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Analysis and Interpretation of Interactions in Agricultural Research

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ABSTRACT

When reporting on well-conducted research, a characteristic of a complete and proper manuscript is one that includes analyses and interpretations of all interactions. Our purpose is to show how to analyze and interpret interactions in agronomy and breeding research by means of three data sets comprising random and fixed effects. Experiment 1 tested wheat (*Triticum aestivum* L.) at two N and four P fertilizer rates in two soil types. For this data set, we used a fixed-effect linear model with the highest order (three-way) interaction considered first and then worked down through the lower order interactions and main effects to illustrate the importance of interactions in data analysis. Experiment 2 evaluated maize (*Zea mays* L.) hybrids under four rates of N for 3 yr. For this data set, we used a linear mixed model and partitioned the four N rates into orthogonal polynomials. Experiment 3 evaluated genotypes in six environments where the objective was to show how to study genotype × environment interactions. Researchers must analyze all interactions, determine if they are due to changes in rank (crossover) or only to changes in scale, and then judge whether reporting on significant main effects or interactions would best explain the biological responses in their experiments. In an experiment with more than one factor, complete and correct analysis of interactions is essential for reporting and interpreting the research properly.

The presence of a treatment × environment interaction (TE) in agronomy experiments and a genotype × environment interaction (GE) in breeding trials is expressed either as inconsistent responses of some treatments relative to others due to treatment rank change (crossover interaction [COI]) or as changes in the absolute differences between treatments without rank change, meaning a scale change or non-crossover interaction (NCOI). The most important interaction in agriculture is due to rank change or COI, given that NCOI does not prevent making general recommendations at the level of one factor across all levels of the other factors. Several models and displays are commonly used for describing the mean response of treatments across environments and for studying and interpreting TE and GE.

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Three-way interactions may arise in agricultural experiments that include treatments established in several environments across years. Also, three-way and four-way interactions can arise for other conditions or treatments applied by the researcher, such as sowing date, fertilizer rate, and/or plant density. Although years themselves may not be intrinsically interesting, they provide the environmental conditions in which the crop is grown, and different responses of environments in years may be relevant for identifying stable treatments across geographical regions and years. McIntosh (1983) provided guidance for analyzing experiments combined across multiple locations or years. Agronomy experiments may also include different treatments in environments under different plant densities evaluated across several years. In these experiments, three- and four-way interactions can be dissected by combining graphical displays and statistical analyses that can reveal COI or NCOI among levels of factors. Milliken and Johnson (1992) provided a comprehensive assessment of how to examine and study two-way fixed-effect treatment interactions in agriculture and other fields of research. A vast review of fixed and mixed linear–bilinear models for assessing and studying interaction effects in the context of plant breeding, genetics, and genomics has been provided by Crossa (2012).

Suppose a researcher needs to know if there is a common structure underlying locations (environments) with respect to years and how the various treatments respond across the structure formed by environments and years. Some treatments

Abbreviations: COI, crossover interaction; GE, genotype × environment interaction; GY, grain yield; HWCI, half width confidence interval; NCOI, non-crossover interaction; TE, treatment × environment interaction.

may respond similarly in some environments and differently in others, and some environments may be more associated with others in some years. An approach for gaining insight into three-way interactions is important and useful and, when possible, this type of approach is more useful than condensing three-way data into two-way data. A less useful approach is to explicitly exclude years and analyze the two-way TE array in each year separately. The limitations of this approach are that it does not explain the significance of the overall TE across years and that there is a loss of information because the data are not analyzed in their original format, which is the three-way interaction (Varela et al., 2006, 2009).

When analyzing data from experiments, researchers need to answer questions related to different hypotheses; for example, how does each treatment affect the response variable? What kind of interaction is there between treatments? Other important questions are whether conclusions regarding the main effects can be reached despite the existence of significant interactions, and whether or not there are specific levels of treatment effects causing the interaction. Crossa et al. (2014) gives a detailed account of how to deal with interactions in several practical situations related to agriculture, plant breeding, and other fields of research using linear fixed- and mixed-effect models.

Usually when levels of a factor are discrete, mean comparisons among them are used (Carmer, 1976; Carmer and Swanson, 1971, 1973; Saville, 2014), whereas polynomial orthogonal contrasts are recommended for quantitative levels of a factor (Swallow, 1984; Little, 1978; Saville, 2014). For multiple comparisons among means, a recommended procedure for researchers who are equally concerned about Type I and Type II errors, and also about comparison-wise rather than experiment-wise error rates, is the unprotected least significant difference (LSD) (Glaz and Dean, 1988: Saville, 2014).

This study analyzed agronomic and breeding experimental data sets with random and fixed effects. We selected data sets that will be meaningful to a large number of agricultural researchers; our examples are meant to serve as useful and practical resources for many researchers as they analyze their data. The analyses of two agronomic experiments (Exp. 1 and 2) and one multienvironment breeding trial with unstructured sets of wheat lines are described to illustrate some of the basic analyses that can be performed for studying and dissecting TE (Exp. 1 and 2) and assessing and studying GE (Exp. 3).

BASELINE MODELS

Two-Way Model without Interaction

The basic two-way main fixed-effect linear model considers that the empirical mean response, $\bar{y}_{ij}$, of the ith treatment ($i = 1, 2, ..., I$) in the jth environment ($j = 1, 2, ..., J$) with n replications in each of the $I \times J$ cells is expressed as

$$\bar{y}_{ij} = \mu + \tau_i + \delta_j + \bar{\varepsilon}_{ij} \qquad [1]$$

where μ is the grand mean across all treatments and environments, τ_i is the additive effect of the ith treatment, δ_j is the additive effect of the jth environment, and $\bar{\varepsilon}_{ij}$ is the average error of the ith treatment in the jth environment assumed to be normally, identically, and independently distributed (IID) $N(0,\mathbf{I}_n\sigma^2/n$, where σ^2 is the within-environment error and $\mathbf{I}_n$ is the identity matrix of order $n \times n$. The possible existence of significant TE or GE is ignored and its effects are estimated together with the average error.

Two-Way Model with Interaction

Taking the model given in Eq. [1] as a starting point, the inclusion of the interaction term in the basic two-way fixed-effect linear model for TE or GE analyses becomes

$$\bar{y}_{ij} = \mu + \tau_i + \delta_j + (\tau\delta)_{ij} + \bar{\varepsilon}_{ij} \qquad [2]$$

where $(\tau\delta)_{ij}$ is the nonadditive fixed interaction (TE or GE) effect of the ith treatment in the jth environment.

For a random model, it is assumed that τ_i, δ_j, and $(\tau\delta)_{ij}$ are IID normally distributed with zero expectations and variances $\mathbf{I}_I\sigma_\tau^2$, $\mathbf{I}_J\sigma_\delta^2$, and $\mathbf{I}_{IJ}\sigma_{\tau\delta}^2$, respectively (where $\mathbf{I}_I$, $\mathbf{I}_J$, and $\mathbf{I}_{IJ}$ are the identity matrices of order $I \times I$, $J \times J$, and $IJ \times IJ$, respectively). This is the most restrictive random model, for it considers equal variances for all levels of treatments and environments as well as no covariances among the different levels of treatments and environments. These restrictions can be relaxed under more realistic situations that consider heterogeneity of variances and different correlation structures among the levels of treatments and environments.

Three-Way Model with Interaction

Now consider a situation in which the trial data of the model represented in Eq. [2] are performed across several years (i.e., treatment × environment × year) ($k = 1, 2, ..., K$). Usually in agronomy and/or breeding trials, years are considered random effects. Assume that the main effects of treatments and environments, as well as their interactions, are considered as fixed effects. The linear model represented in Eq. [2] is now extended to include the random effects of years and their two-way and three-way random interactions with treatment and environments:

$$\begin{aligned}\bar{y}_{ijk} = \mu + \tau_i + \delta_j + \zeta_k + (\tau\delta)_{ij} + (\tau\zeta)_{ik} \\ + (\delta\zeta)_{jk} + (\tau\delta\zeta)_{ijk} + \bar{\varepsilon}_{ijk}\end{aligned} \qquad [3]$$

where ζ_k is the random effect of the kth year assumed to be IID $N(0,\mathbf{I}_K\sigma_\zeta^2)$, where σ_ζ^2 is the variance between years and $\mathbf{I}_K$ is the identity matrix of order $K \times K$, $(\tau\zeta)_{ik}$ is the random interaction effects of the ith treatment in the kth year assumed to be IID $N(0,\mathbf{I}_{IK}\sigma_{\tau\zeta}^2)$, where $\sigma_{\tau\zeta}^2$ is the variance of the treatment × year interaction and $\mathbf{I}_{IK}$ is the identity matrix of order $IK \times IK$, $(\delta\zeta)_{jk}$ is the interaction of the jth environment in the kth year assumed to be IID $N(0,\mathbf{I}_{JK}\sigma_{\delta\zeta}^2)$, where $\sigma_{\delta\zeta}^2$ is the variance of the environment × year interaction and $\mathbf{I}_{JK}$ is the identity matrix of order $JK \times JK$, and $(\tau\delta\zeta)_{ijk}$ is the three-way random interaction of the ith treatment in the jth environment and the kth year assumed IID $N(0,\mathbf{I}_{IJK}\sigma_{\tau\delta\zeta}^2)$, where $\sigma_{\tau\delta\zeta}^2$ is the variance of the three-way interaction treatment × environment × year interaction and $\mathbf{I}_{IJK}$ is the identity matrix of order $IJK \times IJK$.

Table 1. Experiment 1: Main effects, two-way, and three-way interactions ANOVA for grain yield (Mg ha^{-1}) in an experiment with two soil types, two N rates, and four P rates. Phosphorus rates are partitioned into linear, quadratic, and cubic orthogonal polynomial contrasts.

Source	df	Sum of squares	*F* value	Pr > *F*
Model	17	8.3123	39.03	<0.0001
Soil	1	5.9426	474.34	<0.0001
Replicate(Soil)	2	0.0263	1.05	0.3766
N	1	0.5434	43.37	<0.0001
P	3	0.8466	22.52	<0.0001
Linear P	1	0.7765	61.98	<0.0001
Quadratic P	1	0.0679	5.42	0.0354
Cubic P	1	0.0021	0.17	0.6902
Soil × N	1	0.2398	19.14	0.0006
Soil × P	3	0.5230	13.92	0.0002
Linear P × soil	1	0.5120	40.87	<0.0001
Quadratic P × soil	1	0.0080	0.64	0.4385
Cubic P × soil	1	0.0031	0.25	0.6279
N × P	3	0.1725	4.59	0.0194
Linear P × N	1	0.0020	0.16	0.6975
Quadratic P × N	1	0.0167	1.33	0.2676
Cubic P × N	1	0.1538	12.28	0.0035
Soil × N × P	3	0.0182	0.48	0.6993
Soil × N × P linear	1	0.0042	0.34	0.5699
Soil × N × P quadratic	1	0.0004	0.03	0.8677
Soil × N × P cubic	1	0.0136	1.08	0.3159
Error	14	0.1754		
Total	31	8.4877		

EXPERIMENTAL DATA

Three data sets were used to illustrate methods for studying TE and GE. The first data set (Exp. 1) is from an experiment conducted on high-latitude rainfed spring wheat in northern Kazakhstan at two sites with distinct soil types (Chestnut and Black), two rates of N fertilizer (0 and 30 kg ha^{-1}), and four unevenly spaced P fertilizer rates (0, 50, 150, and 250 kg ha^{-1}), with trait grain yield (GY, Mg ha^{-1}) measured in one wheat genotype in 2007. This experiment was conducted using a randomized complete block design with two replicates at each of the two sites.

The second data set (Exp. 2) is from an agronomy trial conducted in Mexico that comprised eight maize hybrids representing 8 yr of their release (1–8) evaluated at four N rates (0, 75, 150, and 300 kg ha^{-1}) for 3 yr with the objective of studying the responses of historical maize hybrids under different N rates. Each hybrid × N rate treatment was arranged in a randomized complete block design with three replicates each year.

The third data set (Exp. 3) came from a breeding trial conducted in Mexico with the objective of evaluating the response of seven wheat lines (1–7) sown in two low-yielding environments (1 and 2) and four intermediate-yielding environments (3–6). The seven wheat lines were arranged in a randomized complete block design with two replicates in each environment.

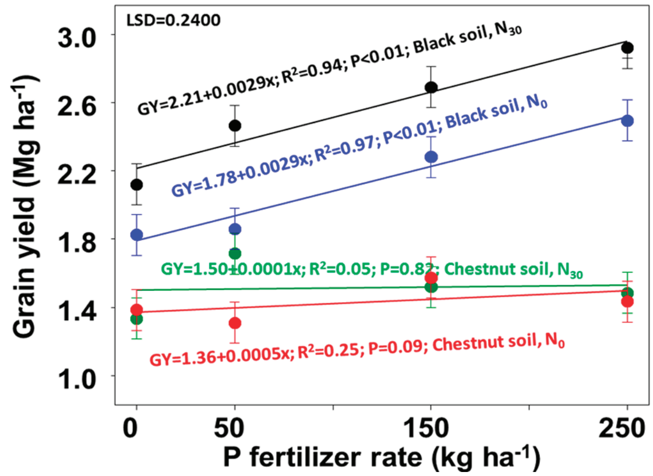

Fig. 1. Linear responses of grain yield (GY) to P fertilizer for soil × N × P interactions in Exp. 1.

RESULTS AND DISCUSSION

Experiment 1

The researcher in Exp. 1 might ask: How do I investigate the response patterns of main effects in the presence of significant interactions? Is it possible to reach conclusions about the main effects of soil, N, and P when several of these factor levels showed significant interactions? Are there COI or NCOI patterns revealed? To dissect and interpret high-order complex interactions is challenging and not straightforward.

The three-way soil × N × P interaction was not significant and neither were any of its linear, quadratic, or cubic polynomial responses (Table 1). The nonsignificant three-way interaction may have been due to the almost parallel response of GY in the Black soil for N_{30} and N_0 at all P rates and the similar responses at all P rates for each of the two N rates. The nonsignificant interaction between the two soil types and the two N rates for all P rates is clearly depicted in Fig. 1.

It must be noted that more complicated three- and four-way interactions may arise in agronomy experiments conducted at different sites and/or years, and untangling such interactions may be even more complicated than the case presented in Exp. 1. This may be especially true when more factor levels are involved. Nevertheless, the example given here illustrates an approach for dissecting and partitioning complex interactions. Aided by proper partitioning of all factorial combinations and graphic displays of significant effects, one can search for and identify COI and NCOI interactions. Then, significant interactions that are caused by NCOI can be studied, and conclusions about the main effects can often be appropriately drawn. Other conclusions regarding only main effects or combinations of main effects cannot be drawn when pertinent COI are significant. Under these circumstances, the researcher must interpret the effects of the interactions and their subsequent inferences to report on the research completely and correctly.

Dissecting Two-Way Interactions

Attempting to interpret interactions is a challenge. As mentioned above, a descriptive method of tackling the complexity of higher order interactions in the context of a fixed-effect model is to present the information in a graphic

Table 2. Experiment 1: Least significant difference ($LSD_{0.05}$) means comparison for the main effects of soil, N, and P for grain yield (Mg ha^{-1}) and their interactions (soil × N, soil × P, N × P, and soil × N × P) of an experiment including two soil types (Black and Chestnut), two N rates (N_0, and N_{30}), and four P rates (P_0, P_{50}, P_{150}, and P_{250}).

Effect	Comparison			
Soil	Black		Chestnut	
	2.331 A†		1.470 B	
N	N_0		N_{30}	
	1.771 B		2.031 A	
P	P_0	P_{50}	P_{150}	P_{250}
	1.666 C	1.837 B	2.016 A	2.083 A
Soil × N	N_0		N_{30}	
Black	2.115 B		2.548 A	
Chestnut	1.426 C		1.513 C	
Soil × P	P_0	P_{50}	P_{150}	P_{250}
Black	1.972 D	2.162 C	2.485 B	2.707 A
Chestnut	1.360 F	1.512 EF	1.547 E	1.460 EF
N × P	P_0	P_{50}	P_{150}	P_{250}
N_0	1.605 D	1.585 D	1.927 C	1.965 BC
N_{30}	1.727 D	2.090 ABC	2.105 AB	2.202 A
Soil × N × P	P_0	P_{50}	P_{150}	P_{250}
Black × N_0	1.825	1.860	2.280	2.495
Black × N_{30}	2.120	2.465	2.690	2.920
Chestnut × N_0	1.385	1.310	1.575	1.435
Chestnut × N_{30}	1.335	1.715	1.520	1.485

† All means in the same row followed by a different letter are significantly different based on the unprotected LSD (0.05).

display and see whether the interactions are COI (rank change), NCOI (scale change), or a mixture of both. In this data set, all the two-way interactions (soil × N, soil × P, and N × P) were significant, including the linear responses of P in the soil × P interaction and the cubic responses of P in the N × P interaction (Table 1).

First, the significant cubic trend of P in the N × P interaction shows higher GY for N_{30} than for N_0 with a NCOI trend (Fig. 2; Table 2). Second, the significant linear soil × P interaction (Fig. 3) shows a much higher linear response to P at all rates in the Black soil than in the Chestnut soil; the NCOI interaction between soil and P is clearly depicted. The reason for the soil × P interaction was that GY increased linearly at the rate of 0.0029 Mg ha^{-1} with each incremental 1 kg ha^{-1} increase in P fertilizer on the Black soil, while GY increased linearly at the much slower rate of 0.0003 Mg ha^{-1} with each incremental increase in P on the Chestnut soil from 0 through 250 kg P ha^{-1}. This NCOI was an important interaction to report because it indicates that although both GY increases were linear and significant, GY increases on the Black soil were much more substantial than GY increases on the Chestnut soil. Depending on the cost of P fertilizer, it may not be economical to apply it on the Chestnut soil. By partitioning the two-way interactions, we have shown that grain yield responses were significant on both soils, but the linear response on the Black soil was significantly more positive than on the Chestnut soil. Key information for the researcher was identified by including two-way interactions and the polynomial contrasts of P in the model.

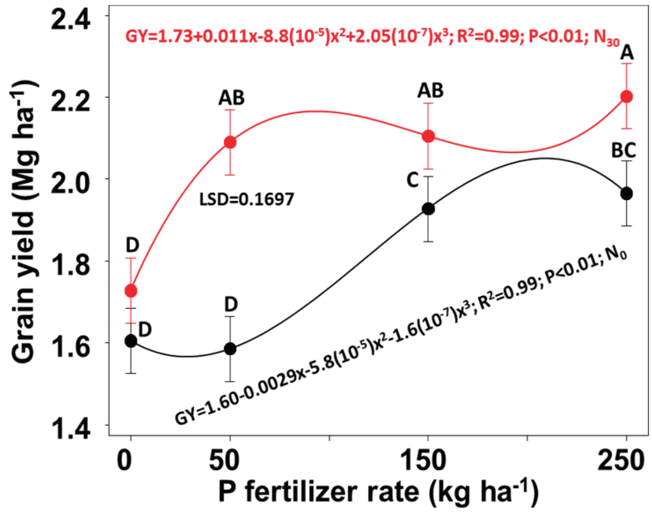

Fig. 2. Cubic responses of grain yield (GY) to P fertilizer for the N × P interaction combining Black and Chestnut soils, Exp. 1. Means with the same letter are not significantly different at the 0.05 probability level.

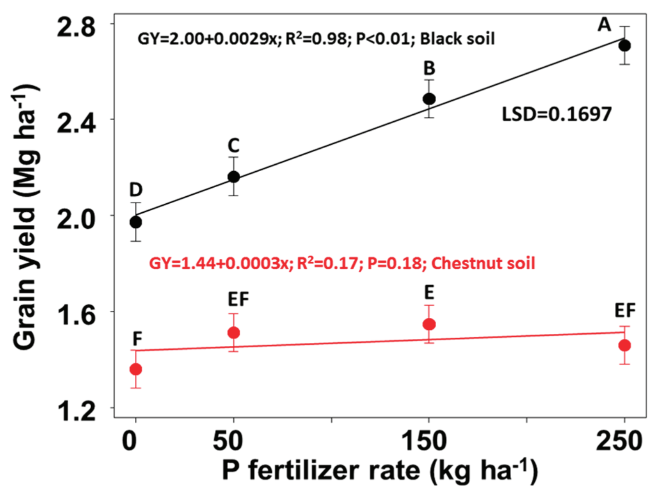

Fig. 3. Linear response of grain yield (GY) on two soils to four P fertilizer rates in soil × P interactions in Exp. 1 averaged across two N rates. Means with the same letter are not significantly different at the 0.05 probability level.

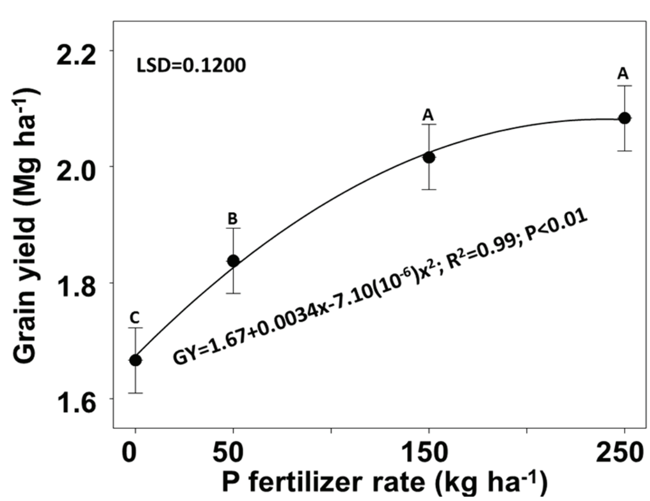

Fig. 4. Quadratic response of grain yield (GY) to four P fertilizer rates in Exp. 1. Means with the same letter are not significantly different at the 0.05 probability level.

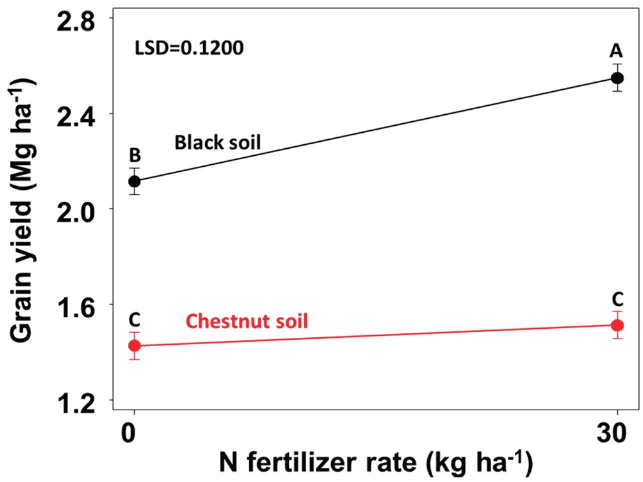

Fig. 5. Response of grain yield on Black and Chestnut soils at two N fertilizer rates averaged across four P fertilizer rates for soil × N interaction in Exp. 1. Means with the same letter are not significantly different at the 0.05 probability level.

Main Effects

The main effects of soil, N, and P and the linear and quadratic orthogonal decomposition of P were significant (Table 1). Grain yield production on the Black soil was significantly higher than on the Chestnut soil, and N_{30} gave significantly higher grain yield than N_0 (Table 2). The quadratic trend response of P indicates that P_{150} and P_{250} did not differ between themselves but had a significantly higher grain yield than P_0 and P_{50} (Fig. 4).

Had we analyzed only the main effect of P in this study, we would have concluded that GY responded positively and similarly to P on both soils. In fact, the soil × P interaction indicated that farmers with Black soil could expect a much more robust response to P than farmers with Chestnut soil. A similar conclusion can be reached regarding the response to N on each soil (Table 2; Fig. 5). Had we analyzed only the main effects, we would have told all farmers to apply the higher rates of N and P. However, based on the significant interactions, it is much clearer that most of the positive response for each element was due to the response on the Black soil. Thus, we can recommend that it is crucial for farmers with Black soil to add each fertilizer at the higher rates but that only marginal yield increases would be realized on the Chestnut soils.

Appendices for SAS Codes

The three appendices show SAS codes applied to the Exp. 1 data set for: (i) computing the coefficients of orthogonal polynomials when the levels of one factor are unevenly spaced (levels of P) (Appendix A); (ii) computing and graphing the LSD of least squares means for interactions (Appendix B); and (iii) directly computing and graphing the confidence intervals of the least squares means for main effects and interactions (Appendix C).

Coefficients of Orthogonal Polynomials: The SAS codes to develop the coefficients for orthogonal polynomials for unequally spaced levels of factors are given in Appendix A. The coefficients for orthogonal polynomials when the levels of factors are equally spaced are easy to obtain directly from tables in books on statistics. For example, Steel and Torrie (1980, Section 15.7, Table 15.11) for two-, three-, four-, five-, and six-factor levels and Kuehl (1999, Section 6.5, Table 6.12 and Display 6.3) for equally spaced and equal replications (no missing data, must be balanced) provided a fairly detailed explanation of how to calculate the regression coefficients. However, when the factor levels are unevenly spaced (for instance, in the Exp. 1 data set there are four unevenly spaced P rates: 0, 50, 150, and 250 kg ha^{-1}), it is helpful to use software to compute those coefficients. The SAS codes shown in Appendix A are also useful for equally spaced factor levels.

The SAS codes for Computing and Graphing Least Significant Difference for Interactions: Because the LSD = $(t_{dfe,\alpha/2})[2\sqrt{(2\text{MSE}/V)}]$ and the half width of the confidence interval HWCI = $(t_{dfe,\alpha/2})[\sqrt{(\text{MSE}/V)}]$, where $t_{dfe,\alpha/2}$ is Student's t quantile with error degrees of freedom and significance level $\alpha/2$, MSE is the mean square error from the analysis of variance, and V is the number of replications multiplied by the number of factor levels not involved in that LSD value (or the number of observations for those means), it is easy to see that LSD = $\sqrt{(2)}$ × HWCI.

For the main effects, SAS Proc GLM directly calculates and outputs the LSD value. However, SAS does not directly calculate the LSD values for the interactions. Using the above relationship between the LSD and HWCI, it is possible to obtain the confidence interval for the least squares adjusted means (lsmeans).

The above relationship is valid only for balanced data. For unbalanced data, the LSD expression becomes LSD = $(t_{dfe,\alpha/2})$ $\sqrt{[\text{MSE}(1/v_i + 1/v_j)]}$, where v_i and v_j are the number of observations involved in the corresponding levels i and j of the factor being analyzed. If the cell sizes are unequal, SAS uses the harmonic mean of the cell sizes to compute the critical ranges in the mean statement and for the confidence limits for the individual lsmeans. This approach is reasonable if the cell sizes are not too different, but it can lead to liberal tests if the cell sizes are highly disparate. Because it is difficult to automatically compute the harmonic mean in the SAS data step, we have included a correction factor given by the ratio of the number of real observations used (OU) in the analysis of variance divided by the total number of observations read (OR). This ratio is the coefficient CF = OU/OR. When the data is balanced, CF = 1.

Graphing the Interaction Profiles in Factorial Experiments Using the GLIMMIX Procedure: An easy way to obtain the graphs for the mean response profiles for main effects and interactions in a factorial experiment is through the generalized linear mixed models procedure (GLIMMIX). The SAS code for the Exp. 1 data set is shown in Appendix C. Also, the response profile and 95% confident interval (CI) for the combination level of lsmeans of soil, N, and P are given in Fig. C1 of Appendix C. The difference between the intervals for the interactions obtained from Appendix B using GPLOT and those computed in Appendix C using the GLIMMIX procedure are twofold: (i) GLIMMIX does not allow a curvilinear polynomial fit to the data but rather only depicts the lsmean profile (and its 95% confident interval) at each level of the factor plotted in the x axis, while the GPLOT procedure will fit the curvilinear polynomial and the LSD at each lsmean point; and (ii) the GPLOT produces a computer graphics metafile (CGM) that

Table 3. Experiment 2: Estimate of variance components of random effects, standard error, approximate Z value, and their approximate probability ($Pr > Z$) for grain yield (Mg ha^{-1}) in an experiment with eight historical maize hybrids evaluated at four N rates for 3 yr, and fixed main effects of N and genotype, and their two-way interaction, their numerator and denominator degrees of freedom, and probability of F values ($Pr > F$). Rates of N are partitioned into linear, quadratic, and cubic orthogonal polynomial contrasts.

	Random effects			
Covariance parameters	Estimate	Standard error	Z value	$Pr > Z$
Year	0.1560	0.2076	0.75	0.2262
Replicate(year)	0.0738	0.0527	1.4	0.0807
Year × N	0.0798	0.0595	1.34	0.0900
Year × Hybrid	0	–	–	–
Year × N × Hybrid	0	–	–	–
Residual	0.5577	0.0507	11	<0.0001
	Fixed effects			
	Numerator df	Denominator df	F value	$Pr > F$
N	3	6	65.43	<0.0001
Linear N	1	6	163.71	<0.0001
Quadratic N	1	6	31.6	0.0014
Cubic N	1	6	0.97	0.3619
Hybrid	7	14	41.65	<0.0001
N × hybrid	21	42	3.89	<0.0001
Linear N × hybrid	7	42	9.15	<0.0001
Quadratic N × hybrid	7	42	1.42	0.1989
Cubic N × hybrid	7	42	0.34	0.9355

can be easily edited and modified; the graphs produced by GLIMMIX are not easy to edit and modify.

Experiment 2

The mixed linear model considers year and its two- and three-way interactions with N and the maize hybrid as random effects. The magnitude of these variance components is shown in the upper part of Table 3. Results indicate that after the residual variance component, year was the most important factor in terms of variance components, followed by a year × N interaction. Two-way year × hybrid and three-way year × N × hybrid interactions were negligible. However, the year variance component had the largest p value for the hypothesis of variance equal to zero by using the approximate Z test. In addition, the zero (or negative) variance components of year × hybrid and year × N × hybrid could be due to an artifact of the optimization algorithm that includes a non-negative constraint; a negative variance component could also represent competition effects between adjacent plots in the same block in the field.

The fixed main effects of N and their linear and quadratic orthogonal contrasts were significantly different from zero, indicating that hybrids had a curvilinear response at higher levels of N fertilizer. The significant quadratic response of the combined eight maize hybrids to the four N rates is clearly depicted in Fig. 6 and shown in the means separation in Table 4. Also, as expected, highly significant differences among historical maize hybrids were detected, as indicated by their mean separation (Table 4).

Table 4. Experiment 2: Least significant difference ($LSD_{0.05}$) means comparison for the fixed main effects of N and genotype for grain yield (Mg ha^{-1}) of an experiment including four N rates (N_0, N_{75}, N_{150}, and N_{300}) and eight historic maize hybrids (1–8) evaluated for 3 yr.

Main effect	Mean
N level	
N_{300}	5.974 A†
N_{150}	5.340 A
N_{75}	4.447 B
N_0	2.530 C
Hybrid	
7	5.400 A
8	5.274 AB
5	5.059 ABC
6	4.996 BC
4	4.845 C
3	4.012 D
2	3.836 D
1	3.161 E

† All means for the same main effect followed by a different letter are significantly different based on the unprotected LSD (0.05).

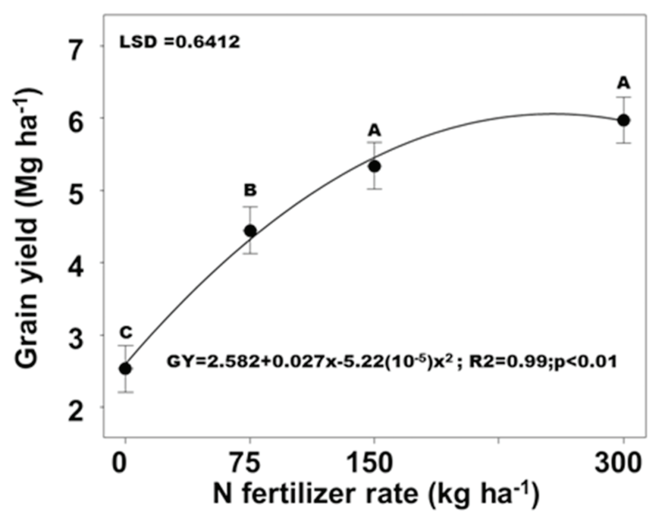

Fig. 6. Linear response of grain yield (GY) to four N fertilizer rates in Exp. 2. Means with the same letter are not significantly different at the 0.05 probability level.

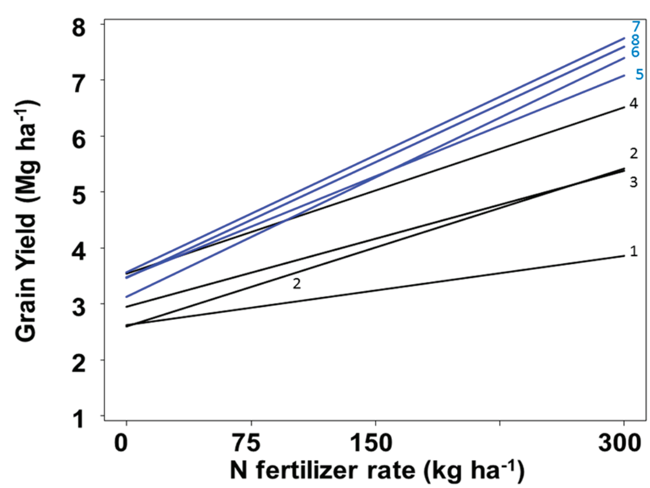

Fig. 7. Linear response of grain yield of eight wheat lines to four N fertilizer rates in Exp. 2. The number at the end of each line refers to the genotype number. Early released hybrids (1–4) are depicted by black lines and more recently released hybrids (5–8) are depicted by blue lines.

Table 5. Experiment 3: Main effects and two-way interaction ANOVA for grain yield (Mg ha^{-1}) of an experiment with seven wheat lines evaluated in six environments (Environ. 1–6). Decomposition of the sum of squares of genotype × environment interaction based on factorial regression contrasts is shown in the lower part of the table. Probability of *F*-values (*Pr* > *F*).

Source	df	Sum of squares	Mean square	*F* value	*Pr* > *F*
Environments	5	57.619	11.524	158.97	<0.0001
Environ. 1 + 2 vs. 3–6	1	56.851	56.851	784.26	<0.0001
Other	4	0.778	0.194	2.29	<0.0500
Replicate(environment)	6	0.772	0.129	1.77	0.1323
Line	6	4.925	0.821	11.32	<0.0001
Line × environment	30	7.155	0.238	3.29	0.0004
Error	36	2.610	0.072		
Total	83	73.081			
Factorial regression of line × environment interactions for three contrasts†					
Line × (Environ. 1 and 2 vs. 3–6)	6	2.182	0.364	2.88	0.0157
Line × (Environ. 1 vs. 3–6)	6	2.468	0.411	5.41	0.0002
Line × (Environ. 2 vs. 3–6)	6	2.913	0.486	6.84	<0.0001

† Factorial regression contrasts are performed separately and their sum of squares cannot be added.

Dissecting the Two-Way Interaction

The test of the linear response on the N × hybrid interaction was significant (lower part of Table 3) because the more recently released hybrids (5–8, depicted by blue lines in Fig. 7) had higher yields than hybrids released earlier (1–3, black lines in Fig. 7) across N rates, while Hybrids 4 through 8 had similar yields at N_0 but Hybrids 5 through 8 had higher yields than Hybrid 4 at higher N rates (N_{150} and N_{300}). Thus the rank change of Hybrid 4 with respect to Hybrids 5 through 8 from low to high doses of N was the major cause of the significant linear N × hybrid interaction. The researcher should also note that the earlier releases (Hybrids 1 to 3) had less pronounced responses to N than Hybrids 5 to 8. Also, Hybrids 2 and 3 had similar productivity at N_{300} but the yield of Hybrid 2 was less than that of Hybrid 3 at N_0; this change in Hybrid 2 productivity throughout the four rates of N compared with that of Hybrid 3 is a NCOI that also contributed to the linear N × hybrid interaction indicated.

In general, the significant linear N × hybrid interaction shown in Table 3 and depicted in Fig. 7 is due to a scale change in the productivity of the eight historical hybrids from low to high N rates. At N_0, grain yield ranged from 2.5 to 3.5 Mg ha^{-1}, whereas at N_{300}, grain yield production ranged from about 2.95 to 7.85 Mg ha^{-1}. However, it should be noted that the only COI was the relationship of Hybrid 4 with Hybrids 5 through 8 across the N rates, as described above.

Table 6. Grain yields of fixed main effects (six environments and seven wheat lines) in Exp. 3.

Main effect	Grain yield
	Mg ha^{-1}
Environment	
4	3.68 A†
6	3.59 AB
5	3.41 AB
3	3.41 B
1	1.79 C
2	1.77 C
Wheat line	
2	3.23 A
1	3.21 A
7	3.02 AB
6	2.96 B
5	2.94 B
4	2.69 C
3	2.51 C

† All means for the same main effect followed by a different letter are significantly different based on the unprotected LSD (0.05).

Experiment 3

In Exp. 3, there were two fixed effects: environment and wheat line. There were seven wheat lines, but they were not structured. There were six environments, but the researcher had enough prior knowledge to structure them into two categories: one containing two low-yielding environments (1 and 2) and the other containing four intermediate environments (3–6).

Fixed effects, environments and wheat lines, as well as their interactions were highly significant (Table 5). The comparison between the two low grain yielding environments and the four intermediate grain yielding environments explained almost 98.6% of the environment main effect sum of squares. The means of the main effects of environment clearly show the differential productivity of Environments 1 and 2 compared with Environments 3 through 6 (Table 6). In terms of the yield of the wheat lines, Lines 1 and 2 had significantly higher GY than Lines 3 and 4 across environments. However, the researcher may be interested in examining the performance of the wheat lines under the low- and intermediate-yielding environmental groups to possibly detect lines with high yields in one or the other environmental group vs. lines that yield well across all environments. Any of these lines may be of value to farmers; the key is for the researcher to identify and communicate their value properly.

Dissecting the Two-Way Interaction

Because the factor wheat line was unstructured, there was no previous indication as to how the wheat lines could be grouped to dissect the interaction. However, the factor environment, with six levels, can be further studied because it has one group with two low-productivity environments and a second group with four environments having intermediate productivity levels. Furthermore, a great deal of the variation among environments can be explained by a single contrast: the mean of Environments

1 + 2 vs. the mean of Environments 3 to 6. This suggests a strategy for partitioning the interaction into contrasts that is related to the natural structure of the data. Therefore, we describe two strategies for dissecting a two-way interaction: singular value decomposition and factorial regression contrasts.

Singular Value Decomposition of the Two-Way Interaction

In Table 7, wheat lines in Environments 1 and 2 had lower grain yield than in other environments. Wheat Line 2 was the most productive line (2.88 Mg ha^{-1}) in Environment 2, and Line 4 was the highest yielding line in Environment 1 (2.26 Mg ha^{-1}). Concerning the response of the lines in the more productive environments, Line 1 was the highest yielding in Environments 4 and 6, whereas Line 2 had high yields in Environment 4. However, this table is dense and not easy to use for extracting the response patterns of wheat lines in environments.

A much more useful and complete description of the performance of the wheat lines in the different environments can be achieved by fitting the fixed-effect linear–bilinear model called the site regression model (SREG) (Cornelius et al., 1996; Crossa and Cornelius, 1997). In the SREG model, the effect of lines (genotypes) plus the genotype × environment interactions are subjected to singular value decomposition. Site regression is a useful model for analyzing multienvironment trials and studying the response patterns of genotypes and environments. The mixed linear–bilinear SREG can also be used for modeling the interaction in these types of trials (Crossa et al., 2014).

The biplot obtained from the SREG model describes in one graph (Fig. 8) the complete response patterns of the wheat lines in the six environments as presented in the mean separation of Table 7. The two low-yielding environments (Environments 1 and 2) are clearly separated in the lower quadrants of Fig. 8, whereas the intermediate-yielding environments (3–6) are located in the upper right quadrant. The biplot additionally shows that Environment 2 caused much more interaction than Environment 1; this conclusion is based on the much more negative location on the *y* axis of Environment 2. In the environments with intermediate grain yields, Environments 3 and 4 caused most of the interaction with the wheat lines––in this case, based on their higher positive location on the *y* axis.

Regarding the performance of the wheat lines in the environments, the SREG biplot (Fig. 8) indicates that Line 4 was the best in Environment 1, whereas Line 2 was the most productive in Environment 2; this graphically confirms what we concluded using mean separation and LSDs above. On the other hand, Line 1 performed well at the intermediate-yielding Environments 3 through 5. Based on these results, the researcher can conclude that Lines 1 and 2 had good performance in one low-yielding environment each. On the other hand, the GY of Lines 6 and 7 is highly dependent on the environment because these lines had low grain yield in Environments 1 and 2 but good yield performance in

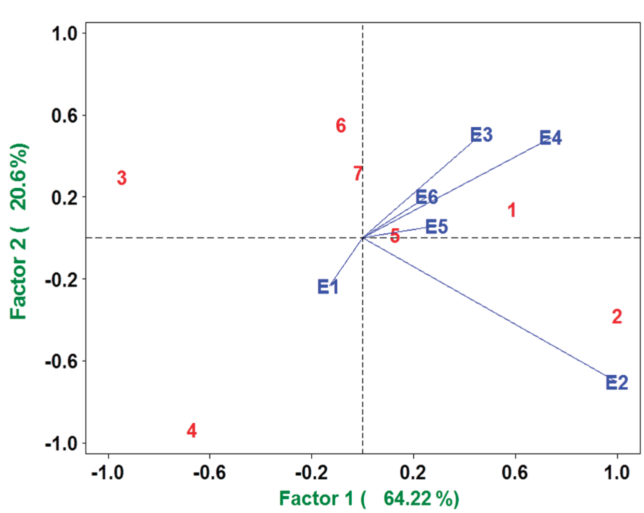

Fig. 8. Biplot of the site regression (SREG) model of seven wheat lines (1–7) evaluated in six environments (E1–E6) in Exp. 3.

Table 7. Grain yields of seven wheat lines (1–7) in six environments (1–6) in Exp. 3.

Environment	Wheat line	Grain yield
		Mg ha^{-1}
4	1	4.13
4	2	4.09
6	1	3.95
4	6	3.87
4	7	3.87
4	5	3.80
6	6	3.79
3	7	3.78
5	2	3.73
3	2	3.66
3	1	3.67
6	2	3.58
6	5	3.56
3	6	3.56
6	7	3.55
5	5	3.53
5	1	3.53
5	6	3.45
6	4	3.37
3	5	3.33
5	3	3.31
6	3	3.31
5	7	3.19
5	4	3.14
4	3	3.12
3	3	3.12
4	4	2.89
2	2	2.88
3	4	2.75
1	4	2.26
1	7	2.21
2	1	2.18
2	5	1.86
1	1	1.82
1	6	1.76
2	4	1.75
2	7	1.56
1	5	1.55
1	2	1.47
1	3	1.42
2	6	1.35
2	3	0.80

Environments 3, 4, and 6 (Table 7; Fig. 8). Line 3 showed poor grain yield performance in all the environments.

Factorial Regression Contrasts for Two-Way Interaction

The factorial regression model can be efficiently applied in the context of any two-way table to define sets of covariables that can be used for analyzing and interpreting interactions (Crossa et al., 2014). Factorial regression models are ordinary linear models that approximate the interaction effects by using external covariables or defining specific contrasts between levels of a factor in its interaction with another factor. The use of defined covariable contrasts can help dissect interactions between factors and detect possible levels or combinations of levels of one factor that interact with levels of the other factor. By using the factorial regression model with a stepwise procedure for variable selection, the important covariables affecting the interaction can be identified and quantified. The use of covariables to define contrasts can be approached with a linear fixed-effects model or a linear mixed-effects model.

For the data in Exp. 3, there was a clear separation of environments into those with low and intermediate productivity. This can be done by defining the interaction contrast [(Environments 1 + 2 vs. Environments 3–6) × lines], which gives a comparison with six degrees of freedom that is significant (F value = 2.88, $P < 0.0157$) (Table 5). This interaction contrast used 20% of the degrees of freedom of the interaction and explained 30.49% of the interaction sum of squares. Similarly, the researcher may be interested in studying the interaction of the seven wheat lines with one low-productivity environment when compared with the intermediate grain yield environments; this was evaluated by defining the interaction contrast (Environment 1 vs. Environments 3–6) × lines, which was highly significant and explained 34.49% of the total interaction sum of squares. Furthermore, the interaction contrast (Environment 2 vs. Environments 3–6) × lines was highly significant and explained 40.72% of the total interaction sum of squares (Table 5).

These results indicate that the covariables in the factorial regression model are useful for rapidly and efficiently partitioning the interaction into contrasts that explain portions of its complexity. When several covariables are defined, a variable selection procedure can be used for determining the most important interaction comparisons (Crossa et al., 2014). Furthermore, the results of the factorial regression are in agreement with the dissection of the interaction performed using the singular value decomposition approach when fitting the linear–bilinear model SREG. The SREG biplot determined that Environments 1 and 2 caused most of the interaction among wheat lines; this is especially clear for Environment 2, which discriminated the lines with a different pattern than Environment 1, as shown by the magnitude of the scores of the second eigenvector represented by the abscissa (Factor 1) and by the coordinate (Factor 2) in Fig. 8.

CONCLUSIONS

It is essential to analyze and interpret all possible interactions among treatments in experiments with more than one treatment. The researcher should report on the significance of all the interactions in the study and explain the nature of the significant interactions. Then, he or she should strategize on how best to communicate the results of the study. There are no rules of statistics that mandate how the results must be described based on which interactions were significant. The researcher's final explanation may focus on two-way interactions when higher order interactions were significant, or it may focus on main effects when two-way interactions were significant. Often it will be necessary to use the highest order interactions that were significant. Our most important message to the researcher is that all of the meaningful research should be analyzed by interpreting all possible interactions; all meaningful interactions that are statistically significant should be reported, and meaningful results should be reported properly and fully while seeking to do so in a simplified manner. Final reports of results and conclusions should be based on biological knowledge and interpretation of statistical procedures that were properly conducted and interpreted.

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Appendix A

It is necessary to install the Interactive Matrix Language (**IML**) procedure for calculating the orthogonal polynomial coefficients. In the **Plevels** statement you express how many and which levels of the variable you wish to calculate. For instance, in the Example 1 data set, phosphorus has 4 unevenly spaced rates: 0, 50, 150, and 250 kg ha^{-1}. The **Orpol** function is used for obtaining the required coefficients; since there are three degrees of freedom, we can calculate three polynomial coefficients—lineal, quadratic and cubic—which are assigned to macro variables that are later used in the data step in the three-way analysis of variance and contrasts.

In the main effects contrasts, we need only positive values of the coefficients, but in the two- and three-way contrasts, a combination of positive and negative coefficients are required, depending on the levels of each of the factors involved in those interactions. These positive and negative coefficients are assigned to the **PosCoefGrade** and **NegCoefGrade** macro variables, respectively, using the **Call Symput** function. The order of the polynomial contrasts is obtained using the **%do** - **%end** cycle. Note that the appropriate number and grade of the coefficients are automatically determined in the **ncoef=ncol(coeff)-1** statement. This program can be easily modified for different numbers and/or levels of factors.

SAS macro program

```
**** Reading data ****;

Data Raw ;
Infile "C:\Yield & Gluten Data 2007.csv" dlm="," firstobs=3 ;
informat Soil$ 10. ;
input Soil N P Rep Yield Gluten ;
Datalines ;
Run ;

**** Here begins the macro code for calculating the orthogonal polynomial
     Contrasts in an automatic way ****;

%Macro Coefficients;
Proc IML;
     Plevels= {0, 50, 150, 250} ;
     coeff = Orpol(Plevels,3);
     ncoef=ncol(coeff)-1;
     call symputx("ncoef", ncoef);
     %do K=1 %to &ncoef;
          CoefGrade&K= t(Coeff[,&K+1]);
          PosCoefGrade&K = rowcat(char(  CoefGrade&K ,15,10));
          NegCoefGrade&K = rowcat(char(-(CoefGrade&K),15,10));
          call symputx("PosCoefGrade&K", PosCoefGrade&K);
          call symputx("NegCoefGrade&K", NegCoefGrade&K);
     %end;
quit;
Run;

Title1 "Three-way ANOVA, decomposing df for P into three contrasts";
Proc GLM data = Raw;
     class Soil N P Rep  ;
     model Yield = Soil N P
                   Soil*N Soil*P N*P
```

```
                  Soil*N*P / ss3;
**** P main effects contrasts ****;
contrast "Linear P"    P  &PosCoefGrade1 ;
contrast "Quadratic P" P  &PosCoefGrade2 ;
contrast "Cubic P"     P  &PosCoefGrade3 ;
**** Soil × P two way interaction contrasts ****;
contrast "Linear Soil*P"    Soil*P  &PosCoefGrade1 &NegCoefGrade1 ;
contrast "Quadratic Soil*P" Soil*P  &PosCoefGrade2 &NegCoefGrade2 ;
contrast "Cubic Soil*P"     Soil*P  &PosCoefGrade3 &NegCoefGrade3 ;
**** N × P two way interaction contrasts ****;
contrast "Linear N*P"    N*P     &PosCoefGrade1 &NegCoefGrade1 ;
contrast "Quadratic N*P" N*P     &PosCoefGrade2 &NegCoefGrade2 ;
contrast "Cubic N*P"     N*P     &PosCoefGrade3 &NegCoefGrade3 ;
**** Soil × N × P three way interaction contrasts ****;
contrast "Linear Soil*N*P"    Soil*N*P &NegCoefGrade1 &PosCoefGrade1
                                       &PosCoefGrade1 &NegCoefGrade1;
contrast "Quadratic Soil*N*P" Soil*N*P &NegCoefGrade2 &PosCoefGrade2
                                       &PosCoefGrade2 &NegCoefGrade2;
contrast "Cubic Soil*N*P"     Soil*N*P &NegCoefGrade3 &PosCoefGrade3
                                       &PosCoefGrade3 &NegCoefGrade3;
Run;

%Mend;
%Coefficients;
Run;
```

Appendix B

The output delivery system (ODS), **ODS Output LSMeanCL = LsmeansCI** statement is useful for creating a virtual file with only the information that will be needed later. **The ODS listing exclude** is for suppressing the additional information that SAS writes by default, which isn't necessary. For generating the different variables containing information for the regression lines associated with each combination of the Soil × P interaction (Fig. 3), we created the new variables **Y_black** and **Y_chest** from the yield values. Similarly, cycles **if – then do – end** are used for obtaining the information needed in the upper and lower LSD bars. The codes can be adapted if four regression lines need to be depicted with their corresponding LSD bars.

For Fig. 3 of Example data set 1, we are interested in the ANOVA for all the main effects and two- and three-way interactions; thus all those terms should be included in the model statement when computing the correct LSD and/or the confidence interval. In the LSMeans statement, we have included only the interactions that are of interest for graphing the LSD values, thus simplifying the output.

```
** Data Reading from an external CSV File **;
Data Raw;
Infile "C:\Yield & Gluten Data.csv" dlm="," firstobs=3;
Informat Soil$ 10.;
Input Soil N P Rep Yield Gluten;
Datalines;
** Three-way ANOVA, including all two-way and three-way interactions **;
Proc GLM Data = Raw  ;
     Class Soil N P Rep ;
     Model Yield = Soil Rep(Soil) N P
                   Soil*N Soil*P N*P Soil*N*P / SS3 ;
     LSMeans Soil*P / Adjust = T Lines CL;
     ODS output LSMeanCL = LsmeansCI NObs = Nobserv ;
     ODS listing exclude CLMeansInfo Diff CLMeans LSMeans LSMeanCL
                         LSMeanDiffCL ;
Run;
** Calculating the Correction Factor for an unbalanced data set **;
Data Nobs;
     Set Nobserv;
     CF0 = NobsUsed / NobsRead ;
     Call symput("CF", CF0);
Run;
 ** LSD for interactions through half width confidence interval (HWCI),
    generating a macro variable using the call symput function **;
Data LSD;
     Set LSMeansCI ;
     HWCI = LSmean - LowerCL;
     LSD = Sqrt(2*&CF)*(HWCI);
     P2 = P + 0.0 ;
     Drop P;
Proc Sort Data = LSD ;
     By Soil P2 ;
***** Calculating the means of the interaction Soil x P to be used in the
      regression curves and graph *****;
Proc Sort Data = Raw  ;
     By Soil P ;
Proc Means Data = Raw noprint;
     By Soil P ;
     output out = Raw_Means mean = ;
     var Yield;
```

```
***** Generating the different curves to be used in graphing the LSD bars
*****;
Data Graph;
     Merge LSD (rename = (P2 = P)) Raw_Means ;
     By Soil P ;
     HLSD = LSD/2 ;
       if Soil = 'black'    then Y_black = Yield;
       if Soil = 'chestnut' then Y_chest = Yield;

       if Soil = 'black' then do;
          Yield1 = Yield;         output;
          Yield1 = Yield - HLSD;  output;
          Yield1 = Yield + HLSD;  output;
       end;

       if Soil = 'chestnut' then do;
          Yield2 = Yield;        output;
          Yield2 = Yield - HLSD; output;
          Yield2 = Yield + HLSD; output;
      end;
   Drop _Type_ _Freq_;
Run;
** Graphic options for creating a CGM file as output ** ;
** Here you must to change the route and the name of CGM file ** ;
FileName Figures 'C:\Multi Factorial Interactions\Figure 3.CGM';
Goptions Device = CGMOF97L GSFName = Figures GSFMode = Replace;

Proc GPlot Data = Graph ;
     Plot (Y_black Y_chest)*P (Yield1 Yield2)*P / frame overlay
                    vaxis = axis1 haxis  = axis2 nolegend;
Symbol1 v=dot  cv=black  h = 2.3   l=1 w=15   i=RL     ci=black ;
Symbol2 v=dot  cv=red    h = 2.3   l=1 w=15   i=RL     ci=Red ;
Symbol3                            l=1 w=2    i=hiloct ci=black;
Symbol4                            l=1 w=2    i=hiloct ci=red;

axis1 length=6.0 in     order=(1.2 to 2.8 by 0.4)
      label=(f=hwcgm002 h=2.0 a=90 'Grain yield (Mg ha-1)')
      value=(f=hwcgm002 h=2.0) offset=(1) minor=none;
axis2 length=9.0 in     order=(0 to 250 by 50)
      label=(f=hwcgm002 h=2.0 'P fertilizer rate (kg ha-1)')
      value=(f=hwcgm002 h=2.0) offset=(3) minor=none;
Title1 f=hwcgm002 h=0.2 ' ';
Run;
```

Appendix C

```
Data Raw ;
     Infile "C:\Yield & Gluten Data.csv" dlm="," firstobs=3 ;
     Informat Soil$ 10. ;
     Input Soil N P Rep Yield Gluten ;
     Datalines;
Run;

ODS graphics on;
ODS Select CovParms Tests3 MeanPlot;

Proc GLIMMIX Data=Raw;
     Class Rep Soil N P ;
     Model Yield = Soil Rep(Soil) N P Soil*N Soil*P N*P Soil*N*P;
     Lsmeans Soil N P / plot = mean (join cl);
     Lsmeans Soil*N Soil*P / plot = mean (sliceby = Soil join cl);
     Lsmeans N*P / plot = mean (sliceby = N join cl);
     Lsmeans Soil*N*P / plot = mean (sliceby = Soil*N join cl);
Run;

ODS graphics off;
Run;
```

The **ODS Graphics** is used to generate by default several graphics related to the **lsmeans** statement. The **meanplot** option or simply **mean** requests displaying the least squares means. For example, in the line Lsmeans Soil N P / plot = mean (join cl), the lsmeans response profiles are requested for all the main effects Soil, N, and P, simultaneously. The **meanplot-options** controls the display of the least squares means. **Join** or **connect** connects the least squares means with lines. **CL** displays upper and lower confidence limits for the least squares means. By default, 95% limits are drawn. The confidence levels can be changed with the **alpha**= option.
The statement Lsmeans Soil*N Soil*P / plot = mean (sliceby = Soil join cl) is used for simultaneously requesting the response profiles for the Soil×N and Soil×P interactions. **Sliceby**=fixed-effect specifies the Soil effect by which to group the means in a single plot, and the levels for the N and P effects to be drawn in the horizontal axis, because Soil is a qualitative factor, while N and P are quantitative factors.

Similarly, the statement Lsmeans N*P / plot = mean (sliceby = N join cl) is used to draw the individual response profiles for each N level for each P level in the horizontal axis.

Finally, the statement Lsmeans Soil*N*P / plot = mean (sliceby = Soil*N join cl) is useful for drawing the four response profiles for the combination of the levels of factors Soil and N, both of them with two levels, and for each level of the P factor in the horizontal axis. The graphs obtained with the above code program are depicted in Fig. C1.

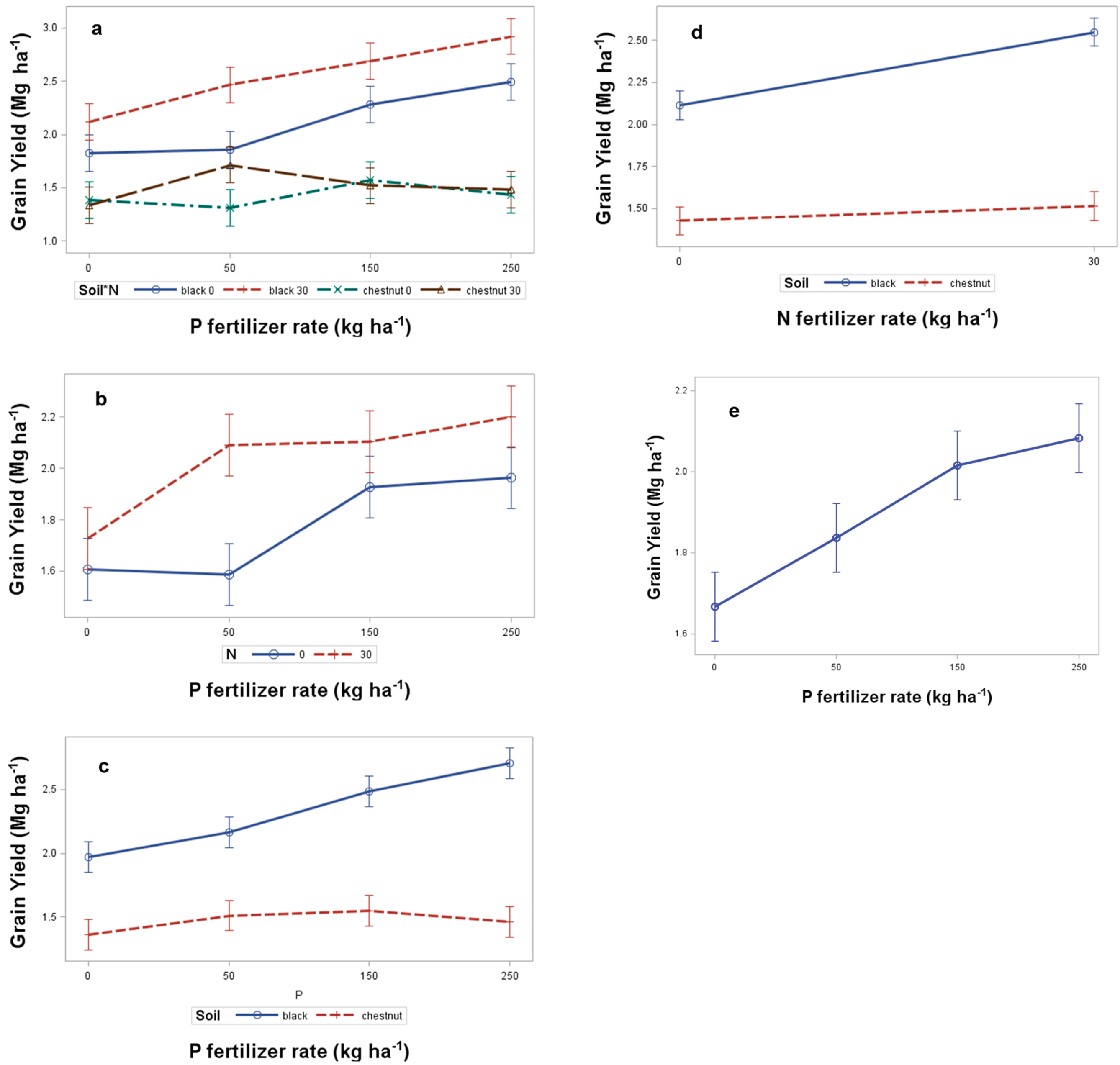

Fig. C1. Response profiles and 95% confidence interval (CI) for the means of various combinations of levels of the soil factor (Chestnut and Black), two levels of N (0 and 30 kg ha^{-1}), and four unevenly spaced P fertilizer rates (0, 50, 150, and 250 kg ha^{-1}): (a) grain yield using two N rates (0 and 30 kg ha^{-1}) on Black and Chestnut soils; (b) grain yield using two N rates (0 and 30 kg ha^{-1}) and four unevenly spaced levels of P fertilizer; (c) grain yield on two soil types, Chestnut and Black, and four unevenly spaced levels of P fertilizer; (d) grain yield using two N levels (0 and 30 kg ha^{-1}) and four unevenly spaced levels of P fertilizer; and (e) grain yield using four unevenly spaced levels of P fertilizer.