THE CHROMOSOMAL LOCATION OF *GAI 1* AND *RHT 1*, GENES FOR GIBBERELLIN INSENSITIVITY AND SEMI-DWARFISM, IN A DERIVATIVE OF NORIN 10 WHEAT

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SUMMARY

The gene conferring gibberellin insensitivity, *Gai 1*, was located on chromosome 4A. This gene is associated with the semi-dwarf habit and consequently it was concluded that *Rht 1* is carried by 4A. No recombination was found between *Gai 1* and *Gai 3*, the gibberellin insensitivity gene in "Tom Thumb"-type dwarf wheats, indicating that these genes are either alternative alleles at the same locus or are closely linked.

1. INTRODUCTION

NORIN 10-BREVOR 14 (NB 14) was derived from a cross between the Japanese dwarf and an American variety. This line is the major source of the semi-dwarfing character common to many present-day commercial wheat varieties. NB14 carries two alleles that confer "insensitivity" of aerial plant parts to application of the phytohormone, gibberellic acid (GA). One of these alleles, *Gai 2*, has been shown to be located on chromosome 4D by Gale *et al.* (1975*b*). They also showed that the segregation of *Gai 2/gai 2* was closely associated with variation in height in a GA-insensitive (GAI) semi-dwarf $\times$ GA-responsive tall wheat cross.

The positive association between GAI and height reduction has been shown for both *Gai 1* with *Rht 1* and *Gai 2* with *Rht 2* in crosses involving Norin 10 derivative semi-dwarfs (Gale and Marshall, 1973; Hu, 1974) and for *Gai 3* with *Rht 3*, the genes controlling GAI and dwarfism in "Tom Thumb"-type dwarf wheats (Gale *et al.*, 1975*a*; Fick and Qualset, 1975). Whether the nature of this association is pleiotropic, *i.e.* the *Gai* and *Rht* genes are, in fact, the same, or due to linked loci, as suggested by Konzak *et al.* (1973), it is clear that the chromosomal locations of *Gai 1*, *Gai 2* and *Gai 3* are the same as those of *Rht 1*, *Rht 2* and *Rht 3*. The location of *Gai 3* (Gale *et al.*, 1975*a*) and *Rht 3* (Morris *et al.*, 1972) has already been ascertained as chromosome 4A. Only *Gai1/Rht 1* remains unmapped.

So far only *Rht 2* has been exploited in British semi-dwarfs such as Maris Fundin and its successors. There is some evidence to suggest that *Rht 1* may have some agronomic advantages and be a better genetic base for semi-dwarfs than *Rht 2*, which produces the shorter straw (Allan, 1970). Knowledge of the location of *Gai 1*, and thus *Rht 1*, will assist in its future incorporation into adapted lines and in assessing the agronomic worth of such derivatives. This would allow the gene(s) to be manipulated by standard aneuploid techniques (Law and Worland, 1972) and be recognised and followed by its qualitative GAI phenotype rather than the quantitative effect on height.

This paper describes experiments that have led to the determination of the chromosomal location of *Gai 1*.

2. GENOTYPES AND METHODS

The varieties used include SD1, a Norin 10 derivative kindly supplied by Dr R. E. Allen, Washington State University; Norin 10-Brevor 14 (NB14) a short derivative from the cross Norin 10 $\times$ Brevor extracted by Dr O. E. Vogel; Hobbit, a semi-dwarf variety bred by Dr F. G. H. Lupton and known to carry *Rht 2* and *Gai 2* (Gale *et al.*, 1975b); Olsen, an extreme dwarf that is known to carry both *Gai 1* and *Gai 2* (Gale, unpublished) as well as another unidentified height reducing gene (Fick and Qualset, 1973) and Tom Thumb and Minister Dwarf, dwarf lines carrying *Rht 3* and *Gai 3* on chromosome 4A. The monosomic series of Bersee and Cappelle-Desprez (Law and Worland, 1972) were used for F_2 monosomic analysis (Sears, 1953) of the GAI genes in SD1 and Olsen respectively.

GA-response tests were carried out under controlled environments as described in Gale *et al.* (1975b).

3. RESULTS

The results of F_2 GA-response analyses from crosses of SD1 with three testers are shown in table 1. These confirm that SD1 carries only *Gai 1*.

TABLE 1

F_2 GA-response analyses to determine the *Gai* allele carried by SD1. Known parental genotypes, Chinese Spring—gai 1 gai 2; NB14—Gai 1 Gai 2; Hobbit—gai 1 Gai 2. I, GA insensitive and R, GA-responsive

Cross	I:R	Ratio	Inference concerning SD1
SD1 $\times$ Chinese Spring	72:28	3:1	Carries a single <i>Gai</i> gene . . . which is
SD1 $\times$ NB14	100:0	—	either <i>Gai 1</i> or <i>Gai 2</i> . . . and is different
SD1 $\times$ Hobbit	95:5	15:1	from <i>Gai 2</i> . . . therefore SD1 carries <i>Gai 1</i>

Eighteen of the possible 21 monosomics of Bersee were crossed with SD1 using the aneuploid as the female parent. Duplicate F_1 monosomic ($2n-1$) hybrids, each carrying a hemizygous chromosome from SD1, were grown and grain collected to produce F_2 plants. This grain was germinated and, after the removal of root tips for subsequent cytological examination, transferred to vermiculite for a GA response test.

The results for the monosomic families combined over duplicates and for the disomic controls are shown in table 2. The critical chromosome, that carrying *Gai 1*, is expected to deviate from the ratio 3 insensitive:1 responsive found in the control by an increase in the insensitive fraction. Two families, 4A and 4B, deviated in this way. For all families, the root tips of a sample of responsive and insensitive seedlings were checked for chromosome number, while for 4A and 4B the families were checked in greater detail. In all families except Bersee 4A/SD1 the GA response was independent of chromosome number.

A further sample of F_2 seedlings from Bersee 4A/SD1 was grown and the results, combined with those of the first sample, are shown in fig. 1, where the GA response, shown as the length of the second leaf-internode after exposure

TABLE 2

GA response of F_2 monosomic families of *Bersee* $\times$ *SD1*. I, *GA* insensitive (*Gai* 1.) and R, *GA* responsive (*gai* 1 *gai* 1)

Group	Genome					
	A		B		D	
	I	R	I	R	I	R
1	25	10	24	9	33	7
2	—	—	18	3	11	4
3	25	10	9	3	28	10
4	37	1***	34	6*	32	8
5	27	11	12	4	30	7
6	29	11	29	9	27	11
7	—	—	30	6	—	—
Euploid control	36	11				

χ^2 deviation from 3:1 ratio.

* $P = 0.05$; *** $P < 0.001$.

FIG. 1.—*GA*-response and chromosome number in *Bersee* 4A/*SD1* F_2 monosomic seedlings.

to GA, is plotted against chromosome number. Clearly the extension of this part of the seedling is associated with the different doses of chromosome 4A from SD1 in 42, 41 and 40 chromosome seedlings.

This result was checked by a similar experiment involving F_2 seedlings from Cappelle-Desprez 4A/Olsen. Since Olsen carries *Gai 1* and *Gai 2* the expected F_2 euploid and non-critical monosomic segregation will be 15 insensitive:1 responsive. For the critical monosomic F_2 all the progeny should be insensitive except those nullisomics homozygous for *gai 2*. In the 4A monosomic family, 80 seedlings were tested and only two of these were responsive. Although this does not differ from the expected disomic segregation ($\chi^2_{(1)} = 1.9$, NS) the responders were found to have chromosome numbers of 40 and 39. The 39 chromosome plant may reasonably be assumed to have been a 4A nullisomic which was monosomic for another, non-critical chromosome. This result confirms chromosome 4A as the location of *Gai 1*.

Chromosome 4A is also the carrier of *Gai 3*, the strong insensitivity gene present in Tom Thumb dwarfs (Gale *et al.*, 1975a; Gale and Marshall, 1975). Thus the observation that the same chromosome carries *Gai 1* necessitated a close look at the relationship of these two genes in segregating families. Grain from bagged F_1 spikes, in which the possibility of outcrossing was absent, was available from hybrids of either Tom Thumb or Minister Dwarf (*Gai 3*) with NB14 or Olsen (*Gai 1 Gai 2*) i.e. TT $\times$ Olsen, MD $\times$ Olsen, TT $\times$ NB14 and MD $\times$ NB14. Assuming independent assortment these F_2 's would be expected to segregate 63 insensitive:1 responsive in a GA response test. Among 1600 such seedlings no full responders was observed indicating that *Gai 1* and *Gai 3* are either alternative alleles at the same locus or closely linked.

Of course, in a three-gene duplicate system such as this, the F_2 is a very inefficient estimator of linkage. Although the estimate of linkage from this experiment is zero, it may be shown that a recombination value as high as 17.3 per cent will give a similar result with a probability of 5 per cent. Clearly then, the F_2 results establish linkage between *Gai 1* and *Gai 3* on chromosome 4A and suggest, but by no means prove, that they may be alternative alleles at the same locus.

In order to reach a more definite conclusion 20 grains from the tallest field grown F_2 's from the same families were progeny tested. The tallest were chosen since these would be the most likely to carry fewest *Gai/Rht* alleles. Among the 42 families sampled from a total of 560, four were found to contain a single partially responsive seedling as might be expected of the *Gai 1 gai 1* heterozygote. No fully responsive plants were observed. It seems likely that, in the absence of any "all-responsive" families or any segregating 3I:1R or 15I:1R that the partial responders were the result of outcrossing. In this case the zero estimate of recombination has a 5 per cent confidence limit of 0.7 per cent which considerably reinforces the possibility that *Gai 1* and *Gai 3* are alleles.

4. DISCUSSION AND CONCLUSIONS

The exact nature of the relationship between gibberellin insensitivity and height reduction is of practical importance to the bread wheat, macaroni wheat and triticale breeders who are using the Norin 10 and Tom Thumb

sources of dwarfism. Clearly a linkage relationship whereby the *Gai* and *Rht* genes can be manipulated independently is more attractive. Thus, as the relative agronomic advantages and disadvantages of either phenotype produced by any of the alleles become known, the genes may be exploited more fully. However if *Rht* semi-dwarfs are short because of their lack of ability to utilise endogenous gibberellins, *i.e.* the *Rht* and *Gai* phenotypes represent pleiotropic expression of a mutation at a single locus, then the breeder's possibilities are restricted to accepting GA-insensitivity and short straw as a package.

The main problem involved in ascertaining the genetic relationship between the pairs of *Gai* and *Rht* genes is that the difference between the *Gai* and *gai* phenotypes is qualitative while the difference between the *Rht* and *rht* phenotypes is quantitative and is usually considerably confounded by the other genetic differences affecting the plant height phenotype in segregating plants or families. Thus in a segregating family, although plant height is clearly strongly associated with *Gai* genotype, it is not always clear whether the variation in plant height within plants of known *Gai* genotype is due to segregation of the *Gai* and *Rht* loci, to other modifying genes effecting height in the presence of a single *Rht* genotype or even to environmental effects on plant height.

The clearest situation should be in the segregating progeny from tall $\times$ "Tom Thumb" type crosses since the height reduction effects of *Rht* 3 are so large as to be considered almost qualitative. In more than 1000 F_2 individuals we have observed no tall *Gai* 3 homozygotes or dwarf *gai* 3 homozygotes indicating either extremely tight linkage between *Rht* 3 and *Gai* 3 or more probably that both phenotypes are controlled by the same gene. However the observation by Hu (*loc. cit.*) that two F_3 families from a similar cross, that were considered to be homozygous for height, segregated for GA-insensitivity in a seedling test indicates that recombination may indeed be possible.

Other circumstantial evidence is available for both hypotheses. In favour of pleiotropy, the relative potencies of the *Gai* alleles in terms of GA-insensitivity exactly coincides with the relative height reducing effects of their respective *Rht* alleles—in potency *Gai* 3 > *Gai* 2 > *Gai* 1 and in degree of dwarfness produced *Rht* 3 > *Rht* 2 > *Rht* 1. Also, all of the semi-dwarf lines produced in breeding programmes at this Institute and those that we have tested from the CYMMIT programme in Mexico display the GA-insensitive reaction. This could, however, indicate agronomic advantages associated with the *Gai* alleles rather than the *Gai* and *Rht* genes being the same. On the other hand, given the observation that *Gai* 1 and *Gai* 3 are either at the same locus on 4A or, at most, less than one map unit apart, the several reports of independence between the "Tom Thumb" type of dwarfism and the two Norin 10 dwarfing genes (e.g. Piech, 1968; Chaudhry, 1973; Fick and Qualset, 1973) would appear to indicate that *Rht* 1 and *Rht* 3 are separate and therefore confirm that at least one may be separated from its *Gai* allele.

This conclusion is open to criticism now that the chromosomal locations of all three *Rht* genes are known. The basis of the genetic independence has, in each case, been based upon the observation of transgressive segregation in an F_2 , *i.e.* plants with longer straw than that of the taller parent have been recorded. Since Norin 10 and Norin 10-Brevor 14 carry two additive *Rht* genes and Tom Thumb carries only a single strong *Rht* gene, transgressive segregation would be expected whether *Rht* 1 and *Rht* 3 were independent *i.e.*

Rh 1 Rht 2 rht 3 $\times$ *rht 1 rht 2 Rht 3* or allelic i.e. *Rht 1 Rht 2* $\times$ *Rht 3 rht 2*. The difference would lie in that the first possibility would yield taller segregants, i.e. $\frac{1}{4}$ *rht 1 rht 2 rht 3*, than the second, i.e. $\frac{1}{8}$ *Rht 1 rht 2*.

The data shown in fig. 2 represent the pooled F_2 distributions of two types of cross, in which *Rht 1* and *Rht 2* segregate with *Rht 3* (type 1) or without *Rht 3* (type 2). If *Rht 1* and *Rht 3* were independent, plants as tall as the tallest from type 2 would be expected in type 1 but with a quarter of

FIG. 2.— F_2 plant height distributions—comparison of combined data for *Rht 1 Rht 2* $\times$ *Rht 3* (type 1) and *rht 3* $\times$ *Rht 1 Rht 2* (type 2). Parents: *Rht 1 Rht 2* Norin 10-Brevor 14, *Rht 3* Tom Thumb and Minister Dwarf; *rht* Cappelle-Desprez and Chinese Spring.

the frequency. No such plants are observed even though the number of individuals is in excess of 650. Of course, another reason for the apparent lack of tall plants in the type 1 crosses could be the presence of dispersed genes for tallness present only in type 2. This, while not impossible, seems unlikely since NB14 (*Rht 1 Rht 2*) is common to all crosses and the tall parents in type 2, Chinese Spring and Cappelle-Desprez are only of standard height. Also, similar F_2 height frequency distributions were obtained by Fick (1971) from type 1 and 2 crosses involving different parents indicating that the lack of tall plants in type 1 is more likely to be due to the spatial arrangement of the *Rht* alleles than to the presence of height promoting gene combinations only in type 2.

The type 1 crosses are those from which the F_3 progeny tests discussed earlier were taken, in which no GA-responsive plants or families were found. Taken together these results are most compatible with the hypothesis that the *Gai* and *Rht* alleles are the same, at least for *Gai/Rht 1* and *Gai/Rht 3*.

These experiments conclude the chromosomal mapping of the known *Gai* alleles in hexaploid wheat. It was, perhaps, not surprising that the two loci definitely established are carried within a single homoeologous group. The genes are qualitatively similar, if quantitatively dissimilar, in their action (Gale and Marshall, 1975) and consequently it is probable that they form part of a homoeologous series across the group 4. Thus there is wide scope for the discovery of other alleles either on the apparently vacant chromosome 4B or as alternative alleles at the other loci. Considering the success following the exploitation of *Gai/Rht 1* and *Gai/Rht 2* in commercial semi-dwarfs and the potential use of *Gai/Rht 3* for the production of low α -amylase quality wheats, a search for other similar genes among collections or in mutation experiments may be well worthwhile.

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