. dicoccoides*×*Ae. longissima*) colch.

e) *T. carthlilongissima*=(*T. carthlicum*×*Ae. longissima*) colch.

f) *T. dicolongissima*=(*T. dicoccum*×*Ae. longissima*) colch.

g) *T. durolongissima*=(*T. durum*×*Ae. longissima*) colch.

h) *T. timolongissima*=(*T. timopheevi*×*Ae. longissima*) colch.

Fig. 1. Synthesised wheat : 1∼4 $A_{Em.}A_{Em.}B_{Em.}B_{Em.}B_{Slt.}B_{Slt.}$, 5∼6 $A_{Em.}A_{Em.}B_{Em.}B_{Em.}D_{squ.}$ $D_{squ.}$, 1) *T. dicospeltoides*, 2) *T. carthlispeltoides*, 3) *T. durolongissima*, 4) *T. dicoidlongissima*, 5) *T. durosquarrosa*, 6) *T. timosquarrosa*

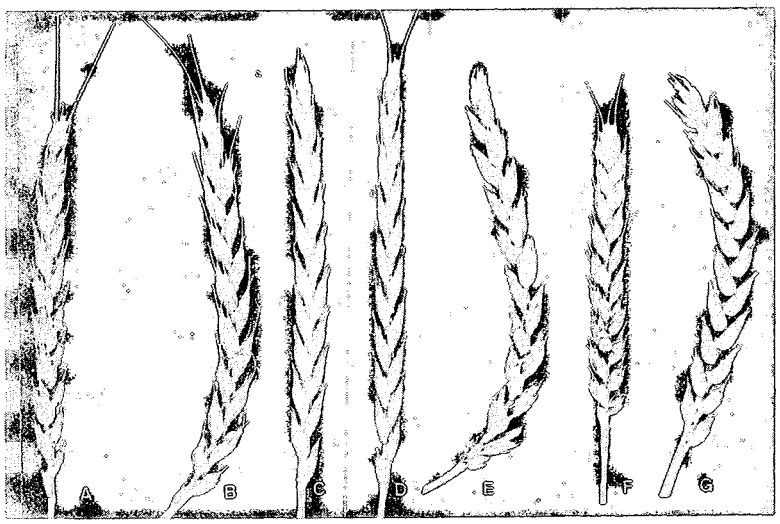

Fig. 2. F_1- and R_1-plants from synthesised wheat × *T. aestivum.* A) *T. carthlispeltoides* × "Opal" B) *T. durosquarrosa* × "Opal" C) *T. timosquarrosa* × "Opal" D) *T. dicoidlongissima* × "Opal" E) A × "Opal" × "Opal", F) B × "Opal" × "Opal", G) *T. aestivum* ("Opal")

i) *T. durosquarrosa* = (*T. durum* × *Ae. squarrosa*) colch.

j) *T. timosquarrosa* = (*T. timopheevi* × *Ae. squarrosa*) colch.

6. The F_1-plants resulting from the crossings between $A_{Em.}$ $A_{Em.}$-$B_{Em.}$ $B_{Em.}$ $B_{long.}$ $B_{long.}$ and $AABB_{ltmo.}$ $DD_{squ.}$ with *Triticum aestivum* (AABBDD) were sterile and suggest a new possibility for the breeding of hybrids, where as crossings between $A_{Em.}$ $A_{Em.}$ $B_{Em.}$ $B_{Em.}$ $B_{Ae.\ spelt.}$ $B_{Ae.\ spelt.}$ with wheat AABBDD generally resulted in a less positive fertility.

7. The R_1-plants show on the whole a resemblance to *T. aestivum;* the shape of the ear and the grain show substantially more characteristics from cultivated wheat and posses resistance to mildew and yellow rust. It may therefore be possible to use therse forms in the breeding of resistant varieties.

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Chromosome constitution of callus tissues from tetra-5A, -5B and -5D of Chinese Spring wheat

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Cytological observation of callus tissues from seeds of tetra-5A, -5B and -5D was done in the fourth successive culture of calluses. The results obtained are summarized in the following table.

Chromosome number of callus cells varied considerably as is usually the case with callus tissues.

It is tentatively assumed that the chromosome aberrations listed in the table (*, **) are due to extra dosage of the chromosome 5B which brought centromere instability, and chromosome breakage and reunion. The former resulted in a telocentric chromosome and the latter resulted in dicentric chromosomes.

Chromosome number	Tetra-5A	Tetra-5B	Tetra-5D
	no. of cells (%)	no. of cells (%)	no. of cells (%)
36		1 (2)	
37		1 (2)	
38	2 (4)	1 (2)	
39			1 (2)
40		1 (2)	2 (4)
41	2 (4)	3 (6)	
42	1 (2)	6 (12)**	2 (4)
43	4 (8)		3 (6)
44	38 (76)	31 (62)*	36 (72)***
45	1 (2)		3 (6)
47			2 (4)
55		1 (2)	
65			1 (2)
73	1 (2)		
80	1 (2)		
83		2 (4)	
85		2 (4)**	
88		1 (2)	
Total	50	50	50

* 1 cell had 1 telocentric chromosome.
** 1 cell from tetra-5B callus with 42 chromosomes had 1 dicentric chromosome. 2 cells from tetra-5B with 85 chromosomes had 2 dicentric chromosomes.
*** 1 cell had some chromosome variation. Its nature was not clear.

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II. Editorial Remarks

Announcement for future issues

WIS No. 41 will be planned for publication in March 1976. Manuscripts for this issue are accepted any time, not later than September 31, 1975.

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:

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Raise of Membership Fee

Due to the economic situations, the yearly membership fee has been raised up to ¥1,000 for foreign member and ¥700 for Japanese member from the fiscal year beginning April 1975. The money should be paid by the Foreign Postal Money Order, otherwise 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. 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. 1~39, and valuable contributions for the present issue. Increased support would be appreciated.

The Managing Editor

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Remarks : Figures in boldface indicate numbers, and figures in parentheses indicate pages.

Explanation of the Figure on the Cover

Fig. 1. Spike of vavilovoid mutant induced by 0.03% ENU in *durum* wheat cultivar Vijay. (Ref. R.M. DESAI and C.R. BHATIA, p. 13~14, Present No. of WIS)