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Integration of Statistical and Physiological analyses of Adaptation of near-isogenic barley lines

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Abstract. Seven near-isogenic barley lines, differing for three independent mutant genes, were grown in 15 environments in Spain. Genotype × environment interaction (G × E) for grain yield was examined with the Additive Main Effects and Multiplicative interaction (AMMI) model. The results of this statistical analysis of multilocation yield-data were compared with a morpho-physiological characterization of the lines at two sites (Molina-Cano et al. 1990). The first two principal component axes from the AMMI analysis were strongly associated with the morpho-physiological characters. The independent but parallel discrimination among genotypes reflects genetic differences and highlights the power of the AMMI analysis as a tool to investigate G × E. Characters which appear to be positively associated with yield in the germplasm under study could be identified for some environments.

Key words: Genotype × environment interaction – Addtitive main effects and multiplicative interaction (AMMI) model – Mutant barley lines

Introduction

Genotype × environment interaction (G × E) is differential genotypic expression across environments. It reduces the association between phenotypic and genotypic values, and may cause selections from one environment to perform poorly in another, forcing plant breeders to examine genotypic adaptation in addition to mean yield. Measuring G × E is important in order to determine an optimum strategy for releasing genotypes with adequate adaptation to target environments.

There are two conceptually different approaches for studying G × E and adaptation. The more common is empirical and statistical and involves relating observed genotypic responses, usually in terms of yield, to a sample of environmental conditions. It has proven useful where G × E appears intractable because environmental factors discriminating among germplasms are poorly understood (Romagosa and Fox 1993). The Additive Main Effects and Multiplicative Interaction (AMMI) analysis (Gauch and Zobel 1988; Zobel et al. 1988) has been applied to large genotype × environment × replicate tables without missing values (Zobel et al. 1988; Crossa et al. 1990, 1991). AMMI first extracts genotype and environment main effects, then uses principal component analysis (PCA) to extract pattern from the G × E, or residual, matrix. Zobel et al. (1988) provided a scale for PCA scores which allows estimation of specific G × E interaction terms. By contrast, the ecological approach defines G × E in terms of biotic and abiotic stresses.

The present study relates empirical patterns of adaptation for grain yield derived by the AMMI analysis to three independent genes and to phenotypes which confer selective advantages for grain production in specific environments. The biplot procedure (Zobel et al. 1988) from AMMI analysis facilitates integrating the performance patterns, the morpho-physiological data, and the genes involved. Such integration combines the empirical and ecological approaches to studying G × E.

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Materials and methods

Plant material

Three recessive mutants (M01, M02, M03), induced in a two-rowed barley variety 'Beka', as well as the three binary recombinants (M12, M13, M23) and 'Beka', were described physiologicaly by Molina-Cano et al. (1990). Compared with 'Beka' (Table 1), Mutant 1 has a smaller leaf area at anthesis and is earlier; mutant 2 has denser spikes and more leaves at maturity and mutant 3 has a shorter grain filling period. Mutant 3 has been shown (Molina-Cano 1982) to involve a mutation at the *ert-d* locus described by Persson and Hagberg (1969).

Field methods

Fifteen yield trials (Table 2) were conducted from 1985 to 1989 in the most important barley-producing regions of Spain. Locations S1, S2, S3, BA, G1 and TO frequently suffer high temperatures during grain filling; G2 generally provides excellent conditions during the latter part of the season; the climate of the two most northern locations (PA and SO) is intermediate between these two extremes.

At each location, a randomized complete block design with four replicates of 12.6 m^2 was sown to measure grain yield. In two contrasting locations, G1 and G2, physiological measurements were taken for 2 years. The following nine morpho-physiological measurements were recorded at each of these four environments: no. of leaves/plant and leaf area at anthesis and physiological maturity; leaf angle; length of the main shoot; days from emergence to anthesis; days from anthesis to maturity; and spike density.

Statistical methods

The AMMI model for the average yield, Y_{ij}, over replicates of the ith genotype in the jth environment is:

$$Y_{ij} = \mu + g_i + e_j + \sum_{K}^{N} \lambda_n \gamma_{ik} \delta_{jk} + \varepsilon_{ij}$$

where μ is the overall mean, g_i and e_j are genotypic and environmental main effects, N is the number of PCA axes considered, λ_n is the singular value of the of the nth PCA axis, γ_{ik} and δ_{jk} are scores for the ith genotype and jth environment on the kth PCA axis and ε_{ij} is the residual term.

AMMI generates a family of models with different values of N. The simplest model, AMMI0 with N equal to zero, considers additive main effects, namely genotypic and environmental means, to explain the data matrix. AMMI0 ranks genotypes identically at each environment, ignoring G × E. The second model, AMMI1, considers main effects as well as one principal component axis to interpret the residual matrix. AMMI2 considers main effects plus two principal components axes for non-additive variation. Subsequent models consider cumulatively an additional principal component axis.

When one PCA axis accounts for most G × E, a feature of AMMI is the biplot procedure. Genotypes and environments are plotted on the same diagram, facilitating inference about specific interactions of individual genotypes and environments by using the sign and magnitude of PCA1 values. Any genotype with a PCA1 value close to zero shows general adaptation to the tested environments. A large genotypic PCA1 scores reflects more specific adaptation to environments with PCA1 scores of the same sign. However, when the best AMMI model includes more than one PCA axis, assessment and presentation of genotypic stability are not as simple as the AMMI1 case.

Table 1. Phenotypic characteristics of the six near-isogenic lines as related to the source variety 'Beka' (Molina-Cano et al. 1990)

Code	Near-isogenic line	Spike density	Height	Earliness	Leaf area	Grain filling period
M01	Mutant-1	Equal	Less than	More than	Less than	Equal
M02	Mutant-2	More than	Less than	Equal	Less than	Equal
M03	Mutant-3	More than	Equal	Less than	Equal	Less than
M12	Recombinant 1 and 2	More than	Less than	More than	Less than	Equal
M13	Recombinant 1 and 3	More than	Less than	Equal	Less than	Less than
M23	Recombinant 2 and 3	More than	Less than	Less than	Equal	Less than

Table 2. Description of the nine sites used

Location		Year	Site trial		Coordinates	Altitude (m)	Annual rainfall (mm)	Average temperatures			Soil fertility
Province	City		Codes	Codes				T_{max}	T_{mean}	T_{min}	
BADAJOZ	Mérida	1989	BA	BA9	38° 54′ N 6° 20′ W	270	450	23.2	16.4	9.6	Medium
GRANADA	Colomera	1987–88	G1	G17-G18	37° 23′ N 3° 42′ W	706	370	22.0	15.5	9.0	Low
GRANADA	Cotilfar	1987–88–89	G2	G27-G28-G29	37° 30′ N 3° 30′ W	912	577	18.6	12.9	7.2	High
PALENCIA	Villarramiel	1988	PA	PA8	42° 02′ N 4° 50′ W	900	378	19.6	12.2	4.9	Medium
SEVILLA	Alcalá del Rio	1985–86	S1	S15-S16	37° 32′ N 5° 58′ W	10	599	25.6	18.4	11.3	High
SEVILLA	Brenes	1988–89	S2	S28-S29	37° 29′ N 5° 49′ W	20	625	24.7	18.0	11.4	High
SEVILLA	Ecija	1989	S3	S39	37° 32′ N 5° 05′ W	112	539	26.1	18.9	11.7	High
SORIA	Almazán	1989	SO	SO9	41° 29′ N 2° 32′ W	938	560	18.0	11.9	5.7	Medium
TOLEDO	Tembleque	1988–89	TO	TO8-TO9	39° 41′ N 3° 30′ W	725	353	23.5	15.4	7.3	Low

Gauch and Zobel (1988) distinguished two processes for determining the number of PCA axes to consider. They used the terms postdictive and predictive accuracy. Postdiction is a descriptive procedure in which the best model was selected on the basis of variation explained by PCA axes. In prediction, individual replicates were allocated at random for each genotype × environment combination to either a data set for modelling or a set for validating the models. The sum of squared differences between validation data and predicted values for the different AMMI models, across the data matrix, was then divided by the number of validation observation. The square root of this quantity is the root mean square predictive difference (RMS PD), for which smaller values indicate more accurate prediction. On the basis of the average of the RMS PD values (Crossa et al. 1991), the best model was selected and re-applied to the data including all replicates. In assessing the number of PCA axes to consider, another model, the so-called DATA model, similarly considers the ability of means of replicates of modelling data, for each genotype × environment combination, to predict the corresponding validation data, namely the other replicate.

Results and discussion

Genotype and environment main effects and their interaction were statistically significant (Table 3), suggesting a broad range of genotypic diversity and environmental variation. Although significant, differences among genotypes accounted for less than 3% of the total variation. In contrast, the environment sum of squares exceeded 80% and G × E accounted for close to 10% of the total variation (Table 3).

The first PCA axis accounted for 72% of the interaction sum of squares, the second axis explained 14% and the third 7% of the G × E sum of squares (Table 3).

Two major trends have emerged from AMMI analyses of yield trials (Romagosa and Fox 1993). First, DATA is of low predictive accuracy compared with the other models. Second, after a certain number, additional PCA axes, decrease rather than increase predictive accuracy. PCA appears to extract pattern in the first few axes, with subsequent axes being associated with noise. For this reason, predictive modelling is preferred since it considers fewer axes than postdiction (Gauch and Zobel 1988).

Table 3. Combined analysis of variance for grain yield using the AMMI model for the seven barley genotypes at 15 locations

Source of variation	*df*	Sum of squares	R^{2a}	F-value[b]
Total	419	1071.2		
Environment (E)	14	859.7	80.3	115.34**
BLQ (site)	45	23.9	2.2	
Genotype (G)	6	31.4	2.9	4.53**
G × E	84	97.0	9.1	5.28**
AMMI01	19	69.4	71.5	8.57**
AMMI02	17	14.0	14.4	2.89**
AMMI03	15	6.9	7.1	2.23*
Residual	33	6.8	7.0	0.94ns
Error	270	59.0	5.5	

[a] Fraction of sum of squares associated to each term or interaction

[b] Hypothesis constructed based on a mixed model, the significance of the AMMI models based on postdiction
ns, *, **: $P > 0.05$; $0.05 > P > 0.01$; $0.01 > P > 0$

Table 4. Average RMS PD (kg/ha) and average of ranks for RMS PD for seven models based on 25 randomizations

Model	RMS PD	Mean rank
AMMI0	687	7.00
AMMI1	572	2.78
AMMI2	572	2.28
AMMI3	579	2.90
AMMI4	581	2.76
AMMI5	591	4.84
DATA	595	5.44

Based on postdiction, the AMMI3 model for this study was statistically significant. However, in the predictive sense, the AMMI1 and AMMI2 models were superior, showing the least deviation from validation data, based on 25 randomizations (RMS PD of 572 kg/ha, Table 4). When comparing the average rank of the RMS PD for each AMMI model across all randomizations, the AMMI2 model was more likely to show a more accurate prediction (average RMS PD rank = 2.28) than the AMMI1 (average of ranks for RMS PD = 2.78). The DATA model and the model considering only main effects, AMMI0, showed the lowest predictive ability (Table 4).

Figure 1 shows the biplot for the AMMI1 model, which explains almost 90% of the total variation. This biplot simultaneously summarizes information on genotypic and environmental main effects and their interaction. Displacements along the abscissa indicate differences in main effects, whereas deviations from zero on the ordinate reflect interactions. Therefore, compared to the others tested, genotypes with PCA1 values close to zero show wider adaptation to the sites used. Genotypes and locations with PCA1 values of the same sign interact positively.

Isogenic lines carrying mutant gene 1 tend, as an average, to yield less in most environments, the rest showing an overall higher-yielding performance. The original variety and the isogenic lines M02 and M23 showed less interaction than the others.

The average rank of the seven genotypes for all 15 environments, and for the specific environments showing positive (PCA1 values for genotypes and locations of the same sign), negative (different signs), and minimal interaction (PCA1 values for locations close to zero), are presented in Table 5. The highest main effect axis value of the biplot, M03, showed the best average rank in all environments, whereas M12 had the worst

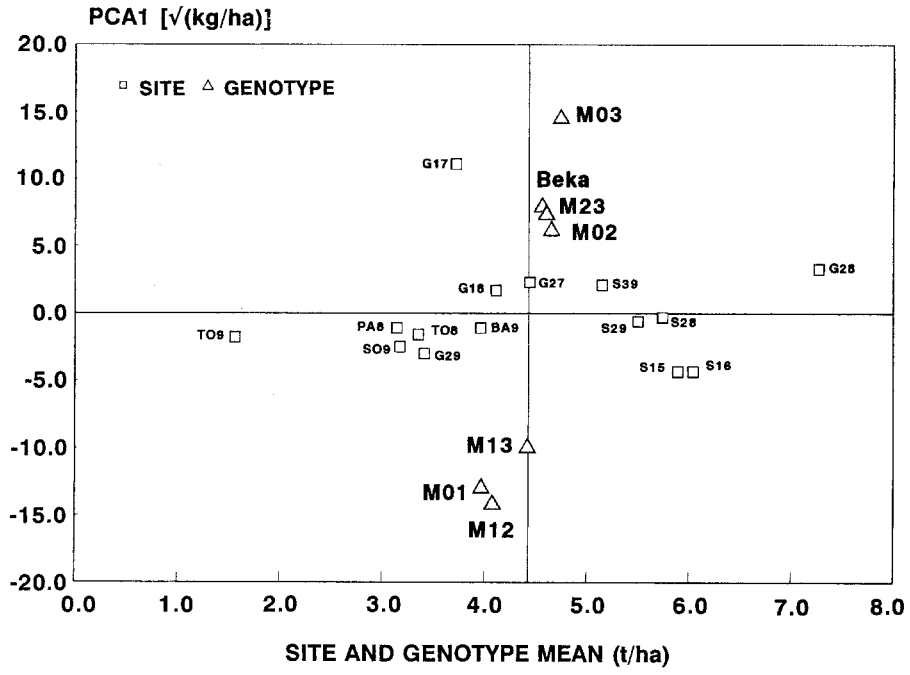

Fig. 1. Biplot of the AMMI model for grain yield of the seven isogenic lines grown at 15 sites. The *vertical line* represents the grand mean of the experiment

Table 5. Average rank of the seven genotypes for 15 environments, and for specific sites showing positive, negative and no G × E interaction

Genotype	All sites	Positive interaction	Negative interaction	Non-interaction
Beka	3.60	2.50	6.00	4.33
M01	5.33	3.50	7.00	7.00
M02	3.13	2.33	4.00	3.00
M03	2.60	1.00	7.00	1.67
M12	5.63	2.00	7.00	5.67
M13	3.93	2.83	5.67	3.67
M23	3.77	1.75	5.42	2.67

ranks across environments. M03 is highly interactive, with the highest absolute PCA1 value (together with M12, Table 5). Its average rank was 2.60. However, its rank in the environments with positive interaction was 1.0 (best overall yield), while its rank was 7.0 (poorest yielder) for all environments with negative PCA1 values. In contrast, M12, with an average rank across sites of 5.63, was the worst line at all environments with positive PCA1 values, but second at sites with negative PCA1 scores.

Based on prediction, the model including the two first principal component axes was selected. The top part of Fig. 2 shows the scattergram of all seven genotypes for the two PCA axes. There is a close relationship between PCA1 and mutant gene 1, and PCA2 and mutant gene 2. All isogenic lines carrying mutant gene 1 had negative PCA1 values, similarly all lines not carrying mutant gene 2 had negative PCA2 values.

Based on ecophysiological measurements taken at two contrasting locations (G1 and G2) during two years (1987, 1988) the seven lines were characterized (Molina-Cano et al. 1990). The data encompassed morphological characteristics related to canopy structure (number of leaves per plant, leaf area at anthesis and physiological maturity, leaf angle and length of the main shoot, spike density) and phenology (days from emergence to anthesis and grain-filling duration). The phenotypic variation produced by the three mutant genes suggests that Mendelian genes may explain quantitative variation. The first three principal components accounted for more than 97% of the total variation. The first principal component, PHYS1, was related to mutant gene 1, as the characteristics possessing the higher loadings are governed by this gene. Consequently, the separation of lines carrying this gene from the rest was made along this axis (bottom part of Fig. 2). These lines showed (Table 1) a smaller leaf area at anthesis and were earlier heading than the others.

The second principal component, PHYS2, was similarly related to mutant gene 2. These lines had denser spikes and greener leaves at physiological maturity than the other genotypes. the third principal component extracted for these physiological measurements, PHYS3, was much less strongly related to mutant gene 3, mainly through the length of the grain-filling period; M03, M13 and M23 needed a shorter period for filling the grains. They also showed the smallest coordinates on the third axis.

The integration of the empirical or statistical approach to G × E with the physiological meausrements has proven fruitful in this study. As mentioned, Fig. 2

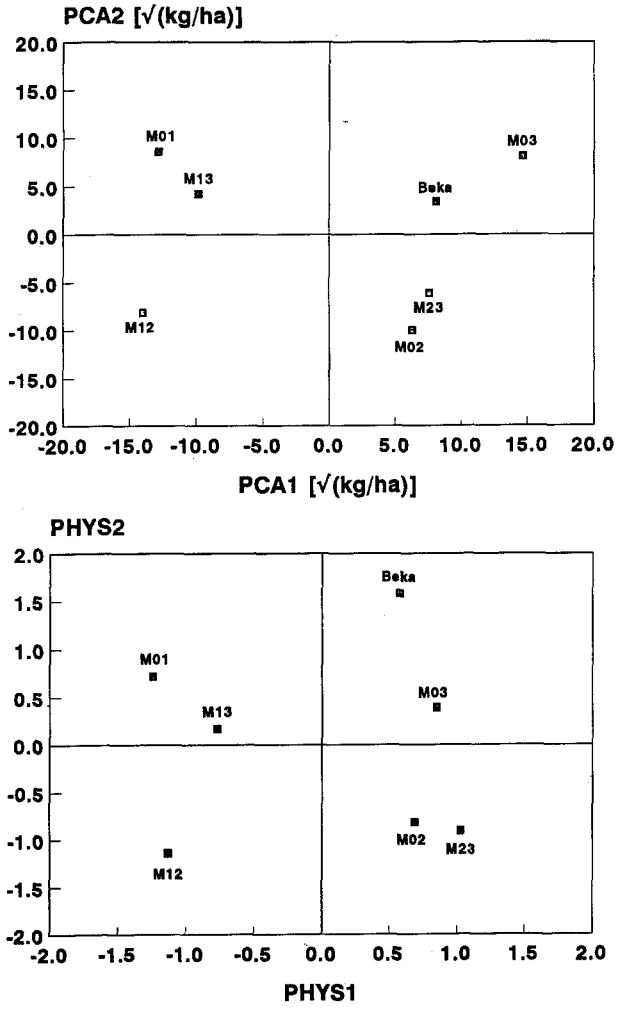

Fig. 2. Two-dimensional grouping of the genotypes obtained from the first two PCA axes of the AMMI2 model for grain yield (*top*) and from principal component analysis of the morpho-physiological data (*bottom*) from Molina-Cano et al. 1990

reveals the genotypic principal component scores for physiological measurements (PHYS1 and PHYS2, Molina-Cano et al. 1990) and for the multiplicative component of the AMMI model (PCA1 and PCA2). Both PCA1 and PHYS1 are closely related to gene 1. The correlation of the scores for both axes for the seven genotypes is 0.97 ($P < 0.001$) Similarly, a close correspondence is shown for PCA2 and PHYS2 ($r = 0.81$, $P < 0.025$). This suggests that the empirical effects of mutant genes 1 and 2, as determined by the G × E analysis, have a precise biological background as defined by PHYS1 and PHYS2. Furthermore, for those locations with a consistent PCA1 sign across years, such as Toledo or Sevilla, the phenotypical characters associated with the isogenic lines best adapted to them should be of value for selection.

No physiological or genetic explanation was found for the third PCA axes of the AMMI model which, according to the postdiction criterion, was associated not with pattern but with noise. No significant correlation was detected between PCA3 from the AMMI model and PHYS3 from the morphometric data.

Although this study is based on retrospective physiological studies, involving only a few cultivars, it shows the value of integrating some of the plant processes that result in higher yields, as measuredd physiologically, with new statistical techniques such as the AMMI analysis.

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