A genetic study of cold resistance in wheat

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SUMMARY

The variety Chinese Spring of *Triticum aestivum* is susceptible to cold treatment applied at the juvenile plant stage, while the variety Cappelle-Desprez shows resistance to such treatment. By cytological and backcross procedures single homologous pairs of chromosomes from Cappelle-Desprez were substituted for their homologues in Chinese Spring. Assay experiments carried out on each of the 21 possible substitution lines indicated that three chromosomes, 4D, 5D and 7A of Cappelle-Desprez were involved in the determination of cold resistance. The resistance expressed by the three substitution lines carrying these chromosomes was less than the reaction of Cappelle-Desprez to cold treatment. Also the magnitude of this resistance supported a hypothesis that the action of the three chromosomes was additive on the scale of measurement used. The possible relationships of the genes for cold resistance to the established genes controlling other developmental characters, also carried by these three chromosomes, are discussed.

1. INTRODUCTION

During recent years a methodology of genetic analysis has developed, using the hexaploid wheat, *Triticum aestivum*, which allows the recognition and location, often in terms of linkage maps, of the genetic factors responsible for continuous variables. This methodology depends upon (i) the ability of the hexaploid wheats to tolerate chromosomal deficiencies so that aneuploid lines specific for particular chromosomes can be produced, and (ii) the presence of an easily distinguishable cytological marker, the telocentric chromosome, among these aneuploid lines. The combination of these two features enables single chromosomes from a donor variety to be substituted for their homologues in a recipient variety (Sears, 1953; Unrau, 1950). If all the requisite aneuploid lines are available then it is possible to produce substitution lines for each of the 21 pairs of chromosomes of wheat. Moreover, each of these lines will differ from the recipient variety by a single pair of homologous chromosomes. In this way the standard procedures of 'chromosome assay' which have been used in *Drosophila melanogaster* can be applied to the common bread wheat of agriculture.

This kind of genetic analysis has been applied already to the elucidation of the genetic control of a number of characters in wheat. For instance, it has been possible to map two genetic factors controlling the time to ear-emergence on chromosome 7B (Law, 1966*a*) and to determine the relative positions of factors

also on 7B influencing such characters as grain weight, the number of grains per ear and height (Law, 1967). This investigation of the number and the position of these genetic factors was of course preceded by studies of the differences produced by whole chromosome substitutions. Numerous examples of this kind of investigation, the chromosome assay experiment proper, are available and relate to the study of the chromosomal control of yield and its components, grain quality, vernalization requirement and a wide range of other characters (Kuspira & Unrau, 1957; Morris et al. 1966; Halloran & Boydell, 1967).

The present paper describes two experiments designed to determine the genetic basis of cold resistance. The investigations concern single chromosome substitution lines in which chromosomes from a variety exhibiting cold resistance have been substituted into a variety having only a slight resistance to the cold treatment applied. The study of the behaviour of these inter-varietal chromosome substitutions should help in obtaining an understanding of the genetic control of cold resistance.

2. MATERIALS

The two varieties of hexaploid wheat, *Triticum aestivum* $(2n = 6x = 42)$, used in these experiments are:

(i) Chinese Spring. This is a spring variety which has all the requisite aneuploid stocks for carrying out intervarietal chromosome substitutions, so that it was used as the recipient, that is the variety into which chromosomes were substituted, in the experiments to be described.

This variety also shows a minimal resistance to the cold treatments used in these experiments.

(ii) Cappelle-Desprez. This is a winter wheat widely grown in the U.K. Although this variety has not an extreme resistance to cold treatment, it, nevertheless, has considerably more resistance than the variety Chinese Spring.

3. METHODS

(i) *Cytological*

During the development of substitution lines, it is essential to keep a close check of chromosome constitutions. This was carried out by examining somatic chromosomes obtained from squashes of root-tips pretreated in mono-bromonaphthalene and stained by the Feulgen method. In some instances, meiosis was also studied using Feulgen squashes of pollen mother cells taken from anthers fixed in acetic-alcohol.

(ii) *Cold Treatments*

In carrying out the cold treatment, ten seeds from each line under test were sown in pots containing potting compost topped with a layer of silver sand. The pots were then arranged in a growth chamber in randomized blocks with six replications in each experiment. After germination in the dark for 6 days at 15 °C, the seedlings were hardened by subjecting them to a day length of 10 h and a day

temperature of 10 °C, alternating with a night temperature of 5 °C. Hardening was carried out for a period of 6 weeks, at which point the plants were at the two to three leaf stage. Plants were then removed for freezing.

The freezing technique was based upon the methods described by Kretschmer (1960) and Marshal (1965). After removal from the pots, the seedlings were washed, clipped above and below the meristematic 'crown' region and placed in waxed paper bags. These were then randomized, as in the hardening chamber, in a freezing cabinet. Here, the plants were maintained at 1 °C for 12 h before the temperature was dropped to −3 °C for 6 h and finally to −6 °C for 15 h. After freezing the plants were thawed over a period of 6 h to 1 °C and then, after a further period of 6 h were removed from the freezing cabinet. They were then planted out in trays of moist sand in a glasshouse maintained at 10–15 °C with the natural day light supplemented artificially to give an 18 h day during the winter. Survival counts were made at intervals commencing 8 days after freezing.

4. THE CHINESE SPRING (CAPPELLE-DESPREZ) SUBSTITUTIONS

(i) *Development*

The crossing procedures used for the development of the Cappelle-Desprez substitutions into Chinese Spring are described in Fig. 1. The most efficient method of substituting chromosomes from one wheat variety into another employs the use, as the recurrent female parent, of lines from the recipient variety which carry a telocentric chromosome in the hemizygous condition. These mono-telocentric lines are then hybridized with a donor variety and selection is practised for monosomic individuals, which must carry the substituted chromosome in the hemizygous condition. This selected monosomic is then used as the pollen parent and hybridized with the same mono-telocentric line and the cycle repeated. In this way, the chromosome being substituted will be kept intact whereas the remaining chromosomes of the complement, over a series of backcrosses, will revert by segregation and recombination to the recipient genotype or a genotype very close to it. After a number of backcrosses the selected monosomes are selfed and from the resulting progeny 42-chromosome plants, disomic for the substituted chromosome are isolated.

Unfortunately, in the development of the Cappelle-Desprez substitutions, the requisite mono-telocentric lines of Chinese Spring were not available at the commencement of the programme. Monosomic lines were therefore used for the first three backcrosses until mono-telocentric lines had been developed. A further three backcrosses were then carried out using the mono-telocentric lines.

The use of monosomics as the recurrent female parent is, however, open to criticism since, following the hybridization of two monosomic plants, there is a chance that a 20-chromosome gamete, deficient for the substituted chromosome, will be transmitted through the pollen. When this occurs it is possible to select monosomics, resulting from the fusion of a 20-chromosome pollen with a 21-chromosome egg cell, in which the substituted chromosome has been lost and

replaced by its recipient homologue. This is obviously less efficient than the use of mono-telocentrics where a 'switch' between the complete substituted chromosome and the recipient telocentric-chromosome is immediately apparent.

On the other hand, the frequency of 20-chromosome pollen transmission in crosses between monosomics is likely to be small. Sears (1958) quotes transmission frequencies of 20- and 21-chromosome pollen from monosomics of Chinese

$$
\begin{array}{c}
\text{CS mono-1A} \quad \times \quad \text{Cappelle-Desprez} \\
20'' 1' \qquad\qquad\qquad 21''
\end{array}
$$

BC₁   CS mono-1A   ×   CS (Cap mono-1A)
         20″ 1′   20″ 1′

BC₂   CS mono-1A   ×   CS² (Cap mono-1A)
         20″ 1′   20″ 1′

BC₃   CS mono-1A   ×   CS³ (Cap mono-1A)
         20″ 1′   20″ 1′

BC₄   CS mono-telo-1A   ×   CS⁴ (Cap mono-1A)
         20″ 1′ t   20″ 1′

BC₅   CS mono-telo-1A   ×   CS⁵ (Cap mono-1A)
         20″ 1′ t   20″ 1′

BC₆   CS mono-telo-1A   ×   CS⁶ (Cap mono-1A)
         20″ 1′ t   20″ 1′

CS⁷ (Cap mono-1A)
20″ 1′

Self and select 42-chromosome plant
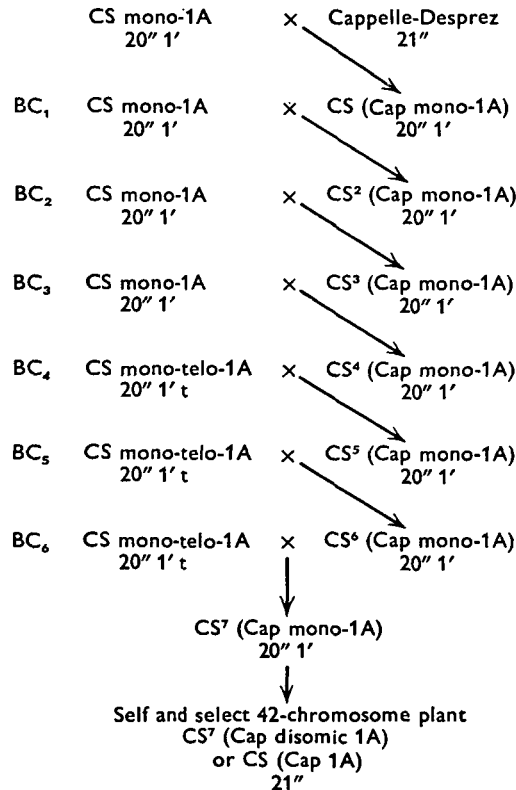CS⁷ (Cap disomic 1A)
or CS (Cap 1A)
21″

Fig. 1. The development of a substitution line carrying chromosome 1A of Cappelle-Desprez (Cap) in Chinese Spring (CS). Identical procedures were used in the substitution of other chromosomes. ″ = bivalent; ′ = univalent; t = telocentric chromosome.

Spring of 4 and 96% respectively, whereas in the female, 20- and 21-chromosome gametes give transmission frequencies of 75 and 25%. Under these circumstances, 1% of the zygotes produced will be monosomics in which 'switch' has occurred, and 72% of the zygotes will be monosomics in which 'switch' has not taken place and which, in the production of chromosome substitutions, will represent the desired genotype. The probability that 'switch' has not occurred after one generation is therefore $\frac{72}{73}$ or 0·9863. Thus, in the development of the Cappelle-Desprez substitutions, the probability that a substitution line is correct after the three generations of backcrossing to the monosomic of Chinese Spring, assuming of course that the probability of 0·9863 will be the same for each of the three generations, is $(\frac{72}{73})^3$ or 0·9595 and that it is incorrect after the same period of back-

crossing is 0·0405. For the development of 21 substitution lines and assuming that these calculations are applicable to each of them, $21 \times 0\cdot0405 \pm (0\cdot0405 \times 0\cdot9595 \times 21)^{\frac{1}{2}}$ or $0\cdot85 \pm 0\cdot90$ would be expected to be wrong after three backcrosses. One and perhaps two of the substitution lines could therefore have undergone 'switch' during the development of the Cappelle-Desprez substitutions.

This of course assumes a transmission rate of 20-chromosome pollen of 4%. It is possible that this may be an underestimate for some monosomics. On the other hand, the observations made by Sears (1954) on the range of 21 monosomics in Chinese Spring give estimates for most monosomics which are in fact less than this.

However, some direct evidence is available which suggests that these calculations, based on the averages of Sears's estimates, are not greatly inaccurate. This refers to situations where genic and cytological differences easily recognizable phenotypically are associated with particular chromosomes of Cappelle-Desprez. Altogether seven chromosomes display differences of this nature; of these 3D, 4B, 5A, 5D and 6B carry readily identifiable genetic markers, whereas 5B and 7B are involved in a reciprocal translocation. It is thus possible to follow these chromosomes through a backcross programme and determine whether 'switch' has occurred or not. In none of these instances had such an event occurred. Evidence presented in this paper also indicates that at least two other chromosomes are unlikely to have undergone a 'switch'.

This suggests therefore that the possible errors introduced by the use of monosomics up to the third generation are not large, and it is probable that only one or at the most two of the lines will have undergone 'switch' to the recipient genotype. Whether this will prove a correct assumption must await further studies of the Cappelle-Desprez substitution lines, in which other character differences are investigated. For the present, the possibility of errors resulting from 'switch' must be kept in mind in the final interpretation of the results.

(ii) *Background effects*

In the absence of 'switch' the substituted chromosome must remain intact. On the other hand, even though a large number of backcrosses have been completed, there is still a possibility that genes from the donor variety on other chromosomes of the complement will be present. When this occurs the derived disomic substitution line could be carrying these background genes in the homozygous or heterozygous state. The difference between the substitution line and the recipient would therefore reflect the genetic differences associated with both the substituted chromosome and these background genes. In other words the effects of the substituted chromosome would be confounded so that a spurious analysis would result.

One way of allowing for this difficulty is to develop substitution lines as duplicates. In this way each duplicate will have the same substituted chromosome, but need not be the same for genes in the background. Any difference between the duplicate lines will consequently make possible the detection of background effects and will enable due allowance to be made for their presence in the analysis.

In the development of the Cappelle-Desprez substitutions, duplicate lines were commenced after the third backcross and were maintained as duplicates in 14 lines. In the substitution lines carrying chromosome 2B, 3B, 3D, 4A, 5A, 7B and 7D the duplicate failed and sibs were used to re-commence the duplicate backcross programme.

5. RESULTS

Due to limitations of space, cold resistance tests could not be carried out on all the substitution lines, duplicates included, at the same time. Two experiments were therefore carried out in which one of the duplicates or sibs from each substitution line was tested in the first experiment and the second duplicate or sib in the second experiment.

Table 1. *Mean survival of plants with chromosomes of Cappelle-Desprez substituted into Chinese Spring*

Chromosome substituted	Experiment 1		Experiment 2	
	Mean survival per 10 plants	Difference from CS	Mean survival per 10 plants	Difference from CS
1A	0·17	− 0·29	2·93	+ 0·33
1B	0·17	− 0·29	3·26	+ 0·66
1D	0·81	+ 0·35	4·09	+ 1·49
2A	0·22	− 0·24	3·37	+ 0·77
2B	0·19	− 0·27	1·87	− 0·73
2D	0·60	+ 0·14	3·28	+ 0·68
3A	0·24	− 0·22	1·44	− 1·16
3B	0·22	− 0·24	4·10	+ 1·50
3D	0·58	+ 0·12	3·64	+ 1·04
4A	0·62	+ 0·16	2·59	− 0·01
4B	0·99	+ 0·53	6·37	+ 3·99**
4D	2·09	+ 1·63*	6·18	+ 3·58**
5A	0·41	− 0·05	3·68	+ 1·08
5B	0·12	− 0·34	3·20	+ 0·60
5D	3·03	+ 2·57**	6·53	+ 3·93**
6A	0·03	− 0·43	2·03	− 0·57
6B	1·62	+ 1·16	3·10	+ 0·50
6D	0·13	− 0·33	4·59	+ 1·99
7A	3·38	+ 2·92**	4·96	+ 2·36*
7B	0·72	+ 0·26	4·21	+ 1·61
7D	0·21	− 0·25	3·25	+ 0·65
Cappelle-Desprez	8·04	+ 7·58**	9·47	+ 6·89**
Hybride du Joncquois	4·91	+ 4·45**	—	—
Vilmorin 27	8·39	+ 7·93**	—	—
Chinese Spring (CS)	0·46	—	2·60	—
Standard error	0·52	—	0·82	—

* P 0·05–0·01,   ** $P < 0·01$.

In the first experiment 21 substitution lines along with the donor and recipient varieties, Cappelle-Desprez and Chinese Spring, and the two parents of Cappelle-Desprez, Vilmorin 27 and Hybride du Joncquois were tested. Scoring was carried out at intervals after the treatment application and the results obtained 14 days

afterwards are given in Table 1. At this stage survival was minimal among the replicate cultures of Chinese Spring.

The analysis indicates that significant differences occur between Chinese Spring and the substitution lines carrying chromosomes 4D, 5D and 7A of Cappelle-Desprez. These three lines are also significantly different from Cappelle-Desprez, so that the level of resistance expressed by these substitution lines is intermediate.

Of the two parents of Cappelle-Desprez, only Vilmorin 27 expresses a high resistance to freezing equivalent to Cappelle-Desprez, whereas Hybride du Joncquois gives an intermediate level of resistance. This could indicate that the major proportion, if not all, of the genetic control of cold resistance of Cappelle-Desprez derives from Vilmorin 27 rather than Hybride du Joncquois.

The second experiment involved the testing of the second set of duplicate and sib lines along with Chinese Spring and Cappelle-Desprez. Neither Vilmorin 27 nor Hybride du Joncquois was included in this test. In this experiment an adequate distinction between Chinese Spring and Cappelle-Desprez was not achieved until 20 days after cold treatment, and even at this stage the average survival was much greater than in the previous experiment. The results obtained at this stage are also presented in Table 1 and indicate that four of the substitution lines are significantly different from Chinese Spring. Three of these lines, 4D, 5D and 7A, are the same as those observed in the first experiment. Also all three lines are true duplicates in the sense that they relate to the duplicate lines set up after the third backcross. They are not sibs. This therefore suggests that the differences noted in the first experiment are attributable to the substituted chromosome, and not to the segregation of genes for cold resistance in the background.

The difference observed in the second experiment for the substitution line carrying chromosome 4B of Cappelle-Desprez remains to be explained. This could have arisen as a result of either background segregation or because the duplicate line used for the first experiment had undergone a 'switch'. The latter possibility however can be discounted, since chromosome 4B of Cappelle-Desprez carries an easily recognizable genetic marker affecting the degree of awning. In both lines this effect is expressed so that 'switch' of one of the lines cannot have occurred. It follows that the difference between the duplicates in the two experiments is a reflection of differences in the genetic background and does not relate to the substituted chromosome.

It is possible therefore to conclude from these experiments that three of the chromosomes carry a gene or genes responsible for the differences in cold resistance between these two varieties of wheat.

6. GENE ACTION

On the assumption that the effects of 'switch' have not resulted in a loss of a chromosome from the donor variety responsible for cold resistance, then the present data can be used to determine whether the genes on chromosomes 4D, 5D and 7A behave in an additive fashion or are not independent in their action.

This may be determined by summing the differences between Chinese Spring and each of the substitution lines and comparing this with the difference between Chinese Spring and Cappelle-Desprez. In the absence of between-chromosome interaction these differences should be the same (Law, 1966b). However, in the present experiments it has been proposed that cold resistance is controlled by three chromosomes only. The relevant comparisons can therefore be based upon the mean of Chinese Spring and those substitution lines which so far as cold resistance is concerned, are identical to Chinese Spring. In this way a more accurate test can be made.

Table 2. *The determination of (i) the sum of the differences between the Chinese Spring phenotype and the three substitution lines exhibiting cold resistance, and (ii) the difference between Chinese Spring and Cappelle-Desprez*

	Experiment 1		Experiment 2	
	Score	Difference from mean	Score	Difference from mean
CS (Cap 4D)	2·09	1·64	6·18	3·00
CS (Cap 5D)	3·03	2·58	6·56	3·38
CS (Cap 7A)	3·38	2·93	4·96	1·78
Mean of remaining 18 substitution lines and CS	0·45	—	3·18*	—
Total of substitution differences	—	7·15 ± 0·97	—	8·16 ± 1·52
Cappelle-Desprez	8·04	7·59 ± 0·73	9·47	6·29 ± 1·15

* Mean based on 17 substitution lines and CS, since in this experiment one of the 18 substitution lines carries a gene or genes in the background affecting cold resistance.

The results of these comparisons are shown in Table 2. Standard errors are also given. It is thus possible to test whether the summation difference is identical or not to the varietal difference. In both experiments the two differences are similar and when tested against their standard errors are not significantly different from each other, so that the evidence is consistent with an additive behaviour of the three chromosomes on the scale of cold resistance used in these experiments.

7. DISCUSSION

(i) *Chromosome 5D*

The effect of chromosome 5D on cold resistance is of considerable interest. Chromosome 5D has a major effect on the control of the time to ear-emergence and growth habit in many of the crosses involving Chinese Spring with a wide range of spring and winter wheats (Sears, 1954; Kuspira & Unrau, 1957; Morrison, 1960; Tsunewaki, 1966; Law, 1968a). It is possible that this control is determined by a single genetic factor located on the long-arm of the chromosome (Sears, 1954; Law, 1968b).

Experiments involving increased dosage of this chromosome in Chinese Spring and substitutions of chromosome 5D from the variety Hope into Chinese Spring have indicated that 5D has a large effect on vernalization requirement (Halloran, 1967; Halloran & Boydell, 1967). It is likely that this response to vernalization is also controlled by the same genetic factor implicated in the determination of ear-emergence time and growth habit.

In the present experiments it is of course not possible to say whether the cold resistance expressed by chromosome 5D is related in any way to the above characters. This is likely, however, since it can readily be envisaged that a large vernalization requirement will in itself reduce susceptibility to cold by delaying floral initiation. In this way, plants at a juvenile developmental stage, which are more resistant, will be exposed to cold rather than plants at adult stages of development, which are more susceptible (Levitt, 1956). Likewise such a requirement will have its correlated effect on the time to ear-emergence. Pleiotropic effects on these other characters of a gene, sensitive to vernalization, can consequently be envisaged.

The results obtained by studying the behaviour of the substitution line carrying the complete chromosome 5D of Cappelle-Desprez do not, however, allow such a hypothesis to be tested. This substitution line is indeed later than Chinese Spring in ear-emergence and also affects growth habit, but this in itself is no proof of pleiotropy. On the other hand, techniques are available which enable the genetic analysis of single chromosome differences to be investigated in detail (Law, 1966a). These involve the production of homozygous recombinant lines derived, using appropriate crossing procedures, from a hybrid between the substitution line and its recipient variety. By this means, a wide range of recombinant lines can be produced and can be analysed to determine whether a single locus is responsible for the control of all these characters or whether different loci are responsible.

This would provide not only a much more exact study of the genetic determination of these characters, but also a much firmer base from which to study the physiological processes involved in their expression. The requisite stocks necessary for carrying out this kind of investigation are now being developed.

(ii) *Chromosome 7A*

So far this chromosome has not been implicated in the control of vernalization response, ear-emergence time, growth habit or response to photoperiodic treatments. Its effect on cold resistance in view of the relationships that can occur between this character and the developmental characters mentioned is therefore of interest.

Monosomic analyses using Chinese Spring and a wide range of European wheats including Cappelle-Desprez have also shown a consistent response of this chromosome to cold treatments applied at the coleoptile stage (Goujon, Maia & Doussinault, 1968). In this instance, however, lines carrying chromosome 7A from the varieties tested were less resistant to cold treatment at the coleoptile stage than the controls in which chromosome 7A of Chinese Spring was present. This is of course the reverse of the effects described in the present paper. Why such a

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difference between the two studies should occur is not yet clear. Monosomic analyses suffer from the disadvantage of having chromosomes in the hemizygous state, which can give rise to complications absent in the case of substitution lines. In addition, apart from chromosome 7A, the other chromosomes involved in cold resistance at the coleoptile stage are not the same as those recognized in the experiments described here. Neither chromosome 4D nor 5D of Cappelle-Desprez has any affect on the coleoptile treatments of Goujon *et al.* whereas chromosomes 1B and 1D have.

This may be a reflection of the different treatment stages used in the two investigations. If so, and if the effects of chromosomes 5D and perhaps 4D are the outcome of a vernalization requirement which holds plants at a developmental stage that is less sensitive to cold treatments, then it must be supposed that the different effects in the experiment of Goujon *et al.* arise because this developmental stage has not yet been reached. Whether this will prove to be a correct assessment can only be decided following further investigations of the type mentioned earlier which seek to determine the true genetic and physiological relationships between these characters and cold resistance.

(iii) *Chromosome 4D*

Chromosome 4D has not been associated as yet with the control of vernalization, photoperiodic response or growth habit. There is, however, some evidence that it is implicated in the control of ear-emergence time (Kuspira & Unrau, 1957). Further studies are consequently necessary in which the effects of substituting chromosome 4D of Cappelle-Desprez into Chinese Spring are analysed in greater detail before the nature of the cold resistance exhibited by this chromosome can be determined.

(iv) *Evolutionary implications*

The two chromosomes 4D and 5D account for a major proportion of the difference in cold resistance between Chinese Spring and Cappelle-Desprez. This agrees with the view that it is the introduction of the D genome which enabled the original hexaploid wheats to expand and colonize more northern latitudes whereas the tetraploids have been confined to regions with higher average temperatures. Similar conclusions have been reached by Tsunewaki (1968), based upon the distribution of growth habit genes among the three genomes of a wide range of synthetic and natural hexaploids.

The present results, however, demonstrate a direct correlation between D genome chromosomes and cold resistance, a character which is central to the above assertion, whereas growth habit need not always be indicative of an ability to survive low temperatures.

(v) *Other considerations*

It is generally considered that the inheritance of cold resistance is complex. The present experiments using inter-varietal chromosome substitutions have

shown that for at least one varietal difference, Chinese Spring versus Cappelle-Desprez, the genetic control as opposed to the physiological control is not over-complex either in numbers of factors involved or in the manner of their action on the scale of measurement used. Whether this will prove to be true when varieties more winter-hardy than Cappelle-Desprez are considered is of course not known. The fact that in the hexaploid wheats triplicated loci are probably involved provides ample grounds for achieving complexity. On the simplest hypothesis of three loci, one to each of the chromosomes isolated in this experiment, six other homoeologous loci could occur. It is not unlikely therefore that for the more cold-resistant varieties, some allelic variation at these loci would be involved, and this does not take into account the different spectrum of chromosome effects observed at the coleoptile stage (Goujon *et al.* 1968).

At present, the requisite chromosome substitution lines are not available for studying this possible complexity. On the other hand, recent developments of aneuploid lines and the commencement of a number of substitution lines involving a wide range of European wheats indicate that the required materials could be available within a few years (Law, 1968c). It is not too much to hope that from these studies a fully comprehensive picture of the genetic and physiological control of cold resistance will emerge and this for a large population of the hexaploid wheats of the world.

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