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The inheritance of resistance to eyespot (*Cercosporella herpotrichoides*) in wheat

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SUMMARY

The genetic control of resistance to the fungus, *Cercosporella herpotrichoides*, causing the eyespot disease of wheat was studied using the Chinese Spring (Cappelle-Desprez) chromosome substitution lines and F_2 monosomic families of hybrids between Cappelle-Desprez and Mara. Chromosome 7A of Cappelle-Desprez was found to increase resistance to eyespot in both types of test. Chromosomes 2B and 5D of Cappelle-Desprez gave increased resistance compared to their homologues in Mara on an F_2 background. When substituted into Chinese Spring neither of these chromosomes appeared to increase resistance. Chromosome 1A of Cappelle-Desprez in a Chinese Spring background increased the level of infection. Dominance was towards resistance and the presence of between-chromosome interaction could be deduced.

1. INTRODUCTION

The facultative fungal parasite, *Cercosporella herpotrichoides*, is responsible for the eyespot disease of the hexaploid wheat, *Triticum aestivum*. This disease causes considerable lodging and reductions in yield among susceptible varieties of wheat. The incorporation of genes for resistance to eyespot is an important aim of wheat varietal improvement. Apart from the resistance of *Aegilops ventricosa* (Kimber, 1967), some of which has been transferred to wheat in the variety VPM1 (Maia, 1967; Doussinault, Koller, Touvin & Dosba, 1974), one of the best sources of resistance is found in the variety Cappelle-Desprez. At present, the genetic basis of this eyespot resistance is unknown. Indeed, very few investigations into the inheritance of this character have been attempted. Varietal differences have been assessed (Macer, 1966), but the analysis of segregating generations which might lead to the estimation of the number of genes involved as well as a description of their effects, has not been made. This is primarily due to the low heritabilities normally obtained using present scoring methods.

The development of cytogenetical techniques in wheat (Sears, 1953; Unrau, 1950; Law, 1968), by which varietal differences can be partitioned into single chromosomal effects, offers an opportunity for carrying out the genetic analysis of this character with a precision not possible using conventional methods. This paper describes a series of investigations, using cytogenetical techniques, into the genetic nature of the resistance of Cappelle-Desprez to eyespot infection.

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2. MATERIALS

Three varieties of hexaploid wheat, *T. aestivum* ($2n = 6x = 42$) were used in the experiments:

(i) *Chinese Spring*. A wide range of aneuploid lines are available in this spring variety and it has been used extensively in cytogenetic studies. It is susceptible to eyespot.

(ii) *Cappelle-Desprez*. This is a winter wheat, bred in France, and grown widely in the UK. Its high resistance to eyespot has already been mentioned.

(iii) *Mara*. This is a short-strawed Italian spring wheat, highly susceptible to eyespot.

3. METHODS

(i) *Genetical*

(*a*) *Chromosome substitution lines*. The 21 inter-varietal chromosome substitution lines of Cappelle-Desprez into Chinese Spring in which single pairs of chromosomes of Cappelle-Desprez replace their homologues in Chinese Spring were used. The development of these lines by means of backcrossing has been described in detail elsewhere (Law & Jenkins, 1970). In each of the experiments duplicates for each of the substitution lines were tested in order to detect genetic variation not associated with the substituted chromosome. Experiments were carried out on each substitution line after four backcrosses and after seven backcrosses.

(*b*) *Monosomic analysis*. Seventeen different monosomics ($2n = 41$) of Cappelle-Desprez were hybridized to the susceptible variety Mara as pollen parent. F_1 monosomics were selected on the basis of chromosome counts of root-tip cells stained using the Feulgen technique. These selected monosomics were selfed and the F_2 seedlings scored for eyespot infection. Each of the 17 F_2 monosomic families is deficient for a different Cappelle-Desprez chromosome. Increased susceptibility of one or more of the F_2 families indicates therefore the chromosome(s) of Cappelle-Desprez responsible for eyespot resistance.

The monosomics of Cappelle-Desprez were developed at the Plant Breeding Institute by recurrent backcrossing to the Chinese Spring monosomic series. At least eight backcrosses were made for each of the monosomic lines used in the crosses with Mara. In every case, the Cappelle-Desprez monosomic line was established as being monosomic for the correct chromosome by crossing with the appropriate ditelocentric lines of Chinese Spring (Law & Worland, 1973).

(ii) *Pathological*

The methods of scoring the degree of eyespot infection were similar to those described by Macer (1966). Six seeds were germinated in $3\frac{1}{2}$ in. plastic pots containing John Innes compost. Each pot represented a replicate and several replicates were grown in each of the experiments. Following germination, inoculation was carried out by placing a straw cylinder, previously infected with *Cercosporella herpotrichoides*, over each emerging coleoptile. Sieved compost was then added to

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cover the straws and the pots were maintained under cool conditions in a glass-house.

At the third to fifth leaf stage at least four seedlings from each pot were scored and the degree of infection ascertained by examining each successive leaf sheath for disease symptoms and classifying it as either infected or penetrated by the fungus. Each seedling was then given one of the following scores (Scott, 1971): seedling uninfected, 0; coleoptile infected, 1; coleoptile penetrated, 2; first leaf sheath infected, 3; first leaf sheath penetrated, 4; second leaf sheath infected, 5; second leaf sheath penetrated, 6; etc.

4. RESULTS

(i) *Chinese Spring (Cappelle-Desprez) substitution lines*

Three experiments were carried out using these substitution lines. The difference between the eyespot scores for each substitution line, averaged over duplicates, and the score for Chinese Spring for each of the experiments is given in Table 1.

Table 1. *The difference between the mean eyespot scores for each substitution line and Chinese Spring (CS) in each of three experiments*

Chromosome	Expt 1 (4th backcross) CS = 6·43	Expt 2 (7th backcross) CS = 6·80	Expt 3 (7th backcross) CS = 5·94
1A	+0·31	+0·42**	+0·99*
1B	−0·01	−0·28	+1·70**
1D	−0·49	+0·17	−0·50
2A	+0·54	+0·12	+0·49
2B	+0·52	−0·13	+0·25
2D	+0·05	+0·13	−0·32
3A	−0·18	+0·24	+0·57
3B	+0·28	+0·14	−0·43
3D	−0·08	0·0	+0·37
4A	+0·07	+0·32*	+0·32
4B	+0·04	−0·05	−0·36
4D	+0·06	+0·01	−0·84
5A	−0·24	−0·25	−0·23
5B	−0·51	−0·22	−0·23
5D	+0·24	−0·25	−0·28
6A	+0·52	−0·12	−0·14
6B	−1·21**	+0·11	−0·28
6D	−1·97**	−0·08	+0·24
7A	−0·84*	−0·39**	−1·11**
7B	+0·59	−0·28	−0·16
7D	−0·25	+0·22	+0·04
Cappelle-Desprez	−0·98*	−1·15**	−0·73
Least significant difference ($P = 0{\cdot}05$)	0·79†	0·29	0·86

* 0·05 > P > 0·01.
** P < 0·01.
† Based upon duplicate variation as error.

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In the first experiment, based upon lines extracted after four backcrosses to Chinese Spring, three of the lines – Chinese Spring (Cappelle-Desprez 6B), Chinese Spring (Cappelle-Desprez 6D) and Chinese Spring (Cappelle-Desprez 7A) – were more resistant than Chinese Spring and indistinguishable from Cappelle-Desprez. However, the variation between the duplicates for each substitution line was also significant, so that some of the differences between the lines could be due to background genes rather than to the effects of the substituted chromosomes.

For this reason, two further experiments were carried out at different times on the same lines produced after an additional three backcrosses to appropriate aneuploid lines of Chinese Spring. The analyses of each of these experiments indicated that duplicate variation was no longer significant, so that background variation can be discounted in making comparisons between the mean eyespot scores of the substitution lines.

In neither of these experiments were Chinese Spring (Cappelle-Desprez 6B) and Chinese Spring (Cappelle-Desprez 6D) significantly different from Chinese Spring. The resistance of these lines in the first experiment must therefore be due to genes for resistance present in their backgrounds at the fourth backcross generation. On the other hand, Chinese Spring (Cappelle-Desprez 7A) was significantly more resistant than Chinese Spring in both experiments. This substitution line has therefore maintained a resistant phenotype throughout all the experiments, which must indicate that chromosome 7A of Cappelle-Desprez is responsible for a major part of the difference in resistance between Chinese Spring and Cappelle-Desprez.

In both experiments a number of other substitution lines showed significant departures from Chinese Spring. Of these only Chinese Spring (Cappelle-Desprez 1A) was consistent and gave increased susceptibility to eyespot in each experiment. So far as the other lines are concerned, the lack of agreement suggests that these differences are due to sampling variation, although the highly significant departure shown by Chinese Spring (Cappelle-Desprez 1B) in Expt 3 may warrant further attention.

The differences between Cappelle-Desprez and Chinese Spring could therefore be explained in terms of two chromosomes alone. The gene (or genes) carried by chromosome 7A of Cappelle-Desprez produces in a Chinese Spring background a level of resistance similar to that of Cappelle-Desprez, whereas in Cappelle-Desprez this chromosome is equally active and epistatic to the susceptible alleles carried by chromosome 1A. This hypothesis is relatively simple and is consistent with the results of Expts 2 and 3. If background effects are taken into consideration, it also agrees with the first experiment in most details except that it does not explain the lack of effect of chromosome 1A.

(ii) *Monosomic analysis of Cappelle-Desprez × Mara*

The differences between the mean eyespot scores for 17 F_2 monosomic families, obtained from crosses of Cappelle-Desprez monosomics with Mara, and the two parental varieties are given in Table 2. It is evident that three families, Cappelle-Desprez 2B/Mara, Cappelle-Desprez 5D/Mara and Cappelle-Desprez 7A/Mara,

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stand out from the other F_2 monosomic families as being significantly different from Cappelle-Desprez but not from Mara and are the most susceptible of all the families studied. This indicates that chromosomes 2B, 5D and 7A of Cappelle-Desprez are involved in the increased resistance of this variety over Mara.

One further conclusion can also be drawn from this experiment and this concerns the direction of dominance exhibited by this character. The remaining 14 F_2 monosomic families can be regarded as providing an estimate of the eyespot score of a normal F_2. The mean of the eyespot scores of these 14 families is 6·58 which is very close to the Cappelle-Desprez value of 6·33. Dominance is therefore in the direction of resistance and is possibly very close to being complete.

Table 2. *Differences between the mean eyespot scores of* F_2 *monosomic families and their parents, Cappelle-Desprez and Mara*

Chromosome	Difference from Cappelle-Desprez = 6·33	Difference from Mara = 8·74
1A	+0·55	−1·86**
1B	+0·27	−2·14**
1D	+0·82	−1·59**
2A	−0·88	−3·29**
2B	+1·68**	−0·73
3B	−0·47	−2·88**
3D	−0·35	−2·76**
4A	+0·87	−1·54**
4B	+0·90	−1·51**
5A	−0·18	−2·59**
5D	+1·84**	−0·57
6A	+0·20	−2·21**
6B	+0·36	−2·05**
6D	+0·37	−2·04**
7A	+1·51**	−0·90
$5B^S$–$7B^S$†	+0·01	−2·40**
7D	+1·05*	−1·36**

Least significant difference ($P = 0·05$) = 0·96.

* $0·05 > P > 0·01$. ** $P < 0·01$.

† Refers to one of the reciprocal translocated chromosomes relative to the primitive structure of wheat, present in Cappelle-Desprez and composed predominantly of the two short arms of chromosomes 5B and 7B.

5. DISCUSSION

The investigations involving three varieties – Chinese Spring, Cappelle-Desprez and Mara – have uncovered chromosomes 1A, 2B, 5D and 7A as being implicated in the control of eyespot resistance. Of these, chromosome 7A of Cappelle-Desprez confers increased resistance in all the investigations, irrespective of the varietal combination used. Chromosomes 2B and 5D of Cappelle-Desprez only emerge to any degree when compared with their homologues in Mara on a segregating Mara–Cappelle-Desprez background. Substituted into Chinese Spring these chromosomes

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appear to have little effect. On the other hand, the Cappelle-Desprez/Mara F_2 monosomics show no effect of chromosome 1A, whereas in the Chinese Spring background it is the Chinese Spring homologue which appears to give resistance.

To what extent the absence of an effect of chromosomes 2B and 5D of Cappelle-Desprez in Chinese Spring is due to epistasis rather than to allelic differences between Mara and Chinese Spring is not clear. Certainly, the study of the Cappelle-Desprez substitution series, which implicates chromosomes 1A and 7A only, suggests that epistasis is a component of the genetic control of this character. On the other hand, the high eyespot score of Mara suggests that it may be more susceptible than Chinese Spring so that some allelic differences between these varieties could exist. Intercrosses between the Chinese Spring (Cappelle-Desprez) substitution lines for chromosomes 2B, 5D and 7A should however enable these possibilities to be examined.

The recognition that chromosome 7A is important in eyespot resistance is of interest, since, in a survey of diploid and tetraploid wheats, high levels of eyespot resistance were associated with anthocyanin pigmentation of the stem (Defosse & Vandam, 1969). Also the resistant variety VPM1, derived by introducing genes from *Aegilops ventricosa* into hexaploid wheat, has strong anthocyanin pigmentation of the coleoptile and stem (Doussinault *et al.* 1974). Although Chinese Spring (Cappelle-Desprez 7A) is non-pigmented, this chromosome and indeed all the chromosomes of homoeologous group 7 have been shown to carry genes for anthocyanin production (Law, 1966; Gale & Flavell, 1971). It is probable therefore that the association between eyespot resistance and pigmentation in these *Triticum* species and VPM1 arises from linkage between genes located on the group 7 chromosomes and controlling the two characters.

The number of chromosomes involved in eyespot resistance indicates that the inheritance and physiological control of this disease can be complex. This is in agreement with the findings of Riley & Macer (1966), who showed that several chromosomes of rye, *Secale cereale*, added separately to wheat, had effects on the level of eyespot resistance. This conclusion need not of course mean that selection for eyespot resistance in varietal improvement will be difficult, since many varietal combinations will already have a concentration of resistance genes in them. Many West European wheats are probably of this type because of their long history of exposure and selection for resistance to the disease. This might explain the apparent ease with which the Cappelle-Desprez resistance can be transferred (J. Bingham, personal communication).

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