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Mixed model for estimating the effects of the *Rht1* dwarfing allele, background genes, CCC and their interaction on culm and leaf elongation of *Triticum aestivum* L., spring wheat

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A model for the effects of a single gene (SG), background genes (BG), an environmental factor (EF) and the effects of their interactions on quantitative traits is developed. It is a mixed model where SG and EF have fixed effects while BG have a random effect. This model is applied to the analysis of the effects of the dwarfing alleles at the *Rht1* locus (SG), interfamily variation (representing BG) and the growth regulant CCC (EF) on coleoptile, leaf and culm length of spring wheat. Culm length of F_7 families was tested in a field experiment in the absence of lodging. Coleoptile and leaf lengths of F_9 seedlings were examined in a growth room at 18°C. Each family was descended from a single F_5 plant, heterozygous at the *Rht1* locus. Within each family the homozygous tall (*rht1*) and the homozygous semi-dwarf (*Rht1*) genotypes were identified. Thus, comparing nearly isogenic genotypes within random families in advanced generations enabled the estimation of all the main effects and interactions between SG, BG and EF. The restricted maximum likelihood (REML) method was used in the analysis of variance.

In all the three organs CCC caused significant shortening which was somewhat greater in the *rht1* than in the *Rht1* genotype and the CCC × *Rht1* interaction effect on culm length was significant. Considerable and significant interfamily variation was found for all three characters. A significant CCC × family interaction effect on the length of the first leaf was obtained. This interaction effect was of a specific trend indicating a distinct increase in the response to CCC with greater leaf length. No CCC × *Rht1* × family or *Rht1* × family interaction effects were detected. The use of two graphical/analytical methods proved to be complementary for a complete evaluation of two-way interactions (CCC × families and CCC × *Rht1* in the present study).

Keywords: environmental factor, interaction, polygene, quantitative trait, single gene.

Introduction

Many quantitative traits (QT) are known to be controlled by one or more single genes of major effect (SG) as well as by variation in background (BG) which is akin to the polygene (PG) system discussed by Falconer (1981) and by Mather & Jinks (1971).

The existence of interactions between genotypes and environmental factors has long been recognized (Fisher & Mackenzie, 1923). Various methods have been proposed for the statistical analysis of these interactions (Freeman, 1973). An experimental design for self-pollinating plants — consisting of individuals, each belonging to a specific F_3 family which segregates for a SG — and the corresponding statistical procedures were developed by Elkind & Cahaner (1986) to estimate the components of a mixed model which includes the effects of SG, PG and their interaction.

A modification of this design for advanced generations has been applied by Beharav *et al.* (1988, 1992). This report presents the application of a mixed model which enables the estimation and the analysis of the main effects and interaction effects of an environmental factor (EF) in addition to those of the SG and BG, which have already been dealt with in a previous report (Beharav *et al.*, 1992). This was achieved in a

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study of the effects of the growth regulant CCC (EF) on leaf and culm elongation of the tall (*rht1*) and semi-dwarf (*Rht1*) genotypes (SG) in each of 25 or 30 advanced generation spring wheat families (representing BG).

CCC (chlormequat) is a growth retardant inhibiting GA biosynthesis (Roberts & Hooley, 1988). Owing to its shortening effect on wheat culms, it is occasionally applied to prevent lodging of this crop. In most modern semi-dwarf wheat cultivars the short stature is due to one of two height-reducing genes, *Rht1* and *Rht2*, introduced from the dwarf Japanese cv. 'Norin 10' (Gale & Youssefian, 1985). Culm length of wheat is furthermore controlled by many polygenes (Allan *et al.*, 1968). A weaker response to CCC of semi-dwarf (*Rht1* or *Rht2*) lines than of their near-isogenic tall lines was demonstrated in a study with winter wheat (Gale & Youssefian, 1983). This study, however, did not enable an estimate of the interactions between CCC, *Rht* alleles and BG and between CCC and BG, which has been attempted in the present investigation.

Experimental population

The families examined were progenies of a random sample of single F_5 plants of 'Mivhor' × 'Lakhish', heterozygous at the *Rht1* locus. The parental cultivars are high-yielding semi-dwarf spring wheats differing in their dwarfing alleles ('Mivhor' (Penjamo Sib × Gabo 55) carrying *Rht1* and 'Lakhish' (Yaktana × Norin 10 − Brevor) × (Florence × Aurore) carrying *Rht2*). Bulked F_2, F_3, F_4 and F_5 populations were grown without any deliberate selection. The heterozygosity of the F_5 plants was confirmed by testing the seedlings, from a sample of F_6 seeds from each F_5 plant, by a GA response test (Gale & Gregory 1977). The assessment in each family of the *Rht* dwarfing allele (whether *Rht1* or *Rht2*) was based on test crossing with the homozygous *Rh2* cv. 'Lakhish'. Due to insufficient amounts of seed the *Rht2* families (in which the semi-dwarf genotype was *rht1rht1 Rht2Rht2*) were excluded from the experiments reported here. In each *Rht1* family the seeds from all the F_6 plants which had been identified by a seedling GA response test as homozygous tall (*rht1 rht1 rht2 rht2*) were bulked separately and likewise all the seeds of the plants which had been identified as homozygous semi-dwarf (*Rht1 Rht1 rht2 rht2*). These bulks comprised the F_7 seed of the respective two *Rht1* genotypes in each family (seeds from the heterozygous F_6 plants were discarded).

Experimental procedures and measurements

Culm lengths of 30 F_7 families were determined at the Lakhish Experiment Farm (1988) in a field which was adequately fertilized and kept free of weeds, diseases and lodging. Seasonal rainfall and irrigation amounted to 582 mm. The experiment consisted of two blocks in a split-plot design. The combinations of the two *Rht1* genotype groups (tall and semi-dwarf) and the two levels of CCC (treatment and control) comprised the four main plots in each block. The sub plots were 30 rows, one of each family, each row 150 cm long at a spacing of 30 cm between rows and 2 cm within rows. CCC (0.4 g m^{-2}) was applied twice in aqueous sprays, at intervals of 5 days during the period of the elongation of the first and the second culm internodes.

Coleoptile and first leaf lengths of seedlings of 25 F_9 families (descendents of the above mentioned F_7 families but excluding five of them) were examined in a growth room at a constant temperature of 18°C and continuous light of 200 μmol m^{-2} s^{-1} PAR at seedling level, in a split-plot design with four randomized blocks. Seeds were sown in 34 × 24 × 7 cm trays filled with vermiculite saturated with 2.75 l of 6.34×10^{-4} M (100 p.p.m.) aqueous CCC solution or 2.75 l water for the control treatment. In each block the four combinations of the two *Rht1* genotype groups and the two CCC treatments were sown in different trays which comprised the main plots. Each tray contained 25 rows of 10 seedlings, one row of each of the 25 families as a subplot. After sowing the trays were kept for 3 days at 3–4°C to ensure uniform germination before being moved to a growth room where they were watered as needed. Seedlings were harvested at the time of emergence of the tip of the third leaf, which occurred at the age of 21 days.

The model

The effects on the traits examined comprised the following components:

$$\mathrm{SG} + \mathrm{BG} + \mathrm{EF} + (\mathrm{SG} \times \mathrm{BG}) + (\mathrm{SG} \times \mathrm{EF}) + (\mathrm{BG} \times \mathrm{EF}) + (\mathrm{SG} \times \mathrm{BG} \times \mathrm{EF}).$$

This model represents a general mixed model which combines the effects of SG, BG and an EF. The SG is assumed to have two alleles (S, s) and three genotypes (*SS*, *Ss*, *ss*), of which the two homozygous ones were considered in this study. The variation among the BG is obtained by random sampling of families from a population segregating for the BG. There should be two or more levels of EF, representing various growth environments or various treatments of a given factor in the same environment. The interactions are tested by a three-way factorial ANOVA with the SG, the families and the EF as factors. The effects of the SG, EF and the interaction between them (SG × EF) are regarded as 'fixed', the aim being to estimate the means of the

various levels of each of them, while the effects of the other components of the model are considered 'random', the aim being to estimate their variances.

In the present study *Rht1* is the SG represented by two genotypes (the semi-dwarf *Rht1/Rht1* and the tall *rht1/rht1*), CCC is the EF (with two levels: treatment and control) and BG are represented by the different families.

Data analysis

A restricted maximum likelihood method (REML) was used in the analysis of variance by program 3V of the BMDP package (Dixon *et al.*, 1985). REML is considered the method of choice for variance component estimation because estimates must fall within the parameter space. Furthermore, in REML estimation all available information can be utilized (Henderson, 1984). The linear model used for analysis was:

$$Y_{ijkl} = \mu + \tau_i + \alpha_j + (\tau\alpha)_{ij} + B_l + E^1_{ijl} + F_k + (\tau F)_{ik} + (\alpha F)_{jk} + (\tau\alpha F)_{ijk} + E^2_{ijkl},$$

where Y_{ijkl} is the quantitative value of the examined trait of the subplot of family k with *Rht* genotype j and CCC treatment i in block 1; μ is the general mean; τ_i is the effect of CCC treatment i; α_j is the *Rht* genotype j effect; $(\tau\alpha)$ is the CCC × *Rht* interaction effect; B_l is the effect of block 1; F_k is the effect of family k; (τF) is the CCC × family interaction effect; (αF) is the *Rht* genotype × family interaction effect; $(\tau\alpha F)$ is the CCC × *Rht* genotype × family interaction effect; E^1 and E^2 are error 1 and error 2 terms, respectively. Error 1 is the pooled variance, over blocks, between the combinations of CCC treatments and *Rht* genotypes. Error 2 represents the variance between subplots within the same CCC × *Rht* combination and it comprises the background variation within family-*Rht* genotype-CCC combinations and the experimental

Table 1 Effect of CCC on culm length in the field (means from 30 F_7 families) and on coleoptile and first leaf lengths of seedlings (means from 25 F_9 families) in *rht1* and *Rht1* genotypes of 'Mivhor' × 'Lakhish' wheat

		Family means			
Trait		*rht1* Average	*rht1* Range	*Rht1* Average	*Rht1* Range
a. Averages and ranges of family means					
Culm length (cm)	Control	129	111–137	107	87–116
	CCC	110	92–125	93	77–108
Coleoptile length (mm)	Control	45	39–49	37	32–41
	CCC	41	37–44	35	31–38
First leaf length (mm)	Control	221	204–240	189	159–203
	CCC	175	166–194	158	139–171

	Culm length		Coleoptile length		First leaf length	
Variance source	σ^2%	P†	σ^2%	P	σ^2%	P
b. Relative values (% of total) of the variance components (σ^2) and their probability estimates (P) in the REML analysis						
CCC		< 0.001		< 0.001		< 0.001
Rht1		< 0.001		< 0.001		< 0.001
CCC × *Rht1*		0.030		0.170		0.140
Block	1.6		36.1		0.5	
Error 1	2.3		9.1		23.7	
Family	54.5	< 0.001	24.5	< 0.001	11.7	0.057
CCC × Family	7.2	0.086	1.0	0.541	10.6	0.016
Rht1 × Family	0.0	—	0.7	0.665	4.7	0.169
CCC × *Rht1* × Family	5.6	0.249	0.1	0.977	0.1	0.975
Error 2	28.2		28.5		48.7	

†$P(F)$ for the 'fixed' effects; $P(\chi^2)$ for the 'random' effects.

variation. The effects of CCC, *Rht* genotype and the interaction between them were regarded as 'fixed' and the null hypothesis that their various levels had no effect was tested by the *F*-test with E^1 as the error term. All other effects were considered 'random' and hypotheses testing was accomplished by calculating the difference between $-2\times\log$ of maximum likelihood of the complete model and that of a model in which the tested component was absent. This difference is distributed as a chi-square with one degree of freedom (Dixon *et al.*, 1985).

In an additional approach to detect and illustrate treatment × family interacton, means of CCC-treated plants within each family were plotted against the means of their control sibs, as suggested by Falconer (1952). The following model was used:

$$Y_j = \alpha + \beta(Y'_j) + E_j,$$

where Y_j is the mean, within family j, of CCC-treated plants; α is the mean effect of the CCC treatment; Y'_j is the mean of the control plants in family j; $\beta(Y'_j)$ is the regression coefficient of treated plants on their control sibs and E_j is the random error term. Rejection of the null hypothesis of a unity slope ($\beta = 1$), tested by *t*-test (SAS Institute, 1985), indicates a significant family × treatment interaction.

Results and discussion

Averages and ranges of the family means, for the three traits tested, of the CCC treated and untreated tall (*rht1*) and semi-dwarf (*Rht1*) genotypes are presented in part a of Table 1. Hypothesis testing and variance-component percentages are presented in part b of Table 1. No significant second-order CCC × *Rht1* × family interaction effect on any of the tested characters was found; nor was there any significant *Rht1* × family interaction.

CCC had a significant shortening effect on all the three organs tested (Table 1a). This effect was somewhat weaker in the semi-dwarf (*Rht1*) genotype than in the tall (*rht1*) genotype. This CCC × *Rht* interaction effect was significant in the case of culm length. The relative mean reductions in culm length of the *rht1* and *Rht1* genotypes, however, were rather similar, amounting to 14.7 per cent and 13.1 per cent, respectively.

High and significant interfamily variation (i.e. genetic background variation) was found for all three traits (Table 1b) and is depicted in Fig. 1 for culm(a) and first leaf(b). A significant CCC × family interaction effect was found in the case of the first leaf, indicating significant interfamily variation in the response of this organ to CCC. This seems noteworthy considering the apparent lack of existing reports on genotypic differences in response to CCC other than those due to the *Rht* alleles (Gale & Youssefian, 1983) or varietal differences which should rather be attributed to differences in their phenological development (Pinthus, 1968).

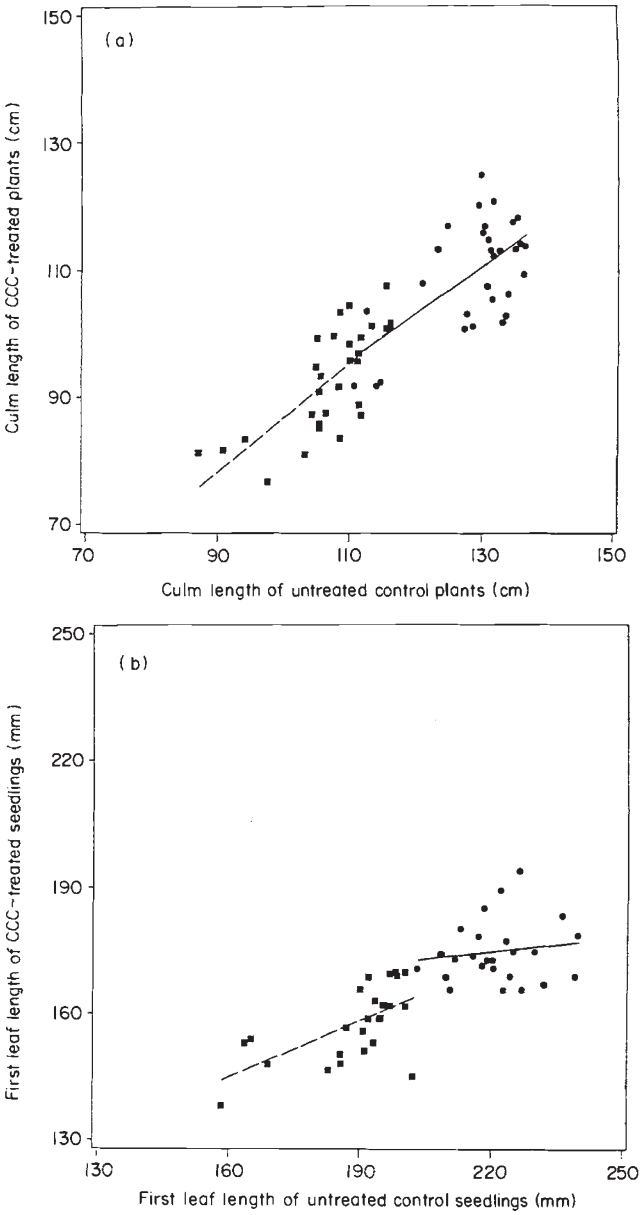

Fig. 1 Regression of mean length parameters of CCC-treated plants on untreated control plants of *rht1* (● ——) and *Rht1* (■ – – –) genotypes within different 'Mivhor' × 'Lakhish' wheat families. (a) Culm length of F_7 plants; (b) first leaf length of F_9 seedlings.

	Regression coefficients (slopes)	
	a	b
rht1	0.73	0.11
Rht1	0.86	0.45

The graphical presentation in Fig. 1 and the corresponding statistical analyses are similar to those used to evaluate genotype × environment interaction (Falconer, 1952). The interaction of the CCC and control treatments with the families could be clearly detected by regressing the means of CCC-treated plants on the means of their control sibs over families (Fig. 1). In the case of the first leaf length, the highly significant rejection ($P<0.001$) of the null hypothesis of $\beta=1$ indicated the interaction between families and CCC treatments in both *Rht1* genotypes (Fig. 1b). In the *rht1* genotype the slope ($\beta=0.11$) was not significantly different from zero, indicating that the first leaf length of CCC-treated tall plants was completely independent of the first leaf length of their untreated sibs (Fig. 1b). In contrast, Fig. 1a clearly demonstrates the lack of CCC × family interaction effect on culm length. There was a good agreement between the ANOVA and the regression analyses, with the latter being more sensitive to the interactions between the fixed effects *Rht* and CCC and the random effects of the families.

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