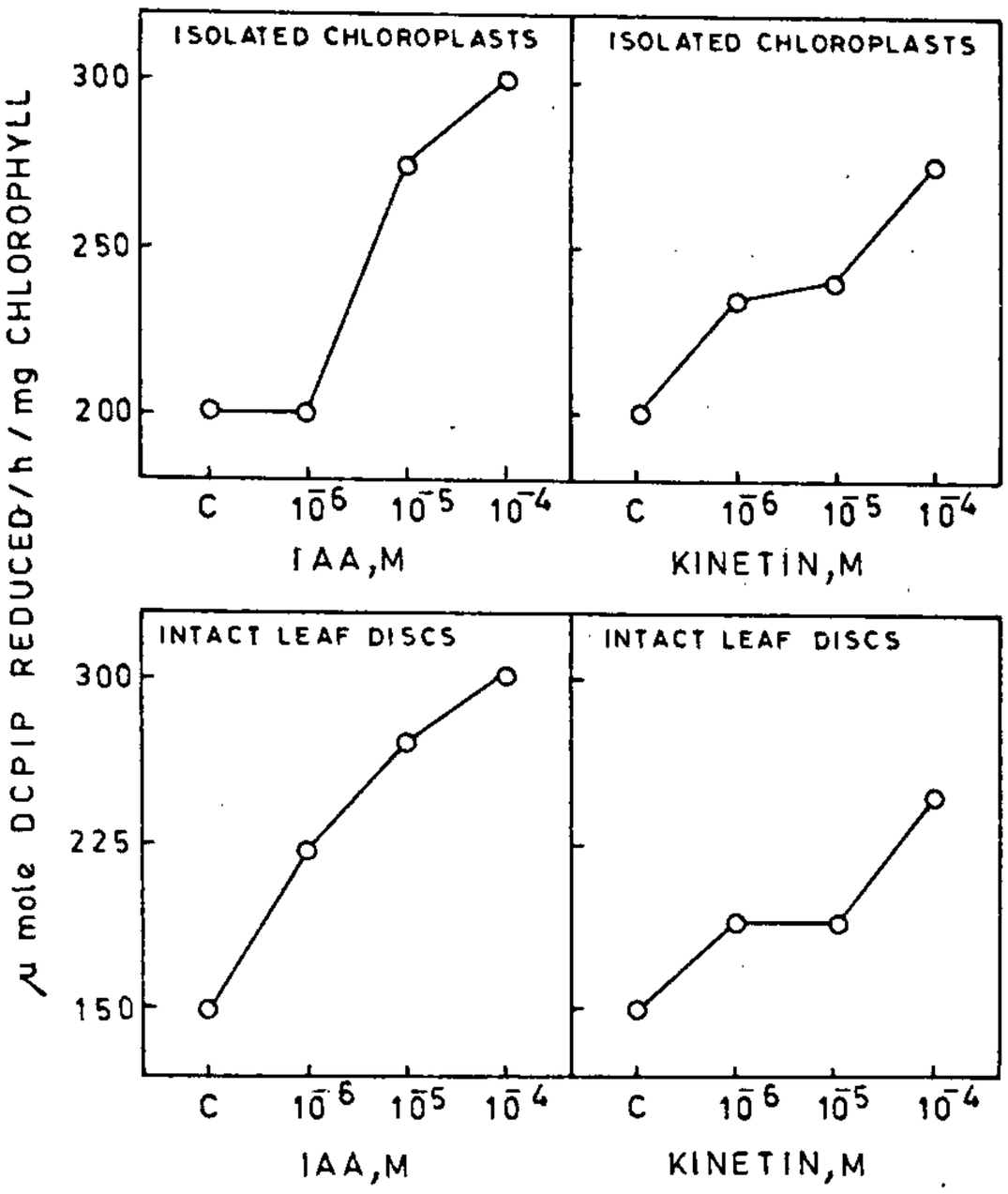

Figure 1. Effect of different concentrations of IAA and kinetin on the reducing power of chloroplasts. Data expressed as µmol DCPIP reduced per h per mg chlorophyll.

that the activity increased with increasing concentrations reaching the peak at 10^{-4} M. However, the DCPIP photoreduction-capacity of chloroplasts of isolated system developed at a faster rate in presence of IAA (55% higher than control at 10^{-4} M), whereas the effect produced by kinetin was relatively less (37% higher than control at 10^{-4} M). In the intact system, on the other hand, the hormonal effect was a little more pronounced, showing 77% and 63% increments with IAA and kinetin respectively.

In the light reaction of photosynthesis, the rate of reduction of the electron acceptor dye DCPIP is directly proportional to the rate of electron flow from the excited chlorophyll molecules in a medium where photolysis of water takes place with generation of electrons. In our experiment, larger rate of reduction in presence of IAA and kinetin by chloroplasts from both sources indicates that they enhance the liberation of electrons and their flow towards the artificial electron acceptor DCPIP. Apparently, it is clear that IAA and kinetin accelerate the photolysis of water both in intact and isolated systems, giving rise to a greater rate of electron production. It is further noted that the hormone-induced development of reducing power of chloroplasts occurs in a more efficient manner in intact system compared to the isolated counterpart.

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Introduction of rye genes into bread wheat by chromosome manipulations

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Monosomic 5B ($2n=41$) of wheat variety Chinese Spring was crossed with a rust-resistant strain of rye (*Secale cereale*, $2n=14$). The 27 chromosome hybrid-lacking chromosome 5B of wheat and showing extensive chromosome pairing between wheat and rye chromosomes was backcrossed to rust susceptible wheat variety Sonalika. In the subsequent segregating generations only rust-resistant plants were selected and in BC_1-F_7 several cytologically stable 21 bivalent-forming plants were identified. Many of these were resistant at the seedling stage to several races of leaf rust (*Puccinia recondita*) and to all the races of stripe rust (*P. striiformis*). These genetic stocks are expected to be a good source of rust resistance in wheat breeding.

WHEAT is today an important cultivated plant to meet human needs and is the only crop with an annual world production of 520 million metric tons[1]. The present study reports our attempts towards transferring rust-resistant rye genes into bread wheat through chromosome 5B manipulations. This has been found necessary to prevent the loss of about 7–20% of grain yield every year amounting to 3.7 to 10.4 million tons of yield per year due to attack of rusts[2]. Chromosome 5B-deficient method was earlier employed for transferring desirable traits into bread wheat from its allied genera[3,4]. However this technique made it difficult to induce homoeologous recombinants between the chromosomes of wheat and rye[5]. Riley and Kimber[5] had colchicined the chromosome 5B-deficient hybrid of wheat and rye to yield a 54 chromosome amphidiploid.

This amphidiploid was repeatedly backcrossed to wheat but none of the 21 bivalent-forming segregant showed any rye character. Joshi and Singh[6] attempted a modified chromosome 5B method to induce recombinants between wheat and rye. In the present study this technique was used for transferring rust resistance into bread wheat from its allied diploid genus *Secale cereale* ($2n=14$) by inducing homoeologous recombination between their chromosomes. Since the chromosomes of wheat and rye do not pair in the presence of chromosome 5B of wheat, recombination breeding between these two genera was not yet possible through conventional breeding procedures.

Monosomic 5B($2n=41$) of cv Chinese Spring was crossed with a rust-resistant strain (R 466 Acca) of rye (*Secale cereale*, $2n=14$) obtained from CIMMYT, Mexico. In the F_1 two types of hybrids, $2n=27$ and $2n=28$ were obtained, the former lacking chromosome 5B of *Triticum aestivum* and the latter having it. The former showed extensive chromosome pairing, whereas the latter had mostly univalents. In the former recombination between the chromosomes of wheat and rye presumably took place and was directly backcrossed to rust-susceptible hexaploid wheat cultivar Sonalika.

Transferring rye genes into bread wheat

The BC_1-F_2 families of mono 5B × rye × Sonalika produced a spectrum of aneuploids (chromosome numbers ranging from $2n=34$ to 44). The field population was infected with a total of about 40 races of stem, leaf and stripe rusts. In these segregating populations only rust-resistant hybrid derivatives were selected for further analyses. In the off-season, the progenies of rust-resistant plants were grown at the Regional Station of the Institute at Wellington (Nilgiris), a "hot spot" for all the three rusts. Rust-resistant plants were selected of which many possessed the desirable agronomic traits like high tiller number, spikelets/spike and larger number of grains per spike.

The procedure of rigorously testing rust-resistant progenies having other desirable traits was continued, and in the BC_1-F_7 generation several rust-resistant disomic plants ($2n=42$) were identified cytologically. These plants when crossed to disomic Chinese Spring showed 21 bivalents, confirming that these plants are indeed homoeologous recombinants.

Seedling test against individual races of leaf rust (Puccinia recondita)

Thirteen wheat-rye recombinants were tested against 15 pathotypes of leaf rust at the seedling stage. Except races 12–2, 104B and 162 the others showed resistance to leaf rust races. All these recombinants were also resistant to leaf rust at the adult plant stage both at Delhi under artificially created epiphytotic conditions and at Wellington, Tamil Nadu, under natural conditions of infection.

The above recombinants were also tested against race 12–1 of leaf rust. Five recombinants were highly resistant to this race, suggesting the presence of genes in addition to *Lr* 26. The leaf rust resistance of most wheat–rye derivatives is attributed to gene *Lr* 26 derived from Petkus rye[7]. This resistance broke down against races 12–1 and 77–1 of leaf rust[8,9]. These five genetic stocks are therefore expected to be useful donors of alien genes for resistance against race 12–1 of leaf rust.

The rye genotype used in the present programme is different from the rye which gave rise to many rust-resistant wheat cultivars[7]. They are either spontaneous 1B/1R substitution lines or 1BL/1RS translocation lines[10]. These lines carry the linked genes *Lr* 26, *Sr* 31, *Yr* 9 and *Pm* 8 and confer effective resistance to the infection of leaf, stem, stripe rusts and powdery mildew respectively[7].

Seedling test against races of stripe rust (Puccinia striiformis)

The same 13 recombinants were also tested against 8 races of stripe rust at the seedling stage. Four were highly resistant to these virulences, of which three, viz. selection WR 76, WR 125 and WR 161 were also resistant to race 12–1 of leaf rust at the seedling stage. This was considered to be an advantage for transferring

Table 1. Characteristic features of promising wheat–rye recombinants.

Wheat–rye recombinants and controls	Plant height (cm)	No. of tillers per plant	Yield/plant (g)	No. of spikelets/ spike	No. grains/ spike	100 grain weight (g)	Seed colour
WR-2*	106.2 ± 6.73	5.8 ± 0.74	16.82	23.0 ± 1.67	54	3.0	Amber
WR-76	96.4 ± 5.98	4.5 ± 0.64	18.38	21.8 ± 1.72	64	3.5	Red
WR-125	109.2 ± 7.32	6.3 ± 0.92	14.66	20.4 ± 1.36	56	3.1	Red
WR-161	102.3 ± 6.87	6.2 ± 0.98	33.08	23.0 ± 1.41	53	2.8	Red
Sonalika	113.6 ± 16.70	10.0 ± 1.34	20.45	23.8 ± 1.72	84	2.4	Amber
Chinese Spring	104.8 ± 8.32	8.0 ± 2.32	12.25	20.4 ± 1.74	60	2.0	Red
Rye	140.6 ± 6.52	12.2 ± 4.62	18.88	36.8 ± 2.31	62	3.1	Red

*Amber seeded genetic stock resistant at adult plant stage against all the three rusts.

alien genes into cultivated varieties which can be effective against more than one pathogen. Normally, genes transferred at the intervarietal level offer resistance only against one pathogen.

The salient features of the most promising wheat–rye recombinants developed in the present programme, as compared to Sonalika and Chinese Spring, are given in Table 1.

The efficacy of chromosome 5B manipulation in transferring desirable traits from rye into bread wheat has been demonstrated[6]. Earlier, a wheat-rye genetic stock (selection 111) was bred[11] which consistently gave 1000 grain weight of 64–68 g (the spike of this selection has about 50 seeds per spike). Such a high test weight is not available in the existing bread wheat germplasm.

The present programme laid emphasis on developing rust-resistant genetic stocks in wheat having rye genes. Since these genetic stocks cross easily with wheat varieties and the F_1s thus produced form 21 bivalents at metaphase I of meiosis, it is envisaged that these stocks could be useful in wheat bree ing programme.

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5-Methylcytosine residues in DNA decrease during ageing

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The degree of methylation of DNA is thought to be linked to many processes involving DNA, including transcriptional activity. Changes in DNA methylation are also believed to be a component of ageing of cells in culture and of organisms. We have estimated 5-methylcytosine in liver DNA of mice of ages between 11 days and 36 months using anti-5mC antibody and biotin–avidin peroxidase. We show that 5mC levels first decrease by 30% between 1 and 6 months of age, remain unchanged thereafter till 26 months, and then decrease again by 17% between 26 and 36 months. In differentiated liver, known to have decreased transcriptional activity, we find hypomethylation of DNA. On the basis of these and earlier data, we suggest that DNA in old liver is less accessible to methylases.

THE fidelity and efficiency of retrieval of genetic information depends on genome integrity, which may be causally related to the nature of lesion and the DNA repair function[1–4]. Furthermore, normal metabolic processes like methylation of cytosine may modify DNA structure[5] and alter DNA:protein interactions[6] In eukaryotes, chromatin proteins seem to control the accessibility of DNA to repair enzymes, so that linker DNA is preferentially repaired[7,8]. The presence of 5-methylcytosine (5mC) increases recombination frequency[9], while 6-methyladenine increases mismatch repair[10]. Razin and Riggs[11] have correlated the extent of methylation to the functional state and integrity of DNA. Transcriptionally active regions of chromatin seem to be undermethylated while inactive chromatin appears to be hypermethylated[12,13]. Wilson and Jones[14] have reported that, unlike in immortal cell lines, 5mC in diploid fibroblast DNA decreases with number of passages of the cultures, and suggested that survival and lifetime of diploid fibroblasts may depend on their ability to maintain a constant level of 5mC. Using an immunochemical assay[15,16], we have estimated 5mC in liver nuclear DNA of mice at several ages covering the entire life-span. We find that 5mC concentration decreases in two steps, first between 1 and 6 months, and later during the last quarter of life (26 to 36 months). This supports our preliminary observation[4].

Liver nuclei were isolated[17] from C57Bl × A/J F_1 hybrid mice at ages 11 days, and 1, 6, 7.5, 18, 26 and 36 months. DNA was purified[18], reextracted after treatment with ribonuclease A, and dissolved in TE (10 mM Tris–HCl, pH 7.8; 1 mM EDTA). The 260/280 absorption ratio was 1.82–1.87. DNA concentration was then adjusted to 100 $\mu g\,ml^{-1}$. It is well established that contaminating RNA raises the 260/280 ratio beyond 2.0, which was not the case in the preparation. Further, no RNA could be detected in these DNA preparations upon electrophoresis[19]. Nonmethylated DNA from bacteriophage lambda (grown in *dcm*⁻ *dam*⁻ *E. coli*, purchased from Sigma) was used as negative control. For each age, pooled liver DNA from six mice (three males and three females) was used.

DNA (100 ng in 1 μl) was spotted on nitrocellulose BA85 paper and baked for 4 hours at 70°C. The paper was incubated with 180 μg of rabbit