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# Identification of the chromosomes involved in a wheat–rye translocation using isozyme markers

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## SUMMARY

Monosomic and disomic substitutions of Imperial rye chromosome *C* for 4*A* of Chinese Spring wheat were analysed for two enzyme markers, alcohol dehydrogenase (ADH) and 6-phosphogluconate dehydrogenase (6-PGD). The gene(s) coding for ADH and 6-PGD were located on the short and long arms of rye chromosome *C* respectively.

Isozyme analyses revealed that the substitute chromosome was a translocation involving alpha arm of wheat chromosome 4*A* and long arm of rye chromosome *C*. Pairing behaviour of the substitute chromosome with rye and wheat chromosomes in hybrids with rye addition line *C* and ditelosomic 4*A* (alpha) confirmed this.

## INTRODUCTION

The homoeologous relationships of wheat and rye chromosomes is fairly well established. Homoeologies between five rye chromosomes, 1, 2, 3, 5 and 6*R* and the corresponding wheat chromosomes have been described earlier (Zeller & Fischbeck, 1971; Sears, 1968; Acosta, 1961; O'Mara, 1946; & Riley, 1965; respectively). In order to assess the homoeologous relationship of the group 4 chromosomes of wheat with chromosome *C* of Imperial rye, we attempted to substitute the Imperial rye chromosome *C* for 4*A* of wheat and found that the substitution line obtained was highly fertile (33% seed set) compared to nulli-4*A* (0% seed set). Since the male fertility genes were located on the alpha arm of 4*A* (Sears, 1954), it was felt that the *C* chromosome of rye can substitute for the loss of them (Prabhakara Rao, 1975).

Koller & Zeller (1976) suggested that the chromosome *CR* of *Secale cereale* L. consists of the short arm of *Secale montanum* Guss. chromosome 4*R*, its centromere region plus a segment of *montanum* 7RS including the Rc (Red coleoptile) locus. They have also shown that 4*A*/*CR*, 4*B*/*CR* and 4*D*/*CR* substitutions were characterized by poor fertility and reduced vigour. In order to resolve this problem we felt that a further check of the chromosome constitution of our substitution line was necessary. The karyotype analysis did not give much information about the chromosome constitution. Hence isozyme markers located on the opposite arms of chromosome *C* were used to infer the chromosome constitution and this was further verified by the analysis of the pairing behaviour of the substitute chromosome.

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## MATERIALS AND METHODS

The pedigree of the wheat–rye substitution used in the present study was as follows:

Chinese Spring (*CS*) monosome $4A \times$ *CS*-Imperial rye disomic Addition line

| | | |
|---|---|---|
| $(20''+1')$ | | $(21''W+1''C)$ |
| $F_1$ | $20''W+1'4A+1'C$ | |
| | | selfed |
| $F_2$ | $20''W+1'C$ (?) | Monosomic substitution |
| $F_3$ | $20''W+1''C$ (?) | Disomic substitution |

The alcohol dehydrogenase (ADH) and 6-phosphogluconate dehydrogenase (6-PGD) isozyme patterns were determined as previously described by Irani & Bhatia (1972) and Narayana Rao & Prabhakara Rao (1980), respectively. The relative intensities of the isozymes were determined using the serial dilution method (Klebe, 1975).

Meiotic analyses were made in Feulgen stained squashed preparations of pollen mother cells from anthers fixed in acetic alcohol (1:3).

## RESULTS

Chinese Spring wheat with an added pair of Imperial rye chromosome *C* was crossed with monosomic $4A$ of Chinese Spring, and the 42 chromosome $F_1$ plants $(20''W+1'4A+1'C)$ were selfed. No pairing was observed between the wheat $(4A)$ and rye $(C)$ chromosomes. The rye univalent could be easily distinguished since it was much larger than the wheat univalent. A 41 chromosome $F_2$ plant $(20''W+1'C(?))$ with one large univalent was selected as monosomic substitution and selfed to get the disomic substitutions $(20''W+1''C(?))$.

## ALCOHOL DEHYDROGENASE ZYMOGRAM

The ADH zymogram of the 42 chromosome substitution plants showed three bands corresponding in mobility and relative intensities (1:4:4) with the hexaploid wheat zymogram (Fig. 1*b*, *c*). The gene for the fast moving dimer (band 1) of this triplet was

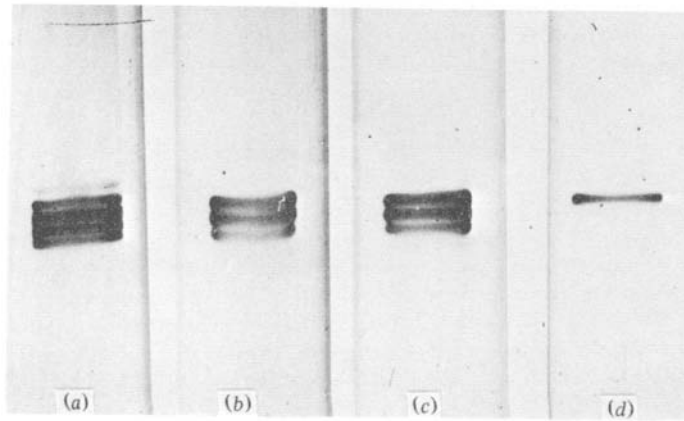


Fig. 1. Alcohol dehydrogenase (ADH) zymogram phenotypes of (*a*) Triticale (Chinese Spring–Imperial rye); (*b*) Chinese Spring wheat; (*c*) $4A$ (alpha)/$CR^L$ translocation discussed; in this paper; (*d*) Chinese Spring wheat Nulli-4A.

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located on the alpha arm of chromosome 4*A* of wheat (Hart, 1970). The gene(s) coding for ADH was located on the short arm of *CR* of Imperial rye (Mahajan, 1975). The three band pattern obtained in the substitution plants as against the single band found in the nulli-4*A* plants (Fig. 1*d*) suggests that the 4*A* alpha arm of wheat is present, and the short arm of *CR* is absent. This eliminates the possibility of these plants carrying a complete rye chromosome, and the presence of 4*A* alpha arm explains the high fertility observed.

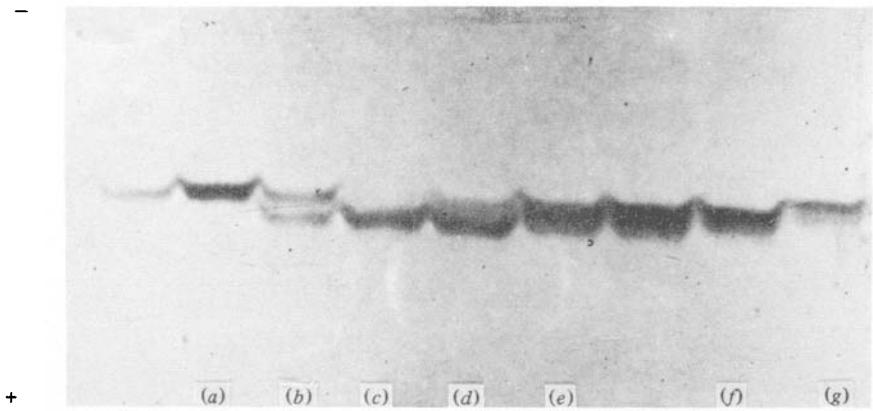


Fig. 2. 6-phospho gluconate dehydrogenase (6-PGD) zymogram phenotypes of (*a*) Chinese Spring wheat; (*b*) Chinese Spring wheat + Imperial Rye mixture; (*c*) Imperial rye; (*d*) Triticale (Chinese Spring–Imperial + Imperial rye mixture); (*e*) Triticale (*CS* + Imperial); (*f*) 4*A* (alpha)/$CR^L$ translocation discussed in this paper; (*g*) *CS* + *C*: disomic addition line.

## 6-PHOSPHOGLUCONATE DEHYDROGENASE (6-PGD) ZYMOGRAM

The 6-PGD zymogram phenotypes of the substitution line showed three bands (Fig. 2*f*) corresponding to the wheat (6-PGD-3), rye (6-PGD-1) and the hybrid (6-PGD-2) dimers, suggesting that the rye gene(s) coding for this enzyme are also present. The genes coding for this enzyme have been located on the long arms of chromosomes *C* and *F* of Imperial rye (Narayana Rao & Prabhakara Rao, 1980). There is no possibility of the long arm of the chromosome *F* being present (since the starting material was *CS* + *C* addition) it is inferred that a part of the long arm of *C* chromosome of rye is present.

The above zymogram analyses indicates that the substitute chromosome carried segments of wheat chromosome 4*A* and rye chromosome *C*.

## PAIRING BEHAVIOUR OF THE SUBSTITUTE CHROMOSOME

In a cross between the ditelosomic 4*A* (alpha) and the disomic substitution, a heteromorphic bivalent was observed (Fig. 3), confirming the presence of the 4*A* alpha arm in both the parents. In a cross of the disomic substitution with the addition line *CS* + *C*, a trivalent was observed confirming that the substitute chromosome has homology with the chromosome *C* of rye as well.

The above biochemical and cytological analyses reveal that the substitute chromosome

was not complete *CR* as reported earlier (Prabhakara Rao, 1975), but is a 4*A* (alpha)/$CR^L$ translocation. The cytological analyses also suggest that this chromosome has segments of 4*A* and *CR* large enough to form chiasmata.

The substitution line originated from the $F_1$ carrying wheat (4*A*) and rye (*C*)

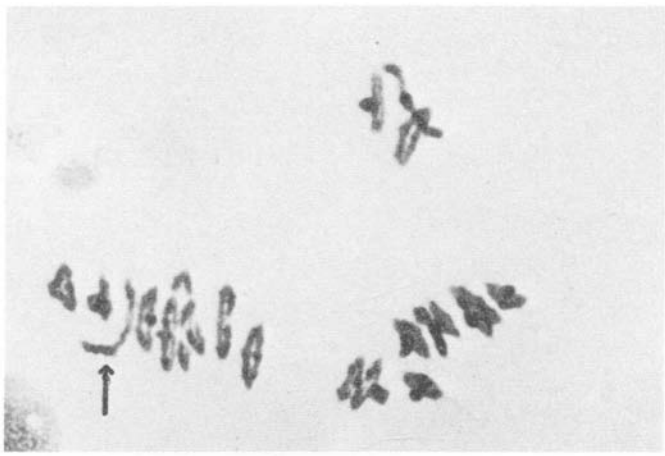

Fig. 3. Mataphase-*I* of $F_1$ from a cross ditelosomic 4*A* (alpha) × 4*A* (alpha)/$CR^L$ showing a heteromorphic bivalent (arrow).

univalents. Since wheat and rye univalents are known to misdivide at the centromere (Sears, 1952), it can be assumed that breakage and reunion has occurred at the centromere region. Alternatively, it could be the result of a crossing over between the wheat and rye chromosomes. It would be possible to infer the break points more precisely if more isozyme marker are available for these two chromosomes.

## DISCUSSION

Isozyme markers are being routinely used in determining the chromosome constitution of mammalian somatic cell hybrids and addition lines (Ruddle & Nichols, 1971; Mouse News Letter, 1975). They are being used mainly to confirm the cytological data. It was suggested that the enzyme markers can be used to determine the chromosome constitution of materials not amenable to cytological analysis (Tang & Hart, 1975). In this study we have demonstrated the utility of isozyme markers in the determination of chromosome constitution of wheat rye translocations. Since this was the first attempt in plants, we have verified the results through cytological analysis. In principle, segments of alien chromosomes not detectable by pairing behaviour can be identified by the use of isozyme markers. For this to be possible a number of isozymes have to be analysed and the genes coding for these isozymes located on to the chromosomes. Presently nine out of the fourteen (homoeologous) arms of wheat and of rye are marked with at least one enzyme marker (Hart, 1979).

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* Original not seen.