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Use of the additive main effects and multiplicative interaction model in QTL mapping for adaptation in barley

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Abstract The additive main effects and multiplicative interaction (AMMI) model has emerged as a powerful analytical tool for genotype × environment studies. The objective of the present study was to assess its value in quantitative trait locus (QTL) mapping. This was done through the analysis of a large two-way table of genotype-by-environment data of barley (*Hordeum vulgare* L.) grain yields, where the genotypes constituted a genetic population suitable for mapping studies. Grain yield data of 150 doubled haploid lines derived from the 'Steptoe' × 'Morex' cross, and the two parental lines, were taken by the North American Barley Genome Mapping Project (NABGMP) at 16 environments throughout the barley production areas of the USA and Canada. Four regions of the genome were responsible for most of the differential genotypic expression across environments. They accounted for approximately 50% of the genotypic main effect and 30% of the genotype × environment interaction (GE) sums of squares. The magnitude and sign of AMMI scores for genotypes and sites facilitate inferences about specific interactions. The parallel use of classification (cluster analysis of environments) and ordination (principal component analysis of GE matrix) techniques allowed most of the variation present in the genotype × environment matrix to be summarized in just a few dimensions, specifically four QTLs showing differential adaptation to four clusters of environments. Thus, AMMI genotypic scores, when the genotypes constituted a population suitable for QTL mapping, could provide an adequate way of resolving the magnitude and nature of QTL × environment interactions.

Key words *Hordeum vulgare* · Quantitative trait loci · Genotype × environment interaction · Pattern analysis · Molecular marker-assisted selection

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Introduction

Genotype × environment interaction (GE) is differential genotypic performance across environments. It reduces the association between phenotypic and genotypic values, and thus selections that perform well in one environment may perform poorly in another (Romagosa and Fox 1993; Fox et al. 1996). GE is considered quantitative, or non-crossover, when ranking of genotypes does not change from one environment to another. Non-crossover interactions are less important to plant breeders than crossover, or qualitative, ones in which genotypes change rank across locations. When mapping quantitative trait loci (QTLs) with data from different locations, GE interaction is shown by variable levels of significance of QTL effects across sites. Crossover interactions are present when contrasting favorable alleles are shown in different environments. If present, the design of alternative molecular marker-assisted selection (MMAS) schemes for different sites may be warranted. In MMAS, the total number of QTLs that may be used for selection should be balanced by the high cost of genotyping segregating populations. Such balance requires the use of statistical tools to identify the QTLs that determine most of the adaptation to a set of environmental conditions.

The additive main effects and multiplicative interaction (AMMI) model has emerged as a powerful analytical tool to interpret large complete GE data sets (Gauch 1992), often providing parsimony, effectiveness, and insight into GE. It first extracts genotype and environmental main effects and then uses principal component

analysis to explain the pattern in the GE matrix, that is the residuals after removal of the main effects. AMMI analysis generates a family of models with different numbers of principal component axes (PCAs) retained. The simplest model, AMMI0, which only considers additive main effects, without GE interaction, ranks genotypes identically at each environment. AMMI1 considers main effects and one PCA (PCA1) to interpret the residual matrix. For every entry in the model (both genotypes and environments) AMMI1 gives an average value and a first principal component score. AMMI2 considers main effects plus was two PCAs (PCA1 and PCA2) for GE. Subsequent models consider additional PCAs. Simultaneous examination of the magnitude and sign of PCA scores for genotypes and sites facilitates inferences about specific interactions. Any genotype with PCA scores for yield close to zero shows general adaptation to the tested environments. Large genotypic scores reflect specific adaptation to environments with PCA scores of the same sign.

AMMI tends to extract 'pattern' in the first PCA axes, with subsequent axes being associated with 'noise' (Gauch 1992). This feature could be used to identify QTLs with a sufficiently large effect to be considered for MMAS. If patterns of adaptation have a genetic origin, QTL mapping of the scores for the different genotypes could identify those regions of the genome responsible for the differential genotypic expression across environments.

The objective of the present study was to assess the value of AMMI analysis in QTL mapping. This was done through the analysis of a large two-way table of genotype-by-environment data of barley (*Hordeum vulgare* L.) grain yields, where the genotypes constituted a genetic population suitable for QTL mapping.

Materials and methods

Plant material and agronomic data

The genotypic (markers and map information) and phenotypic (grain yield) data used in this study were gathered by a consortium of barley geneticists and breeders in the USA and Canada, as part of the North American Barley Genome Mapping Project (NABGMP). A population of 150 doubled haploid lines (DHLs), derived from 'Steptoe'/'Morex' (S/M) F_1s by the *Hordeum bulbosum* technique as modified by Chen and Hayes (1989), and the two parents were studied. Steptoe is a broadly adapted high yielding six-row 'Coast'-type feed barley. Morex, a 'Manchurian'-type, is the six-row malting quality standard in the US. Yield data were collected throughout the US and Canadian barley production areas described by Hayes et al. (1993a, 1994). The trial sites, years, and codes used are: Crookston, Minnesota, 1992 (MN92); Ithaca, New York, 1992 (NY92); Guelph, Ontario, 1992 (ON92); Pullman, Washington 1992 (WA92); Brandon, Manitoba, 1992 (MA92); Outlook, Saskatchewan, 1992 (SKo92); Goodale, Saskatchewan, 1992 (SKg92); Kcfr, Saskatchewan, 1992 (Skk92); Tetonia, Idaho, 1992 (ID92); Bozeman, Montana, irrigated, 1992 (MTi92); Bozeman, Montana, dryland, 1992 (MTd92); Aberdeen, Idaho, 1991 (ID91); Klamath Falls, Oregon (OR91); Pullman, Washington, 1991 (WA91); Bozeman, Montana, irrigated, 1991 (MTi91); Bozeman, Montana, dryland, 1991 (MTd91).

In 1991, two replications of 50 DHLs plus the two parents and a single replicate of the other 100 DHLs was used at five sites. In 1992, yield data was collected from eight randomized block design trials with two replications and from three non-replicated trials. For all statistical analyses, given that only three sites were used for the 2 years, site × year combinations identified different environments. Given the non-orthogonal nature of the data taken in 1991, the best linear unbiased estimator for each DHL was estimated at each site using the residual maximum likelihood method from *proc MIXED* of SAS/STAT (SAS Institute 1992). All subsequent analyses were carried out using these values.

Additive main effects multiplicative interaction model

The AMMI model (Gauch 1992) for the yield of the ith genotype in the jth environment is:

$$y_{ij} = \mu + G_i + E_j + \sum_{n=1}^{N} \lambda_n \gamma_{ni} \delta_{nj} + \varepsilon_{ij},$$

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 eigenvalue of the nth PCA axis, γ_{ni} and δ_{nj} are the genotype and environment scores for the nth PCA axis, and ε_{ij} is the residual term. As a convenient scaling for the PCA scores, both genotype and environment scores are expressed as a unit vector times the square root of the eigenvalue. Multiplication of a genotype PCA score by an environment score then gives the estimated interaction directly. Thus, the predicted value of the ith genotype and jth environment, for the AMMI model retaining three interaction axes, AMMI3, would be the sum of the G_i and E_j main effects, plus the sum of the three cross products between the ith genotype and jth environment PCA scores for PCA1, PCA2 and PCA3. If a genotype has positive PCA values, it would be particularly adapted to those environments with positive PCA scores and poorly adaptated to those environments with negative PCA scores. AMMI analyses were carried out using SAS procedures (SAS Institute 1988).

QTL mapping

For each entry in the trial, 152 genotypes and 16 environments, AMMI analysis produced a mean yield and a set of PCA scores. The mean grain yields and genotypic scores were included as phenotypic data for QTL mapping, along with a 222-point S/M base map developed by Mather (1995) from a comprehensive map of about 500 molecular markers (Kleinhofs et al. 1993; Kleinhofs 1995). Analyses were conducted with MQTL (beta version 0.93 for DOS, Tinker and Mather 1995a,b), which does simple interval mapping (SIM) and simplified composite interval mapping (sCIM) for multi-location data sets. The test statistics (TS) provided by MQTL is that described by Haley and Knott (1992). For similarity to the LOD scores reported by Mapmaker/QTL (Lander et al. 1987; Lincoln et al. 1992), TS can be divided by 2·ln10 (Tinker and Mather 1995a). The statistical error rate was controlled by means of 1000 permutation tests, as described by Churchill and Doerge (1994), implemented in MQTL.

Pattern analysis: clustering of environments

The term 'pattern analysis' (Williams 1976) describes the parallel use of multivariate classification and ordination techniques to present the maximum variation from GE data sets in a few dimensions. Environments can be considered in a multi-dimensional space with each dimension a genotype and, thus, can be grouped based on the relative similarities of the yields of the different genotypes. Pattern analysis was done by the simultaneous use of AMMI, as an ordination method, and cluster analysis, as a classification technique. Cluster analysis of the standardized mean yields for the DHLs carrying the same QTL were done as suggested by Fox and Roseille (1982). To group environments that rank genotypic classes similarly, and thus provide similar screening information, yields were standardized for

each environment with a mean of zero and a unit phenotypic standard deviation. The Ward or incremental sum of squares method (Romagosa and Fox 1993) was used as a fusion strategy. At each fusion, the group formed was the one that minimized the increment in the within-groups sums of squares.

Results

Ge and AMMI analysis

Mean grain yields ranged from 7.5 t/ha at two irrigated sites (ID91 and Sko92) to 3.2 t/ha at Montana (rainfed) 1991. The proportions of the total sum of squares in the combined analysis of variance due to differences among environments, genotypes, and GE were 70, 7, and 23%, respectively. AMMI was used to partition GE interaction. The first seven PCA axes (the so-called AMMI7 model) were significant based on post-diction (Gauch 1992; Fox et al. 1996), and together explained more than 75% of GE. The first four axes, PCA1–PCA4, explained 65% of GE (Table 1).

Mapping AMMI genotypic scores

Scans for the sCIM test statistics given by MQTL are shown for the combined analysis (Fig. 1) and for the genotypic main effects and individual PCA scores (Fig. 2). In the multiple environment MQTL analysis, a single QTL on chromosome 3 (QTL1), with a TS peak for both SIM and sCIM in the ABG396–BCD828 interval with a length of 3.5 cM, explained over 35% of yield differences, with a yield advantage of 0.5 t/ha attributable to Steptoe (top Fig. 1, Table 1; Hayes et al. 1993a,b). Two other regions, on chromosomes 1 and 4, showed peaks just above the significance levels. The multiple environment sCIM showed peak for the test statistics at every single chromosome which interacted with the environments (bottom Fig. 1). Although no control of the statistical error rate for the GE interaction when using sCIM is currently available (Tinker and Mather 1995a), most of these peaks exceeded the threshold level defined for SIM.

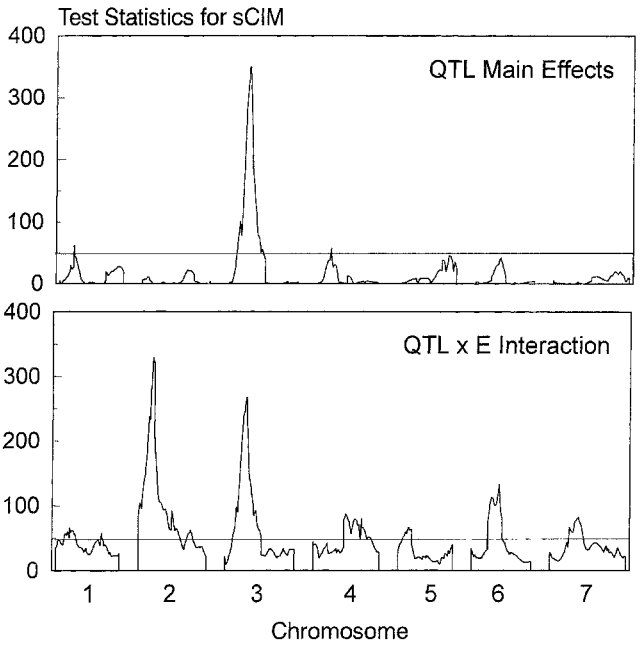

Fig. 1 Scans for the sCIM test statistic given by MQTL used to make inferences about the presence and position of QTLs for barley grain yield main effects (top) and QTL × environment interaction. *Horizontal lines* show approximate threshold levels that give 5% experiment-wise type-I error rates

When the genotypic PCA scores were used as phenotypic data in mapping, four chromosomal regions showed significant QTLs (Fig. 2, Table 1). PCA1 identified a QTL within the same interval on chromosome 3 as the one identified using average grain yields across sites. When mapping PCA2 and PCA3 genotypic scores, a

Table 1 Yield QTLs for main effects and interaction PCA axes of the AMMI model

Additive main effects			QTL mapping: MQTL					
			Chromosome	Interval	TS[a]SIM/sCIM	Yield difference S-M (t/ha)	Variance explained (%)	Designation
			3	ABG399-BCD828	68/101	0.50	36	QTL1
Multiplicative interaction			QTL mapping: MQTL					
Axes	SS of GE explained (%)		Chromosome	Interval	TS[a]SIM/sCIM	Difference S-M	Variance explained (%)	Designation
	Individual	Cumulative						
PCA1	22	22	3	ABG399-BCD828	43/61	0.34	25	QTL1
PCA2	18	40	2	ABC156A-ABG358	19/55	0.23	12	QTL2
			3	ABG399-BCD828	17/36	0.21	11	QTL1
			6	CDO497-BCD340E	22/36	−0.24	14	QTL3
PCA3	14	54	2	ABC156A-ABG358	63/78	0.36	34	QTL2
PCA4	10	64	7	ABC324-ABC302	47/48	0.30	27	QTL4

[a] MQTL test statistic for single interval mapping and simplified composite mapping. TS thresholds for $\alpha = 0.05$ were approximately 13 and 21 for SIM and sCIM respectively

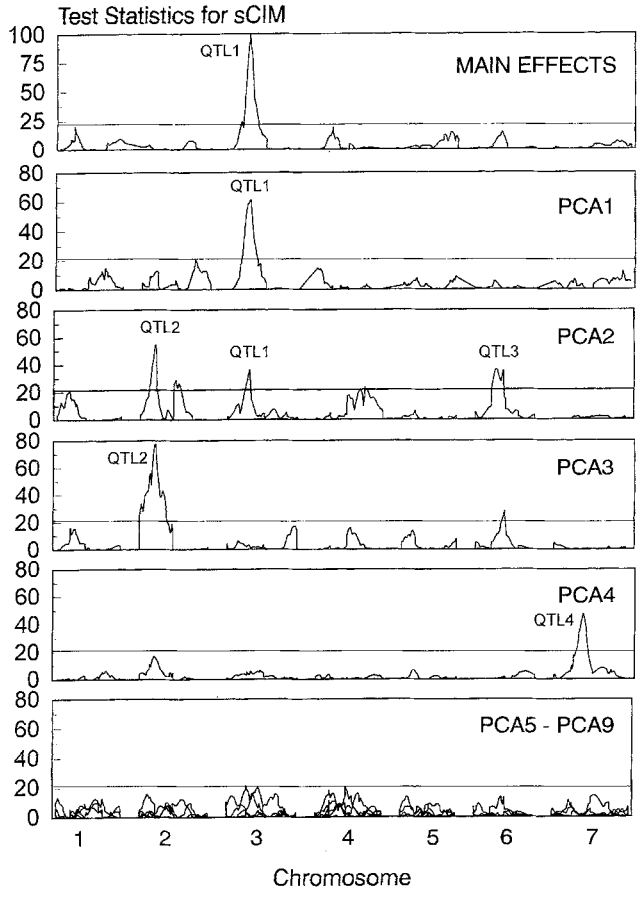

Fig. 2 Scans for the sCIM test statistic given by MQTL used to make inferences about the presence and position of QTLs for barley grain yield for the genotypic main effects and the individual PCA scores produced by the AMMI9 model. *Horizontal lines* show approximate threshold levels that give 5% experiment-wise type-I error rates

second QTL (QTL2) was found on chromosome 2, with a peak interval at ABC156A–ABG358 of 5 cM. QTL2 explained more than one-third of the differences among DHLs for PCA3 and a small fraction of PCA2. A third QTL (QTL3) was also identified when mapping PCA2 on chromosome 6, with a TS peak in a 5.4-cM interval between CDO497–BCD340E. Finally, QTL4 was identified on chromosome 7 accounting for 31% of the differences among genotype PCA4 scores. Its flanking markers are ABC324 and ABC302, with a distance of 7.6 cM. No QTLs were found in PCA5 to PCA9.

The analysis of a subset of 130 entries in which there were no crossovers between any of the flanking markers showed that close to 50% of the genotypic and 30% of the GE sums of squares were accounted for by just these four chromosome regions (Table 2). It was not known if a single QTL could be simultaneously responsible for main effects and the differential adaptation of genotypes to the environments, as suggested in this analysis. However, such a hypothesis seemed plausible particularly in the case of non-crossover interactions. In this way, the effect of a gene substitution, although positive in favor of a given allele, may change in size from one environment to the next. Similarly, it was also unknown if a single QTL could partially control more than one interaction axis. For simplicity such an assumption was made in this study. Thus QTL1, QTL2, QTL3 and QTL4 were believed to be unique QTLs at each of the identified chromosome regions. For the subsequent analysis they were treated as such.

Assessing adaptation

Based on the standardized grain-yield means of DHLs with the same genotypic constitution for QTL1–QTL4, environments were clustered into two main groups (Fig. 3), each with two distinct subsets. All Western Canadian sites and OR91 were grouped together. Un-

Table 2 Partition of genetic main effects and GE for barley grain yield of 130 DHLs not segregating between the flanking markers of four yield QTLs, grown at 16 environments clustered as shown in Fig. 3. The only significant epistatic interaction between QTLs is shown

Source of variation	*df*	Sums of Squares	Partial R^2(%)	Mean Squares
G:	129	375.22		
Genotypes [Geno]	15	181.24	48.3	12.08**
QTL1	1	145.80	80.5	145.81**
QTL2	1	0.80	0.4	0.80
QTL3	1	9.39	5.2	9.39*
QTL4	1	0.93	0.5	0.93
QTL1*QTL2	1	6.26	4.3	6.26*
DHL (Geno) (error a)	114	193.98	51.7	1.70
GE:	1935	1206.90		
Environments*Geno	225	350.08	29.0	1.56**
Clusters*Geno	45	165.00	47.1	3.67**
Clusters*QTL1	3	54.08	32.8	18.03**
Clusters*QTL2	3	65.80	39.9	21.93**
Clusters*QTL3	3	13.97	8.5	4.66**
Clusters*QTL4	3	6.20	3.8	2.07**
Env*DHL (Geno) (error b)	1710	856.82	71.0	0.50

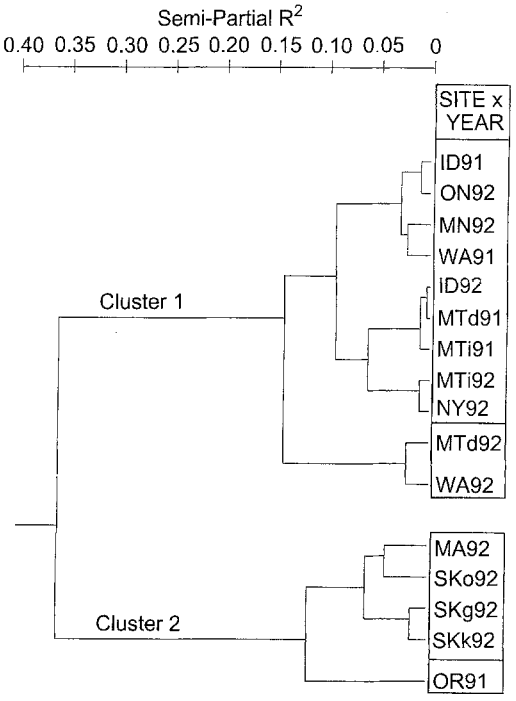

Fig. 3 Dendrogram of relationships among environments using, as classification variables, standardized mean grain yields for DHLs carrying the same alleles at each of four QTLs

like the main effect QTL for grain yield, the S-M differences listed in Table 1 for the different QTLs are not immediately interpretable. Both their magnitude and sign have to be related to the scores of the different environments, which facilitate inferences about adaptation of genotypes to distinct environments. Means of genotype scores for DHLs carrying a specific genotypic combination for QTL1–QTL4 and PCA scores for the environments are shown in Table 3. Since QTL1 was identified using PCA1 scores, lines carrying the *M* allele at this locus showed a different PCA1 sign from those carrying the *S* allele (Table 3). DHLs carrying the *M* allele at QTL1, with negative PCA1 scores, had lower yields in the first cluster of environments, which tend to have positive PCA1 scores. The cross products of these genotype and environment scores are negative and, therefore, the first interaction term substracts a given amount to the main-effect total. Lines with the *M* allele had relative higher yields in the second cluster of environments, particularly at Klamath Falls in 1991 (OR91) with the largest negative score and, therefore, a positive and large interaction term. PCA2 was associated with three QTLs (QTL1, QTL2 and QTL3; Table 1, Fig. 2). Its genetic interpretation is not as simple as for PCA1. The genotypes with the largest absolute values for the PCA2 score were *MMMMSSSS* and *SSSSMMMM*. They showed a differential yield response in the 16 environments. Overall, *SSSSMMMM* DHLs significantly outyielded *MMMMSSSS* DHLs in most sites belonging to the first group of environments and in OR91, with the opposite being true in most sites with negative PCA2 scores.

PCA3 was specifically related to QTL2 (Table 1). The *M* allele was particularly favored in the Western Canadian sites. Differential adaptation of genotypes with the *S* and *M* allele at QTL4 was responsible for the fourth interaction axis, PCA4 (Table 3). The effect of an allele substitution for QTL4 was observed in certain sites which were not particularly associated with any environmental grouping. Both sites with positive and negative PCA4 scores were found within either cluster.

Discussion

Based on independent mapping of grain yields at each individual location, Hayes et al. (1993a) reported that the number of QTLs influencing yields in the Steptoe × Morex data set which interacted with the environment was quite high. Using Mapmaker/QTL with a threshold LOD score of 2 (Lander et al. 1987; Lincoln et al. 1992), Hayes et al. (1993a) found 14 QTLs across all seven barley chromosomes. Only one QTL was shown when mapping genotypic means across sites (top of Fig. 1), and apparently seven (one per chromosome) significantly interacted with the environment (bottom of Fig. 1) when using MQTL (Tinker and Mather 1995a). Mapping of the genotypic scores from AMMI using any available QTL software, such as Mapmaker/QTL, could improve our knowledge of the magnitude and nature of the QTL × E. Mapping of the genotypic scores for the first four PCAs, AMMI4, identified peaks for the test statistic given by MQTL in four chromosome regions revealed by both independent site and QTL × E combined analyses (Fig. 2).

By simultaneous examination of the PCA scores for the different environments, AMMI could identify the environments where the interactions were occurring, in a similar way to running independent maps at each site. For example, ID91 and OR91 had the extreme absolute values among all environments for PCA1 (Table 3). When individual analyses were done, these sites also showed the greatest and the least absolute effects on yields associated with allelic substitution at QTL1 (Hayes et al. 1993a,b). No QTLs were detected in PCA5–PCA9 (Fig. 2), all QTLs were identified in just the first four PCA axes, which confirms the suggestion that AMMI tends to extract pattern (or true GE interaction) in the first axes and noise in the others (Gauch 1992). Thus, mapping of genotypic PCA scores from large two-way GE tables could provide most of the linkage information contained in the data, without the need for individual analyses.

Different types of QTL × E were present in this data set. QTL1 showed non-crossover interaction. Steptoe (*S*) alleles were associated with higher yields. Ranking of genotypes did not dramatically change from one environment to another, the differential response was primarily a matter of scale. Crossover interactions were found for QTL2, which also showed an epistatic interaction with QTL1 (Table 2). When the *M* allele was pres-

Table 3 Average mean yield and PCA1 to PCA4 scores for DHLs with the same genotypic constitution for QTL1 to QTL4 and for the 16 environments grouped into the four clusters defined in Fig. 3

Genotype	No. lines	Site-year	Mean yield	PCA1	PCA2	PCA3	PCA4
MM MM MM MM	7		5.03	−0.19	0.00	−0.20	−0.20
MM MM MM SS	10		5.13	−0.27	−0.05	−0.12	0.07
MM MM SS MM	7		4.93	−0.31	−0.20	−0.11	−0.04
MM MM SS SS	12		4.94	−0.15	−0.41	−0.07	0.02
MM SS MM MM	8		5.16	0.05	0.02	0.18	−0.10
MM SS MM SS	14		5.25	−0.11	0.13	0.12	0.27
MM SS SS MM	5		5.08	−0.04	−0.01	0.18	−0.13
MM SS SS SS	8		5.00	−0.21	−0.08	0.26	0.27
SS MM MM MM	5		5.83	0.12	0.10	−0.24	−0.35
SS MM MM SS	8		5.68	0.18	0.03	−0.34	0.09
SS MM SS MM	7		5.81	0.13	−0.06	−0.31	−0.33
SS MM SS SS	6		5.29	0.25	−0.05	−0.18	−0.05
SS SS MM MM	8		5.61	0.19	0.39	−0.03	−0.03
SS SS MM SS	6		5.57	0.15	0.20	0.20	0.08
SS SS SS MM	8		5.53	0.35	0.05	0.24	−0.20
SS SS SS SS	11		5.58	0.24	0.09	0.30	0.18
		ID91	7.49	1.60	1.71	−0.71	0.72
		ON92	3.28	−0.01	0.45	−0.04	−0.33
		MN92	4.90	0.49	0.02	−0.02	−0.05
		WA91	5.50	1.34	0.38	−0.70	−2.40
		ID92	4.94	0.62	0.59	1.32	0.36
		MTd91	3.20	0.66	0.54	0.87	0.02
		MTi91	5.85	0.65	0.34	0.92	0.19
		MTi92	5.97	0.44	−0.27	0.47	0.67
		NY92	5.74	−0.43	−0.49	0.60	0.34
		Mean cluster 1.1	**5.21**	**0.59**	**0.36**	**0.30**	**−0.05**
		MTd92	5.01	0.11	−1.20	0.95	0.90
		WA92	3.55	−0.67	−1.04	0.36	−0.07
		Mean cluster 1.2	**4.28**	**−0.28**	**−1.11**	**0.66**	**0.41**
		MA92	6.91	−0.21	−0.72	−0.58	−1.18
		SKo92	3.73	−1.05	−1.33	−0.39	−0.41
		SKg92	7.48	0.22	−0.29	−2.75	1.49
		SKk92	5.14	−0.73	−1.07	−0.24	−0.16
		Mean cluster 2.1	**5.82**	**−0.44**	**−0.85**	**−1.00**	**−0.07**
		OR91: cluster 2.2	**5.92**	**−3.00**	**2.37**	**−0.06**	**−0.10**

ent at QTL1, the presence of Steptoe alleles at QTL2 seemed to confer a yield advantage in the first group of environments. The converse was true in the second group of environments, where the presence of the *S* allele at QTL2 decreased grain yields.

Overall, a superiority of DHLs with the *SSMM--MM* (*S* at QTL1, *M* at QTL2 and QTL4 and either *S* or *M* at QTL3) genotype was found for most sites, while a superiority for the *SSSSSSSS* lines was seen in others, particularly in the first cluster of environments (Table 3). Figure 4 shows the average rank and standard deviation across sites for every DHL with the three genotypes noted above, in the two main environmental clusters. Low average rank is associated with yield superiority; whereas low standard deviation of ranks indicates yield stability. DHLs with a relative low average rank across sites and a low standard deviation of ranks, such as those carrying *SSMMMMMM*, showed general adaptation. DHLs with the *SSSSSSSS* genotype were specifically adapted to the first cluster of environments.

The assessment of adaptation of these 152 genotypes to the 16 environments was done in this study according to 'Pattern Analysis' (Williams 1976; Romagosa and Fox 1993). It was based upon the parallel use of classification (cluster analysis of environments) and ordination (principal component analysis of GE matrix, i.e., AMMI models) to present the maximum variation from the 152 × 16 GE matrix in just a few dimensions: four QTLs showing differential adaptation to four groups of environments. The Clustering of environments could have been done in different ways. Environments can be considered in a multi-dimensional space with each dimension a genotype. Grain yields for each individual DHL could have been used, but these estimates were based on just one or two replicates per site. Environments could also be defined by the estimates of the additive effect of each significant QTL at each individual site, or their PCA scores from AMMI. However, since the final goal of MMAS is the identi-fication of superior genotypes, standardized mean yields for the 16 genotypes defined by QTL1–QTL4 were preferred.

AMMI is an empirical or statistical approach relating observed phenotypic responses, in terms of yield, to a sample of often unknown environmental conditions. However, it may allow for an analytical or physiological assessment of the value of any genotype at any specific site. AMMI has often unveiled specific patterns of adaptation, by examining the yield of certain well-known genotypes at specific sites, which could be *a posteriori* related to previously unknown biotic or abiotic factors

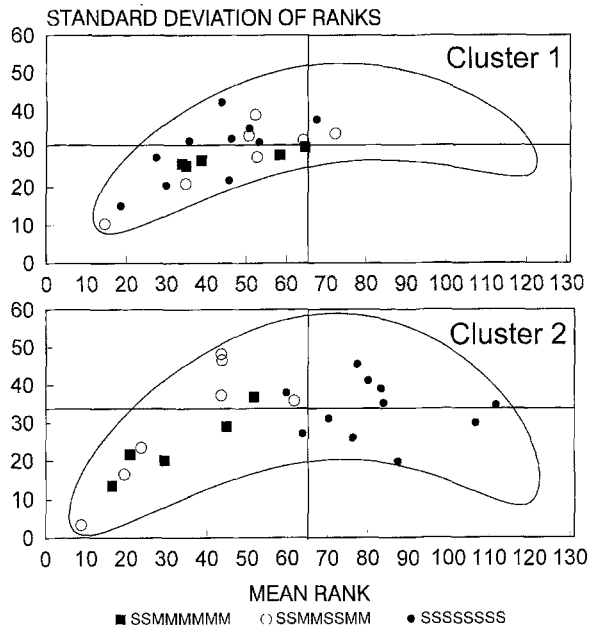

Fig. 4 Relative rank performance of DHL with the *SSMMMMMM*, *SSMMSSMM* and *SSSSSSSS* genotypes in the two main regions identified in Fig. 3. The *horizontal lines* represent the average standard deviation of ranks, the *enclosing lines* the approximate boundaries of variation for the 130 DHLs

(Royo et al. 1993). Unfortunately, the grouping of locations by their PCA scores could not allow for the identification of distinct agronomic or unique geographic patterns of adaptation. Environments were associated with two independent clusters (Fig. 3). The second cluster includes all the northern-most sites and OR91. OR91 was very peculiar, showing extreme scores for both PCA1 and PCA2. It is a high-elevation site that may behave in some respects similarly to the Western Canadian sites. QTL3, as also revealed by the independent site analysis, was specifically associated with yield differences at this site. Had the specific agronomic particularities of this site been known, specific adaptation patterns associated to this QTL could have been investigated. The biological determinant of yields may only be speculated for QTL2. As described by Hayes et al. (1993a), QTL2 seems to be coincident with a significant QTL for heading date.

The S/M cross was made as a compromise between the need for adequate DNA-level polymorphism for linkage mapping and the need to generate meaningful QTL information. Such a cross, between the 'Coast' and 'Manchurian' germ plasm pools, would not be generally attempted in a direct breeding effort aimed at cultivar development particularly for malting types (Ozdemir 1994). However, some DHLs have acceptable malting quality and showed wide adaptation out-yielding Steptoe, the high-yield parent. Thus, MMAS could be of some direct use in breeding within this germ plasm pool. Based on these analyses, the simplest strategy would be to genotype those breeding lines for the four chromosomal regions corresponding to the identified QTLs, selecting lines with the *SSMMMMMM*, *SSMMSSMM* or *SSSSSSSS* constitution. Multi-location evaluation of these lines should render the highest probability of recovering superior genotypes, and would limit the number of entries in the trials. Such an appraisal is currently underway for an independent set of DHLs, produced from the S/M cross, which were not used in the mapping process.

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