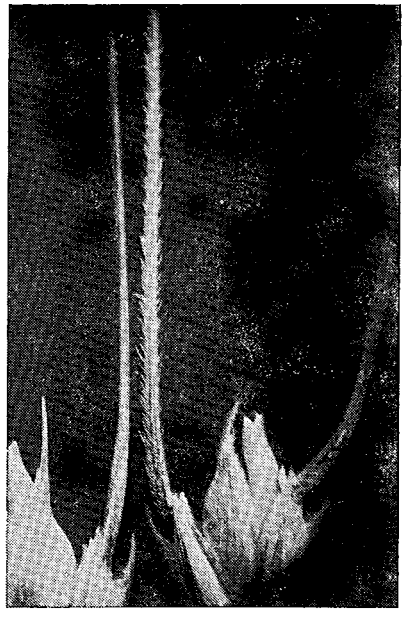
WHEAT INFORMATION SERVICE



No. 44 March, 1977

Wheat Information Service
Kihara Institute for Biological Research
Misima, Japan

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Explanation of the Figure on the Cover

Enlarged portions of (A) normal and (B) super-barbed awns.

(Cf. Fig. 1, p. 3, present issue of WIS, V.S. Prakasa RAO, M.D. BHAGWAT and V.P. PATIL)

No. 44 March, 1977

I. Research Notes

Spring wheats under the conditions of their old home, Sapporo, after their fifty year culture in Kyoto as winter crop

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Introduction

It is generally considered that the spring and winter habits of wheats are genetically determined. If the habits are really of genetical, they should not be changed unless a mutation occurs in the gene locus controling the habits. Neverthless, there are some reports which argue the presence of the habit shifts (for example, GLOUSCHCHENKO, I.E., WIS 41–42, 1976).

I have been continuously using those spring wheats as my research materials since I moved from Sapporo to Kyoto. Although those materials were grown as spring wheats in Sapporo before I actually began my research work in Kyoto, they have been grown wheats thereafter. This switch-over was done because the flowering as winter time of them meets with the rainy season, *"tsuyu"*, in Kyoto, if grown as spring wheats.

Since then half a century has passed, and it seemed to be a good timing to test whether or not this duration was long enough to alter their genetical spring habit to the adapted winter habit. To substantialize this idea, the seeds of the above mentioned species of wheats of the Kyoto strains were recently sown in Sapporo to check any alteration in their original spring habit.

Material and Method

All the strains of the following five species of wheats are originally spring wheats from Sapporo. They have been grown as winter wheats in Kyoto and inbred since 1926.

2x species: *T. monococcum*
4x 〃 : *T. durum, T. polonicum*
6x 〃 : *T. vulgare (aestivum), T. spelta*

For the control, the same five species which were inbred in Sapporo during the past 50 years by growing as spring wheats, were used. As the control for winter wheat, an American winter wheat variety "Gaines" which has been grown in Misima was used to see the response to spring growing in Sapporo.

All the above materials were sown at the Hokkaido University in Sapporo in heat sterilized soil on April 4, 1976, and the seedlings were planted in rows there on April 21.

Results and Discussion

1. Shooting:

Except a strain, "Gaines", all the tested strains normally grew and shooted ears. There was also no difference in the mode of ear development between the test strains and the control strains. "Gaines", which requires vernalization as a winter wheat, stayed in rosette form in this spring sowing test.

2. Pollen fertilities and seed fertilities:

The pollen and seed fertilities of the five species used in the present test could be compared between the conditions of Sapporo and Kyoto where they were grown as winter wheats. Table 1 shows the results obtained in Sapporo through the present test and the results obtained in Misima, grown as winter wheats.

From the comparison of the results, it is understood that the five species once adapted to winter sowing in Kyoto and Misima showed their original habit when switched back to Sapporo, their old home.

Table 1. Seed fertilities of tested five species of wheat in Sapporo and Misima

Species	Seed fertility (bagged) in	
	Sapporo (spring growing)	Misima (winter growing)
	%	%
T. monococcum vulgare	67.3	36.4
T. durum reichenbachii	92.9	95.6
T. polonicum vestitum	73.9	77.3
T. vulgare erythrospermum	92.9	92.1
T. spelta duhamelianum	94.9	92.7
"Gaines"	no shooting	89.0

3. Discussion:

It has been revealed from the observations on ear differentiations that the autumn sowing for 50 years in Kyoto did not alter their habit of tested five species of wheat, from the original "spring" to the adapted "winter". Namely, the Kyoto strains of the five species differentiated their ears normally under the spring sowing condition in Sapporo, while the proper winter strain, "Gaines", stayed in rosette form under the same condition.

The combination of the subjects described above may justify to consider that a 50 year treatment of our spring wheats with successive winter growing and associated inbreedings

did not alter the habit of those strains. Although half a century would not be long in the sense of evolutional chronology, the present experiment may support the concept that adaptation can not change any genes, but only mutations can do.

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(Received Feb. 10, 1976)

Super-barbing — A new marker character in wheat

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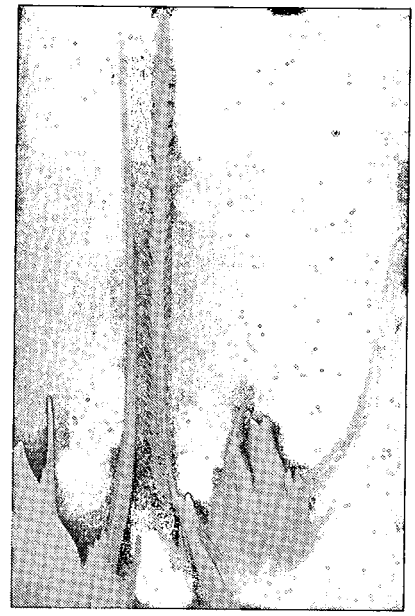
Earlier wheat workers have identified a large number of characters useful for varietal identification and studied their inheritance (reviewed by AUSEMUS, MCNEAL and SCHMIDT 1967). Among these are several awn characteristics like awn development, awn colour and awn barbing. Barbing of the awn is predominant in most of the wheats and is responsbile for the scabrid nature of the awns and results from the presence of uni-cellular thick-walled cells with fine points directed upwards. The base of the awns was almost smooth in *durum* wheats (PERCIVAL 1921). However, smooth-awned wheats have been described from Syria and Palestine (FLAKESBERGER 1929), from Spain (SANCHEZ-MONGE and VILLENA 1951), and from a line of the cross Marquis × Iumillo (SIGFUSSON 1929). In crosses with smooth-awned wheats SIGFUSSON (1933) reported cumulative factors respon barbing. Later KNOWLES (1943) identified a major gene and a minor gene for barbing in *durum* wheats. The major factor is responsible for usual roughness while the second factor when homozygous in the absence of major pair produces an intermediate type of barbing



A B

Fig. 1. Enlarged portions of (A) normal and (B) super-barbed awns.

characterised by scattered barbs from tip to base.

A variant of the barbing was observed in the course of investigations in progress at this Institute, in a plant of the culture RT 838, derived from the F_5 generation of (*T. dicoccum* var. KDH × *T. pyramidale*) F_{11} × *T. durum* var. Mx 0.16. In this type, apart from the regular barbing, additional felt-like outgrowths extend over the entire awn surface (Fig. 1B) and are so well marked as to be noticed even from a distance. Microscopic observations and cross sections have revealed no structural differences between the barbs described earlier and the additional outgrowths present in this culture. This character appears to be due to the abnormal elongation of the barbs. Because of the structural similarity of the trait to the barbing on wheat, this character has been named as Super-barbing and assigned the gene symbol B^s.

In order to study the inheritance of this character, true breeding lines have been isolated and crossed with the sib-culture with normal awns and also with other *durum* wheats with typical barbed awns. The results are presented in Table 1.

Table 1. Segregation of super-barbing in F_2 generation

Cross	F_2 segregations				Ratio	P
	super-barbed	inter-mediate	normal	Total		
T. durum-N 59 × RT 838 (super-barb)	184	374	175	733	1:2:1	0.70 - 0.80
RT 838 (normal) × RT 838 (super-barb)	216		75	291	3:1	0.80 - 0.90

The super-barbing on F_1 plants was of a lesser intensity compared to the (super-barbed) parent suggesting thereby incomplete dominance of this character. In F_2 generation of the cross *T. durum*-N 59 × RT 838 (super-barb) apart from the super-barb and normal awned plants there were intermediate types characterised by sparcely elongated barbs. Hence, three classes – normal, intermediate and super-barb were made. In 9 populations studied there was no heterogeneity indicated and hence the data were pooled. The segregation indicated a close fit to 1:2:1 ratio. In the cross RT 838 (normal) × RT 838 (suber-barb), however, the classification of barbed condition into super-barb and intermediate types was not possible due to poor development. Hence, only two classes – super-barb and normal were recorded. This also gave a good fit to 3:1 ratio.

Thus, the super-barbing trait appears to be governed by a single partially dominant gene. Further investigations regarding its relation to the smooth awned condition are in progress at this Institute.

The authors are grateful to Dr. G.B. DEODIKAR, Director, M.A.C.S. for guidance and facilities. The work was conducted as a part of the All India Coordinated Projects on Wheat and Fruit Improvement, of the I.C.A.R.

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Considerations on the involvements of genetical differences with callus formation in wheat

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Tissue culture of wheat and the related genera is not so advanced as compared with that of dicotyledonous plants and several monocotyledons. The conditions for callus initiation or subculturing have been reported by several workers (GAMBORG and EVELEIGH 1968; TRIONE *et al.* 1968; SHIMADA *et al.* 1969; UTSUMI 1969). However, there have been few reports indicating the redifferentiation conditions from wheat calluses. OUYANG *et al.* (1973) reported a successful haploid production from anther-derived calluses in hybrids of common wheat. In barley, haploid production by both anther and ovary culture method has been reproted (KASHA and KAO 1970; CLAPHAM 1973). Nevertheless, the most important step, i.e., redifferentiation conditions for practical plant breeding is still in question, in wheat and the related species.

Probably due to various difficulties in wheat tissue culture, several studies of the other direction has been elaborated recently concerning the influence of genetic factors on callus formation. The genetic role of individual chromosomes or chromosome arms in callus formation has been reported, in anther culture of A genome aneuploids of common wheat (SHIMADA and MAKINO 1975). Cytoplasm effects on callus induction from wheat anthers have been reported, using the cytoplasm substitution lines of Chinese Spring wheat (OGURA and TSUNEWAKI 1974). These attempts may be useful to know the genetical factors involved in callus formation. Since wheat and a related genus *Aegilops* contain many species, their phylogenetical relationships have been revealed, and many kinds of hybrids, some sets of aneuploid series in Chinese Spring wheat and the cytoplasm substitution lines are available, wheat and the related genera seem to be one of the most suitable materials to

investigate the genetical effects on callus formation. However, few studies have been known, on the influence of genome constitution on callus formation.

In order to know which genome gives the highest callus formation, in response to the same auxin concentration, the writer exmained the degree of callus formation from the seedling roots and the anthers of several wheat, *Aegilops* and rye species. The protocols of the experiments are as follows: Sterilized soaked seeds of several wheat, *Aegilops* and rye species were placed on agar slant medium aseptically and cultured *in vitro* on an RM–1964 medium supplemented with 0.1 mg/l of 2,4-D, for the 2,4-D concentration applied was reported to be a threshold concentration of callus initiation from the seedling roots of wheat and *Aegilops* (UTSUMI 1969) and the validity of his result was ascertained before the present experiment. Anthers of these species and varieties were also inoculated on an RM–1964 medium containing 3 mg/l of 2,4-D, as described by OGURA and TSUNEWAKI (1974). The seeds were incubated in the dark and the anthers under constant fluorescent illumination of about 1,000 luxes, both were cultured with the constant temperature of 25±1°C. The pH of the media was adjusted to 5.8±0.1 before autoclaving.

Callus formation from germinated seedling roots was observed about 4 weeks after the initiation of the experiment. In the case of anther culture, callus formation was firstly observed about 6 weeks. The preliminary results of callus formation from seeds and

Table 1. The numbers of calluses induced from the seedling roots and the anthers of wheat, *Aegliops* and rye. Data were taken eight weeks for the seedling roots and about ten weeks for the anthers, after the initiation of the experiment, respectively

Species & varieties	Genome formula	No. seeds germinated	No. seedl. roots with callus (%)	No. anthers inoculated	No. anthers with callus (%)
T. monococcum flavescens	AA	20	16(80)	152	0(0.0)
T. durum reichenbachii	AABB	20	5(25)	87	2(2.3)
T. timopheevi typicum	AAGG	19	2(11)	154	0(0.0)
T. dicoccoides spontaneo-nigrum	AABB	21	6(29)	146	0(0.0)
T. aestivum cv. Chinese Spring	AABBDD	—	—	172	0(0.0)
〃 cv. Norin 26	AABBDD	20	1?(5)	98	0(0.0)
〃 strain Salmon	AABBDD	15	1(7)	105	0(0.0)
ABD-1 (*T. dicoccoides spont.* × *Ae. squa.* typica No. 2)	AABBDD	23	1(4)	—	—
Ae. squarrosa typica	DD	16	0(0)	117	0(0.0)
Ae. caudata typica	CC	12	0(0)	68	0(0.0)
Ae. spelotides	SS	—	—	79	0(0.0)
S. cereale cv. Imperial	RR	—	—	134	2(1.5)
S. cereale cv. King II	RR	—	—	152	0(0.0)

anthers are presented in Table 1. The data on callus formation from seedling roots of wheat can roughly be interpreted that the feasibility of callus formation is in the following order; diploid, tetraploid and hexaploid.

Callus formation from anthers was observed only in *T. durum* cv. *reichenbachii* and *S. cereale* cv. Imperial, the calluses of the latter being shown in Fig. 1. A marked effect on growth of anther-derived callus is seen in this figure. It seems that there are no relation-

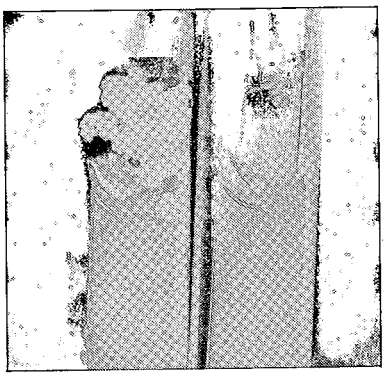

Fig. 1. Anther-derived calluses of *Secale cereale* cv. Imperial. Subcultured in the medium added with 3 mg/l of 2,4-D (left) and with no auxin (right). 2,4-D effect on callus growth is evident in the former.

ships or parallelism between the data of seedling roots and the anthers. Supposing that some genetic factors are closely related to callus formation, the callus formation results from seedling roots, anthers or other parts of wheat plants should parallel. The present preliminary results are not clear-cut and of small scale. However, tissue culture studies of this direction should be advanced further, since genome level studies on callus formation in wheat may be more advantageous than other materials.

(Recieved May 1. 1976)

The effect of nucleo-cytoplasmic interaction on the endosperm protein in wheat (preliminary)

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KIHARA (1963, 1973) suggested that "cytoplasmic heterosis" of nucleo-cytoplasm hybrids can be used for plant breeding as a new source of genetic variability. In this regard, different alien cytoplasmic ditelocentric Chinese Spring lines are being developed for the study on the effect of nucleo-cytoplasmic interaction on the endosperm protein quantity and quality in wheat. As a preliminary step to this program, the effect of alien cytoplasms was studied on the grain protein content (%) in the wheat variety, Chinese Spring.

One hundred and eight plants of 8 alien and one original cytoplasmic Chinese Spring euploid lines were grown in three replications having 4 plants randomised within each replicate block in the field at Tottori in 1975–1976. The Kjeldahl protein analysis of seeds obtained by bagging was made for each plant. For the male sterile Chinese Spring lines with *T. timopheevi* or *Ae. caudata* cytoplasm, seeds obtained by hand made pollination were

Table 1. The Kjeldahl % grain protein of 8 alien and one original cytoplasmic lines of Chinese Spring grown in the field at Tottori in 1975–76

Origin of cytoplasms[1)]	*caud.*	*timo.*	*umbel.*	*cereale*	*spelt.*	*ovata*	*variab.*	*squar.*	Cns.	Sig. diff.
% protein[2)] (N% × 5.83)	23.1*	22.6*	19.3*	18.4*	15.8	15.5	15.3	14.9	14.6	2.9
Corrected % protein[3)]	18.9	18.4	16.9	17.5	17.9	16.9	18.1	18.5	17.6	3.0

1) *caud., timo., umbel., cereale, spelt., ovata, variab., squar.* and Cns. stand for *Ae. caudata, T. timopheevi, Ae. umbellulata, S. cereale, Ae. speltoides, Ae. ovata, Ae. variabilis, Ae. squarrosa* and Chinese Spring, respectively. As for the introducers of these cytoplasms, except rye's, into common wheat, refer to TSUNEWAKI *et al.* (1976).
The rye cytoplasm source line was provided by Dr. T. LELLEY, Göttingen University, FRG.

2) 11.0% moisture basis.

3) Based on the regression of the % grain protein (Y) and on the grain number per spikelet (X), $\hat{Y}=17.7-3.209\ (X-1.5)$.

*: Significantly higher than 14.6% for Cns at the 5% level. Significant difference $=S\bar{x}(0.58) \times Q^{9}_{18}(5.03)=2.9$ (%).

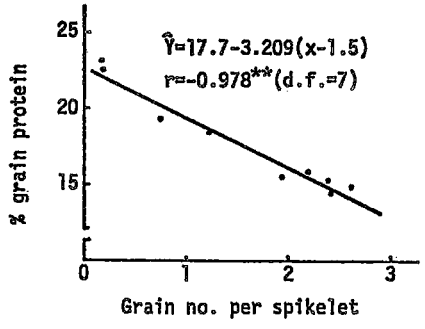

Fig. 1. Regression of % grain protein on grain number per spikelet (line mean basis).

used. The variance analysis was made for 27 mean values consisted of 3 block means for each material line.

The Kjeldahl % grain protein (N% × 5.83) of 9 different Chinese Spring lines are shown in Table 1. The differences among lines are significant for the % grain protein, and those lines which have *T. timopheevi, Ae. caudata, Ae. umbellulata* or *S. cereale* cytoplasm were higher in the % grain protein than the original Chinese Spring line. However, these high protein lines were in general low in seed fertility and yield, short in culm length. And, the % grain protein were highly correlated negatively to those characters, but positively to 1000-grain weight (Table 2 and Fig. 1). The latter relation suggests that the bran protein did not contribute to the higher % grain protein of lower seed fertility lines. The relationships of this type shown in Table 2 between the % grain protein and the other characters seem to be quite general in a certain material having a large seed fertility variation. The same relationships were also observed for the 11 ditelocentric Chinese Spring lines grown in the glass-house in the same season (unpublished). The analysis of

Table 2. The correlation between the % grain protein and 7 characters of 8 alien and one original cytoplasmic lines of Chinese Spring grown in the field at Tottori in 1975–76

Character	Culm length	Tiller number	Spikelet no./spike	Seed fertility[1)]		1000-grain weight	Yield/plant
				A	B		
Correl. coef. (r)	−.672*	−.580	−.254	−.978**	−.965**	.503	−.818**

1) Seed fertility A=grain number/spikelet; seed fertility B=% fertile grain for 1st and 2nd florets.

*,**: Significant at the 5% and 1% level, respectively.

variance showed that in this experiment differences between the original and alien cytoplasms are not statistically significant for the corrected % grain protein based on the regression of the % grain protein (Y) and grain number per spikelet (X), $\hat{Y}=17.7-3.209\ (X-1.5)$.

These results suggest that (1) under the certain nitrogen source and culture conditions, smaller sinks such as shorter culm, fewer tillers, seeds and yield cause higher accmulation of protein (nitrogen) into the seed sink making seeds heavier, and (2) the cytoplasm affects less directly on the % grain protein, but much indirectly through the other yield component characters. However, that those lines with the *caudata*, *squarrosa* or *timopheevi* cytoplasm are still higher than the line with the original cytoplasm in the corrected % grain protein, though the differences are not significant, requires a further study in detail on the effect of alien cytoplasms on the grain protein quantity and quality in wheat. An analysis for amino acids composition of the grain protein are under way.

Acknowlegements: We are grateful to Drs. H. FUKASAWA, M. MURAMATSU, K. TSUNEWAKI and T. LELLEY for providing us with the source line seeds of alien cytoplasms. The work has been supported in part by a Grant-in-Aid (No. 1668/RB) from the International Atomic Energy Agency (IAEA).

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Unscheduled DNA synthesis induced by gamma-radiation in radicle meristem cells of *Triticum aestivum* L.

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DNA damages by physical or chemical agents can be repaired through various processes such as photoreactivation, excision repair, post-replication repair and *de novo* synthesis,[22,24] and these repair processes, unseparably connected with other fundamental metabolism of DNA, i.e. replication and recombination, are essential to living cells, and casually related to mutagenesis.[1,8,21] Also, it has been well demonstrated that DNA synthesis can be induced in cells at the non-DNA replicative phase by ionizing or ultraviolet radiation, or chemical mutagens for unicellular organisms[3,23,26,28] as well as for mammalian cultured cells,[4,7,9,11,27] and that this unscheduled DNA synthesis is commonly associated with the performance of DNA repair replication in excision repair process, one of the most universal repair processes.[3,5,6,27,29] The existence of such synthesis in plant cells, however, is problematical, and it has been suggested that green plant cells in general might lack excision repair process.[25,30,32,33,35,36] To examine the universality of repair synthesis in plants, a series of experiments have been conducted[15] and the present report deals with unscheduled DNA synthesis in cells *in situ* in the radicle meristem of germinating seeds of hexaploid wheat after gamma-ray irradiation.

Germinating seeds of *Triticum aestivum* L. variety "Chinese spring" (2n=42) were irradiated with a sublethal dose, 5.5 kR, of ^{60}Co gamma-radiation at 55 kR/h at 25°C.[14] Seeds were previously surface-sterilised by submersion in 1% sodium hypochlorite for 10 min followed by rinsing thoroughly in distilled water and germinated on moist filter paper in petri dishes containing aqueous solution of 0.1 mg/ml chloramphenicol and 1 mg/ml penicillin in the dark for 3 days at 25°C. Immediately after irradiation, the radicles were submerged in (methyl-^{3}H) thymidine solution (^{3}H-TdR, 45 Ci/mM, 10 μCi/ml) containing 10^{-2} M hydroxyurea, a DNA replication inhibitor,[31] for one or two hours, and then rinsed free of the radio-isotope in cool water and in a solution of unlabelled thymidine. Parallel controls with non-irradiated radicles were also run. The radicle tips were then fixed in a solution of acetic-alcohol (3:1 v/v). The Feulgen-stained radicle tips were used for preparing autoradiographs by the dipping technique.[16,18] The number of labelled nuclei were counted for each of three radicles per lot on the autoradiograms after exposure and development of the emulsion. The synthesis of DNA was also monitered by measuring the radioactivity of ^{3}H-TdR incorporated in to TCA (trichloroacetic acid)-insoluble fractions. Fixed roots were homogenized with a glass homogenizer and the homogenates were suspended into 10% cold TCA solution in test tubes, which were kept in ice-bath for at least one hour. The precipitates were trapped onto glass fiber discs and washed twice with 5% cold

TCA solution, once with cold 95% ethyl alcohol and acetone. After drying, tritium radioactivity was counted in Packard Tri-Carb liquid scintilation spectrometer.

The autoradiogram obtained from the exposed radicle showed two types of nuclei, i.e. heavily labelled nuclei indicating the cells in the S phase, and lightly labelled nuclei representing the cells undergoing unscheduled DNA synthesis, which is typical of unscheduled DNA synthesis.[7]

The frequencies of labelled nuclei were significantly increased in radicles exposed to gamma-radiation (Table 1, Fig. 1). The fraction of labelled nuclei was up to four times more than in the control for the first one hour, and about three times more for the first two hours of the post-irradiation periods. In unirradiated control, the labelled cells, in the presence of hydroxyurea, was 7% for one hour and 10% for two hours, a difference of less than two-fold, of the postirradiation periods. As seen in Fig. 1, the fraction of labelled nuclei induced by gamma-radiation increased in the first hour of the post-irradiation period and remained virtually constant in subsequent another one hour.

This could result from the accelerated onset of DNA synthesis in some cells of the radicle meristem at the nonreplicative phase of the cell cycle. This unscheduled DNA synthesis was also revealed by enhanced ^{3}H-TdR uptake into their DNA in the state of inhibited normal scheduled DNA synthesis after gamma-ray irradiation (Fig. 2).

Both the increased frequency of labelled nuclei and the enhanced uptake of ^{3}H-TdR

Table 1. Unscheduled DNA synthesis in radicle cells of gamma-irradiated wheat seeds

Treatment		No. cells observed	Labelled cells	
γ ray	^{3}H-TdR		Number	%
0 kR	1 hr	829	61	7.4
		733	41	5.6
		823	44	5.3
		2385	146	6.1
0 kR	2 hr	696	74	10.6
		1077	163	15.1
		738	8	1.0
		2511	245	9.8
5.5 kR	1 hr	565	152	26.9
		612	158	25.8
		649	165	34.1
		1826	475	26.0
5.5 kR	2 hr	589	156	26.5
		785	271	34.5
		775	239	30.8
		2149	666	31.0

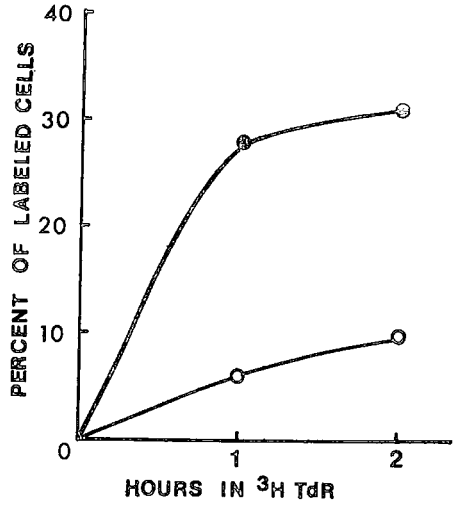

Fig. 1. Dependence of unscheduled DNA synthesis in radicle cells of gamma-irradiated wheat seeds on post-irradiation labelling period. O unirradiated control; ● irradiated.

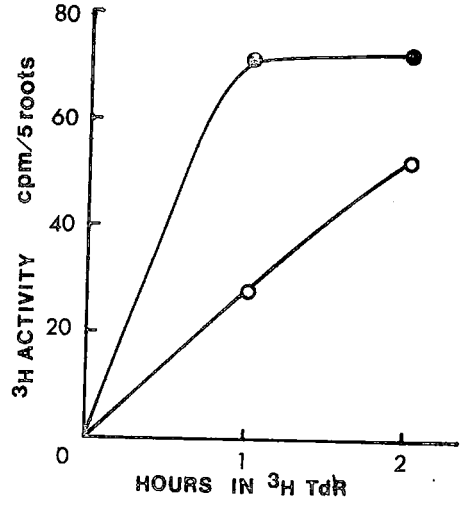

Fig. 2. Dependence of ^{3}H-TdR incorporation into radicle cells of gamma-irradiated wheat seeds on post-irradiation labelling period. O unirradiated control; ● irradiated.

into DNA were seen mainly for the first one hour after irradiation, and the amount of newly synthesized DNA were not increased more in the later postirradiation periods. The increase and enhancement of DNA synthesis in the radicle meristem cells after gamma-radiation seems to be more clearly seen by autoradiographic method than by biochemical method involving the direct measurement of the radioactivity. One of inhibitors of normal semiconservative DNA replication, hydroxyurea, did not affect this unscheduled DNA synthesis induced by gamma-radiation.

It seems almost certain, thus, that unscheduled DNA synthesis observed here may not be a reflection of stimulated normal DNA replication, but a consequence of the performance of repair replication in the excision repair process after gamma-radiation. It seems also clear that wheat seed embryo cells possess excision repair system for gamma-radiation-induced lesions in DNA, which can function after gamma-irradiation.

The present results, along with evidence from onion,[15] barley.[34,37] carrot,[12,13] pea[10] or *Euglena*[19] cells showing excision reapir activity, strongly suggest that excision repair process seems to be widespread in plant species. However, the phylogenic distribution of this capability among plant cells has more and yet more to be determined to generalize this to most plant cells, because there are other reports indicating an absence of excision repair activity in *Nicotiana*,[32] *Haplapoppus*,[32] *Vicia*,[25,35,36] *Ginkgo*[33] or *Chlamydomonas* nuclear and chloroplast DNAs.[30]

The role of excision reapir system in the rejoining of chromosome breaks or in the formation of chromosome aberrations is also the type of experiment which is to be done in the future.[2,17,20,35]

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Origin of *Triticum monococcum* L.

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The cultivated einkorn *Triticum monococcum* L. is commonly believed to have been domesticated from wild einkorn *T. boeoticum* BOISS. em SCHIEM. They differ from each other largely with respect to fragility of the rachis. In *T. boeotium* individual spikelets disarticulate at maturity. According to HALBAEK (1966) West Central Anatolia was the primary center of conscious selection of *T. monococcum*. Various sites of excavations such as Ali KOSH (Iran), Catal HÜYÜK and HACILAR (Turkey), from where specimens of *T. monococcum* have been recovered (HALBAEK 1959, 1966), are within the general area of distribution of *T. boeoticum* (HARLAN and ZOHARY 1966; JOHNSON 1975).

Recently another wild diploid wheat *T. urartu* Tum. – heretofore considered as an obscure Armenian endemic – is found distributed aboundantly in South Eastern Turkey and Lebanon and sporadically in Northern Iraq and Western Iran (JOHNSON 1975). The distribution of *T. urartu* covers the probable area of domestication of *T. monococcum*. *Triticum urartu* is reproductively isolated from *T. boeoticum* throughout the range of their sympatric distribution (JOHNSON and DHALIWAL 1976). *Triticum monococcum*, therefore, could have been domesticated from *T. urartu* or *T. boeoticum* or both at one or at several places of their wide sympatric distribution.

Morphological and cytological evidence reported here suggests that *T. monococcum* was presumably domesticated only once from a population of *T. boeoticum* with a limited introgression from *T. urartu*.

Materials and Methods

The *T. urartu* × *T. monococcum* F_1 hybrids and the reciprocal crosses were made between 14 accessions of *T. urartu* and 12 accessions of *T. monococcum* to cover the variability in the two species. A few *T. monococcum* × *T. boeoticum* hybrids including their reciprocals were also made. Observations on size, appearance and germination of the F_1 hybrid seeds were made as reported by JOHNSON and DHALIWAL (1976) for *T. boeoticum* × *T. urartu* hybrids. Sterile *boeoticum* (♀) × *urartu* (♂) hybrids including the reciprocal crosses (JOHNSON and DHALIWAL 1976) were backcrossed with *T. boeoticum* and *T. urartu* to obtain first backcross (BC_1) progenies (Table 2). Morphological attributes (Table 3) were recorded from herbarium specimens and field growing plants of several accessions of each species.

Results and Discussion

Hybrids involving *T. boeoticum* and *T. urartu* gave germinable seeds only when *T.*

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boeoticum was used as the female parent (Table 1). The reciprocal crosses with *T. urartu* as the female parent gave completely shrivelled and non-germinable seeds (JOHNSON and DHALIWAL 1976). The F_1 hybrid seeds from reciprocal crosses involving *T. boeoticum* and *T. monococcum*, although differed from each other with respect to size, were fully developed and germinable (Table 1) supporting the earlier view that *T. monococcum* was just a domesticated type of *T. boeoticum*. The *monococcum* (♀) × *urartu* (♂) F_1 hybrid seeds were small, plump and completely germinable like that of the *boeoticum* (♀) × *urartu* (♂) hybrids. However, the *urartu* (♀) × *monococcum* (♂) hybrids gave only partially shrivelled and germinable seeds (Table 1) unlike that of the *urartu* (♀) × *boeoticum* (♂) hybrids. *Triticum monococcum*, therefore, differs from *T. boeoticum* in their reciprocal crosses with *T. urartu*. Only partial shrivelling and good germination of *urartu* (♀) × *monococcum* (♂) hybrid seeds suggests that *T. monococcum* might have been domesticated from a *T. boeoticum* population with introgression from *T. urartu*.

The *boeoticum* × *urartu* hybrids, irrespective of the female parents, were completely self-sterile (JOHNSON and DHALIWAL 1976). Similarly, all the *monococcum* × *urartu* hybrids (Table 1) without regard to the female parent were self-sterile indicating that *T. monococcum* like *T. boeoticum* is reproductively isolated from *T. urartu*. Hybrids involving *T. monococcum* and *T. boeoticum* were completely fertile suggesting that the *urartu* genes responsible for the *boeoticum* × *urartu* hybrid sterility were not introgressed in the parental population of *T. monococcum*. It suggests further that only a very limited amount of the *urartu* genome

Table 1. Morphology and germination of F_1 hybrid seeds from reciprocal crosses among *T. boeoticum*, *T. urartu* and *T. monococcum*; and fertility of the F_1 hybrids

Hybrid	No. of crosses	Seed size	Seed appearance	% germination	F_1 hybrid fertility
urartu × *monococcum*	20	normal	partially shriv.	45	sterile
monococcum × *urartu*	10	reduced	plump	100	〃
urartu × *boeoticum**	32	normal	completely shriv.	0	〃
boeoticum × *urartu**	85	reduced	plump	100	〃
〃 × *monococcum*	8	normal	〃	100	fertile
monococcum × *boeoticum*	4	〃	〃	100	〃

* Data from JOHNSON and DHALIWAL (1976).

Table 2. Fertility of first backcross (BC_I) progenies obtained by crossing the sterile *boeoticum* × *urartu* hybrids including reciprocal crosses with either parental species

Progeny	No. of plants	% pollen viability	% seed set
(*boeoticum* × *urartu*) × *boeoticum*	60	3-100	1-100
(*urartu* × *boeoticum*) × 〃	9	6- 56	7- 82
(*boeoticum* × *urartu*) × *urartu*	11	0	0
(〃 × 〃) × 〃	1	43	5
(*urartu* × *boeoticum*) × 〃	1	0	0
(〃) × 〃	1	35	12

is represented in *T. monococcum*.

Fertility of the BCI progenies (*boeoticum* × *urartu*) × *boeoticum* and (*boeoticum* × *urartu*) × *urartu* (Table 2) suggests that the introgression would have been possible only from *T. urartu* to *T. boeoticum* rather than in the opposite direction. Plants from the BCI progeny (*boeoticum*×*urartu*) × *urartu* were essentially completely male and female sterile while that from (*boeoticum* × *urartu*) × *boeoticum* had very low to very high fertility (Table 2). Presumably due to segregation or further backcrossing of the BCI progeny (*boeoticum* × *urartu*) × *boeoticum* with *T. boeoticum* only a fraction of the *urartu* genome was retained. The facts that under natural conditions viable hybrid seeds are possible only with *T. boeoticum* as the female parent and substitution of the *urartu* nucleus in the *boeoticum* cytoplasm gives male sterile plants (DHALIWAL 1976) indicate further that the introgression of *T. boeoticum* to *T. urartu* could not have possibly occurred.

With respect to certain plant and spikelet characteristics such as leaf pubescence, seed colour and spike density (Table 3) *T. monococcum* resembles *T. urartu* rather than *T. boeoticum* indicating further that the *boeoticum* population from which *T. monococcum* was domesticated involved introgression of *T. urartu*. However, a close resemblance of *T. monococcum* to the contemporary cultivated tetraploid wheat *T. dicoccum* (Table 3) suggests that *T. monococcum* might have originated as a result of introgression of the tetraploid to *T. boeoticum*. Alternatively, *T. monococcum* was domesticated first and *T. dicoccum* originated as a results of introgression of *T. monococcum* into wild tetraploid wheat. Available archaeological evidence (HALBAEK 1959, 1966) does not provide answer as to which of the two was domesticated first. Experimental evidence (DHALIWAL, unpublished), however, suggests that the introgression from diploid to tetraploid wheats can occur easily while it is almost impossible in the opposite direction.

Table 3. Morphological characteristics of *T. urartu*, *T. boeoticum*, *T. monococcum* and *T. dicoccum*

Character	*T. urartu*	*T. boeoticum*	*T. monococcum*	*T. dicoccum*
Leaf pubescence	glabrous	pubescent	glabrous	glabrous
Leaf sheath pub.	〃	〃	〃	〃
Glume pub.	〃	pub./glab.	〃	pub./glab.
Spike density	dense	lax	dense	lax
Seed colour	amber	bluish-amber	amber	amber
Awns	equal	dimorphic	dimorphic	equal
Rachis	fragile	fragile	tough	tough
Rachis hairiness	dense	dense	sparse	sparse
Seed length	long	long	short	short
Seed shape	slender	flattened	cylindrical	cylindrical
Palea	split	split	split	intact

At present *T. monococcum* is cultivated on a limited scale in Yugoslavia, Asia Minor, Transcaucasia and North Africa. However, in the past it was cultivated over a very large area extending beyond the distribution of wild einkorn. *T. monococcum* accessions from

different sources are remarkably similar in their growth habit and morphological characteristics. They have erect growth habit, pale green colour, dense spike, dimorphic awns, glabrous-leaves, -leaf sheaths and -glumes, and identical seed shape. The wild diploid wheats vary considerably over the area of their distribution. Uniformity of *T. monococcum* suggests that it was probably domesticated only at one place.

Conclusion: Evidence from morphology and cross-compatibility of diploid wheats and fertility of the F_1 hybrids among them suggests that *T. monococcum* was presumably domesticated only once from a population of *T. boeoticum* involving introgression from *T. urartu*. Experimental evidence suggests that the introgression could have been possible only from *T. urartu* to *T. boeoticum* but not in the opposite direction. (Supported by D.F. JONES' postdoctoral fellowship to the author)

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Cuticular waxes in the tribe Triticinae

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In the tribe Triticinae, species belonging to *Triticum, Secale, Hordeum, Avena* and other genera often show varietal differences in respect of presence or absence of cuticular waxes in the form of powdery white, "waxy bloom". This character serves as a marker for varietal characterisation. By reducing transpiration, it contributes to drought resistance (FRUWIRTH 1923; HALL and JONES 1961). This waxy coat also prevents or retards the germination of rust and other spores or prevents their entry through stomata, thus serving as one of the factors for rust resistance (COBB 1891; VAVILOV 1950). Thus this "waxy bloom" has practical importance. The authors are not aware of any data on chemical composition of these waxes. Data collected by us have been summarised in this preliminary note:

Material and Methods

Following seven species belonging to different genera of the tribe Triticinae were grown during 1975–75 and utilized for these studies:

1. *T. durum* var. Wells CI–13333 (2n=28)
2. *T. durum* var. MACS–9 (2n=28)
3. *T. aestivum* var. NI–747–19 (2n=42)
4. *Secale cereale* var. OD–182 (2n=14)
5. *Triticale* var. JNKV 67040 (2n=42)

6. *Hordeum vulgare* var. Hiproly (2n=14)
7. *Avena sativa* var. Rapida (2n=14)

Cuticular wax of the above was scrached by scalpel from (a) leaf sheaths, (b) peduncles, and (c) spikes at proper stage, scrappings collected in diethyl ether and separated by thin layer chromatography (TLC). The TLC plates were prepared from Silica gel 'G' (E. Merk) of 0.2 mm thickness and activated at 110°C for half an hour. The components of cuticular wax were separated by using petrolium ether; diethyl ether; acetic acid (80:20:1 by volume) and visualised by iodine vapours. The chromatogram was compared with a standard chromatogram and the components identified. Confirmation of the results has been done by repeating the experiments thrice.

Results and Discussion

Qualitative estimation of different components of cuticular waxes of the above varieties has been summarised in the Table 1.

Table 1. Estimation of wax components in the tribe Triticinae

Species	Leaf sheath						Peduncle						Spike					
	ME	TG	FA	AL	ST	MG	ME	TG	FA	AL	ST	MG	ME	TG	FA	AL	ST	MG
1. *T. durum* var. Wells CI-13333 (2n=28)	+	−	+	+	−	+	+	+	+	+	−	+	−	+	+	+	−	−
2. *T. durum* var. MACS-9 (2n=28)	+	+	+	−	−	+	+	−	+	+	−	+	+	+	+	+	−	+
3. *T. aestivum* var. NI 747-19 (2n=42)	+	+	+	+	+	+	+	+	+	+	−	+	Not determined					
4. *Secale cereale* var. OD-182 (2n=14)	+	+	+	+	+	+	−	+	+	−	−	−	−	+	+	−	−	+
5. *Triticale* var. JNKV 67040 (2n=42)	+	+	+	+	−	+	+	+	+	−	−	−	+	+	−	+	−	−
6. *Hordeum vulgare* var. Hiproly (2n=14)	+	+	+	+	−	+	Not determined						+	+	+	+	−	−
7. *Avena sativa* var. Rapida (2n=14)	+	+	+	−	−	+	〃						+	+	+	+	−	+

Remarks: ME=Methyl esters of fatty acids; TG=Triglycerides of fatty acids; FA=Fatty acids; AL=Alcohol; ST=Steroids; MG=Monoglycerides; +=Presence of the component; −=Absence of the component

Wax deposition on cuticle is due to the excretion of the required precursors through the peripheral cell walls (Lee and Priestley 1924; Mazliak 1968). This deposition helps in avoiding spore germination through its physiological, biochemical or mechanical properties (Joshi 1957). It will be seen from the above table that components of the cuticular waxes such as fatty acids are present in waxes from leaf sheath, peduncle and spike in all the seven types studied. Such constancy is also observed for triglycerides, except in waxes from leaf sheath (in var. Wells CI-13333) and peduncle (in var. MACS-9). Other components vary in their presence on different parts in these seven types. Thus there is constancy in

certain components (fatty acids, triglycerides, methyl ester) on leaf sheath, peduncle and spike irrespective of the ploidy level. Constancy within species has also been observed for methyl esters, fatty acids and alcohols though other components vary in different parts even within the same species (*T. durum*). Except for the occurrence of methyl esters, monoglycerides on leaf sheath, fatty acids on peduncle and triglycerides on spike, the other components differ in their presence on other parts, e.g. leaf sheath wax components did not show presence of triglyceride in *T. durum* var. Wells, alcohol in MACS-9 and in oats, where as steroids were noticed only in *T. aestivum* var. NI-747-19 and in rye. Similar variations for peduncle and spike waxes have been noticed as shown in Table 1. Further qualitative and quantitative analysis is in progress at this Institute and will be reported in due course.

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Evidence of heterochromatin polymorphism through crossing-over[1)]

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Polymorphism in heterochromatic chromosome segments has been reported in *Anemone* (MARKS and SCHWEIZER 1974), *Tulipa* (FILION 1974), and rye (WEIMARCK 1975).

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SINGH and ROBBELEN (1975) also found a wide variation in pattern of heterochromatic bands, both between and within species of *Secale*. Since the SAT-chromosome was most easily recognized, four structural types of it could be distinguished by their different Giemsa banding pattern in twenty seeds of *Secale africanum*; one of these types was always more frequent than the others.

In order to obtain more information on the mechanism, by which changes in banding pattern of chromosomes occur, one plant of *Secale africanum* with known banding pattern was allowed to self-pollinate. Spikes were bagged to avoid contamination with foreign pollen, although *S. africanum* is an autogamous species. From the resulting seeds several types of Giemsa banding of SAT-chromosomes were determined in root tips (Fig. 1). All plants with a particular banding pattern were grown in the greenhouse; root tips were recollected 26 days after the first analysis of primary roots was made, in order to check whether bands were changing during somatic cell division.

If meiotic crossing-over is assumed not to occur within the distal heterochromatic regions (NATARAJAN and GROPP 1971), but only in the median euchromatic parts of the SAT-chromosomes, a total of 10 banding types is expected from a heterozygous plant (Fig. 1). With designation of the parental banding types as A and B and the crossing-over types as C and D, there will be 4 homozygous (AA, BB, CC, DD) and 6 heterozygous (AB, AC, AD, BC, BD, CD) combinations of SAT-chromosomes.

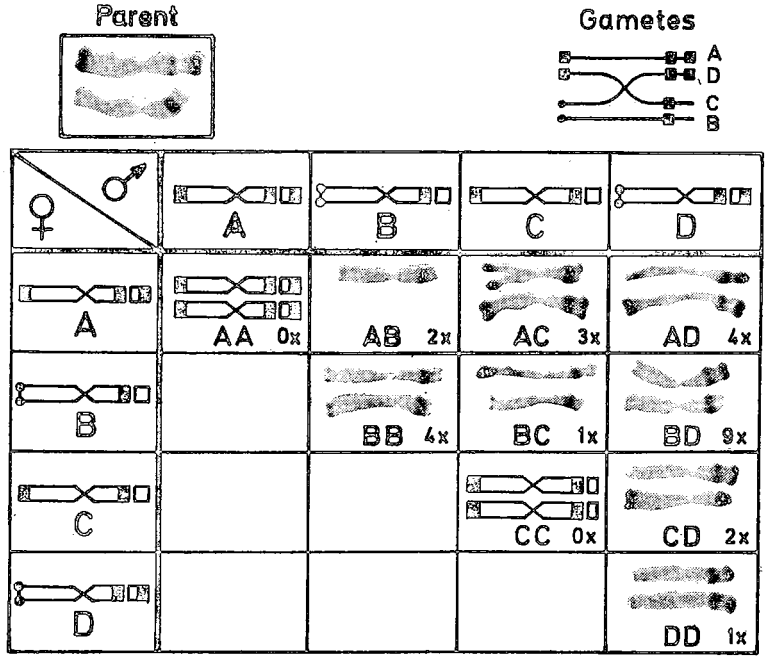

Figure 1: Segregation through crossing-over in Giemsa pattern of SAT-chromosomes determined in the progeny after self-pollination of a structurally heterozygous plant in *Secale africanum*.

Twenty six seeds were studied with Gimesa, and all of the expected heterozygous and two homozygous (BB, DD) types were observed in various frequencies. AA and CC types were not detected within the limited number of analyzed individuals (Fig. 1). The maximum frequency in heterozygous types was for BD (9), and the lowest for BC (1). The occurrence of one type in higher frequency than others indicates preferential transmission through the gametes. No somatic instability was ascertained. Thus, the study provides direct cytological evidence that meiotic crossing-over causes polymorphism in heterochromatin of rye chromosomes.

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Haploidy in the genus *Aegilops*

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Because of their low spontaneous frequency, haploids have been reported only in the diploid species *Aegilops caudata* (CHAPMAN and RILEY 1964) and *Ae. longissima* (RILEY and CHAPMAN 1957) and in the tetraploid *Ae. ovata* (MATSUMURA 1940). Recently, however, high frequencies of haploids of barley (KASHA and KAO 1970) and of wheat (BARCLAY 1975; MILLER and CHAPMAN 1976) have been obtained from crosses with *Hordeum bulbosum*. This report describes the application of this method to the production of haploids in tetraploid *Ae. triuncialis* and hexaploid *Ae. crassa*.

Emasculated ears of *Ae. triuncialis* (2n=28) and *Ae. crassa* (2n=42) were pollinated with pollen from plants of tetraploid *H. bulbosum*. Grains resulting from these pollinations were removed after 14 days. Embyros which had developed in some grains were cultured on standard orchid agar medium. Three haploid plants of *Ae. crassa* and one of *Ae. triuncialis* were established by this procedure.

Table 1. Mean chromosome pairing at first metaphase of meiosis (30 cells per plant)

Haploid	I	II	III	IV
Ae. triuncialis	9.17	2.10	0.17	0.03
Ae. crassa	16.10	2.20	0.17	—

KIHARA (1954) by genome analysis concluded that the two putative parents of *Ae. triuncicalis* were *Ae. caudata* (C) and *Ae. umbellulata* (C^u) and assigned to it the genome formula CC^u. In a hybrid between these two diploid species the mean pairing at meiosis was much higher than that now found in the haploid *Ae. triuncialis*. By the use of a similar analysis KIHARA *et al.* (1959) also found that in hexaploid *Ae. crassa* there were two closely related genomes which had originated from diploid *Ae. squarrosa* (D). The meiotic analysis of haploid *Ae. crassa* does not provide evidence to support the proposed genome formula DD^2M^{cR}. In the haploid there was a much lower frequency of bivalents than might have been expected to result from the pairing of two closely related genomes.

In both haplid *Ae. triuncialis* and *Ae. crassa* the meiotic pairing levels were lower than would have been predicted from the evidence of genome analysis. Either the genomes within the two species are less closely related than suggested by genome analysis or a form of genetic control of chromosome pairing restricts the pairing between homoeologous chromosomes of the genomes.

The wheat and barley haploids were formed by the elimination of *H. bulbosum* chromosomes in the early mitotic divisions of the hybrid embryos. If, as seems likely, the haploids in this experiment resulted from a similar procedure of chromosome elimination it may be possible to obtain haploids from all the polyploid species in the genus *Aegilops*. Such haploids would provide evidence which would assist the understanding of genome relationships and the control of meiotic chromosome pairing in the Triticinae.

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Effects of gamma rays on some wheat characters

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The present investigations are aimed at providing reliable and scientific information on the effect of ionizing radiations during second mutation generation (M_2) on four hexaploid wheat varieties namely Wisconsin supremo, No. 43, Kenya plume and Kenya hunter with respect to leaf length/Breadth ratio and chaff or glume colour.

Leaf Length/Breadth ratio

Table 1. Leaf length/breadth ratio of gamma irradiated M_2 populations of four kenya wheat varieties compared against their control

Culture		Treatements			
Variety	Statistical constants	Control	25 kR	35 kR	45 kR
Wisconsin supremo	Mean	15.41±0.18	16.30±0.38	15.56±0.46	16.80±0.57
	C.V.	14.50±0.90	17.40±1.45	20.85±1.75	10.91±2.10
No. 43	Mean	13.90±0.10	14.10±0.20	14.00±0.18	15.50±0.80
	C.V.	12.30±0.78	12.80±0.90	15.90±1.50	11.95±3.50
Kenya plume	Mean	18.20±0.31	18.90±0.30	19.01±0.38	17.76±0.36
	C.V.	15.88±0.95	14.50±0.87	16.67±1.10	18.20±1.52
Kenya hunter	Mean	16.80±0.35	17.80±0.48	18.45±0.53	20.80±1.10
	C.V.	19.10±1.28	19.80±1.65	18.68±1.92	25.20±3.40

The character leaf length/breadth ratio, which affects the Carbon-Nitrogen ratio and ultimately the productivity, in present studies displayed an erroneous trend suggesting that it is not very much affected by radiation in any regular fashion (Table 1). Changes have been noticed which, though positive, were irregular. I feel inclined to accept the views of other workers like GREGORY (1956), OKA *et al.* (1958) and SCRASCIA (1965) that in characters, polygenic in nature a near equal number of beneficial and deterimental mutation is caused.

The observation is reflective of the compensating tendency by these biotypes to moderate the damage incurred to them through gamma-rays enabling them to evade the threatened extinction. Leaf lengths were elongated or shortened while leaf breadths were narrowed or broadened reciprocally. It may be assumed that histological observation might have revealed almost an equal number of stomata etc., in the irradiated and control population, the factors essential for the execution of vital plant metabolic activities.

In case of cereal crops where tillering is one of the yield components and it covers

nearly the whole of the field, for the small distance kept between rows and plants, the lesser the vegetative (leafy) growth the better, it is, for the photosynthetic activity. It has been seen that fields with very dense populations, though are promising for yields, lodge very badly due to the weakness of stem. This weakness is ascribable to the etiolation effect resulting into and enlarged development of the internodes. The etiolation in its turn is the consequence of the lack of sunlight penetration, so very neccessary for the photosynthetic activity by the mesophyll cells of the leaf which are present throughout the length of culm. Reduction therefore, in the area of the leaf or rather reduction in vegetative growth would, though reduce the area of photosynthetic activity per leaf, ultimately increases the number of leaves involved in the manufacture of carbohydrates, enlarging the photosynthetic activity of the plant which would restore the vigour of the culms and would reduce the chances of lodging.

Chaff or glume colour

Genetic studies have indicated the dominance of red or brown glume colour over white. (Both mono-genic and digenic factors have been proposed by genetic workers).

Recessive mutations are generally known to servive and hence easily detectable as against the dominant ones which are deleterious. The fact that white chaff of variety Kenya Hunter was transformed into red (a dominant character mutant) conveys that the chaff character is controlled through the inter-action of genes. The variety Kenya hunter during the present studies displayed a mutation from white chaff to red chaff appearance in M_2. This is change from recessive character to a dominant one and can only happen when the original genotype of the parent is dominant associated with an inhabitory factor preventing the expression of the dominant trait. In the present case, it seems that the variety Kenya hunter carries the genes for redness '*RR*' but fails to express it because of inhibitory gene '*II*' and hence its expression is white chaff '*IIRR*.' The radiated population with white chaff character seems to have undergone deletion, loosing the inhibitory factor or the inhibitory gene might have got dissolved with the result that the mutated progeney is left with only the genes for redness '*R*' that found their expression in the absence of the epistatic gene '*II*'. Glume colour alterations have been reported by ARNASON *et al.* (1952) and SWAMINATHAN *et al.* (1958).

Abnormalities in the development of glume, as observed in the present study, have often been reported by other workers like MACKEY (1954), KIHARA and TSUNEWAKI (1963). This change would be expected in lieu of the pleiotropic gene '*Q*' which probably gets disturbed in speltoid compactoid expressions. Other segmental changes can not be ruled out.

It seems that the microspores of the same spike or floret arise from diverse cells during micro-sporogenesis. This assumption is derived from the fact the PMC of the same anther bore hetermorphic aberrant chromosomes. GAUL (1964) also reported this experience.

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II. Errata

In the article, "Persistent modifications and their genetic importance for spring wheat breeding, Part I and Part II". by Dr. I.E. GLOUSHCHENKO, WIS Nos. 41–42, 1976, the following errata should be noted.

Page 21, line 11 from the bottom. It is written (I), but it should be read *BRIGGS, F. and P. NOWLS.*

Page 22, line 9 from the top; it is written *in spring wheat,* but it should be read *in winter wheat.*

Page 23, line 14 from the bottom; it is written *a little earlier,* but it should be read *much earlier.*

Page 23, line 8 from the bottom; it is written *a little higher,* but it should be read *much higher.*

Page 24, the first passage, line 2; it is written *1968,* but it should be read *1969.*

III. News

Proceedings of The Fourth International Wheat Genetics Symposium

This 1973 hard-bound volume of 955 pages contains all the contributions at the symposium (137 papers on genetics, breeding, disease resistance, triticale, cytogenetics, induced and natural variation, cytoplasmic and hybrid wheat, biochemical genetics and evolution, plus a list of gene symbols).

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IV. Editorial Remarks

Announcement for future issues

WIS No. 45 will be planned for publication in December 1977. Manuscripts for this issue are accepted any time, not later than September 30, 1977.

WIS is open to all contributions regarding methods, materials and stocks, ideas and research results related to genetics and cytology of *Triticum*, *Aegilops*, *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 cm²). 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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Membership Fee

Due to the economic situations, the yearly Membership Fee has been raised up to ¥ 1,000 for foreign members and ¥ 700 for Japanese members from the fiscal year beginning April 1975. The money should be paid by the Foreign Postal Money Order, otherwise considerable loss (¥ 1,000 or more per 1 item) is caused due to the bank charges. Back numbers are available.

Acknowledgment

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The Managing Editor

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