Griffing and Hayman diallel analyses of protein and lysine content of spring triticale

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Data on the percent protein, protein per grain, and lysine content of grain protein in a 6 × 6 diallel cross of triticale (× *Triticosecale* Wittmack) were analyzed by both the Griffing and the Hayman diallel methods. Results agreed with those of a previous analysis of the same data by the gene effects method. No advantage of diallel analysis over gene effects analysis was indicated. Parallel use of the Griffing and Hayman analyses illustrated the relationship between these two diallel methods. Gene action was mainly additive for the three traits, although some dominance was detected for percent protein and protein per grain. Dominance for protein per grain was in the direction of higher protein. Variation in lysine content was largely attributable to variation in percent protein, protein per grain, and grain plumpness. Selection of parents on the basis of the Griffing general combining ability effects would be similar to selection on the basis of parental phenotype.

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Les données présentées dans ce travail se rapportent au pourcentage de protéine, à la protéine totale et au contenu en lysine de la protéine des grains. Un modèle de croisement diallèle de triticale (× *Triticosecale* Wittmack) 6 × 6, fut utilisé et les analyses ont été effectuées selon les deux méthodes de diallèle de Griffing et de Hayman. Les résultats obtenus concordent avec ceux qu'on obtient par l'analyse des mêmes données selon la méthode de l'effet des gènes et de ce fait on ne peut démontrer aucun avantage de l'analyse diallèle sur l'analyse par la méthode de l'effet des gènes. L'effet des gènes est surtout de type additif pour les trois caractères étudiés. Quelques effets de dominance ont été notés pour le pourcentage de protéine et le contenu en protéine. Dans le cas du contenu en protéine, la dominance agit dans le sens de la haute teneur en protéine. La variation dans le contenu en lysine fut surtout expliquée par la variation du pourcentage de protéine, du contenu protéique des grains et de la plénitude des grains. La sélection des parents sur la base de l'aptitude générale à la combinaison de Griffing devrait donner à peu près les mêmes résultats que la sélection sur la base des phénotypes parentaux.

[Traduit par le journal]

Introduction

In a previous study (Mather and Poysa 1983), protein and lysine contents of the F_2 and F_3 grain of a 6 × 6 diallel cross in triticale (× *Triticosecale* Wittmack) were analyzed by the gene effects method (Mather and Jinks 1971). First degree statistics (means) were employed in the analysis of the 15 crosses of the diallel.

There may, however, be advantages to the use of second degree statistics (variances and covariances) in the analysis of such a diallel arrangement of crosses. First, since the terms of second degree statistics are squared before summation, there is no balancing effect owing to sign (Mather 1971). Genes of opposite effect at different loci cannot balance each other. Secondly, analysis of such data as a diallel set of crosses, rather than as 15 individual crosses, recognizes the relationships among the crosses, by treating them as members of arrays sharing common parents.

Several methods have been proposed for the analysis of data from diallel crosses (Hayman 1954*a*, 1954*b*; Griffing 1956; Gardner and Eberhart 1966). Sokol (1976) has demonstrated the basic similarity among these methods.

The approaches of Griffing (1956) (method 1, model I) and Hayman (1954*a*, 1954*b*), which were used in this study, are statistically similar. They differ, however, in the genetic assumptions and interpretations which are associated with them. In their analyses of variance, the Griffing general combining ability (GCA) component is mathematically identical to the Hayman additive component (a). Griffing employs one specific combining ability (SCA) component and one reciprocal effects component, while Hayman subdivides these into three dominance components (b_1, b_2, and b_3), and two reciprocal effects components (c and d), respectively. The Griffing analysis is a strictly statistical treatment of main effects (GCA) and interactions (SCA) (John 1971), whereas the Hayman analysis incorporates genetic assumptions. The Griffing method involves only analysis of variance and estimation of GCA and SCA effects. The Hayman method may include statistical and graphical analyses of array variances and covariances, and the estimation of a number of genetic parameters.

The Hayman method appears to extract more genetic information than the Griffing method does from the

TABLE 1. Pedigrees and mean values of percent protein, protein per grain, and adjusted DBCP values for the six parental triticale lines

Parental line	Pedigree or source	Origin	% protein	Protein per grain (mg)		Adjusted DBCP (milligrams of dye bound per gram of protein)
				Guelph	Elora	
P1	Tcl Arm S-54N-1B-OY	Mexico	22.76 *a*	8.00 *a*	6.30 *ab*	285 *a*
P2	(UM 940 SxMy 64) × 1039-14M-1Y -16M-1Y-OM	Mexico	20.44 *b*	8.09 *a*	6.70 *a*	274 *bc*
P3	Octa−Hexa OY	Canada	20.22 *b*	5.63 *b*	4.40 *c*	290 *a*
P4	Tcl E3 Arm S H277-69-1Y-1B-1Y -101B-1Y-4B-OY	Mexico	18.75 *c*	6.44 *b*	6.40 *a*	273 *c*
P5	Jenkins 209	U.S.A.	18.09 *c*	8.61 *a*	5.73 *ab*	289 *a*
P6	TA × 1648-4N-8M-OY	Mexico	16.45 *d*	6.18 *b*	5.24 *bc*	284 *ab*

NOTE: DBCP values were adjusted for percent protein, protein per grain, and seed plumpness. Means followed by the same letter are not significantly different ($P = 0.01$).

TABLE 2. Griffing and Hayman analyses of variance of percent protein in diallel crosses of spring triticale

Griffing (6 × 6 diallel)			Hayman (5 × 5 diallel)		
Source	*df*	Mean square	Source	*df*	Mean square
Blocks	7	1.48	Blocks	7	0.61
Genotypes	35	27.23**	Genotypes	24	30.87**
GCA	5	165.78**	*a*	4	153.18**
SCA	15	6.58**	*b*	10	8.67
			b_1	1	2.16
			b_2	4	16.02*
			b_3	5	4.08
Reciprocal	15	1.69	*c*	4	4.66**
			d	6	3.81**
Error	220	1.25	Error	147	0.86

*Significant at the 0.05 level of probability.
**Significant at the 0.01 level of probability.

same data. However, the validity of this information depends on the validity of the genetic assumptions of the analysis. Hayman (1954*a*, 1954*b*) assumes the following: (1) diploid segregation; (2) parental homozygosity; (3) no reciprocal differences; (4) no multiple alleles; (5) independent distribution of genes among the parents; and (6) independent action of nonallelic genes (no epistasis). Despite criticism of these assumptions (particularly of Nos. 5 and 6) as being unjustifiable and biologically unrealistic (Gilbert 1958; Sokol and Baker 1977; Baker 1978), breeders and geneticists continue to use the Hayman diallel analysis, perhaps because of a paucity of alternative methods for obtaining genetic information on quantitative traits.

Diallel methods have been employed for analysis of protein content in wheat (Arcioni *et al.* 1974; Halloran 1975; Kraljevic-Balalic *et al.* 1982) and rye (Plarre and Fischer 1975; McLeod 1979), of lysine content in barley (Olsen 1979), and of trypsin inhibitor activity in triticale (Tanner *et al.* 1981). The authors are not aware of any previous reports of diallel analyses of protein or lysine contents in triticale.

In the present study, data on percent protein, protein per grain, and lysine content of protein, as measured by the dye binding capacity of the protein (DBCP, milligrams of acid orange 12 dye bound per gram of protein) (Mossberg 1969; Munk 1976) in triticale grain were analyzed by both the Griffing and Hayman methods.

Materials and methods

The material used, the F_2 and F_3 grain of a 6 × 6 diallel (parents and reciprocals included) of spring triticale, and the methods for determining protein content by near-infrared reflectance, and DBCP by the Udy method (Udy 1971) have been described elsewhere (Mather and Poysa 1983). DBCP was adjusted for variation in percent protein, protein per grain, and grain plumpness. Pedigrees, and mean values of percent protein, protein per grain, and adjusted DBCP for the six parental lines are given in Table 1. Genotype by environment ($g \times e$)

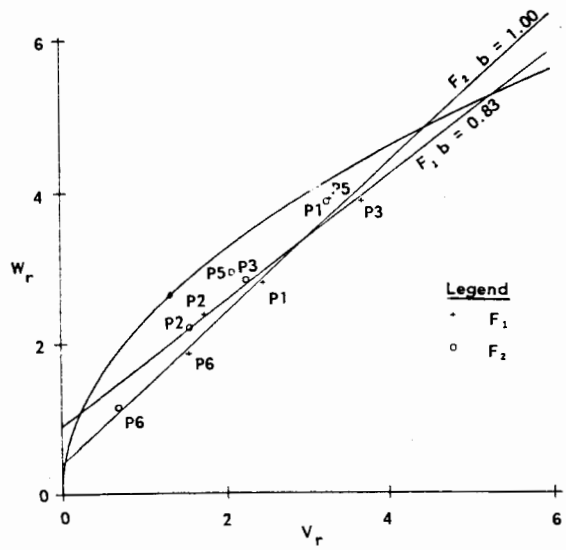

FIG. 1. W_r,V_r graph for percent protein in the F_1 and F_2 of a 5 × 5 diallel cross.

interaction was detected only for protein per grain. Therefore, the data for percent protein and adjusted DBCP were pooled across the two locations used (Guelph and Elora, Ontario).

The Griffing (method 1, model I) and Hayman diallel analyses of variance were performed with the aid of SAS (Statistical Analysis System) procedures which were based on the original models and assumptions of Griffing (1956) and Hayman (1954*a*), and which are capable of handling unbalanced data (owing to missing observations) and performing covariate adjustment. In the Griffing analysis, GCA and SCA effects were estimated. For the Hayman analysis, the array variances (V_r's) and the covariances of each array with the nonrecurrent parent (W_r's) were calculated. Tests of significance of ($W_r + V_r$) and ($W_r - V_r$) values of arrays, and (W_r,V_r) graphic analyses were performed.

Diallel analyses of variance were performed on data from F_2 seed only. Analysis of W_r and V_r were performed on data from both F_2 and F_3 seed. With reference to the inheritance of percent protein and protein per grain, these generations are referred to as F_1 and F_2, since these traits were considered to be controlled by the maternal plant genotype (Favret *et al.* 1970; Piano *et al.* 1976).

Adjusted DBCP was considered to be controlled by the F_2 and F_3 seed embryo genotypes (Favret *et al.* 1970). DBCP may in fact be controlled by the $3n$ seed endosperm genotype, but $2n$ and $3n$ variances should not differ greatly in these generations (Greenberg 1977).

Results and discussion

In the Hayman analyses of percent protein and protein per grain (Elora), the (W_r,V_r) regression line differed significantly ($P = 0.01$) from unit slope, indicating a failure of one or more assumptions. Removal of crosses with P4 resulted in 5 × 5 diallels with (W_r,V_r) regression line slopes not significantly different from unity. For protein per grain (Guelph), Hayman diallel analysis was not performed because neither the 6 × 6 nor any of the six possible 5 × 5 diallels had (W_r,V_r) regression lines with a unit slope.

In the analyses of percent protein (Table 2) the mean squares for GCA and *a* are very much larger than those for SCA and *b*. This apparent predominance of additive gene action agrees with the results of the gene effects analysis (Mather and Poysa 1983). In the Hayman diallel analysis, the reciprocal components (*c* and *d*) are significantly ($P = 0.01$) different from zero, suggesting cytoplasmic influences on percent protein. Cytoplasmic effects on protein content have previously been noted by Hsam and Larter (1974) in triticale and by Piano *et al.* (1976) in wheat. The array values for ($W_r - V_r$) were homogeneous across parental arrays.

TABLE 3. Griffing and Hayman analyses of variance of protein per grain in diallel crosses of spring triticale grown at the Guelph and Elora locations

Griffing (6 × 6 diallel)				Hayman (5 × 5 diallel)		
Source	*df*	Mean square (Guelph)	Mean square (Elora)	Source	*df*	Mean square (Elora)
Blocks	3	1.46*	0.17	Blocks	3	2.01**
Genotypes	35	3.17**	5.54**	Genotypes	24	4.37**
GCA	5	16.02**	21.22**	*a*	4	7.60
SCA	15	1.39**	4.12**	*b*	10	4.58
				b_1	1	19.30*
				b_2	4	1.26
				b_3	5	4.29
Reciprocal	15	0.67	1.73	*c*	4	4.84**
				d	6	1.54**
Error	103	0.49	0.95	Error	52	0.31

*Significant at the 0.05 level of probability.
**Significant at the 0.01 level of probability.

indicating lack of epistasis, while the $(W_r + V_r)$ values were heterogeneous across arrays, indicating presence of dominance. The positive W_r intercept of both the F_1 and F_2 regression lines in the graphic analysis (Fig. 1) indicates partial dominance. The distribution of the (W_r,V_r) points does not clearly indicate the direction of dominance. This is consistent with the lack of significance of the directionality of dominance (b_1) component (Table 2).

In the Griffing analysis of protein per grain, the GCA mean squares are five times as large as the SCA mean squares (Table 3). However, in the Hayman analysis, the additive component (a) mean squares are less than twice as large as the dominance component (b) mean squares (Table 3). In the Hayman diallel analysis of protein per grain at the Elora location, the reciprocal effects (c and d) are significant. Epistasis was not detected by the $(W_r - V_r)$ test, while the $(W_r + V_r)$ test detected dominance in the F_1 but not in the F_2. Dominance should be more difficult to detect in the F_2, because of reduced heterozygosity. In the graphical analysis (Fig. 2), this is illustrated by the clustering of the F_2 points around the point $(1/2\ V_p,\ 1/4\ V_p)$ for no dominance. The F_1 points are more evenly distributed along the regression line, and their order indicates dominance for high protein per grain. The points for the three higher protein parents P5, P2, and P1 (Table 1) are nearer to the origin, indicating that these parents carry dominant alleles, while the lower protein parents P3 and P6 are further from the origin. The F_1 regression line intercepts the W_r axis near the origin, indicating partial to complete dominance. The F_2 intercept is above the F_1 intercept, as is expected when there is

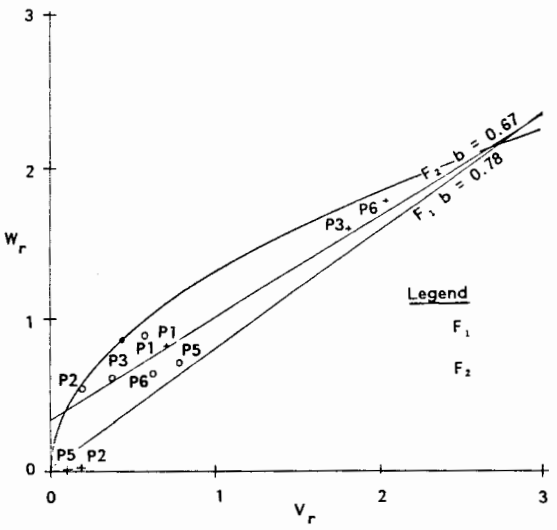

FIG. 2. (W_r,V_r) graph for protein per grain in the F_1 and F_2 of a 5 × 5 diallel cross at the Elora location.

dominance. The Hayman diallel analysis of protein per grain at the Guelph location was not performed as neither the 6 × 6 nor any of the possible 5 × 5 diallels had (W_r,V_r) regression lines with unit slope, indicating that the assumptions for diallel analyses are not valid for this case. These differences between the Guelph and Elora location in the Hayman analysis are consistent with the detection of significant genotype by environment interaction for this trait (Mather and Poysa 1983). The Hayman diallel analysis, however, does not provide any specific information about the nature of this interaction; it only confirms that the expression of inheritance of

TABLE 4. Griffing and Hayman analyses of variance of DBCP, with adjustment for variation in percent protein, protein per grain, and grain plumpness in a 6 × 6 diallel cross of spring triticale

Griffing			Hayman		
Source	*df*	Mean square	Source	*df*	Mean square
Blocks	1	846.41**	Blocks	7	846.41**
Percent protein	1	33 235.15**	Percent protein	1	33 235.15**
Protein per grain	2	3 124.25**	Protein per grain	2	3 124.25**
Grain plumpness	1	7 446.49**	Grain plumpness	1	7 446.49**
Genotypes (adjusted)	35	233.67**	Genotypes (adjusted)	35	233.67**
GCA	5	978.71**	a	5	978.71**
SCA	15	131.85*	b	15	131.85*
			b_1	1	125.62
			b_2	5	101.33
			b_3	9	149.49*
Reciprocal	15	63.81	c	5	31.43
			d	10	79.99
Error	216	71.11	Error	216	71.11

*Significant at the 0.05 level of probability.
**Significant at the 0.01 level of probability.

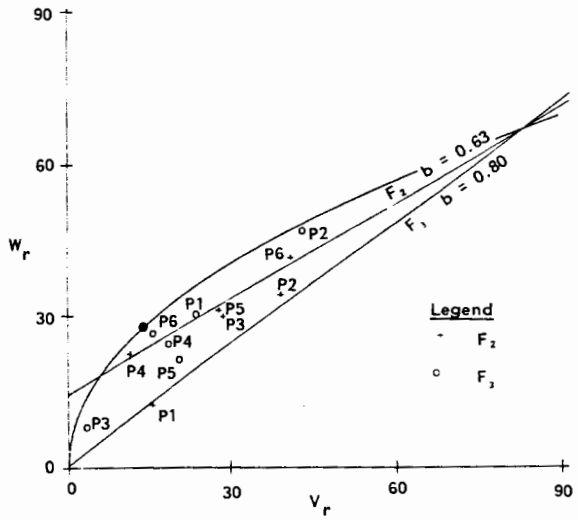

FIG. 3. (W_r,V_r) graph for adjusted DBCP in the F_2 and F_3 of a 6 × 6 diallel cross.

this trait differs between the two locations.

The analyses of adjusted DBCP (Table 4) provide an opportunity for direct comparison of the Griffing and Hayman methods, since both were performed on the full 6 × 6 diallel. Comparisons of the degrees of freedom and the mean squares reveal the equivalence of GCA with *a*, SCA with *b*, and Griffing's reciprocal effect with the sum of *c* and *d*.

The magnitude of the covariate components, percent protein, protein per grain, and grain plumpness (Table 4), indicates the extent to which variation in unadjusted DBCP results from variation in these traits. The Griffing and Hayman analyses indicate the predominance of additive effects in adjusted DBCP. Both the ($W_r + V_r$) and ($W_r - V_r$) values were homogeneous across parental arrays, indicating lack of epistasis and dominance. The F_2 regression line intersects the W_r axis near the origin (Fig. 3) indicating presence of dominance, but this intercept has a large standard error (10.15) associated with it. Both the F_2 and F_3 points are generally clustered around the point (1/2 V_p, 1/4 V_p) for no dominance. The F_2 intercept is above the F_3 intercept, indicating lack of dominance.

Estimates of Griffing GCA effects were calculated for each of the parents for percent protein, protein per grain, and adjusted DBCP (Table 5). The ranking of the parents according to GCA effects closely corresponds to their ranking according to parental means (Table 1) for all three traits. Only P3 (low GCA values) for percent protein and P4 (high GCA) for protein per grain at Guelph are exceptions. In all other cases choice of parents based on GCA effects would be similar to selection based on parental phenotype.

From the results reported here, one may conclude that: (*i*) gene action in these crosses is mainly additive for the traits studied, although there is some dominance for percent protein and protein per grain; (*ii*) the dominance for protein per grain is in the direction of higher protein; (*iii*) reciprocal differences due to cytoplasmic effects may be present for percent protein and protein per grain; (*iv*) most of the variation in DBCP is due to variation in percent protein, protein per grain, and grain plumpness; (*v*) parent selection on the basis of GCA effects would be similar to selection on the basis of parental phenotype.

Thus, the results of the diallel analyses are in agreement with the results of gene effects analyses (Mather and Poysa 1983), yet they provide little if any new genetic information. The genetic interpretation of the diallel analyses is rendered uncertain by failure to fully satisfy the Hayman assumptions. In the analysis of the 5 × 5 diallel of percent protein and protein per grain, significant reciprocal effects were identified, violating assumption No. 3. The validity of assumption No. 6 is also questionable. Although the Hayman tests detected

TABLE 5. Estimates of Griffing general combining ability effects for percent protein, protein per grain, and adjusted[a] DBCP of the six parental spring triticale lines

Parental line	% protein	Protein per grain (mg)		Adjusted[a] DBCP (milligrams of dye bound per gram of protein)
		Guelph	Elora	
P1	1.82	0.22**	0.41**	1
P2	0.86	0.65**	0.53	−3*
P3	−0.30**	−0.91**	−0.93**	4**
P4	−0.24*	0.17	−0.24	−6**
P5	0.12	0.36**	0.82**	5**
P6	−2.26**	−0.49**	−0.59**	−1

[a]Adjusted for variation in percent protein, protein per grain, and grain plumpness.
*Estimate is significantly different from zero at the 0.05 level of probability.
**Estimate is significantly different from zero at the 0.01 level of probability.

no epistasis, epistasis was detected in some crosses by gene effects analysis (Mather and Poysa 1983). Failure to meet these assumptions may also affect the validity of any genetic interpretations of the Griffing GCA and SCA.

While there are theoretical advantages to the analysis of second degree statistics, these are not illustrated by the results reported here. The results serve to support (but not augment) those reported for the analysis of first degree statistics (Mather and Poysa 1983), to illustrate the relationship between the Griffing and Hayman diallel analysis methods, and to demonstrate the difficulties that may be encountered in trying to satisfy the assumptions of the Hayman diallel analysis.

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