THE GENETIC CONTROL OF GIBBERELIC ACID INSENSITIVITY AND COLEOPTILE LENGTH IN A "DWARF" WHEAT

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SUMMARY

In hexaploid wheat (*Triticum aestivum*) analysis of the F_2 generation of the cross of Minister Dwarf and Chinese Spring showed that insensitivity to gibberellic acid, a characteristic of Minister Dwarf and other dwarf wheats, was determined by a single gene, *Gai*₃. The short coleoptiles, which are also a feature of these dwarf wheats, was also identified as being predominantly controlled by a single gene which was probably identical to the gene *Gai*₃.

F_2 monosomic analysis located *Gai*₃ on chromosome 4A and demonstrated that the allele of *Gai*₃ for insensitivity was an active allele rather than a deletion or a null allele, since plants nullisomic for chromosome 4A were not insensitive. The transmission rates of 20 chromosome gametes deficient for chromosome 4A was found to be higher than normally expected.

*Gai*₃ was also epistatic to genes for increasing coleoptile length carried by other chromosomes than 4A, indicating that selection for acceptable coleoptile lengths in breeding programmes may be difficult in the presence of *Gai*₃.

1. INTRODUCTION

GIBBERELIC ACID (GA) insensitivity for plant height in dwarf wheats, *Triticum aestivum* ($2n = 6x = 42$), has been described by Allan, Vogel and Craddock (1959), and has been shown to be associated with high endogenous levels of the hormone by Radley (1970). Other aspects of the GA insensitive reaction were described by Gale and Marshall (1973*a*) who showed that the dwarf varieties, Norin 10-Brevor 14, Tom Thumb and Minister Dwarf displayed a similar lack of response to GA in plant height, but an increased response in tiller production, the reverse of the reduced tillering response found in tall GA-sensitive varieties. Also both Minister Dwarf and Tom Thumb failed to produce α -amylase in germinating grain in response to GA treatment, whereas Norin 10-Brevor 14, like most other wheat genotypes, was stimulated to produce the enzyme.

Norin 10-Brevor 14 has been used as a source of dwarfism in the development of many of the successful semi-dwarf wheat varieties which are widely grown throughout the world. Probably all the semi-dwarf wheats derived from this source are GA-insensitive in aerial tissues, so that an understanding of the genetic and physiological relationships which exist between dwarfism, GA-insensitivity and other associated characters is of considerable significance in plant breeding.

This paper describes experiments designed to investigate the inheritance of the GA-insensitivity of Minister Dwarf and the relationships of this character to short coleoptiles which is another correlated feature of dwarf wheats.

2. MATERIALS AND METHODS

Minister Dwarf (MD) and the monosomic series of Chinese Spring (CS), a variety sensitive to exogenous GA, were used in these studies.

MD was obtained from a hybrid of *Triticum spelta* and "Tom Pouce", backcrossed on to the variety Minister (P. Derenne, personal communication). "Tom Pouce" was derived from an old English variety "Hybrid Carter G" as described by Zeven (1969).

The method of monosomic analysis (Sears, 1953) was employed to study the genetic control of the differences in response to GA expressed by MD and CS. This method permits the recognition of the chromosome carrying the gene or genes responsible for a character difference by comparing, in the case of dominant genes, the segregation of each of the 21 F_2 monosomic families with the normal or control F_2 . For the critical F_2 monosomic family, derived from an F_1 monosomic in which the hemizygous donor chromosome carries the gene or genes under investigation, most of the F_2 progeny should have the donor varieties phenotype. For the non-critical F_2 monosomic families, the segregation of this gene or genes will be as in a normal F_2 . Departures from the normal F_2 therefore indicate the chromosome carrying the gene or genes concerned.

The method has worked well in the analysis of character differences which are under the control of a few genes whose effects are readily recognisable (Unrau, 1950; Macer, 1966).

MD was crossed to each of the 21 monosomic lines of CS and monosomic F_1 hybrids were selected by means of chromosome counts made on root-tip squashes stained by the Feulgen procedure. The resulting F_1 monosomics, as well as F_1 disomic hybrids were then selfed and F_2 grains obtained from all of the lines except CS 2B/MD which was sterile.

The monosomic analysis experiment was carried out on plots of 50 unselected grains from each of the 20 F_2 monosomic families, seven F_2 control families and the two parents, MD and CS. The grain was sown in vermiculite, moistened with culture solution (Austin and Maclean, 1972) and grown for 3 days at 2°C to ensure even germination and then for 6 days at 18°C and 3000 lux. Coleoptile lengths were scored at this time and then the culture solution was supplemented with GA (Berelex, Plant Protection Ltd.) at 10 ppm. Seedlings were scored as responsive or insensitive after a further 10 days.

3. RESULTS

(i) GA insensitivity

Following treatment with GA, plants could be classified readily into responders and insensitives. Responders (R) gave a distinctive phenotype and produced thin stems with elongated internodes and leaves.

A preliminary experiment in which 99 F_2 progeny of the cross MD $\times$ CS gave 79 insensitive (I) : 29 responsive (R) seedlings, indicated that the character was under the control of a single dominant gene, henceforth designated as *Gai*₃ ($\chi^2_{(1)} = 0.19$, $P > 0.2$). In a further experiment to assess the relationship of GA insensitivity with coleoptile length, embryos from 40 F_2 seeds from the MD $\times$ CS cross were grown in the dark on agar, scored for coleoptile length after 6 days and then transplanted into pots. Embryos

were preferred for this investigation since previous experiments (Gale and Marshall, 1975) had shown that the GA-insensitivity of MD can affect coleoptile and early seedling growth via an effect on mobilisation of endosperm reserves during germination.

Measurement of coleoptile lengths indicated that the seedlings could be classified into two distinct groups of 30 short (30-60 mm) to 10 long (75-95 mm) indicating the segregation of a single gene affecting coleoptile length. On applying GA, only the 10 seedlings with long coleoptiles responded to give elongated internodes, whereas all the short coleoptile seedlings were

TABLE 1

GA-responsive (R) and Ga-insensitive (I) progeny and coleoptile lengths (mm) of F₂ control and F₂ monosomic families

Family	R : I	$\bar{x}_R$	$\bar{x}_I$	Chromosomal effects	Interaction effects
Control F ₂	71 : 251	25.39	19.31	—	—
CS1A/MD	13 : 35	26.00	20.20	1.49	-0.28
CS1BM/D	10 : 40	26.80	20.83	2.92	-0.11
CS1D/MD	18 : 32	28.17	19.91	3.37**	2.18
CS2A/MD	7 : 43	24.14	19.60	-0.95	-1.55
CS2D/MD	17 : 32	23.82	15.94	-4.94***	-1.80
CS3A/MD	9 : 39	30.44	21.56	7.30***	2.79**
CS3B/MD	11 : 32	26.18	19.34	0.82	0.75
CS3D/MD	13 : 31	27.77	20.71	3.78**	0.97
CS4A/MD	5 : 43*	29.00	19.71	—	—
CS4B/MD	7 : 43	25.29	20.23	0.82	-1.03
CS4D/MD	15 : 35	22.93	15.86	-5.91***	0.98
CS5A/MD	14 : 33	31.50	21.30	8.10***	4.11***
CS5B/MD	14 : 35	30.71	20.74	6.45***	3.88***
CS5D/MD	9 : 39	31.00	21.59	7.89***	3.32
CS6A/MD	13 : 37	27.92	20.73	3.95**	1.10
CS6B/MD	11 : 35	27.64	21.83	4.77***	-0.28
CS6D/MD	14 : 31	28.07	20.35	3.72	1.63
CS7A/MD	12 : 37	25.33	19.38	0.00	-0.14
CS7B/MD	14 : 35	28.07	19.69	3.06**	2.29
CS7D/MD	14 : 34	28.86	19.26	1.42	1.51
CS	—	27.55	—	—	—
MD	—	—	15.35	—	—

* $\chi^2_{(3;1)}$, $P < 0.02$; ** and *** "t" tests, $P < 0.01$ and < 0.0001 .

insensitive. There is thus an association between GA response and coleoptile length which is independent of the effect of GA on endosperm mobilisation. The complete association also suggests that the single genes affecting these two characters are either the same or are closely linked.

The results obtained from the F₂ monosomic analysis, classified as the number of R and I progeny within each monosomic family and the mean coleoptile lengths of these two types of progeny, are given in table 1.

Since a single dominant gene *Gai*₃ is responsible for the distinction between R and I seedlings, then only one of the F₂ monosomic families should show an excess of I progeny compared with the control F₂. For this critical F₂ monosomic family the only R progeny expected, assuming that *Gai*₃ is expressed in the hemizygous condition, would be nullisomic and deficient for the chromosome carrying *Gai*₃. Normally such nullisomics are rare and in CS constitute about 3 per cent of the progeny of a monosomic (Sears, 1953).

Examination of the segregation into R and I progeny indicates that none of the monosomic families depart from the control F_2 in showing an excess of insensitives. Only three families stand out, CS2A/MD, CS4A/MD and CS4B/MD, as having low frequencies of responders and none of these families depart significantly from the control, although CS4A/MD gives a ratio which is significantly different from the expected 3 : 1 ratio ($\chi^2_{(1)} = 5.44$, $P < 0.02$). This can only be interpreted to mean that either Gai_3 is not completely effective in the hemizygous condition or the transmission of 20 chromosome gametes giving rise to responsive nullisomics is much higher than expected in the critical F_2 monosomic family.

To test this latter possibility the R progeny in these three families were scored for their chromosome numbers. For CS2A/MD and CS4B/MD both disomic and monosomic progeny were found, whereas for CS4A/MD the four R progeny successfully scored had 40 chromosomes and were therefore presumably nullisomic for chromosome 4A. A repeat experiment on the CS4A/MD F_2 family was consequently carried out and gave 35 I : 12 R in which the 10 successfully scored plants from the R group were found to be nullisomic.

It is clear therefore that Gai_3 is located on chromosome 4A and that the high incidence of responders in the F_2 monosomic for this chromosome is due to an abnormally high transmission of nullisomic gametes.

(ii) *Coleoptile length*

Gai_3 or a closely linked gene also accounts for a major part of the variation in coleoptile length between the two varieties. Other genes carried by chromosomes other than 4A may however affect this character and contribute to the differences between the varieties.

The location of some of these genes may be attempted by comparing the mean coleoptile lengths of each of the monosomic F_2 families with the mean of the control F_2 . Any differences that occur between these means would indicate that either different alleles or alterations in chromosome dosage or both were responsible.

A second comparison which is perhaps more interesting and informative can also be made from the separation of coleoptile lengths of untreated progeny into the two groups, R and I, based upon their behaviour following treatment with GA. This separation permits a test to be made of the effect of segregation at the Gai_3 locus on the variation within each of the monosomic families. If the R and I groups within any monosomic F_2 have larger or smaller differences in mean coleoptile length than the R and I groups in the control, then this indicates that Gai_3 must interact with the genes affecting coleoptile length on the particular chromosome under test irrespective of whether the differences from the control are due to chromosome dosage or allelism.

The mean coleoptile lengths of untreated plants at 6 days partitioned according to their subsequent classification as R or I are shown as $\bar{x}_R$ and $\bar{x}_I$ in fig. 1. The first comparison, which estimates chromosomal effects due to allelic variation and/or chromosome dosage, is given by the mean of an F_2 monosomic family minus the F_2 control family, *i.e.* $(\bar{x}_R + \bar{x}_I - \bar{x}_{RC} - \bar{x}_{IC})$ where $\bar{x}_{RC}$ and $\bar{x}_{IC}$ refer to the mean of the R and I progeny of the control F_2 respectively. The second or interaction comparison is derived from

$\bar{x}_R - \bar{x}_I - \bar{x}_{RC} + \bar{x}_{IC}$. The values and significance of both these comparisons for each monosomic family are presented in table 1.

In determining the levels of significance for each of these comparisons, within family variances for each of the F_2 monosomics were used except in the case of the F_2 disomic control where seven separate families were grown. The within family variances for each of these control F_2 's were however homogeneous whereas the between family differences were found not to differ from the within family variances. A pooled estimate of between and within family variances was therefore used in the case of the control F_2 . Throughout a modified "t" test (Cochran, 1964) was applied in cases of borderline significance and, because of the large number of comparisons, an acceptable level of significance of $P < 0.01$ was adopted.

FIG. 1.—Mean coleoptile lengths for responsive and insensitive classes within F_2 monosomic families, compared with control F_2 means. (Note: * $P < 0.01$ and ** $P < 0.001$.)

Eleven of the F_2 monosomic families departed significantly from the control F_2 in mean coleoptile length. Only CS2D/MD and CS4D/MD gave mean coleoptile lengths less than the control. It is possible therefore that these chromosomes of MD carry alleles which reduce coleoptile length compared with their alleles in CS. On the other hand, the marked increases in coleoptile length obtained for the remaining significantly different families suggest that chromosome dosage rather than allelic differences are implicated. If this is correct then it is reasonable to suppose that each of these chromosomes carry genes which inhibit coleoptile length and it is their removal which results in the increased coleoptile lengths observed.

Only three chromosomes, 3A, 5A and 5B, gave significant interactions with the segregation at the Gai_3 locus, although the high interaction estimate for chromosome 5D points to all the chromosomes of this group being involved. Moreover, all the interactions were positive indicating that Gai_3 reduces the effects of the genes, carried by these chromosomes, for increasing coleoptile length. This could arise either from the enhanced expression of promoters in the absence of Gai_3 or the enhanced expression of inhibitors in its presence. Since, as mentioned in the previous paragraph, reduced

chromosome dosage would appear to be responsible for the increases in mean coleoptile length exhibited by these families, the enhanced effect of inhibitors is probably correct.

4. DISCUSSION AND CONCLUSIONS

The monosomic analysis clearly indicates that a gene (*Gai*₃) for insensitivity to GA is located on chromosome 4A. The association of this character with coleoptile length indicates that these characters could be controlled by the same gene and suggest that the reduced coleoptile lengths could arise as a consequence of abnormal GA-metabolism.

However, the unequivocal proof that associations between two or more characters are due to the pleiotropic effects of a single gene is extremely difficult to establish. For the sample sizes used in the experiments described, it can be shown that recombinant lines need not appear even though two linked genes, having a recombination frequency between them of 5 per cent, were responsible for the separate control of the two characters under investigation. The results presented here are therefore inconclusive on this point.

However, other data available from crosses of MD with tall wheat varieties, in which over a 1000 progeny were classified with respect to coleoptile length and GA insensitivity, gave no evidence of a breakdown in the association between these two characters. A combination of all the data relating to MD crosses reduces the admissible recombination value on the hypothesis of two genes to a value as low as 0.025 per cent. This is very suggestive that pleiotropy rather than linkage is responsible for the association.

On the other hand, whilst it is clear that a genetic explanation of the association between these two characters is forthcoming from these experiments, it is not clear how these characters relate to the dwarf nature of adult MD. Coleoptile length and final plant height are often highly correlated (Allan and Vogel, 1964). Also the location of the dwarfing gene in Tom Thumb, a variety which is similar in phenotype to MD, on chromosome 4A by Morris, Schmidt and Johnson (1972) suggests that all these characters could be causally related and determined by the gene *Gai*₃. Hu (1974), however, from a study of dwarf × tall wheat crosses involving Tom Thumb, found two *F*₄ lines that were apparently homozygous for the genes for dwarfism but which segregated for GA insensitivity. He thus concluded that the two characters were under separate control. There is thus some evidence which suggests that the "dwarf" character has a separate genetic control from GA-insensitivity and coleoptile length and it may be possible to produce "dwarf" and tall plants which are sensitive and insensitive respectively.

It is also evident from the results presented here, that *Gai*₃ has an epistatic effect on genes, distributed on chromosomes other than 4A, for increasing coleoptile length. Positive selection for coleoptile length in the presence of *Gai*₃ will consequently not be as effective as in its absence. This could have important consequences on crop establishment if GA insensitivity proves to be an advantage in varietal improvement. It could be even more important if *Gai*₃ has a similar effect on genes for increasing final plant height, since the dwarfness of varieties such as MD and Tom Thumb appears to be too short for present agricultural requirements.

A question regarding the mode of action of Gai_3 raised by Gale and Marshall (1973b), is answered by the present results. It is clear that Gai_3 is almost completely dominant and that in the hemizygous state the gene is as potent as in either the homozygous or heterozygous conditions. Moreover, the absence of Gai_3 as in plants nullisomic for 4A, produces a response to GA, indicating that insensitivity is an active effect rather than the lack of some essential biosynthetic apparatus for processing the hormone as might be expected in the case of a deletion.

Finally a point emerges from this work concerning the theoretical basis for monosomic analysis. As mentioned earlier, it has generally been assumed that F_2 families monosomic for the critical chromosome carrying a dominant gene will deviate from the expected 3 : 1 ratio of the control F_2 since only the nullisomics, normally expected to be about 3 per cent, will express the recessive phenotype. However, it is evident in the experiment described here, that the critical monosomic family had a frequency of nullisomic progeny of about one-quarter indicating a male transmission rate much higher than previous experience has indicated (Sears, 1953). Interestingly, Morris *et al.* (1972) obtained similar high frequencies for the same chromosome in the monosomic analysis of dwarfism in Tom Thumb. It is possible that this high transmission rate of nullisomic gametes may be associated with Gai_3 or a different locus carried by chromosome 4A. If, however, this proves not to be the case and if such transmission rates were to occur frequently then conventional monosomic analysis may fail to locate other single dominant genes unless efforts are made to check the chromosome counts of F_2 monosomic progeny.

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