# A CYTOGENETIC STUDY IN HEXAPLOID WHEAT OF CHARACTERS DERIVED FROM *AEGILOPS SQUARROSA*.

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ABSTRACT

Monosomics and telocentrics of hexaploid wheat, Triticum aestivum ssp. vulgare were used to establish the chromosome association, chromosome arm location and crossover distance from the centromere of genes controlling characters introduced into synthetic hexaploid wheat (2n=42=AABBDD) from Aegilops squarrosa (2n=14=DD). One of the two synthetic hexaploids investigated, RL 5404, originated from a cross between Tetra Canthatch (the AABB component of the common wheat cultivar Canthatch) and Ae. squarrosa var. strangulata RL 5271. The second synthetic, RL 5406, was produced from Tetra Canthatch and Ae. squarrosa var. meyeri RL 5289. The chromosome arm location and the crossover distance from the centromere of each gene studied are as follows: RL 5404 - brown glumes, 1DL, 13.3 $\pm$ 3.3%; tenacious glumes, 2D$\alpha$, 39.4 $\pm$ 4.9%; inhibitor of waxy foliage, 2D$\alpha$, 52.5 $\pm$ 5.0%; adult-plant leaf rust resistance, 2D$\alpha$, 63.6 $\pm$ 4.8%; purple coleoptile, 7DS, 10.3 $\pm$ 2.8%. RL 5406 - brown glumes, 1DL, 1.7 $\pm$ 1.0%; tenacious glumes, 2D$\alpha$, 42.9 $\pm$ 4.6%; inhibitor of waxy foliage, 2D$\alpha$, 58.9 $\pm$ 4.6%; purple coleoptile, 7DS, 9.8 $\pm$ 2.8%. The gene for seedling leaf rust resistance found in RL 5406 is located on chromosome 1D.

Substitution of chromosome 5A of Rescue with chromsome 5A of RL 5404 produced a substitution line that was free-threshing and semi-square headed, indicating that the synthetic hexaploids RL 5404 and RL 5406 possess the Q factor on chromosome 5A and that their non free-threshing character is due to the glume tenacity gene on chromosome

arm 2D$^{\alpha}$. The synthetic hexaploids produced from Triticum turgidum ssp. carthlicum x Ae. squarrosa hybrids were also non free-threshing. These results suggest that the first hexaploid wheat must have been non free-threshing. The evolutionary implications of this finding are discussed.

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# 1. INTRODUCTION

Triticum aestivum (L.) Thell. is an allohexaploid wheat (2n=6x=42) with the genomic formula AABBDD. It is the ultimate product of the hybridization of three wild, diploid members of the Triticum-Aegilops complex (Figure 1). They are Triticum monococcum L. ssp. boeoticum (Boiss.) MK., n. comb. (2n=14=AA), Aegilops speltoides Tausch or Aegilops mutica Boiss. (2n=14=BB), and Aegilops squarrosa L. (2n=14=DD) (Morris and Sears, 1967).

The hexaploid group was, however, the last of the wheats to be brought under cultivation. Neolithic man first domesticated the diploid T. monococcum ssp. monococcum from its wild form ssp. boeoticum and the tetraploid Triticum turgidum (L.) Thell. ssp. dicoccum (Schrank) Thell. from its wild relative ssp. dicoccoides (Korn.) Thell. sometime prior to 7,000 B.C. (Helbaek, 1966). It was not until the cultivated tetraploids came into contact with Ae. squarrosa that the hexaploid wheats appeared. The earliest dating of T. aestivum by Helbaek is approximately 5,800 B.C.

It is beyond doubt that the first hexaploid arose from the hybridization of a tetraploid wheat with Ae. squarrosa. Unknown, however, is what tetraploid was involved; the geographical location of the hybridization; the characteristics of the first primitive hexaploid, and whether the hexaploids are of a mono- or polyphyletic origin. These questions are difficult to answer, because generally the genetical theories that have been put forward to account for the wide variation in form found among the hexaploids have not been substantiated by the

ORIGIN AND EVOLUTION OF TRITICUM (WHEAT)

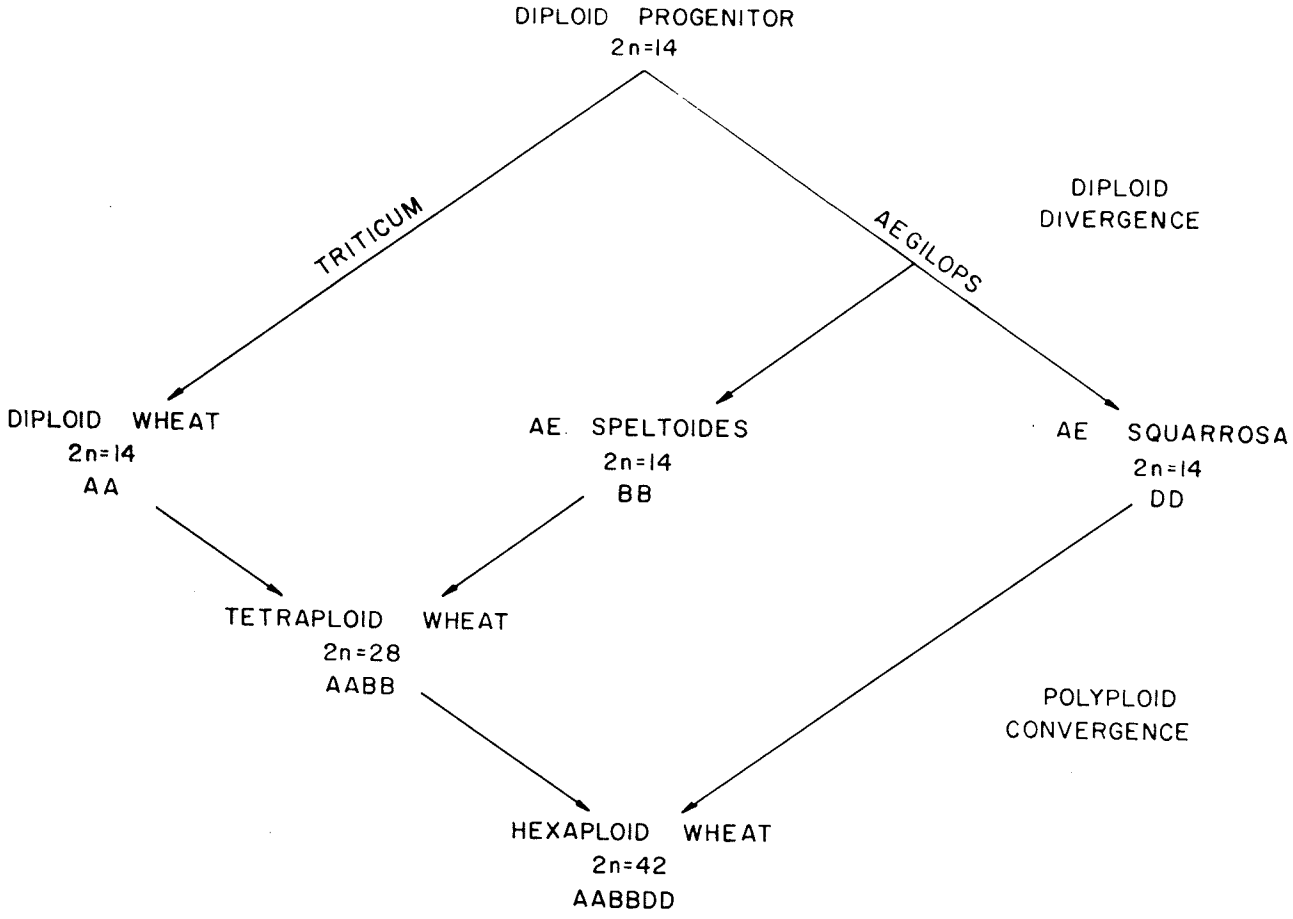



FIGURE 1

meager archeological finds.

To gain a better understanding of the evolutionary processes which took place in the origin of common wheat, numerous workers have produced and studied synthetic hexaploids obtained by crossing tetraploid wheat with Ae. squarrosa. These have also been useful in providing a practical method of transferring desirable characters from tetraploid wheats and Ae. squarrosa to breeding stocks of hexaploid wheat.

The purpose of this study was to examine two such synthetic hexaploids with three objectives in mind. First, to shed further light on the evolution of hexaploid wheat, particularly with regard to the origin of the free-threshing character; second, to determine gene-chromosome associations of characters derived from Ae. squarrosa; third, to map these genes with the aid of telocentrics.

# 2. LITERATURE REVIEW

## 2.1 The free-threshing character and the origin of hexaploid wheat

One way that a wild plant species ensures its survival is by its ability to spread over a wide area. This is true of the primitive wild members of Triticum and Aegilops. They have a fragile spike axis, the rachis, which breaks up on maturing and seeds which remain invested in the spikelets due to the tenacious glumes enclosing them. The spikelets then become attached to passing animals by their barbed awns resulting in wide seed dispersal for these species. However, it would have been an advantage for Neolithic man to obtain plants that had a non-brittle rachis and less tenacious glumes if he wished to maximize his yield of chaff-free seed. Helbaek (1959) considers that this was done by the unconscious selection of free-threshing wheat plants during the harvesting and threshing process, since they would contribute most of the chaff-free seed.

In wheat, a spike which upon vigorous rubbing does not yield naked seed is considered non free-threshing. That is, the glumes are so tough that they retain the seed within the spikelet. In free-threshing wheats a minimal amount of pressure will cause the glumes to break up and the naked seed fall free from the lemma and palea. From genetic studies it has become evident that numerous minor and two major mutations had to be selected for to produce the free-threshing hexaploids. First, there is the polygenic system scattered through all three genomes which counteracts rachis brittleness and tough glumes

(MacKey, 1966); second, the Q factor on chromosome 5A which also suppresses these primitive tendancies (MacKey, 1954); and third, an allele of the gene for tenacious glumes, apparently introduced with the D genome from Ae. squarrosa (Kerber and Dyck, 1969), which evidently must be present to impart complete free-threshability.

Of all three systems, the Q factor, which is hemizygous ineffective, has the greatest visible effect on the hexaploid wheat plant. When T. aestivum ssp. vulgare (Vill.) MK, is monosomic or nullisomic for chromosome 5A the plant is taller than normal and the spike is longer and laxer, resembling T. aestivum ssp. spelta (L.) Thell. in appearance. The glumes are also much tougher and the rachis is more brittle (MacKey, 1954; Sears, 1954). The Q factor, with one exception, is found only in the hexaploid wheats, while the diploid and tetraploid members of Triticum apparently carry the recessive q allele. A classification list of the species of Triticum mentioned in this study along with the allele carried at the 5A speltoid locus is found in Table I.

Muramatsu (1963) presented evidence that Q is likely a triplicate of its allele q, as was suggested by Sears (1954). Muramatsu found that five doses of the long arm of chromosome 5A of ssp. spelta, which carries q, produced the normal squareheaded condition in the ssp. vulgare cultivar Chinese Spring. Doses of less than five produced a speltoid spike. From these results it appears that MacKey's (1954) hypothesis that Q is similar to the Bar locus in Drosophila, in that it was built up by unequal crossing-over, is likely correct. By itself,

TABLE I

Classification of *Triticum* and presence of Q or q allele[1].

| | | Diploid group 2n=14=AA | Q or q allele | Tetraploid group 2n=28=AABB | Q or q allele | Hexaploid group 2n=42=AABBDD | Q or q allele |
|---|---|---|---|---|---|---|---|
| Wild forms | | *T. monococcum* L. ssp. *boeoticum* (Boiss.) MK., n. comb. | q | *T. turgidum* (L.) Thell. ssp. *dicoccoides* (Korn.) Thell. | q | | |
| Cultivated forms | Non free-threshing | *T. monococcum* ssp. *monococcum* | q | *T. turgidum* ssp. *dicoccum* (Schrank) Thell. | q | *T. aestivum* (L.) Thell. ssp. *spelta* (L.) Thell. | q |
| | | | | | | *T. aestivum* ssp. *macha* (Dek. et Men.) MK. | q |
| | | | | | | *T. aestivum* ssp. *vavilovii* (Tum.) Sears | q |
| | Free-threshing | | | *T. turgidum* ssp. *turgidum* conv. *durum* (Desf.) MK., n. comb. | q | *T. aestivum* ssp. *vulgare* (Vill.) MK. | Q |
| | | | | *T. turgidum* ssp. *carthlicum* (Nevski) MK., n. comb. | Q | *T. aestivum* ssp. *compactum* (Host) MK. | Q |
| | | | | | | *T. aestivum* ssp. *sphaerococcum* (Perc.) MK. | Q |

[1] After MacKey (1966)

however, Q cannot completely suppress rachis brittleness and glume toughness. It appears to be interwoven with an older polygenic system that has been derived from the free-threshing tetraploids (MacKey, 1954; 1966). MacKey believes that many minor mutations which increase rachis toughness and glume fragility in the tetraploids were selected for under cultivation. That many genes are involved is shown by the differences in rachis fragility he has found in speltoid mutations in various varieties of ssp. vulgare. Muramatsu (1963) suggests that all chromosomes in homoeologous group 5 have an effect in reducing glume toughness. Sears (1954) described the nullisomics of group 1 as having stiffer glumes than normal.

Swaminathan (1966) and Tsunewaki (1966), on the other hand, are of the opinion that there may be different strengths of Q in the free-threshing tetraploids. Tsunewaki in particular speculates that the free-threshability of T. turgidum ssp. turgidum conv. durum (Desf.) MK., n. comb. is due to a less effective Q allele. One tetraploid is known, however, that apparently owes its free-threshing character to the same gene that is found in the hexaploids; this is T. turgidum ssp. carthlicum (Nevski) MK. n. comb. McFadden and Sears (1946) crossed ssp. carthlicum with ssp. spelta and recovered free-threshing hexaploid segregates. Also, MacKey (1966) crossed a speltoid mutant with ssp. carthlicum and obtained some plants that resembled ssp. vulgare. On the basis of these results it would seem that the Q factor is present in ssp. carthlicum.

Ae. squarrosa apparently carries a dominant gene that results in non free-threshing plants at the hexaploid level. This did not become evident until Kerber (1964) extracted the AABB component of the ssp. vulgare variety Canthatch and crossed it with two forms of Ae. squarrosa to produce synthetic hexaploids. These two synthetics have been designated RL 5404 (Tetra Canthatch x Ae. squarrosa var. strangulata RL 5271) and RL 5406 (Tetra Canthatch x Ae. squarrosa var. meyeri RL 5289). Even though both apparently carry the Q factor they are non free-threshing (Kerber and Dyck, 1969; Dyck and Kerber, 1970). As far as is known all strains of Ae. squarrosa carry this gene for glume tenacity.

Morris and Sears (1967) classified five of the hexaploid sub-species into four groups on the basis of three genes; the Q factor on chromosome 5A, the compactum gene C on chromosome 2D, and the recessive sphaerococcum gene s on chromosome 3D. The groups are genetically designated as follows:

| | | |
|---|---|---|
| ssp. spelta, ssp. macha | qq cc SS | speltoid spike |
| ssp. vulgare | QQ cc SS | square-headed |
| ssp. compactum | QQ CC SS | compact spike |
| ssp. sphaerococcum | QQ cc ss | compact spike, spherical seeds |

Thus, ssp. vulgare is only one mutation removed from the other three groups. T. aestivum ssp. vavilovii (Tum.) Sears is not closely related to these groups (Morris and Sears, 1967) and does not seem to be of major importance in wheat evolution.

McFadden and Sears (1946) crossed the tetraploid ssp. dicoccoides with Ae. squarrosa. The resulting synthetic hexaploid resembled ssp. spelta in appearance and this led them to postulate that ssp. spelta was the original hexaploid, resulting from a cross between ssp. dicoccoides or ssp. dicoccum, which carry the q allele, and Ae. squarrosa. Schiemann (1951) took exception to this theory, pointing out that T. antiquorum, which from archeological remains appears to be a variety of the hexaploid T. aestivum ssp. compactum (Host) MK., is found in Europe prior to ssp. spelta. Helbaek (1966) places T. antiquorum in Europe about 3,000 B.C. and ssp. spelta at 2,000 B.C. Schiemann claims that ssp. spelta resulted from the crossing of the tetraploid ssp. dicoccum with ssp. compactum, a theory supported by MacKey (1966) who recovered speltoid segregates from this cross.

McFadden and Sears (1946), however, had considered T. antiquorum to be the tetraploid forerunner of the modern day ssp. carthlicum and that both ssp. vulgare and ssp. compactum resulted from crosses between this wheat and ssp. spelta. This theory was advanced to account for the fact that free-threshing hexaploids were not thought to have existed at an early enough date to account for T. antiquorum being a variety of ssp. compactum. Kuckuck (1965) also maintains that T. antiquorum could have been a tetraploid as he has found compactoid mutants in irradiation experiments on the tetraploid ssp. dicoccum. Since then Helbaek (1966) has reported finding evidence that ssp. vulgare occurred in the middle-east as early as 5,800 B.C. Consequently, Morris

and Sears (1967) now consider T. antiquorum to have been a hexaploid.

Schiemann (1951) had based her argument that ssp. spelta was of European origin on the fact that ssp. spelta had never been found outside Europe. Subsequently, Kuckuck (1959) reported finding ssp. spelta growing in Iran. Because of these Asian finds he considered that all spelt wheats had a common origin in western Asia (Kuckuck, 1964). This gave strength to the hypothesis of McFadden and Sears (1946) and later to that of Andrews (1964) that ssp. spelta was brought into Europe from western Asia as a small component of a mixture of emmer and einkorn wheats. It was not until it reached southwestern Germany and northern Switzerland that a favourable climate allowed it to emerge as a major crop.

A study of the distribution of necrosis genes in wheat led Tsunewaki (1971) to the conclusion that European and Iranian spelts are of different origins. On the basis of these necrosis genes he differentiated both spelt and free-threshing hexaploid wheats into Asian and Western types. Considering the mutational probabilities of the various necrosis alleles he postulated that European ssp. vulgare has developed from the Asian ssp. vulgare and that European ssp. spelta is the result of hybridization between the European ssp. vulgare and the tetraploid ssp. dicoccum. He stated that ssp. compactum could not be the hexaploid involved, as was suggested by Schiemann (1951) and MacKey (1966) because ssp. spelta does not possess the C gene. However, Schiemann has pointed out that ssp. compactum was found in Europe prior to ssp. vulgare in a mixture of einkorn and emmer wheats. MacKey (1954) had considered ssp.

compactum to be the oldest hexaploid but Morris and Sears (1967) view this to be unlikely as no form of Ae. squarrosa is known that carries the C gene which is found on chromosome 2D. It is doubtful that it could be tolerated at the diploid level.

Riley et al. (1967) crossed the ssp. vulgare cultivar Chinese Spring with ssp. dicoccoides and Ae. squarrosa. They observed no multivalents at metaphase I in these hybrids and thus concluded that Chinese Spring had the primitive chromosome structure of hexaploid wheat. They then crossed Chinese Spring with a number of subspecies of T. aestivum and concluded that only ssp. vulgare or ssp. spelta could have been the first hexaploid as both had members with the primitive chromosome structure. They found that among the spelts only those of Iranian origin had the primitive arrangement, whereas interchanges were present in hybrids with European spelts.

Transcaucasia and northern Iran are considered the two most probable areas of origin of the hexaploids. Tsunewaki (1966), studying genes for waxiness and growth habit in the diploid and tetraploid progenitors of the hexaploids, concluded that the southwestern coastal area of the Caspian Sea in northern Iran is the likely area of origin. This is the only region in which members of Ae. squarrosa are found that are waxy and possess a strong winter habit. Both of these characters are found in present day hexaploids. Zohary et al. (1969) also concurred that this is the area of origin because it is a primary habitat of Ae. squarrosa whereas Transcaucasia is not, although extensive stands of Ae. squarrosa have been found in this area.

Kuckuck (1964) and Dorofeev (1966), on the other hand, are of the opinion that the hexaploids first appeared in Transcaucasia. Dorofeev also states that T. aestivum ssp. macha (Dek. et Men.) MK. was the progenitor of the other hexaploids, although Tsunewaki (1971) considered this unlikely due to the nearly complete isolation barrier between ssp. macha and other hexaploids caused by chlorosis genes. Kuckuck (1970), however, reported that Dekaprelovich has found a wild variety of ssp. macha growing in Transcaucasia, and since no wild hexaploid has previously been known he considered this as further evidence for Transcaucasia being the center of origin of the hexaploids.

MacKey (1954; 1966) speculated that ssp. carthlicum, the only Q bearing tetraploid, may be the tetraploid progenitor of the hexaploids. This idea is further strengthened by the knowledge that ssp. spelta has never been found in prehistoric finds in western Asia (Helbaek, 1966). Also, ssp. carthlicum is found growing in the same area as Ae. squarrosa (Morris and Sears, 1967) and triploid hybrids of these two closely resemble ssp. vulgare (Kihara and Lilenfeld, 1949). MacKey (1966), however, also points out that the alternative view put forward by McFadden and Sears (1946), that ssp. vulgare arose from hybridization of ssp. spelta and ssp. carthlicum, is just as realistic.

Since ssp. carthlicum is found only in the Russian state of Georgia (Transcaucasia), Morris and Sears (1967) have suggested that this indicates a much more recent origin for this group than for the hexaploids. It may be a segregate from a hybrid of some tetraploid and a free-threshing hexaploid. MacKey (1966) has obtained segregates from

a cross between ssp. vulgare and ssp. dicoccum that resemble ssp. carthlicum. Further evidence against ssp. carthlicum being the tetraploid progenitor is the finding by Riley et al. (1967) that it does not have the primitive chromosome structure.

Zohary et al. (1969) reported that hybridization of tetraploids and hexaploids with Ae. squarrosa can be detected in Iran today. They speculated that because of this fact the hexaploids are likely the result of more than one hybridization event. A number of races and forms of tetraploids and Ae. squarrosa probably were involved. This theory of a polyphyletic origin of the hexaploids is also supported by Swaminathan (1966) and Kuckuck (1964).

## 2.2 Leaf rust resistance

Kerber and Dyck (1969) reported the finding of a partially dominant gene for seedling resistance to leaf rust, Puccinia recondita Rob. ex. Desm., in the synthetic hexaploid RL 5406. This gene, which was derived from its Ae. squarrosa parent, gave a type 0;1 reaction to leaf rust races 1, 5, 9, 11, 15, 30, 58, and 126a. This gene is different from seven other known seedling leaf rust resistance genes, Lr1, Lr2, Lr3, Lr10, Lr16, Lr17, and Lr18.

Subsequently, Dyck and Kerber (1970) found a partially dominant gene for adult-plant leaf rust resistance in the synthetic hexaploid, RL 5404. This gene was derived from a different variety of Ae. squarrosa. It gave a type $1^+$ reaction to leaf rust races, 1, 5, 9, 15, and 126a and is different from two other known adult-plant

resistance genes, Lr12 and Lr13.

## 2.3 Glume colour

Glume colour shows up only after the wheat plant has matured. Genes controlling black, brown, or red glumes are dominant over those for white glumes and have been found on chromosomes1A (Ausemus et al., 1967) and 1B (Unrau, 1950). Metzger and Silbough (1970) reported a gene for brown glumes on chromosome 1B of cultivar P.I. 178383 that is linked by $2.00 \pm .30$ recombination units with a gene for stripe rust resistance. Kerber and Dyck (1969), working with the synthetic RL 5406 had previously found a gene for brown glumes linked by $3.1 \pm 1.1$ recombination units with a gene controlling seedling leaf rust resistance.

## 2.4 Waxy bloom

Waxy bloom is found on most cultivated tetraploids and hexaploids. The wax is usually most evident on the flag-leaf sheath, the peduncle, and the spike. Allan and Vogel (1960) found a gene for waxiness on chromosome 2B in a heavily waxy durum selection. Driscoll (1966), using telocentrics, located an epistatic inhibitor of waxiness between 42.3 to 50 crossover units from the centromere on the non-standard arm of chromosome 2B. Tsunewaki (1966) reported finding a dominant gene controlling waxy foliage on chromosome 2B of Chinese Spring. He also located an epistatic inhibitor of waxiness on chromosome 2D of a non-waxy synthetic hexaploid that was produced by crossing a waxy tetraploid with a non-waxy Ae. squarrosa strain. Furthermore,

he concluded that there must be a gene for waxiness in the D genome as there are waxy strains of Ae. squarrosa. In a later paper (Tsunewaki, 1968) he placed this dominant waxy gene on chromosome 2D, although no evidence was given.

Following Tsunewaki's (1968) system of nomenclature, there are two epistatic inhibitors of waxiness, $I_1W$ (2B) and $I_2W$ (2D), and two dominant waxy genes, $W_1$ (2B) and $W_2$ (2D). Most forms of ssp. vulgare carry the genes $W_1$, $W_2$, $i_1W$, and $i_2W$ and are waxy. Chinese Spring which is slightly waxy, is $W_1$, $w_2$, $i_1W$, and $i_2W$. The tetraploid conv. durum is $W_1$ and $i_1W$ and is heavily waxy while ssp. dicoccoides is $w_1$ and $I_1W$ and is non-waxy.

Kerber and Dyck (1969) and Dyck and Kerber (1970) reported that the synthetics RL 5404 and RL 5406 are both non-waxy. Since this character is inherited from Ae. squarrosa it is likely determined by the same epistatic inhibitor, $I_2W$, found by Tsunewaki on chromosome 2D. In RL 5404 this gene was found to be linked with the genes for adult-plant leaf rust resistance and non-free threshability by $15.6 \pm 2.5$ and $17.4 \pm 2.5$ recombination units, respectively. In RL 5406 the non-waxy gene was linked with the non-free threshing gene by $15.1 \pm 2.6$ recombination units.

## 2.5 Coleoptile colour

The red or purple colour of coleoptiles in some varieties of wheat is caused by anthocyanins. Sears (1954) found that the variety Hope carried a gene for red coleoptile on chromosome 7A.

Gale and Favell (1971) also found that chromosome 7B of Hope was involved in pigment production. Jha (1964) found a gene for purple coleoptile on chromosome 7D of a synthetic hexaploid of ssp. dicoccoides x Ae. squarrosa. Kerber and Dyck (1969) concluded that the gene for purple coleoptile in the synthetics RL 5404 and RL 5406 is probably the same as that found by Jha. Tahir and Tsunewaki (1969) located genes for coleoptile pigmentation on chromosome 7A and 7D in a variety of ssp. spelta.

# 3. MATERIALS AND METHODS

## 3.1 Plant material

The classification of Triticum proposed by MacKey (1966) will be followed in this study (Table I). MacKey's system was chosen over that of Morris and Sears (1967) because they combined Aegilops with Triticum. Since most workers still refer to Aegilops, it was felt that the classification of Morris and Sears would cause some confusion. Furthermore, Morris and Sears group ssp. spelta and ssp. macha under T. aestivum var. spelta but Tsunewaki (1971) found that there was a partial isolation barrier between ssp. macha and all other forms of the hexaploids.

The following plant material was used in this investigation.

a) Tetra Canthatch (TC). The tetraploid (2n=28=AABB) component extracted from ssp. vulgare cv. Canthatch (Cth) (Kerber, 1964).

b) RL 5404. A synthetic hexaploid produced by combining Tetra Canthatch with the Ae. squarrosa var. strangulata RL 5271 (Dyck and Kerber, 1970).

c) RL 5406. The synthetic hexaploid of Tetra Canthatch x Ae. squarrosa var. meyeri RL 5289 (Kerber and Dyck, 1969).

d) Ssp. vulgare cv. Rescue (Rsc).

e) Ssp. vulgare cv. Chinese Spring (CS).

f) The complete monosomic series of Rescue.

g) The D genome monosomics of Chinese Spring.

h) Chinese Spring ditelocentric 1DL, ditelo-2D$\alpha$, ditelo-$\beta$ monotelo-$\alpha$ 2D, ditelo-7DS, and double ditelo-2D, all of which were supplied by Dr. E. R. Sears.

i) Ssp. carthlicum varieties RL 5415, RL 5205, RL 5320, and RL 5414.

j) Ae. squarrosa varieties typica RL 5261, strangulata RL 5271, and meyeri RL 5289.

k) The synthetic hexaploid ssp. carthlicum RL 5415 x Ae. squarrosa var. strangulata RL 5271.

l) A spontaneous speltoid mutant of ssp. vulgare cv. Manitou which was found to be monosomic for chromosome 5A.

m) The $F_1$ pentaploids (2n=35=AABBD) of Canthatch x Tetra Canthatch and Canthatch x conv. durum cv. Stewart.

Table II shows the important characters of the parental stocks used in this study. The aneuploids of Rescue and Chinese Spring are identical with their respective disomics for these characters. The leaf rust infection was classified using the system described by Stakman et al. (1962).

The following temporary gene symbols are used. Adult-plant leaf rust resistance, Lra; seedling leaf rust resistance, Lrs; brown glumes, Gc; glume tenacity, T; inhibitor of waxy foliage, $I_2W$; purple coleoptile, P.

## 3.2 Cytological techniques

In mitotic investigations root tips were either collected from

TABLE II

Seedling reaction to leaf rust race 1, adult-plant reaction to leaf rust race 76, waxiness, threshability, glume colour and coleoptile colour of parental stocks used in cytogenetic studies.

| Variety | Leaf rust reaction | | Waxiness | Threshability | Glume colour | Coleoptile colour |
|---|---|---|---|---|---|---|
| | seedling (race 1) | adult plant (race 76) | | | | |
| RL 5404 | - | 1+ | non-waxy | non free-threshing | brown | purple |
| RL 5406 | ;1,;2 | - | non-waxy | non-free-threshing | brown | purple |
| Rsc | 3+ | - | waxy | free-threshing | white | purple |
| CS | 4 | 3 | waxy | free-threshing | white | green |

seedlings that had been germinated on moist blotting paper or from plants after they had begun to tiller. The excised root tips were then placed in ice-water for 20-24 hours after which they were fixed in Farmer's fluid (3 85% ethyl alcohol:1 glacial acetic acid) for at least two days. After removal from the fixative the root tips were hydrolized in 1N HCl for 10 minutes, transferred to vials containing Feulgen solution, and stained for at least 10 minutes. A piece of the stained meristematic tip was cut away and placed on a slide with a drop of aceto-carmine. A cover slip was placed over the specimen and the cells were broken up by tapping with an eraser pencil. The cells were squashed by heating the slide and applying pressure on the coverslip. The chromosome number was recorded from observations of cells at the metaphase stage.

For meiotic studies young spikes were fixed in Carnoy's fluid (6 85% ethyl alcohol:3 chloroform:1 glacial acetic acid) for a minimum of three days. An anther was then placed on a slide along with a drop of aceto-carmine, broken open, and examined under low power for cells at metaphase I of meiosis. If cells at metaphase I were found, a cover slip was placed over the specimen and pressure applied to squash the cells. The number of chromosomes and their pairing behavior was then recorded.

### 3.3 Monosomic analysis

The monosomic method as outlined by Sears (1953) was followed. The complete set of 21 monosomics of Rescue and the seven monosomics

of the D-genome of Chinese Spring were crossed with the synthetic hexaploids RL 5404 and RL 5406. Monosomic $F_1$ plants of these crosses were cytologically selected and harvested. Disomic Rescue and Chinese Spring were also crossed with the synthetics.

In the spring of 1969 approximately 125 $F_2$ seeds of each monosomic and disomic cross, plus the parents were space seeded at the Canada Department of Agriculture Experimental Farm near Winnipeg. The only exceptions were crosses involving Rescue monosomics 5B, 2D, 7D and the cross Rescue 5A x RL 5406. They were planted in the spring of 1970. The $F_2$ populations involving Chinese Spring were heavily infected by stem rust in 1969 and had to be discarded. They were again planted in 1970 and protected from stem rust by application of the fungicide Maneb.

In the field, individual plants in each $F_2$ population were classified as waxy or non-waxy just after heading. The $F_2$ populations involving the disomic and monosomics of Rescue were classified as either resistant (;1,2) or susceptible (3,4) to natural inoculum of leaf rust by comparison with the parents. At maturity all monosomic and disomic populations were harvested by pulling individual plants which were then examined in the laboratory for glume colour and threshability. Threshability of the spikes was determined by the use of a rubbing board as described by Kerber and Dyck (1969).

The $F_2$ populations of the Rescue monosomics x RL 5406 and the Chinese Spring monosomics x RL 5406 were also tested for seedling leaf rust reaction in the greenhouse. Seedlings at the one or two leaf stage were wetted down with water to which a drop of Tween 20 had

been added. Spores of leaf rust race 1 were mixed with talcum powder and puffed onto the seedlings. The inoculated pots were placed in an inoculation chamber for approximately 24 hours. High humidity in the chambers was maintained by periodic spraying with water. The seedlings were classified as either resistant or susceptible 12-14 days after inoculation.

The $F_2$ data was tested for goodness of fit to a 3:1 ratio using the Chi-square test corrected for continuity. The disomic $F_2$ populations were also tested for linkage of the various characters using a Chi-square test for independence. The recombination values were determined by the product method as outlined by Immer (1930).

Since each one of the characters under examination was known to be controlled by a single dominant or partially dominant gene, only one critical monosomic $F_2$ population per character was expected. However, in the 1969 field plots more than one monosomic $F_2$ population appeared to be significant for the segregation of some of the characters. In 1970 approximately 50 random $F_3$ head rows were planted of each population that deviated significantly from the expected ratio in the $F_2$. Unfortunately, just after emergence most of the plot area was flooded by a heavy rain storm that destroyed many seedlings. The plot area remained very wet for the rest of the summer. Due to the small number of plants in some $F_3$ lines, classification of all lines was on the basis of the $F_2$ phenotype.

To test for coleoptile colour approximately 50 random $F_3$ lines of each Chinese Spring monosomic cross were used. A minimum of 20 seeds

per $F_3$ line were placed on moist filter paper in petri dishes and grown at a temperature of 60°F. A relatively cool temperature seemed to enhance the coleoptile pigmentation. The lines were classified as homozygous purple, segregating, or homozygous green. The results were analyzed using the Chi-square test for a 1:2:1 ratio.

3.4 Telocentric method

After the genes had been associated with their respective chromosomes, it was possible to use Sears' (1966) telocentric method to determine on what arm these genes were located and their linkage distance from the centromere (Figure 2). Chinese Spring ditelo-1DL, ditelo-2D$\alpha$, ditelo-$\beta$monotelo-$\alpha$ 2D, and ditelo-7DS were crossed as the female to the synthetic hexaploids RL 5404 and RL 5406. Because of their male sterility, the $F_1$ plants were used as the female in back-crosses to Chinese Spring. Telocentrics of 1DS and 7DL were not available.

Since both arms of chromosome 2D were used, a preliminary test was carried out to determine on which arm the genes for glume tenacity and inhibition of waxy foliage were located. It was assumed that these genes and their chromosome arm locations were common to both RL 5404 and RL 5406. Chinese Spring ditelo-2D$\beta$ could not be used directly because ditelocentrics of this line are unstable unless accompanied by the monotelosome-2D$\alpha$. Thirty-three $BC_1F_1$ plants of (Chinese Spring ditelo-2D$\alpha$ x RL 5406) x Chinese Spring and thirty $BC_1F_1$ plants of (Chinese Spring ditelo-$\beta$ monotelo-$\alpha$ 2D x RL 5406) x

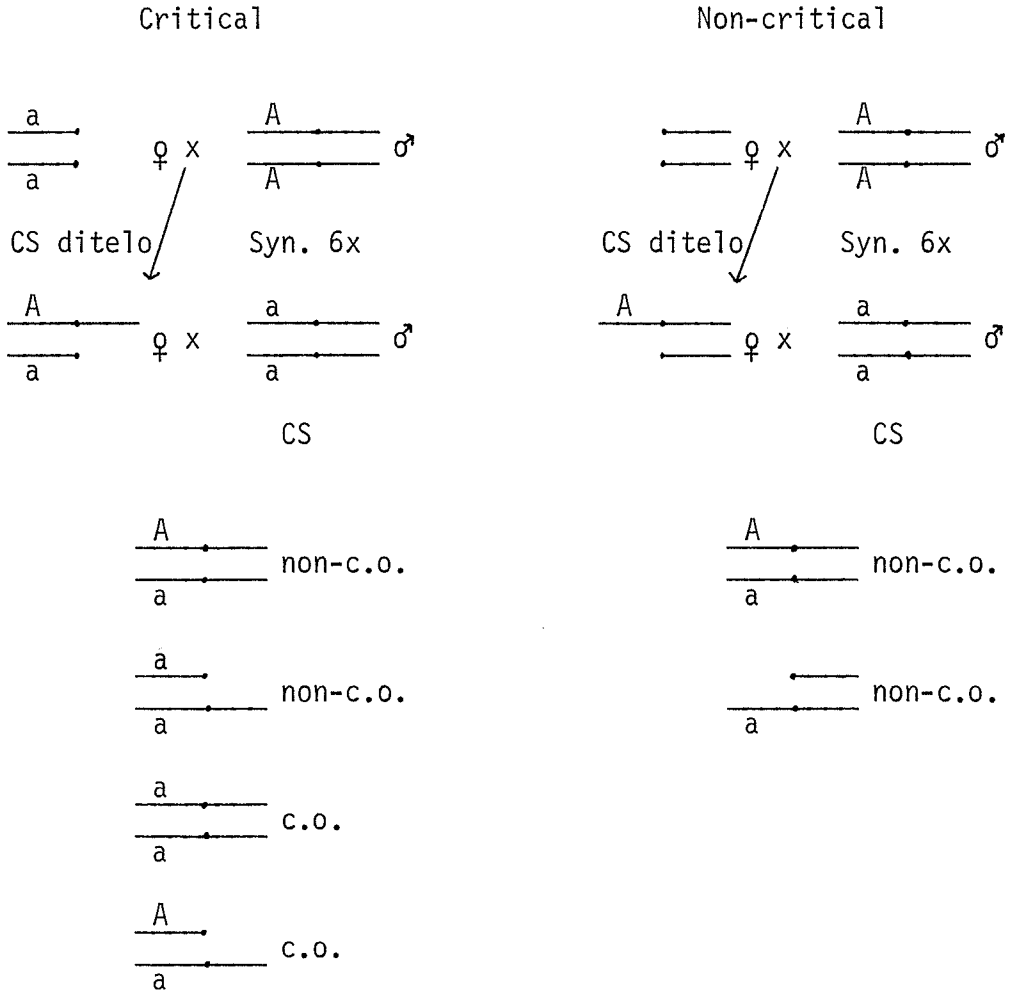



FIGURE 2

Method used for determining which arm carries the gene and the segregation products expected.

Chinese Spring were grown. All plants were checked for chromosome number and classified for waxiness and threshability.

Approximately 200 $BC_1F_1$ seeds of each of (Chinese Spring ditelo-1DL x RL 5404) x Chinese Spring, (Chinese Spring ditelo-1DL x RL 5406) x Chinese Spring, (Chinese Spring ditelo-2D$\alpha$ x RL 5404) x Chinese Spring, and (Chinese Spring ditelo-2D$\alpha$ x RL 5406) x Chinese Spring were planted in the greenhouse. The chromosome number of at least a half of the plants from each $BC_1$ population was determined. $BC_1$ populations involving 1DL were classified for glume colour while those derived from 2D were classified for waxiness and threshability.

$BC_1F_1$ seedlings of (Chinese Spring ditelo-1DL x RL 5406) x Chinese Spring were inoculated with leaf rust race 1, as previously described, and classified resistant (;,;2) or susceptible ($2^+$,4). $BC_1F_1$ adult plants of (Chinese Spring ditelo-2D$\alpha$ x RL 5404) x Chinese Spring were tested for adult plant leaf rust resistance using leaf rust race 76. Race 76 was used because it will differentiate between the adult plant resistance of RL 5404 and that of Chinese Spring. The flag leaf of three stems of each plant were wetted with water and inoculated with rust spores by fingering. The plants were then placed in inoculation chambers for approximately 24 hours. Plants which scored $2^{\pm}$ or less were classified as resistant and those scored 3 or higher were considered susceptible.

Backcross $F_1$ plants that could not be properly classified for a specific character were progeny tested to determine their phenotype. A minimum of 10 plants from each doubtful $BC_1F_1$ plant were grown and

classified as previously described.

For determining the distance of the purple coleoptile gene from the centromere, $BC_1F_1$ seeds of (Chinese Spring ditelo-7DS x RL 5404) x Chinese Spring and (Chinese Spring ditelo-7DS x RL 5406) x Chinese Spring were grown as described for the monosomic method except that individual seedlings were classified for colour and their chromosome constitution was determined from root tips.

The data gathered from each of the $BC_1F_1$ populations was organized into 2 x 2 contingency Tables and a $X^2$ test applied to determine if the genes in question were inherited independently of the centromere.

3.5 Substitution of chromosome 5A of the synthetic hexaploids into Rescue

It was possible that the non free-threshing character of the synthetic hexaploid was due to the absence of the Q factor on chromosome 5A of their tetraploid parent Tetra Canthatch. In order to verify that RL 5404 and RL 5406 do carry the Q factor, chromosome 5A of Rescue was substituted with the corresponding chromosome of these synthetics. These substitution lines should be as free-threshing as Rescue if RL 5404 and RL 5406 do in fact possess Q on chromosome 5A. Rescue monosomic 5A was crossed as the female to each of the synthetics. Monosomic $F_1$ plants were then used as the male in backcrossing to Rescue monosomic 5A. Approximately 96% of the male pollen will have 21 chromosomes (Sears, 1953), which includes chromosome 5A from the

synthetics. After each backcross 41 chromosome plants were selected and backcrossed as the male to Rescue monosomic 5A. After seven backcrosses the monosomic plants of Rescue$^{8}$ x RL 5404 were selfed and disomic plants were selected in the $F_2$ progeny. Spikes from these plants were then tested for threshability. The Rescue$^{8}$ x RL 5406 monosomic plants were selfed but the $F_2$ progeny had a poorly developed root system so chromosome numbers were not determined. However, 42 chromosome plants had been checked after each backcross for threshability.

In the monosomic method of substitution it is possible that a 20 chromosome male gamete will function and fertilize a 21 chromosome egg. The frequency of this occurrence is approximately 1% (Sears, 1953). If this plant were selected for backcrossing, chromosome 5A of the synthetic would have been lost. Rescue has a solid stem whereas RL 5404 and RL 5406 have a hollow-stem. Since it is known that chromosome 5A of Rescue carries genes for stem solidness (Larson and MacDonald, 1962), 42 chromosome plants from the selfed monosomic $BC_7$ plants should have more hollow stems than Rescue if chromosome 5A of the synthetics has indeed been substituted into Rescue. The main culm of both Rescue and 42 chromosome progeny of selfed monosomic $BC_7$ plants were sliced lengthwise and their stem solidness compared.

### 3.6 Production of synthetic hexaploids

The following synthetic hexaploids were produced to determine if ssp. carthlicum x Ae. squarrosa synthetic hexaploids are in fact free-threshing, as was stated by Tsunewaki (1966).

ssp. carthlicum RL 5320 x Ae. squarrosa RL 5289

ssp. carthlicum RL 5320 x Ae. squarrosa RL 5261

ssp. carthlicum RL 5414 x Ae. squarrosa RL 5261

ssp. carthlicum RL 5205 x Ae. squarrosa RL 5271

The ssp. carthlicum parents were used as the female. Approximately 20 days after pollination embryos of immature seeds were excised and cultured in glass vials containing orchid agar. When the first leaf was approximately 5 cm long the seedlings were transplanted to pots and placed in a growth chamber. These hybrids were cytologically confirmed to be 2n=21=ABD; the chromosome number was doubled using the colchicine cotton-wool method outlined by Bell (1950). After the plants had begun tillering cotton-wool was wrapped around the crown and soaked with a 0.1% aqueous solution of colchicine. A glass jar was then inverted over the plant to prevent evaporation. The cotton-wool was soaked with the colchicine solution every morning for 4 days. All heads were bagged to ensure that no outcrossing would occur. Some of the seed produced by these plants was planted and their chromosome number was checked. After ripening, the heads of $F_2$ 2n=42=AABBDD plants were collected and tested for threshability. The synthetic hexaploid ssp. carthlicum RL 5415 x Ae. squarrosa RL 5271 previously produced was also checked for threshability.

## 3.7 Checking for univalent shift

A particular monosomic line may become monosomic for another chromosome as the result of univalent shift (Person, 1956). When

univalent shift was suspected in this study the particular monosomic line was crossed with the ditelocentrics or double ditelocentrics for that chromosome. Depending on the telocentric used, $F_1$ plants that had 40 + t or 40 + 2t were selected. If univalent shift had occurred the telocentric chromosome would be paired at metaphase I of meiosis. If there had been no shift the telocentric would be unpaired.

# 4. RESULTS

## 4.1 Disomic linkage values

The results from linkage studies of the disomic crosses confirmed the gene associations found by Kerber and Dyck (1969) and Dyck and Kerber (1970) in the synthetic hexaploids RL 5404 and RL 5406. $F_2$ recombination values between the gene for glume tenacity and the inhibitor of waxy foliage in RL 5404 and RL 5406 were calculated from the data in Table III and are as follows: Chinese Spring x RL 5404, 30.9 $\pm$ 4.3%; Rescue x RL 5404, 27.2 $\pm$ 3.6%; Chinese Spring x RL 5406, 21.7 $\pm$ 4.5%; and Rescue x RL 5406, 23.3 $\pm$ 3.4%. Chi-square tests for heterogeneity were applied to the data from Table III. Since the tests were not significant the data for the crosses involving RL 5404 were pooled on the one hand and the data for RL 5406 crosses were pooled on the other. These pooled data gave recombination values between the glume tenacity gene and the inhibitor of waxy foliage of 28.2 $\pm$ 2.7% and 21.6 $\pm$ 2.7% for RL 5404 and RL 5406, respectively. The data from the $F_2$ of Rescue x RL 5404 (Tables IV and V) showed that the gene for adult plant leaf rust resistance is linked with the gene for glume tenacity and the inhibitor of waxy foliage by recombination values of 33.0 $\pm$ 4.0% and 18.3 $\pm$ 2.9%, respectively. The data from Table VI showed the genes for seedling leaf rust resistance and brown glumes were associated by a recombination value of 10.1 $\pm$ 2.2% in RL 5406.

TABLE III

Data used for estimating linkage between the gene for glume tenacity and the inhibitor of waxy foliage in the $F_2$ populations of CS x RL 5404, Rsc x RL 5404, CS x RL 5406, and Rsc x RL 5406.

| | Waxiness | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | CS x RL 5404 | | Rsc x RL 5404 | | CS x RL 5406 | | Rsc x RL 5406 | |
| Threshability | Non-waxy | waxy | Non-waxy | waxy | Non-waxy | waxy | Non-waxy | waxy |
| Non free-threshing | 54 | 10 | 54 | 13 | 29 | 3 | 62 | 15 |
| Free-threshing | 10 | 8 | 13 | 19 | 9 | 10 | 6 | 13 |

TABLE IV

Data used for estimating linkage between the gene for adult-plant leaf rust resistance and the gene for glume tenacity in the $F_2$ population of Rsc x RL 5404.

| | Threshability | |
|---|---|---|
| Leaf rust reaction | Non-free threshing | Free-threshing |
| Resistant | 59 | 20 |
| Susceptible | 9 | 11 |

TABLE V

Data used for estimating linkage between the gene for waxiness and the gene for adult-plant leaf rust resistance in the $F_2$ population of Rsc x RL 5404.

| Leaf rust reaction | Waxiness | |
|---|---|---|
| | Non-waxy | Waxy |
| Resistant | 64 | 16 |
| Susceptible | 4 | 16 |

TABLE VI

Data used for estimating linkage between the gene for glume colour and the gene for seedling leaf rust resistance in the $F_2$ population of Rsc x RL 5406.

| Leaf rust reaction | Glume colour | |
|---|---|---|
| | Brown | White |
| Resistant | 62 | 5 |
| Susceptible | 5 | 25 |

4.2 Monosomic analysis

a) Inhibitor of waxy foliage. The results in Tables VII and VIII clearly show that the gene for inhibition of waxy foliage in RL 5404 and RL 5406 is located on chromosome 2D. In both Tables VII and VIII $F_2$ populations involving chromosome 2D deviated significantly from a 3:1 ratio. Although the $F_2$ population of Rescue monosomic 4A x RL 5404 deviated significantly from the expected ratio, $F_3$ progeny tests confirmed that this chromosome was not involved in wax inhibition in RL 5404. Chinese Spring is only slightly waxy and in the field this light coating may disappear through weathering. This is likely the reason that Chinese Spring monosomic 1D x RL 5406 is significant, as only two misclassified plants would have been necessary for this result.

b) Tenacious glumes. The data in Tables IX and X indicate that segregation for this character deviated significantly from the expected ratio for several of the chromosomes. Populations involving Rescue monosomic 5A were expected to be critical as chromosome 5A carries the Q factor which is hemizygous ineffective. Approximately 75% of the $F_2$ plants would be nullisomic or monosomic for chromosome 5A and therefore be non free-threshing. Of the remaining 25%, all of which are disomic, three-quarters would be expected to be non free-threshing as they would carry the dominant gene for tenacious glumes transmitted from Ae. squarrosa. Segregation of Rescue monosomic 3A x RL 5404 deviates significantly from a 3:1 ratio due to an excess of

TABLE VII

Segregation for waxiness in the $F_2$ of the Rsc monosomics x RL 5404 and Rsc monosomics x RL 5406.

| $F_2$ lines | Rsc x RL 5404 | | | Rsc x RL 5406 | | |
|---|---|---|---|---|---|---|
| | Non-waxy | Waxy | $X^2$ (3:1) | Non-Waxy | Waxy | $X^2$ (3:1) |
| Mono- 1A | 72 | 23 | 0.00 | 88 | 24 | 0.58 |
| " 2A | 61 | 29 | 2.13 | 92 | 25 | 0.64 |
| " 3A | 74 | 25 | 0.00 | 89 | 29 | 0.00 |
| " 4A | 84 | 12 | 7.35** | 79 | 26 | 0.00 |
| " 5A | 73 | 21 | 0.23 | 76 | 18 | 1.42 |
| " 6A | 88 | 23 | 0.87 | 90 | 24 | 0.74 |
| " 7A | 66 | 27 | 0.61 | 80 | 31 | 0.36 |
| " 1B | 70 | 29 | 0.75 | 86 | 20 | 1.81 |
| " 2B | 60 | 22 | 0.06 | 66 | 33 | 3.24 |
| " 3B | 56 | 19 | 0.00 | 82 | 26 | 0.01 |
| " 4B | 58 | 25 | 0.90 | 67 | 28 | 0.79 |
| " 5B | 55 | 17 | 0.02 | 59 | 21 | 0.02 |
| " 6B | 64 | 31 | 2.56 | 83 | 28 | 0.00 |
| " 7B | 73 | 26 | 0.03 | 79 | 21 | 0.65 |
| " 1D | 72 | 21 | 0.18 | 80 | 27 | 0.00 |
| " 2D | 73 | 6 | 11.85** | 96 | 5 | 20.59** |
| " 3D | 68 | 21 | 0.03 | 81 | 24 | 0.16 |
| " 4D | 62 | 14 | 1.42 | 65 | 14 | 1.86 |
| " 5D | 38 | 18 | 1.17 | 76 | 25 | 0.00 |
| " 6D | 72 | 25 | 0.00 | 85 | 26 | 0.08 |
| " 7D | 71 | 20 | 0.30 | 63 | 24 | 0.18 |
| Disomic | 69 | 31 | 1.61 | 75 | 27 | 0.05 |

** Significant at the 1% level.

TABLE VIII

Segregation for waxiness in the $F_2$ of CS D-genome monosomics x RL 5404 and CS D-genome monosomics x RL 5406.

| $F_2$ lines | CS x RL 5404 | | | CS x RL 5406 | | |
|---|---|---|---|---|---|---|
| | Non-waxy | Waxy | $X^2$ (3:1) | Non-waxy | Waxy | $X^2$ (3:1) |
| Mono- 1D | 61 | 16 | 0.52 | 71 | 11 | 5.27* |
| " 2D | 70 | 3 | 15.89** | 76 | 3 | 17.83** |
| " 3D | 48 | 21 | 0.82 | 36 | 12 | 0.03 |
| " 4D | 40 | 14 | 0.00 | 52 | 12 | 1.02 |
| " 5D | 71 | 14 | 2.86 | 32 | 9 | 0.07 |
| " 6D | 56 | 14 | 0.69 | 54 | 16 | 0.08 |
| " 7D | 66 | 18 | 0.40 | 52 | 21 | 0.37 |
| Disomic | 63 | 17 | 0.42 | 39 | 13 | 0.03 |

* Significant at the 5% level.
** Significant at the 1% level.

TABLE IX

Segregation for threshability in the $F_2$ of Rsc monosomics x RL 5404 and Rsc monosomics x RL 5406

| $F_2$ lines | Rsc x RL 5404 | | | Rsc x RL 5406 | | |
|---|---|---|---|---|---|---|
| | Non free-threshing | Free-threshing | $X^2$ (3:1) | Non free-threshing | Free-threshing | $X^2$ (3:1) |
| Mono- 1A | 65 | 21 | 0.00 | 85 | 23 | 0.60 |
| " 2A | 60 | 26 | 0.99 | 98 | 20 | 3.66 |
| " 3A | 56 | 30 | 3.97* | 81 | 26 | 0.00 |
| " 4A | 61 | 19 | 0.02 | 70 | 32 | 1.88 |
| " 5A | 78 | 6 | 13.35** | 90 | 6 | 17.01** |
| " 6A | 75 | 22 | 0.17 | 85 | 29 | 0.00 |
| " 7A | 60 | 18 | 0.07 | 69 | 40 | 7.34** |
| " 1B | 75 | 9 | 8.40** | 88 | 21 | 1.62 |
| " 2B | 54 | 9 | 3.31 | 78 | 20 | 0.87 |
| " 3B | 49 | 19 | 0.18 | 81 | 26 | 0.00 |
| " 4B | 56 | 13 | 1.09 | 66 | 19 | 0.19 |
| " 5B | 72 | 10 | 6.50* | 86 | 20 | 1.81 |
| " 6B | 66 | 15 | 1.49 | 72 | 29 | 0.56 |
| " 7B | 67 | 20 | 0.10 | 71 | 23 | 0.00 |
| " 1D | 63 | 15 | 1.09 | 70 | 29 | 0.76 |
| " 2D | 77 | 1 | 22.15** | 95 | 4 | 22.09** |
| " 3D | 51 | 28 | 4.05* | 73 | 23 | 0.01 |
| " 4D | 59 | 11 | 2.74 | 51 | 10 | 1.97 |
| " 5D | 50 | 6 | 5.36* | 87 | 7 | 14.52** |
| " 6D | 65 | 22 | 0.00 | 80 | 27 | 0.00 |
| " 7D | 73 | 13 | 3.97* | 63 | 24 | 0.19 |
| Disomic | 68 | 32 | 2.32 | 89 | 19 | 2.78 |

* Significant at the 5% level
** Significant at the 1% level

TABLE X

Segregation for threshability in the $F_2$ of CS D-genome monosomics x RL 5404 and CS D-genome monosomics x RL 5406.

| $F_2$ lines | CS x RL 5404 | | | CS x RL 5406 | | |
|---|---|---|---|---|---|---|
| | Non free-threshing | Free-threshing | $X^2$ (3:1) | Non free-threshing | Free-threshing | $X^2$ (3:1) |
| Mono- 1D | 61 | 15 | 0.86 | 64 | 14 | 1.71 |
| " 2D | 65 | 4 | 12.57** | 66 | 5 | 11.27** |
| " 3D | 44 | 10 | 0.89 | 36 | 11 | 0.01 |
| " 4D | 36 | 14 | 0.11 | 47 | 15 | 0.00 |
| " 5D | 57 | 18 | 0.00 | 32 | 7 | 0.69 |
| " 6D | 48 | 16 | 0.02 | 53 | 17 | 0.00 |
| " 7D | 50 | 27 | 3.64 | 45 | 28 | 6.25* |
| Disomic | 65 | 21 | 0.00 | 32 | 19 | 3.46 |

* Significant at the 5% level
** Significant at the 1% level

recessive free-threshing plants rather than to a deficiency and, therefore, chromosome 3A cannot be critical. The same is true for Rescue monosomic 3D x RL 5404, Rescue monosomic 7A x RL 5406 and Chinese Spring monosomic 7D x RL 5406. Rescue monosomics 1B x RL 5404, 5D x RL 5404 and 5D x RL 5406 were not significant when $F_3$ lines were tested. $F_3$ lines of Rescue monosomics 5B x RL 5404 and 7D x RL 5404 were not grown as the $F_2$ populations of these crosses were grown one summer later than the majority of the other $F_2$ Rescue monosomic crosses and therefore $F_3$ seed was not available. However, it is unlikely that either of these chromosomes carries the gene for tenacious glumes as they are only significant in one combination each. Chromosome 2D is the only monosome that is critical in all four combinations. Therefore, chromosome 2D must carry the gene for glume tenacity in both RL 5404 and RL 5406.

c) Leaf rust resistance. In the field, segregation for adult-plant leaf resistance of $F_2$ populations of Rescue monosomics 4A-, 5A-, 7A-, 2B-, 2D-, and 4D x RL 5404 deviated significantly from the expected ratio (Table XI). The ratio obtained from $F_3$ lines of monosomic 4A was significant but only at the 5% level, while that of monosomic 7A was not. Monosomic 2B cannot be the critical chromosome due to the large number of recessive susceptible plants. $F_3$ progeny tests of monosomics 5A and 4D were not carried out as their ratios were only significant at the 5% level and a critical monosomic ratio should deviate more than this. However, in all likelihood the critical chromosome in RL 5404 is 2D as the ratio of resistant to susceptible

TABLE XI

Segregation for adult-plant leaf rust resistance to natural inoculum in the $F_2$ of Rescue monosomics x RL 5404.

| $F_2$ lines | Resistant | Susceptible | $\chi^2$ (3:1) |
|---|---|---|---|
| Mono- 1A | 76 | 19 | 1.01 |
| " 2A | 62 | 28 | 1.48 |
| " 3A | 70 | 29 | 0.76 |
| " 4A | 91 | 5 | 19.01** |
| " 5A | 81 | 13 | 5.67* |
| " 6A | 88 | 22 | 1.21 |
| " 7A | 82 | 11 | 7.92** |
| " 1B | 73 | 26 | 0.03 |
| " 2B | 53 | 29 | 4.16* |
| " 3B | 50 | 25 | 2.35 |
| " 4B | 69 | 14 | 2.51 |
| " 5B | 53 | 21 | 0.29 |
| " 6B | 67 | 28 | 0.79 |
| " 7B | 74 | 25 | 0.00 |
| " 1D | 69 | 24 | 0.00 |
| " 2D | 71 | 9 | 7.35** |
| " 3D | 69 | 20 | 0.18 |
| " 4D | 67 | 9 | 6.33* |
| " 5D | 42 | 14 | 0.02 |
| " 6D | 76 | 21 | 0.42 |
| " 7D | 67 | 21 | 0.02 |
| Disomic | 80 | 20 | 1.12 |

* Significant at the 5% level
** Significant at the 1% level

plants was significant at the 1% level. Also, the gene for adult-plant leaf rust resistance is linked with the genes controlling waxiness and threshability, both of which have been found to be on chromosome 2D of RL 5404. The monosomics of Chinese Spring could not be used due to the good adult-plant leaf rust resistance of this cultivar to natural inoculum. Greenhouse space was not available for the testing of adult-plants.

The results in Tables XII and XIII clearly indicate that the gene for seedling leaf rust resistance in RL 5406 is located on chromosome 1D. Other than for chromosome 1D, monosomic $F_2$ populations of Rescue monosomics x RL 5406 which were critical at the adult-plant stage in the field to natural inoculum were not found to be critical at the seedling stage in the greenhouse to leaf rust race 1. This likely resulted from the greenhouse tests being better controlled and thus more precise. Only 1D of the Chinese Spring monosomics was found to be critical.

d) Glume colour. The expression of glume colour in the field is dependent upon the environment and whether the plant is homozygous or heterozygous for this character. RL 5404 and RL 5406 were found to express the brown colouration under all conditions, although the glumes of RL 5406 were somewhat darker. However, plants that are heterozygous may only show a buff colour and under damp conditions at ripening white glumed plants become a greyish colour, making them difficult to distinguish from the heterozygotes which are also affected by weathering. This is likely the reason for the large number of critical $F_2$ populations

TABLE XII

Segregation for leaf rust resistance in the field to natural inoculum and for seedling resistance in the greenhouse to race 1 of the $F_2$ of Rescue monosomics x RL 5406.

| | Field | | | Greenhouse | | |
|---|---|---|---|---|---|---|
| $F_2$ lines | Resistant | Susceptible | $\chi^2$ (3:1) | Resistant | Susceptible | $\chi^2$ (3:1) |
| Mono- 1A | 81 | 31 | 0.29 | 80 | 30 | 0.19 |
| " 2A | 83 | 34 | 0.82 | 90 | 25 | 0.49 |
| " 3A | 99 | 19 | 4.52* | 78 | 29 | 0.15 |
| " 4A | 80 | 25 | 0.03 | 73 | 25 | 0.00 |
| " 5A | 68 | 24 | 0.01 | 89 | 22 | 1.32 |
| " 6A | 91 | 23 | 1.17 | 91 | 30 | 0.00 |
| " 7A | 89 | 22 | 1.32 | 91 | 29 | 0.01 |
| " 1B | 77 | 29 | 0.20 | 83 | 31 | 0.19 |
| " 2B | 81 | 18 | 2.10 | 75 | 29 | 0.32 |
| " 3B | 82 | 26 | 0.01 | 92 | 28 | 0.10 |
| " 4B | 66 | 29 | 1.27 | 93 | 21 | 2.29 |
| " 5B | 69 | 25 | 0.06 | 77 | 33 | 1.21 |
| " 6B | 82 | 29 | 0.03 | 86 | 33 | 0.34 |
| " 7B | 75 | 25 | 0.01 | 86 | 34 | 0.54 |
| " 1D | 100 | 7 | 18.47** | 104 | 8 | 18.11** |
| " 2D | 81 | 23 | 0.32 | 83 | 31 | 0.19 |
| " 3D | 88 | 17 | 3.89* | 82 | 28 | 0.00 |
| " 4D | 50 | 29 | 5.17* | 85 | 29 | 0.00 |
| " 5D | 74 | 27 | 0.08 | 86 | 31 | 0.07 |
| " 6D | 83 | 28 | 0.00 | 91 | 24 | 0.84 |
| " 7D | 70 | 29 | 0.45 | 90 | 26 | 0.29 |
| Disomic | 70 | 32 | 1.88 | 94 | 28 | 0.17 |

* Significant at the 5% level
** Significant at the 1% level

TABLE XIII

Segregation for seedling leaf rust resistance to race 1 in the greenhouse of the $F_2$ of Chinese Spring D-genome monosomics x RL 5406.

| $F_2$ lines | Resistant | Susceptible | $X^2$ (3:1) |
|---|---|---|---|
| Mono- 1D | 110 | 6 | 23.28** |
| " 2D | 97 | 23 | 1.88 |
| " 3D | 82 | 19 | 1.75 |
| " 4D | 50 | 14 | 0.19 |
| " 5D | 90 | 23 | 1.06 |
| " 6D | 80 | 30 | 0.19 |
| " 7D | 96 | 24 | 1.34 |
| Disomic | 92 | 28 | 0.10 |

** Significant at the 1% level

in the Rescue monosomic crosses (Table XIV).

$F_2$ populations of Rescue monosomics 1A-, 2A-, 3A-, and 1D x RL 5406 and Chinese Spring monosomic 1D x RL 5406 deviated significantly from the expected 3:1 ratio of brown to white glumed plants (Tables XIV and XV). Chromosome 2A cannot be involved as there are too many recessive white glumed plants in Rescue monosomic 2A x RL 5406. Segregation of $F_3$ lines of Rescue monosomic 1A x RL 5406 did not deviate significantly from the expected ratio but segregation of the $F_3$ of monosomic 3A x RL 5406 remained significant. Presumably, however, chromosome 1D of RL 5406 carries the gene for brown glumes as $F_2$ populations of both Rescue monosomic 1D x RL 5406 and Chinese Spring 1D x RL 5406 deviated significantly from the expected ratio. Furthermore, the seedling leaf rust resistance gene found to be on chromosome 1D is linked with the gene for brown glumes and analysis of the data from the disomic crosses involving RL 5406 gave no indication that brown glumes is other than monogenically inherited.

$F_2$ populations of Rescue monosomics 1A-, 2A-, 3A-, 4A-, 5A-, 6A-, 2B-, 3B-, 4B-, 3D-, and 6D x RL 5404 deviated significantly from the expected ratio. $F_3$ progeny tests of Rescue monosomics 1A-, 4A-, 5A-, 3B-, and 6D x RL 5404 confirmed that these chromosomes were not involved with glume colouration in RL 5404. However, all other deviating $F_2$ monosomic populations remained significant when their $F_3$ lines were tested. $F_3$ lines of Rescue monosomic 1D x RL 5404 were not grown as the $F_2$ ratio of brown to white glumed plants was not significant, however, the $F_2$ population of Chinese monosomic 1D x RL 5404

TABLE XIV

Segregation for glume colour in the $F_2$ of Rsc monosomics x RL 5404 and Rsc monosomics x RL 5406.

| $F_2$ lines | Rsc x RL 5404 | | | Rsc x RL 5406 | | |
|---|---|---|---|---|---|---|
| | Brown | White | $X^2$ (3:1) | Brown | White | $X^2$ (3:1) |
| Mono- 1A | 77 | 9 | 8.93** | 61 | 47 | 18.78** |
| " 2A | 73 | 12 | 4.80* | 78 | 40 | 4.52* |
| " 3A | 76 | 10 | 7.50** | 102 | 5 | 22.51** |
| " 4A | 71 | 9 | 7.35** | 80 | 22 | 0.47 |
| " 5A | 75 | 9 | 8.40** | 66 | 31 | 2.15 |
| " 6A | 85 | 12 | 7.59** | 87 | 27 | 0.05 |
| " 7A | 65 | 13 | 2.46 | 85 | 24 | 0.37 |
| " 1B | 71 | 13 | 3.57 | 83 | 26 | 0.03 |
| " 2B | 56 | 7 | 5.76* | 77 | 21 | 0.49 |
| " 3B | 62 | 6 | 8.65** | 80 | 27 | 0.00 |
| " 4B | 60 | 9 | 4.64* | 61 | 24 | 0.32 |
| " 5B | 50 | 14 | 0.19 | 57 | 15 | 0.46 |
| " 6B | 68 | 13 | 3.00 | 67 | 34 | 3.59 |
| " 7B | 71 | 16 | 1.69 | 68 | 26 | 0.23 |
| " 1D | 66 | 12 | 3.35 | 94 | 5 | 19.96** |
| " 2D | 46 | 21 | 1.12 | 75 | 21 | 0.35 |
| " 3D | 68 | 11 | 4.59* | 78 | 18 | 1.68 |
| " 4D | 56 | 14 | 0.69 | 42 | 19 | 0.92 |
| " 5D | 46 | 9 | 1.75 | 76 | 18 | 1.42 |
| " 6D | 77 | 10 | 7.76** | 77 | 30 | 0.38 |
| " 7D | 62 | 13 | 1.96 | 59 | 22 | 0.10 |
| Disomic | 83 | 17 | 3.00 | 77 | 31 | 0.60 |

* Significant at the 5% level
** Significant at the 1% level

TABLE XV

Segregation for glume colour in the $F_2$ of CS D-genome monosomics x RL 5404 and CS D-genome monosomics x RL 5406.

| | CS x RL 5404 | | | CS x RL 5406 | | |
|---|---|---|---|---|---|---|
| $F_2$ lines | Brown | White | $\chi^2$ (3:1) | Brown | White | $\chi^2$ (3:1) |
| Mono- 1D | 64 | 1 | 17.85** | 64 | 3 | 13.98** |
| " 2D | 44 | 14 | 0.00 | 47 | 14 | 0.05 |
| " 3D | 38 | 15 | 0.16 | 28 | 11 | 0.08 |
| " 4D | 37 | 11 | 0.03 | 42 | 17 | 0.28 |
| " 5D | 47 | 17 | 0.02 | 24 | 10 | 0.16 |
| " 6D | 43 | 16 | 0.05 | 49 | 18 | 0.04 |
| " 7D | 53 | 17 | 0.00 | 54 | 14 | 0.49 |
| Disomic | 56 | 22 | 0.27 | 29 | 11 | 0.03 |

** Significant at the 1% level

deviated significantly from the expected 3:1 ratio. In view of the previous results from RL 5406 and from the disomic crosses with RL 5404, which also indicated monogenic inheritance for this character, chromosome 1D of RL 5404 likely carries the gene for brown glumes as well. No positive conclusions, however, can be drawn from these results.

e) Purple coleoptile. The data in Table XVI shows that the gene for purple coleoptile is associated with chromosome 7D in RL 5406. Both chromosome 1D and 7D were significant in the Chinese Spring monosomics x RL 5404. It was not possible to check whether univalent shift may have occurred in either the $F_1$ of Chinese Spring 1D x RL 5404 or 7D x RL 5404. However, monosomic $F_1$ plants of both Chinese Spring 1D x RL 5406 and 7D x RL 5406 were crossed to Chinese Spring ditelo 1DL and ditelo 7DS, respectively. In $2n = 40 + t$ $F_1$ plants of both crosses, the telo remained unpaired and thus no univalent shift had occurred. This indicates that the parental Chinese Spring monosomics 1D and 7D were correct. The disomic cross Chinese Spring x RL 5404 indicated that coleoptile pigmentation is monogenically controlled so that only one chromosome should have been involved. The $F_2$ population of Chinese Spring monosomic 1D x RL 5404 was also critical for the gene for brown glumes but there is no indication from the disomic crosses or from the monosomic results with RL 5406 that the genes for brown glumes and coleoptile colour are linked. The only explanation that can be given is that a large enough population was not used. It is likely, nevertheless, that chromosome 7D of RL 5404 also carries the gene for purple coleoptile

TABLE XVI

Segregation for coleoptile colour in the $F_3$ of CS D-genome monosomics x RL 5404 and CS D-genome monosomics x RL 5406.

| | CS x RL 5404 | | | | CS x RL 5406 | | | |
|---|---|---|---|---|---|---|---|---|
| $F_3$ lines | Purple | Segregating | Green | $\chi^2$ (1:2:1) | Purple | Segregating | Green | $\chi^2$ (1:2:1) |
| Mono- 1D | 13 | 34 | 3 | 10.48** | 13 | 27 | 10 | 0.68 |
| " 2D | 9 | 24 | 16 | 2.02 | 10 | 32 | 8 | 4.08 |
| " 3D | 13 | 25 | 12 | 0.04 | 7 | 21 | 9 | 0.89 |
| " 4D | 9 | 29 | 9 | 2.57 | 7 | 31 | 8 | 5.61 |
| " 5D | 10 | 25 | 15 | 1.00 | 6 | 13 | 11 | 2.20 |
| " 6D | 12 | 24 | 14 | 0.24 | 8 | 29 | 13 | 2.28 |
| " 7D | 27 | 21 | 2 | 26.28** | 20 | 25 | 5 | 9.00* |
| Disomic | 13 | 23 | 14 | 0.36 | 13 | 22 | 8 | 1.19 |

* Significant at the 5% level
** Significant at the 1% level

since this would appear to be the same gene found in RL 5406.

4.3 Telocentric chromosome mapping

a) Ditelo-1DL. Only 14 recombinants in a population of 105 were detected for the region between the centromere and the gene for brown glumes in the test cross of (Chinese Spring ditelo-1DL x RL 5404) x Chinses Spring (Table XVII); this is equivalent to a crossover value of 13.3 $\pm$ 3.3%. Since the gene did not assort independently of the centromere its location on chromosome 1D of RL 5404 was confirmed. In the test cross, (Chinese Spring ditelo-1DL x RL 5406) x Chinese Spring, three questionable recombinants were found between the gene for brown glumes and the centromere. The heads of these plants were not as dark a brown as expected; this variation in colour may have been the result of environmental factors. If these are true recombinants a crossover value of 1.7 $\pm$ 1.0% with the centromere is indicated. It would seem, however, that the gene for brown glumes in RL 5406 must also be on 1DL as it is probably located at the same locus as the gene for brown glumes on 1DL of RL 5404.

In the test cross of (Chinese Spring ditelo-1DL x RL 5406) x Chinese Spring no recombinants between the gene for seedling leaf rust resistance and the centromere were found. This indicates that 1D is the correct chromosome as all resistant plants were 2n=42 while all susceptible plants were 2n=41+t. If the gene for seedling leaf rust resistance were located on another chromosome half of the 2n=42 plants would have been resistant and the other half susceptible.

TABLE XVII

Crossover products in (CS ditelo-1DL x RL 5404) x CS and (CS ditelo-1DL x RL 5406) x CS for the genes determining glume colour and seedling leaf rust resistance.

| Character | (CS ditelo-1DL x RL 5404) x CS | | (CS ditelo-1DL x RL 5406) x CS | |
|---|---|---|---|---|
| | 2n=41+t | 2n=42 | 2n=41+t | 2n=42 |
| Glume colour | | | | |
| Brown | 5* | 42 | 3* | 83 |
| White | 49 | 9* | 86 | 0* |
| Seedling rust reaction | | | | |
| Resistant | | | 0* | 81 |
| Susceptible | | | 95 | 0* |

* Crossover class

The same would have been true for 2n=41+t plants. Because of the tight linkage between the gene for brown glumes and the gene for seedling leaf rust resistance in RL 5406 it would be expected that these genes are on the same arm; consequently, the gene for seedling leaf rust resistance probably is also on 1DL. However, the possibility that linkage between these two genes could be across the centromere cannot be excluded.

b) Ditelo-2D$\alpha$. Preliminary tests of (Chinese Spring ditelo $\beta$-monotelo$\alpha$-2D x RL 5406) x Chinese Spring showed that the genes for inhibition of waxiness and for tenacious glumes were not located on 2D$\beta$. This was evident from the observation that all 2n=42 plants displayed the dominant phenotypes, non-waxy and non free-threshing. Plants of the chromosome constitution 41+t exhibited the recessive phenotypes, waxy and free-threshing. On the other hand, in the test cross of ditelo-2D$\alpha$ x RL 5406 all four possible phenotypes were found associated with 2n=41+t and 2n=42 plants. Because crossing-over had occurred the genes for glume tenacity and wax inhibition must be on the 2D$\alpha$ arm of RL 5406. On the assumption that glume tenacity and waxlessness are determined by corresponding genes on the same arm of RL 5404 as in RL 5406, the gene for adult-plant leaf rust resistance in RL 5404 must also be on 2D$\alpha$ since it is linked with the genes for glume tenacity and wax inhibition.

In the (Chinese Spring ditelo-2D$\alpha$ x RL 5404) x Chinese Spring test cross the data from Table XVIII showed the genes controlling threshability, waxiness, and adult-plant leaf rust resistance were

TABLE XVIII

Apparent crossover products in (CS ditelo-2D$\alpha$ x RL 5404) x CS and (CS ditelo-2D$\alpha$ x RL 5406) x CS for the genes determining threshability, waxiness and adult-plant leaf rust resistance.

| Character | (CS ditelo-2D$\alpha$ x RL 5404) x CS | | (CS ditelo-2D$\alpha$ x RL 5406) x CS | |
|---|---|---|---|---|
| | 2n=41+t | 2n=42 | 2n=41+t | 2n=42 |
| Threshability | | | | |
| Non free-threshing | 13* | 45 | 30* | 38 |
| Free-threshing | 28 | 33* | 23 | 20* |
| Waxiness | | | | |
| Non-waxy | 30* | 47 | 36* | 33 |
| Waxy | 30 | 42* | 21 | 26* |
| Adult-plant rust reaction | | | | |
| Resistant | 24* | 39 | | |
| Susceptible | 16 | 29* | | |

* Crossover class

located 38.7 ± 4.0, 48.3 ± 4.0, and 49.1 ± 4.8 apparent crossover units from the centromere, respectively. To be certain of the gene order, $BC_1F_1$ plants, in which segregation for all three characters had been scored, were grouped for 0, 1, and 2 crossovers in Table XIX. The data so classified confirmed that, beginning at the centromere, the gene order was glume tenacity, wax inhibition, and adult-plant leaf rust resistance. The data in Table XVIII permitted the identification of only the apparent crossover products of the three genes on this arm as they were only taken one at a time. To determine the actual crossover products the data were regrouped in Table XX, taking into account all three genes at once. This allows for the detection of double and triple crossover products. From these data the following crossover values with the centromere were obtained: glume tenacity 39.4 ± 4.9%, wax inhibition 52.5 ± 5.0%, and adult-plant leaf rust resistance 63.6 ± 4.8%. The crossover values for the glume tenacity gene calculated from the data in Tables XVIII and XX should have been the same but the differences in population sizes lead to a small discrepancy.

The (Chinese Spring ditelo-2D∝ x RL 5406) x Chinese Spring test cross confirmed that the gene order for glume tenacity and wax inhibition in RL 5406 was the same as in RL 5404. The data from Table XVIII showed these two genes were 45.0 ± 4.7 and 53.4 ± 4.6 apparent crossover units from the centromere, respectively (Table XVIII); the actual crossover values, calculated from Table XXI, are glume tenacity 42.9 ± 4.6% and wax inhibition 58.9 ± 4.6%. Again, the two crossover values calculated from Tables XVIII and XXI for glume tenacity should

TABLE XIX

Data used to determine the order of the genes for glume tenacity, wax inhibition, and adult-plant leaf rust resistance in (CS ditelo-2D$^{\alpha}$ x RL 5404) x CS.

| $F_1$ ♀ gamete | Type of crossover | Number of $BC_1$ plants |
|---|---|---|
| T $I_2$W Lra | None | 49 |
| t $i_2$W lra | None | 32 |
| t $I_2$W Lra | Single | 11 |
| T $i_2$W lra | Single | 7 |
| T $I_2$W lra | Single | 6 |
| t $i_2$W Lra | Single | 12 |
| t $I_2$W lra | Double | 4 |
| T $i_2$W Lra | Double | 4 |

TABLE XX

Actual crossover products in (CS ditelo-2D$\alpha$ x RL 5404) x CS for the genes determining threshability, waxiness and adult-plant leaf rust resistance.

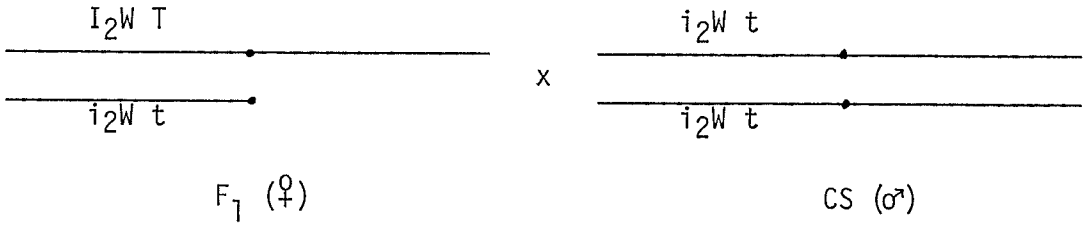
$$\frac{\text{Lra } I_2W \text{ T}}{\text{lra } i_2W \text{ t}} \times \frac{\text{lra } i_2W \text{ t}}{\text{lra } i_2W \text{ t}}$$

$F_1$ (♀) CS (♂)

| Constitution of ♀ gamete | Chromosome number of $BC_1$ plants | Region of crossover | Total number of plants |
|---|---|---|---|
| Lra $I_2W$ T | 42 | None | 26 |
| | 41+t | Centr.-T | 12 |
| lra $i_2W$ t | 42 | Centr.-T | 15 |
| | 41+t | None | 10 |
| Lra $I_2W$ t | 42 | Centr.-T, T-$I_2W$ | 4 |
| | 41+t | T-$I_2W$ | 5 |
| lra $i_2W$ T | 42 | T-$I_2W$ | 3 |
| | 41+t | Centr.-T, T-$I_2W$ | 1 |
| lra $I_2W$ T | 42 | $I_2W$-Lra | 6 |
| | 41+t | Centr.-T, $I_2W$-Lra | 0 |
| Lra $i_2W$ t | 42 | Centr.-T, $I_2W$-Lra | 5 |
| | 41+t | $I_2W$-Lra | 5 |
| lra $I_2W$ t | 42 | Centr.-T, T-$I_2W$, $I_2W$-Lra | 2 |
| | 41+t | T-$I_2W$, $I_2W$-Lra | 2 |
| Lra $i_2W$ T | 42 | T-$I_2W$, $I_2W$-Lra | 3 |
| | 41+t | Centr.-T, T-$I_2W$, $I_2W$-Lra | 0 |

TABLE XXI

Actual crossover products in (CS ditelo-2D$^{\alpha}$ x RL 5406) x CS for the genes determining threshability and waxiness.




$F_1$ (♀) CS (♂)

| Constitution of ♀ gamete | Chromosome number of $BC_1$ plants | Region of crossover | Total number of plants |
|---|---|---|---|
| $I_2W$ T | 42 | None | 30 |
| | 41+t | Centr.-T | 26 |
| $i_2W$ t | 42 | Centr.-T | 16 |
| | 41+t | None | 15 |
| $I_2W$ t | 42 | Centr.-T, T-$I_2W$ | 3 |
| | 41+t | T-$I_2W$ | 9 |
| $i_2W$ T | 42 | T-$I_2W$ | 9 |
| | 41+t | Centr.-T, T-$I_2W$ | 4 |

have been the same but again the difference was due to population size.

The test crosses with the telocentric stocks also allowed additional linkage values to be calculated. For RL 5404 the following recombination values were obtained from the data in Table XIX: glume tenacity and wax inhibitor 20.8 $\pm$ 3.6%, wax inhibitor and adult-plant leaf rust resistance 20.8 $\pm$ 3.6%, and glume tenacity and adult-plant leaf rust resistance 41.6 $\pm$ 4.4%. For RL 5406 the data in Table XXI showed that the genes for glume tenacity and wax inhibition were 22.3 $\pm$ 3.9 recombination units apart.

c) Ditelo-7DS. The data in Table XXII clearly indicate that the gene for purple coleoptile is located on chromosome arm 7DS in both RL 5404 and RL 5406; the locus for coleoptile pigmentation and the centromere did not segregate independently. The coleoptile pigmentation locus-centromere distance in RL 5404 and RL 5406 was 10.3 $\pm$ 2.8 and 9.8 $\pm$ 2.8 crossover units, respectively.

## 4.4 Substitution of chromosome 5A of the synthetic hexaploids into Rescue

When the main culm of Rescue and of the selfed disomic progeny of (Rescue 5A)$^{8}$ x RL 5404, which represents the substitution of 5A of RL 5404 into Rescue, were compared for stem solidness, (Rescue 5A)$^{8}$ x RL 5404 was found to have much less pith than Rescue. Since chromosome 5A of Rescue carries a gene for solid stem and RL 5404 is hollow stemmed, it is evident that the substitution of chromosome 5A of RL 5404 into Rescue was successful. This substitution line will be referred to as

TABLE XXII

Crossover products in (CS ditelo-7DS x RL 5404) x CS and (CS ditelo-7DS x RL 5406) x CS for the genes determining coleoptile pigmentation.

| Coleoptile colour | (CS ditelo-7DS x RL 5404) x CS | | (CS ditelo-7DS x RL 5406) x CS | |
|---|---|---|---|---|
| | 2n=41+t | 2n=42 | 2n=41+t | 2n=42 |
| Purple | 5* | 55 | 4* | 47 |
| Green | 41 | 6* | 45 | 6* |

* Crossover class

Rescue/5A RL 5404.

Spikes of Rescue/5A RL 5404 were morphologically very similar to those of Rescue and were free-threshing. As shown in Figure 3B spikes of RL 5404, Rescue, and Rescue/5A RL 5404 are semi-squareheaded, that is the spikes are somewhat lax at the top so the head is not squared off. Rescue monosomic 5A and monosomic 5A of (Rescue 5A)$^{8}$ x RL 5404 are typically speltoid in appearance having a lax head tapering sharply toward the apex and broad glumes with a blunt secondary tooth. Although the substitution of chromosome 5A of RL 5406 into Rescue could not be verified because the chromosome number of the progeny of selfed (Rescue 5A)$^{8}$ x RL 5406 plants was unknown, the morphological results were very similar to those with RL 5404 (Figure 3A). The disomic plant of (Rescue 5A)$^{6}$ x RL 5406 is squareheaded and free-threshing. Since the Q factor in the homozygous condition produces squareheaded or semi-squareheaded, free-threshing spikes as typified by the two substitution lines, both RL 5404 and RL 5406 must themselves possess the Q factor.

## 4.5 Synthetic hexaploids

The five synthetic hexaploids developed from four varieties of ssp. carthlicum and three varieties of Ae. squarrosa were produced to determine the effect or interaction of the Q factor of ssp. carthlicum with the D genome of Ae. squarrosa. All five synthetic hexaploids were non free-threshing. Figure 4 shows two of these synthetics along with the synthetic RL 5404 and their respective tetraploid parents. The three synthetic hexaploids illustrated have the same Ae. squarrosa parent in common. The spikes of the ssp. carthlicum synthetic hexaploids

FIGURE 3

A. 1) RL 5406. 2) Rescue. 3) Rescue monosomic 5A. 4) monosomic 5A of $(\text{Rescue 5A})^8$ x RL 5406. 5) disomic of $(\text{Rescue 5A})^6$ x RL 5406.

B. 1) RL 5404. 2) Rescue. 3) Rescue monosomic 5A. 4) monosomic 5A of $(\text{Rescue 5A})^8$ x RL 5404. 5) Rescue/5A RL 5404.


A
1
2
3
4
5


B
1
2
3
4
5

FIGURE 4

1) ssp. carthlicum RL 5415. 2) ssp. carthlicum RL 5415 x Ae. squarrosa RL 5271. 3) ssp. carthlicum RL 5205. 4) ssp. carthlicum RL 5205 x Ae. squarrosa RL 5271. 5) Tetra Canthatch. 6) RL 5404.


1
2
3
4
5
6

are laxer than those of RL 5404 but their glumes closely resemble those of ssp. vulgare and certainly are not speltoid in appearance. Morphologically, the other three synthetics derived from ssp. carthlicum also closely resemble ssp. vulgare. From these results it can be concluded that ssp. carthlicum either possesses the Q factor or at least an allele that has a very similar effect and that it is unable to overcome the gene for glume tenacity derived from Ae. squarrosa.

## 4.6 Pentaploid hybrids

The pentaploid $F_1$ (2n=35=AABBD) of Canthatch x Tetra Canthatch (QQ) and of Canthatch x the durum variety Stewart (Qq) were examined for speltoid characters to see what effect Q in the homozygous condition, and in the hemizygous condition, would have at the pentaploid level. Canthatch x Tetra Canthatch was very similar to Canthatch in head morphology (Figure 5). The spike is somewhat denser than that of Canthatch but its partial sterility makes it appear even more dense, and it is predictably free-threshing. Canthatch x Stewart has a slightly laxer head than Canthatch, being closer to RL 5406 in this respect. It does not have the broad glumes and blunt secondary tooth of ssp. spelta spikes which the speltoid of the common wheat cultivar Manitou exhibits even though both are hemizygous for Q. The Manitou speltoid is monosomic for chromosome 5A while the Canthatch x Stewart pentaploid is supposedly Qq. Furthermore, the Canthatch x Stewart pentaploid is free-threshing whereas the Manitou speltoid is non free-threshing. The speltoid of Manitou was used for

FIGURE 5

1) Canthatch. 2) Manitou speltoid. 3) RL 5406.
4) Canthatch x Tetra Canthatch pentaploid.
5) Canthatch x Stewart pentaploid.


1
2
3
4
5

comparison because this cultivar is closely related to Canthatch. These results indicate that the genetic background has an important effect on the expression of the speltoid characters.

# 5. DISCUSSION

## 5.1 Origin of the free-threshing hexaploid wheats

The non free-threshing character of the synthetic hexaploids RL 5404 and RL 5406 could have been due to either a gene introduced from Ae. squarrosa or from the absence of the Q factor. The semi-hollow stem of Rescue/5A RL 5404 verified the substitution of chromosome 5A of RL 5404 (Tetra Canthatch x Ae. squarrosa RL 5271) into Rescue. Since Rescue/5A RL 5404 was semi-squareheaded and free-threshing Tetra Canthatch, the source of 5A, must possess the Q factor. This evidence along with the results of the monosomic analysis of the synthetic hexaploids RL 5404 and RL 5406 substantiates the suggestion of Kerber and Dyck (1969) that the non free-threshing character of these synthetics is due to a single, dominant gene on chromosome 2D derived from their Ae. squarrosa parents.

Kerber (Personal communication) has produced four other synthetic hexaploids by crossing Tetra Canthatch with strains of the Ae. squarrosa varieties anathera, typica, and strangulata. All are non free-threshing. It can be assumed that these strains carry the same gene for glume tenacity (T) that was found in RL 5404 and RL 5406 which had as one of their parents the Ae. squarrosa varieties strangulata and meyeri, respectively. This indicates that all four varietal groups of Ae. squarrosa possess the gene for glume tenacity. This evidence, along with the non free-threshability of the synthetic hexaploids produced from ssp. turgidum (Tsunewaki, 1966) and from ssp. carthlicum, suggests that all hexaploid wheats must originally have been non free-

threshing assuming that all forms of Ae. squarrosa in the past possessed the gene for tenacious glumes as apparently they do at the present. The Q factor present in Tetra Canthatch and ssp. carthlicum is unable to counteract the gene for glume tenacity found in Ae. squarrosa.

Ssp. vulgare (QQ cc SS) differs from the other two free-threshing hexaploids, ssp. compactum (QQ CC SS) and ssp. sphaerococcum (QQ cc ss), by only one gene. The compactum gene C and the sphaerococcum gene s are found on chromosomes 2D and 3D, respectively. Since no form of Ae. squarrosa is known that possesses either of these genes, they must have arisen in the hexaploids (Morris and Sears, 1967). Therefore, ssp. vulgare must be considered the oldest of the free-theshing hexaploids as it retains the primitive genotype of Ae. squarrosa, with respect to these two characters. Furthermore, Riley et al. (1967) considered only ssp. vulgare and ssp. spelta to have the primitive chromosome structure of the hexaploids.

If ssp. spelta was the direct progenitor of the free-threshing hexaploids, as suggested by Tsunewaki (1966), then it must be considered the oldest of the hexaploids as ssp. vulgare is the oldest hexaploid that has so far been found (Helbaek, 1966). This means ssp. spelta would have arisen from a q tetraploid x Ae. squarrosa hybrid and thus have had the genotype qq TT. If the free-threshing ssp. vulgare arose from ssp. spelta then a triplication of the q locus on chromosome 5A and a mutation of the gene for tenacious glumes on chromosome 2D to a less effective allele, would have been required. Furthermore, if the tetraploid parent of this form of ssp. spelta was either ssp. dicoccum

or ssp. dicoccoides, which are both non free-threshing, further mutations to increase rachis toughness and to decrease glume tenacity would have been needed. It is doubtful that a mutation at the 2D locus in this speltoid background would have been recognized by neolithic man or selected for by his harvesting and threshing process, as mutants of ssp. vulgare that lack Q, are non free-threshing and speltoid in appearance (MacKey, 1954) despite the absence of the gene for tenacious glumes on chromosome 2D. Certainly, if a non free-threshing tetraploid was involved the small mutations needed in the A and B genomes to increase rachis toughness and decrease glume tenacity would have had no visible effect on the plant. The appearance of the Q factor in this qq TT form of ssp. spelta would have been recognizable to prehistoric man in the form of a plant that would have had shorter straw and squarer heads. However, the plant would have remained non free-threshing due to the glume tenacity gene on 2D, and unless the Q factor provided some other useful advantage to man it is doubtful it would have been selected for.

It seems certain that the European ssp. spelta is not the product of the chromosome doubling of a tetraploid wheat x Ae. squarrosa triploid as was suggested by McFadden and Sears (1946). They found free-threshing hexaploid segregates from a cross between ssp. carthlicum and an European variety of ssp. spelta. Since the D genome of this hybrid could only have been derived from the ssp. spelta parent, the presence of free-threshing hexaploid segregates indicated that the gene for glume tenacity on 2D was not present. As already mentioned it would

have been unlikely that speltoid plants bearing a mutation at the 2D locus could have been selected by neolithic man; therefore, the suggestion that ssp. spelta from Europe arose from either a ssp. dicoccum x ssp. compactum hybrid (Schiemann, 1951) or a ssp. dicoccum x ssp. vulgare hybrid (Tsunewaki, 1971) is more likely. Ssp. compactum would appear to be the hexaploid involved as it was found in Europe prior to ssp. vulgare (Schiemann, 1951). Tsunewaki's (1971) objection to ssp. compactum being involved because of the absence of the C gene in ssp. spelta is not valid as MacKey (1966) has found ssp. vulgare type segregates from a ssp. dicoccum x ssp. compactum cross.

Since there are no reports of crosses between the Iranian ssp. spelta and ssp. carthlicum it is not known if these spelts also lack the gene for tenacious glumes on 2D. If they do not possess this gene, they too can be removed as a direct progenitor of the free-threshing hexaploids. However, Kuckuck (1970) mentions that some forms of ssp. spelta in Iran have been found that are free-threshing but possess a fragile rachis. This indicates that if enough of the genes for tenacious glumes are removed from the A, B and D genomes of ssp. spelta the Q factor is not necessary for free-threshability. It can then be argued that free-threshing hexaploids could have arisen from ssp. spelta. If this did occur ssp. spelta must have been grown over many centuries to allow man to select plants, through the harvesting and threshing process, that had chaff free seed and thus indirectly select for plants that had the QQ tt genotype. There is no evidence, however, that ssp. spelta was cultivated in prehistoric

western Asia (Helbaek, 1966). This fact alone tends to remove ssp. spelta from the ancestry of the free-threshing hexaploids. The only explanation that can be given for Kuckuck's finding is that these plants are segregates from a cross between a free-threshing tetraploid wheat and ssp. spelta.

Since ssp. carthlicum carries the Q factor (McFadden and Sears, 1946; MacKey, 1966), it would seem logical to consider it as the tetraploid progenitor of ssp. vulgare. A Q bearing hexaploid could have been produced directly by the spontaneous chromosome doubling of a ssp. carthlicum x Ae. squarrosa triploid hybrid (MacKey, 1954) or indirectly as a segregate from a ssp. spelta x ssp. carthlicum pentaploid hybrid (McFadden and Sears, 1946). The evidence presented in this study on the non free-threshability of the ssp. carthlicum x Ae. squarrosa synthetic hexaploids refutes Tsunewaki's (1966) report that they are free-threshing. This implies that if ssp. carthlicum were involved, as has been suggested by MacKey (1954; 1966), the first member of ssp. vulgare must have been non free-threshing as it would have carried the gene for tenacious glumes on chromosome 2D. However, it would only have been one major mutation removed from free-threshability and pre-historic man would have easily selected such a mutation in this background. There is the problem, however, that ssp. carthlicum has never been found in prehistoric remains.

If the Q factor did arise as a mutation at the tetraploid level, how would it have been recognized in a free-threshing tetraploid? Muramatsu (1963) showed that six doses of q transferred from ssp. spelta

into Chinese Spring caused subcompactoid spikes. This fact indicates that the compact spike of Tetra Canthatch (Kerber, 1964) and the compactoid spikes of the extracted tetraploids of Prelude, Rescue, and Thatcher (Kaltsikes *et al.*, 1969) is due to the *Q* factor. However, the *Q* factor in ssp. *carthlicum* does not cause a compact spike. Therefore, it appears that the *Q* factor would have been recognizable only if it occurred in a non free-threshing tetraploid such as ssp. *dicoccum*, making it free-threshing.

There may, however, be a dosage series of *q* in the tetraploids as was suggested by Swaminathan (1966) and Tsunewaki (1966). Muramatsu (1963) indicated that four doses of *q* transferred from ssp. *spelta* into a Chinese Spring background has the effect of softening the glumes, although otherwise the plant is still speltoid in appearance. If some of the free-threshing tetraploids do have a duplication at the *q* locus ($q^2q^2$) and they had taken part in the formation of hybrids with *Ae. squarrosa*, a mutation at the *T* locus on chromosome 2D in this background may then have been recognizable to the early farmers in the form of chaff free seed. Although such plants would not likely have been completely free-threshing the seed would not have been as tightly invested in the glumes as plants of ssp. *spelta*. Further evidence for this possibility is the observation that the Canthatch x Stewart durum pentaploid was free-threshing, suggesting that conv. *durum* may have a duplication at the *q* locus. It can be argued, however, that there is no duplication of *q* in conv. *durum* as there is only a haploid compliment of *D* genome chromosomes in this pentaploid hybrid and therefore,

the Qq genotype would be enough to produce free-threshing, square headed plants. Muramatsu (1963) found, however, that 5 doses of q from ssp. spelta in Chinese Spring produced square headed spikes while 6 doses caused semi-compactoid spikes. The difference between the Canthatch x Tetra Canthatch pentaploid, which is QQ, and that of the Canthatch x Stewart pentaploid was that the former was slightly more compact than the latter. This indicates that there is only a difference of one dose of q between the two pentaploids and that conv. durum possesses a duplication at the q locus. Conv. durum is not likely the tetraploid progenitor as the earliest dating for it is 300 B.C. (Helbaek, 1959).

If a $q^2q^2$ TT hexaploid had existed in the past it would help to explain why ssp. spelta or ssp. carthlicum have never been found in prehistoric remains. As already mentioned, a hexaploid wheat that originally was qq TT would likely have had to been grown over a long period of time in order for the harvesting and threshing process to select the free-threshing genotype, QQ tt. Therefore, remains of ssp. spelta should have been found by now. However, if the original genotype of the hexaploid wheats was $q^2q^2$ TT a mutation to $q^2q^2$ tt would have been selected since these plants would have been at least partially free-threshing and have contributed the bulk of the chaff free seed. Subsequently, a further duplication of $q^2$ to $q^3$ (=Q) in this background would have further decreased glume tenacity. The selection by neolithic man of the QQ tt genotype in this manner would likely have required a relatively short period of time. Thus, the

probability of finding remains of these $q^2q^2$ TT speltoid plants would be greatly reduced as the primitive farmers would have discarded the tough threshing speltoids for the free-threshing QQ tt plants. Ssp. carthlicum could have originated from the natural progression of a $q^2q^2$ tetraploid wheat to QQ or, as suggested by Morris and Sears (1967), as a segregate from the hybridization of a q tetraploid and a Q hexaploid.

The formation in nature of a hexaploid wheat from its parental tetraploid and diploid species must be a fairly rare occurrence. It is known that the frequency of successful hand pollinations between tetraploid wheat and Ae. squarrosa is farily low and that to obtain plants it is often necessary to resort to embryo culturing of immature seeds. In nature this event occurs relatively often as in Iran Zohary et al. (1969) claim to have found hybrids from crosses of Ae. squarrosa with both cultivated tetraploid and hexaploid wheats. These triploid hybrids must then undergo spontaneous doubling of their chromosomes in order to produce the hexaploid state. Kihara et al. (1957) and Kerber (personal communication) have found that the seed-set of triploid hybrids of tetraploid wheat x Ae. squarrosa is from 0 to 50% depending on the parental genotypes. Despite these limiting factors hexaploid wheats appear to have originated more than once; that is they are polyphyletic in origin.

Much of the variation in the hexaploid wheats can be attributed to introgression of tetraploid wheat genes through tetraploid-hexaploid hybrids. MacKey (1966) comments that the Q factor in the

hexaploid wheats interacts with the polygenic system derived from the emmers which results in considerable variation in the degree of threshability among the so-called free-threshing hexaploid wheats. This suggests that a number of forms of the tetraploids are in the background of the present day hexaploid wheats and that probably the majority of these genes were introduced into the hexaploid level through introgression. However, a number of Ae. squarrosa varieties appear to have contributed to the gene pool of the hexaploid wheats as well. This further suggests that a number of hexaploid wheats must have been formed in nature. Kerber and Tipples (1969) speculated that the Ae. squarrosa var. strangulata has contributed genes for bread-making quality to ssp. vulgare, but evidence supplied by Kihara and Tanaka (1958) and Tsunewaki (1966) indicates that strangulata is non-waxy. If this is true, some other variety of Ae. squarrosa must have contributed the waxy character to the hexaploid wheats. Furthermore, strangulata is indigenous to humid areas (Kihara and Tanaka, 1958) but other Ae. squarrosa varieties are found in dry areas suggesting that they have contributed genes for drought resistance to the hexaploids. Also, a strong winter habit gene was acquired by the hexaploid wheats from Ae. squarrosa. Since it is doubtful that one Ae. squarrosa variety could have contributed all of the important genes found in the D genome of the hexaploids, therefore, the hexaploid wheats likely arose in nature more than once. This implies that there probably was more than one area of origin for the hexaploids. Since Ae. squarrosa is found growing in both Transcaucasia and northern Iran and since there

is no evidence to indicate that the area distribution of Ae. squarrosa has changed in the last 8,000 years the hexaploid wheats have likely originated in both regions.

A polyphyletic origin of hexaploid wheat does not mean that the QQ tt genotype has appeared more than once, as some free-threshing plants would segregate from a QQ tt x qq or $q^2q^2$ TT cross. In fact no free-threshing forms of Ae. squarrosa have ever been found, suggesting that the mutation rate at the T locus is very low or possibly that the possession of the t gene would be a selective disadvantage in nature.

In conclusion, the theory that the free-threshing hexaploid wheats arose from a tetraploid that had a duplication at the q locus ($q^2$) would seem to best fit the facts available, as the antiquity of both ssp. spelta and ssp. carthlicum is questionable. Precisely what tetraploid wheat this could have been is unknown since no experiments have been performed to determine if such duplication at the q locus exists in the tetraploid group. It would seem, however, that some intermediate form must have been required to proceed from q to $q^3$(Q).

## 5.2 Homoeologous loci

A study of the nullisomics and compensating nulli-tetrasomics of Chinese Spring allowed Sears (1954 and 1966b) to classify the 21 pairs of chromosomes in hexaploid wheat into seven homoeologous groups of three, each consisting of a chromosome from the A, B, and D genomes, respectively. The compensation in the nulli-tetrasomics of an extra pair of one chromosome for the absence of another pair in the same

homoeologous group showed that they must have many genes in common. Consequently, a function controlled by one pair of genes in a diploid organism could be determined by as many as three in hexaploid wheat. In this study it appears that with the exception of the adult plant leaf rust resistance gene in RL 5404, all the genes located by monosomic or telocentric analysis in RL 5404 and RL 5406 can each be placed in a homoeologous series, evidence of which is as follows.

Jha (1964) found a gene for purple coleoptile on chromosome 7D of a synthetic hexaploid. This must be the same gene found on chromosome 7D of the synthetic hexaploids RL 5404 and RL 5406. The complete homoeologous series is known for this character as Sears (1954) and Gale and Flavell (1971) have found genes for coleoptile pigmentation on chromosome 7A and 7B respectively.

The gene for wax inhibition on chromosome 2D of RL 5404 and RL 5406 must be the same gene found by Tsunewaki (1966) on chromosome 2D of two synthetic hexaploids. The $\underline{I_2W}$ gene in RL 5404 and RL 5406 was found to be $48.3 \pm 4.0$ and $53.4 \pm 4.6$ crossover units from the centromere, respectively. When this is compared to the crossover value of 42.3 to 50% found by Driscoll (1966) for the $\underline{I_1W}$ gene on chromosome 1B and the centromere it would seem that $\underline{I_1W}$ and $\underline{I_2W}$ are on homoeologous loci. There is no known gene for wax inhibition in ssp. boeoticum, ssp. monococcum or the A genome of any of the tetraploid or hexaploid wheats.

The gene for glume tenacity found on chromosome 2D of RL 5404 and RL 5406 has no known equivalent within the cultivated wheats, as

no known gene for tenacious glumes has been found on homoeologous group 2 chromosomes. Sears, (1968) however, reported that when chromosome 2R of rye (Secale cereale) was substituted for chromosomes 2B and 2D of Chinese Spring it compensated for the missing wheat chromosome, indicating that chromosome 2R of rye is at least partially homoeologous to group 2 chromosomes in hexaploid wheat. These substitution lines had much stiffer glumes than Chinese Spring apparently due to a gene on rye chromosome 2R as nullisomics of homoeologous group 2 have thin papery glumes (Sears, 1954). Since rye is related to the diploid members of Triticum and Aegilops this implies that the gene for tough glumes on rye chromosome 2R may be at a locus that is homoeologous to the gene for glume tenacity on 2D of Ae. squarrosa. If this is so, all wild diploid members of Triticum and Aegilops likely carry a gene controlling glume tenacity at this locus. Forms of cultivated tetraploid and hexaploid wheats having mutations at these loci would have been selected by man because of their improved threshability.

Metzger and Silbaugh (1970) reported a gene for stripe rust resistance linked by $2.0 \pm 0.3$ recombination units to a gene for brown glumes on chromosome 1B of the hexaploid wheat variety P.I. 178383. This is very close to the crossover value of $3.1 \pm 1.1\%$ between the gene for seedling leaf rust resistance and the gene for brown glumes found by Kerber and Dyck (1969) in RL 5406. Monosomic and telocentric analysis showed that these genes are on chromosome 1D of RL 5406 and that they are tightly linked. This suggests that the gene for stripe rust resistance and the gene for seedling leaf rust resistance may be

located at homoeologous loci. The gene for brown glumes found on chromosome 1D of RL 5404 and RL 5406 completes the homoeologous series for this character as genes for glume colour have also been found on chromosomes 1A (Ausemus et al., 1967) and 1B (Unrau, 1950).

These examples show how closely related the diploid progenitors of hexaploid wheat must have been. Washington (1971) has proposed that genes found at homoeologous loci be designated homoeoalleles. He argues that these genes are located on chromosomes that have had different evolutionary histories, so should not be termed alleles. Although in many instances such genes are located on different chromosomes they appear to have an identical effect on the phenotype of the plant. To imply, therefore, that they are alleles would seem to be incorrect. If, on the other hand, the gene for stripe rust resistance in P.I. 178383 and the gene for seedling leaf rust resistance in RL 5406 are located at homoeologous loci they could be considered true homoeoalleles.

## 5.3 Linkage values

Most of the recombination values obtained in this study agree reasonably well with those found by Kerber and Dyck (1969) and Dyck and Kerber (1970) for RL 5406 and RL 5404, respectively. A comparison of these values can be found in Table XXIII. The largest discrepancies in recombination values are for the T-Lra region in RL 5404. Dyck and Kerber (1970) found that the genes for glume tenacity and adult-plant leaf rust resistance were separated by $6.0 \pm 1.5$ recombination units while the test cross of Chinese Spring ditelo 2D$\alpha$ x RL 5404 showed these

TABLE XXIII

Comparison of recombination values obtained from crosses of RL 5404 and RL 5406 with each of Cth, Rsc, CS, and CS ditelos.

| Parental combinations | Crossover region and recombination value | | | |
|---|---|---|---|---|
| | T-$I_2$W | $I_2$W-Lra | T-Lra | Gc-Lrs |
| RL 5404 x Cth[1] | 17.4 ± 2.5 | 15.6 ± 2.5 | 6.0 ± 1.5 | |
| " x Rsc | 27.2 ± 3.6 | 18.3 ± 2.9 | 33.0 ± 4.0 | |
| " x CS | 30.9 ± 4.3 | | | |
| " x CS ditelos | 20.8 ± 3.6 | 20.8 ± 3.6 | 41.6 ± 4.4 | |
| RL 5406 x Cth[2] | 15.1 ± 2.6 | | | 3.1 ± 1.1 |
| " x Rsc | 23.3 ± 3.4 | | | 10.1 ± 2.2 |
| " x CS | 21.7 ± 4.5 | | | |
| " x CS ditelos | 22.3 ± 3.9 | | | 1.7 ± 1.0 |

[1] Recombination values from Dyck and Kerber (1970).
[2] Recombination values from Kerber and Dyck (1969).

genes to be separated by 41.6 $\pm$ 4.4 recombination units. Although this was unexpected, it is not without precedent since McIntosh and Baker (1968) have found differences of approximately 45 recombination units between the genes Sr6 and Lr2, which are located on chromosome 2D, when they were in different genetic backgrounds. However, since none of the other linkage values for chromosome 2D of RL 5404 show this large variance neither the genetic background nor the environment in which the $F_1$ plants were grown appear to be a factor in this discrepancy.

The results from the telocentric mapping indicate that the gene order from the centromere on the $\alpha$ arm of chromosome 2D of RL 5404 is I-$I_2$W-Lra, while the data of Dyck and Kerber (1970) suggest that the gene order is I-Lra-$I_2$W. A likely explanation for these results is the presence of an inversion in the $\alpha$ arm of Canthatch, which was used by Dyck and Kerber. This would not only explain the difference in gene order but it also would explain the tight linkage between I and Lra found by Dyck and Kerber. Crossing-over is reduced in inversion heterozygotes due both to the suppression of crossing-over in the inversion loop and to non-functional gametes that result when crossing-over does occur. No definite conclusions can be drawn, however, without a further cytogenetical study of the hybrids and segregating generations of RL 5404 x Canthatch. In this regard it would be helpful if additional markers could be added to 2D$\alpha$ of RL 5404 as this would help to locate the inversion break points if indeed there is an inversion present on 2D$\alpha$ of Canthatch. This may be possible as McIntosh and Baker (1968) list six other genes on chromosome 2D, some of which probably are on the $\alpha$ arm.

Endrizzi and Kohel (1966) observed that the crossover frequency near the centromere in a telocentric chromosome of cotton is reduced from that found in this region of the whole chromosome. This also appears to be the case in hexaploid wheat. There were no recombinants recovered between the centromere and the gene for seedling leaf rust resistance in the test cross of Chinese Spring ditelo-1DL x RL 5406. Furthermore, Sears (1966a) found that the gene for Neatby's virescent on the short arm of chromosome 3B and an awn inhibitor on the long arm of chromosome 6B were within one crossover unit of the centromere. Sears and Loegering (1968) suggested that this indicates reduced crossing-over occurs in the centromere region of telocentrics of wheat. Sears and Loegering also surmise that while chiasmata formation may be inhibited near the centromere in heteromorphic bivalents, the total chiasma frequency remains the same because the chiasmata are distally shifted. The recombination values in Table XXIII do not appear to support this theory as on the whole the values from segregating disomic populations are practically the same as in the telocentric test crosses.

# 6. SUMMARY

1. The synthetic hexaploid wheats RL 5404 and RL 5406 used in this study were produced by combining the extracted tetraploid of Canthatch with the Ae. squarrosa varieties strangulata RL 5271 and meyeri RL 5289, respectively. All characters studied in these synthetics were derived from their Ae. squarrosa parents.

2. The following gene-chromosome associations were established for the synthetic hexaploid wheats RL 5404 and RL 5406 by monosomic and telocentric analysis.

a) RL 5404. Brown glumes (Gc) on 1D; tenacious glumes (T), wax inhibitor ($I_2W$) and adult-plant leaf rust resistance (Lra) on 2D; purple coleoptile (P) on 7D.

b) RL 5406. Seedling leaf rust resistance (Lrs) and brown glumes (Gc) on 1D; tenacious glumes (T) and wax inhibitor ($I_2W$) on 2D; purple coleoptile (P) on 7D.

3. With the exception of the gene for seedling leaf rust resistance the chromosome arm location of these genes and their actual crossover distance from the centromere was determined by telocentric mapping. They are as follows.

a) RL 5404. Brown glumes: 1DL, 13.3 $\pm$ 3.3%; glume tenacity: 2D$\alpha$, 39.4 $\pm$ 4.9%; wax inhibitor: 2D$\alpha$, 52.5 $\pm$ 5.0%; adult-plant leaf rust resistance: 2D$\alpha$, 63.6 $\pm$ 4.8%; and purple coleoptile: 7DS, 10.3 $\pm$ 2.8%.

b) RL 5406. Brown glumes: 1DL, 1.7 $\pm$ 1.0%; glume tenacity; 2D$\alpha$, 42.9 $\pm$ 4.6%; wax inhibitor: 2D$\alpha$, 58.9 $\pm$ 4.6%; and purple coleoptile:

7DS, 9.8 ± 2.8%. The seedling leaf rust resistance gene showed no crossing-over with the centromere (1DL) indicating that it is either tightly linked with centromere or on the short arm of 1D.

4. It was suggested that with the exception of the adult-plant leaf rust resistance gene in RL 5404, all the genes studied in RL 5404 and RL 5406 could each be placed in a homoeologous series.

5. Linkage values obtained from the data of segregating $F_2$ disomic populations are as follows.

a) RL 5404. The gene for adult-plant leaf rust resistance was linked with the genes for glume tenacity and wax inhibition with a recombination value of 33.0 ± 4.0% and 18.3 ± 2.9%, respectively. The genes for glume tenacity and wax inhibition were linked by 28.2 ± 2.7 recombination units.

b) RL 5406. The genes for glume tenacity and wax inhibition were linked by 21.6 ± 2.7 recombination units while the genes for seedling leaf rust resistance and brown glumes showed a recombination value of 10.1 ± 2.2%.

6. Additional linkage values were also obtained from the telocentric backcross data and are as follows.

a) RL 5404. The adult-plant leaf rust resistance gene was linked with the genes for glume tenacity and wax inhibition by 41.6 ± 4.4 and 20.8 ± 3.6 recombination units, respectively. The genes for glume tenacity and wax inhibition were separated by a recombination value of 20.8 ± 3.6%.

b) RL 5406. The genes for glume tenacity and wax inhibition were associated with an estimated linkage value of 22.3 $\pm$ 3.9%. The genes for seedling leaf rust resistance and brown glumes were linked by 1.7 $\pm$ 0.1 recombination units.

7. It was suggested that an inversion was present on the $\alpha$ arm of chromosome 2D of Canthatch, which was used by Dyck and Kerber (1970). This may account for the difference in gene order found in this study and that found by Dyck and Kerber.

8. The data from the telocentric test crosses indicated that there is a reduction in crossing-over in the centromere region of telocentrics of wheat, but it does not appear that the chiasmata are distally shifted as was suggested by Sears and Loegering (1968).

9. All five synthetic hexaploids produced by crossing various varieties of ssp. carthlicum with several Ae. squarrosa varieties were non free-threshing. This disproved Tsunewaki's (1966) claim that synthetics having the AABB genomes derived from ssp. carthlicum are free-threshing.

10. Chromosome 5A of Rescue was substituted with chromosome 5A of RL 5404. Since this substitution line was semi-square headed and free-threshing, the synthetic hexaploids produced from Tetra Canthatch x Ae. squarrosa possess the Q factor.

11. The first hexaploid wheat must have been non free-threshing due to the presence of the gene for tenacious glumes on chromosome 2D derived from the Ae. squarrosa progenitor.

12. The tetraploid progenitor of the free-threshing hexaploids was likely a species that had a duplication at the q locus ($q^2$) on chromosome 5A.

13. European ssp. spelta is probably a segregate from a q tetraploid x Q hexaploid hybrid and not the product of the chromosome doubling of a tetraploid wheat x Ae. squarrosa triploid as was suggested by McFadden and Sears (1946).

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