AN ABSTRACT OF THE THESIS OF

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Title: Evaluation of Hill Plots and Near Infrared Reflectance (NIR) Techniques as Breeding Tools in Spring Malting Barley

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Hill plots and near infrared reflectance (NIR) spectroscopy were investigated as breeding tools to facilitate doubled haploid recurrent selection for malting quality characters in spring barley. Main objectives of this research were to i) compare hill and row plot expression of agronomic and malting quality traits in an array of elite spring growth habit barley germplasm, and ii) compare NIR prediction of grain protein and malt extract in the two plot types.

Twenty-four elite spring barley genotypes were evaluated in separate row and hill plot experiments in three environments. Hill plots were evaluated at four seeding rates. A single seeding rate was used for row plot evaluation.

There was significant genotypic variation for all agronomic traits in hill plots. Genotype response to hill seeding rates was significant and consistent for most agronomic traits. Spearman's rank correlations between hill and row plots were high for all malting quality traits and most agronomic traits, except grain yield. Comparable results were obtained when the percentage of lines in common at 25 and 50% selection intensities was tabulated. Therefore, selection in hill plots should be

effective for most malting quality and agronomic traits, with the exception of grain yield. Differential expression of yield in hill and row plots was attributed to differences in tillering as a consequence of distinct patterns of competition in the two plot types. A seeding rate of 10 seeds per hill was identified as appropriate for preliminary screening of traits amenable to hill plot evaluation. Samples from this seeding rate were used as validation samples to test row-plot derived NIR prediction equations for grain protein and malt extract.

Separate of five and six wavelength calibration equations were selected for grain protein and malt extract, respectively. The multiple correlation coefficient (R) for the protein equation was 0.98 and that for malt extract was 0.88. Three distinct sets of validation samples were used to test the grain protein and malt extract calibrations. Based on these analyses, NIR prediction of protein in hill and row plots should be effective. NIR prediction of malt extract may only be effective in row plots.

Due to differential trait expression in row and hill plots and the error associated with NIR measurement, doubled haploid recurrent selection based on NIR screening and hill plot evaluation will likely only be effective for plant height, yield components other than numbers of tillers per unit area, and grain protein. Selected entries should then be evaluated for grain yield and malt extract in conventional row plots. NIR prediction for malt extract should be effective in row plot evaluations, achieving considerable efficiency compared to complete chemical malt analysis.

EVALUATION OF HILL PLOTS AND NEAR INFRARED REFLECTANCE TECHNIQUES AS BREEDING TOOLS IN SPRING MALTING BARLEY

by

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INTRODUCTION

New barley breeding strategies, such as doubled haploid recurrent selection, rapidly produce more homozygous lines than can be economically evaluated using conventional row plots. Hill plots would allow for the efficient, replicated, multiple environment evaluation of these materials, provided the ranking of quantitative trait expression in the two plot types is comparable. A preliminary screening technique for key malting quality traits - grain protein and malt extract - is needed for efficient use of hill plots in breeding for malting quality. As an example of such a strategy, Frey et al. (1988) recently reported that Near Infrared Reflectance (NIR) screening for grain protein, coupled with hill plot evaluation, allowed for completion of one cycle of recurrent selection per year in spring oats.

Hill and row plots are reported to provide comparable measures of many agronomic traits (Frey, 1965; Baker and Leisle, 1970; Walsh et al., 1976). However, Khadr et al. (1970) reported that hill and row plots were not equally efficient in discriminating among wheat lines for yield. The two plot types were not equally effective in screening for lodging resistance in oats (Jellum et al., 1963).

Concerns regarding competitive effects in hill plots have prompted a number of investigations on hill seeding rate and spacing. Frey (1965) reported that hill seeding rates affected yield and yield components in oats; similar results were reported for grain yield in

barley (Welty and Ramage, 1973). Hill seeding rates did not affect plant height of barley in either 30x30 cm or 45x45 cm hill spacings (Walsh et al., 1976).

The use of NIR for analysis of grains and seeds was originally developed for the measurement of moisture content (Norris, 1964). Requirements for rapid, inexpensive, and practical screening methods in breeding programs led to the application of NIR for prediction of quality characters in a range of crops (Hymowitz et al., 1974; McGuire, 1982; Norris et al., 1976). In barley breeding, grain protein and malt extract are key selection criteria in malting barley improvement. NIR is reported to successfully predict grain protein (Pomeranz et al., 1977) and malt extract (McGuire, 1982; Morgan, 1979).

There are no reports comparing the expression of malting quality traits in hill and row plots, nor has NIR prediction in the two plot types been addressed. Therefore, a primary objective of this study was to compare the expression of quantitatively inherited agronomic and malting quality traits in elite spring barley lines evaluated in hill and row plots. A second objective was to identify an appropriate seeding rate for hill evaluation of quantitatively inherited traits in irrigated spring malting barley. A third objective was to compare chemical and NIR estimate of grain protein and malt extract in hill and row plots. Realization of these objectives will allow for an informed decision to be made regarding the feasibility of doubled haploid recurrent selection for malting quality based on hill plot evaluation and NIR screening.

MANUSCRIPT I

Comparison of Hill and Row Plots for Agronomic and Malting Quality Traits in Spring Malting Barley.

Abstract

Hill plots should be useful for doubled haploid recurrent selection in malting barley provided they reliably predict row plot performance. The primary objective of this research was to compare hill and row plot expression of agronomic and malting quality traits in an array of elite spring habit barley germplasm. A supporting objective was to identify an appropriate seeding rate for hill plot evaluation. Eight-replicate hill plots at four seeding rates (10, 20, 30, and 40 seeds per hill) were compared with adjacent four-replicate row plots in each of three environments. Genotype and genotype x environment interactions were significant for most agronomic traits in both plot types. Significant, linear genotype responses to hill plot seeding rates were observed for most agronomic traits. Seeding rate had no consistent effect on the expression of malting quality. The percentage of lines in common in the two plot types at 25 and 50% selection intensities was the most useful comparison statistic and indicated hill plot selection should be effective for most agronomic and malting quality traits. Although yield heritability estimates were consistently high in both hill and row plots, there was little relationship between trait expression in the two plot types. Differential tillering in response to hill plot competition is likely responsible. A seeding rate of 10 seeds per hill should be appropriate in preliminary screening for traits amenable to hill plot selection in irrigated spring habit malting barley.

Introduction

Hill plots should be useful for screening large small grain germplasm arrays and when area and/or seed are limiting. Concerns regarding the relationship of genotype performance in hill and row plots have prompted investigations in barley (Walsh et al. 1976), oats (Frey 1965) and both common and durum wheats (Baker and Leisle 1970). Comparison studies indicate that the two plot types provide comparable measures of performance of qualitatively inherited characters, i.e. heading date and plant height. Conflicting reports on the utility of hill plots for evaluating quantitatively inherited characteristics, such as grain yield, may be attributed to competition effects in hill plots that do not reflect row plot population dynamics (Smith et al. 1970).

While it would seem that competition relationships in hill plots could be manipulated through seeding rate and plot spacing, differential responses of genotypes to these variables are apparently of little consequence. Significant seeding rate and spacing effects are reported in hill plots of oats (Frey 1965) and barley (Walsh et al. 1976; Welty and Ramage 1973), but the relationship of trait performance in hill and row plots, as measured by several criteria is rarely affected by hill plot seeding rate and spacing.

Hill and row plots have been compared in terms of coefficient of variation (C.V.), range as a percent of mean, number of lines in common at specified selection intensities, heritability, as well as phenotypic, rank and genotypic correlations (Baker and Leisle 1970; Frey 1965; Garland and Fehr 1981; Walsh et al. 1976).

With evidence supporting comparable trait expression in the two plot types, hill plots are used in self-pollinated crop recurrent selection programs where yield (Bregitzer et al. 1987; Patel et al. 1985) and grain protein (Frey et al. 1988) are principal selection criteria. There are no reports comparing the expression of barley malting quality traits in the two plot types.

Before implementing a doubled haploid recurrent selection program for malting quality in barley, we sought to compare the performance of elite lines, under irrigated conditions, in hill and row plots. Our objectives were to i) compare the expression of agronomic and malting quality traits in hill and row plots, and ii) identify an appropriate seeding rate for hill plot evaluation of elite spring malting barley germplasm.

Materials and Methods

Twenty-four elite spring barley lines and cultivars, 18 two-rowed including a quality check cultivar 'Klages' and six six-rowed including a malting quality check cv. 'Morex' and a feed check cv. 'Steptoe', were grown in separate, but adjacent, row and hill plot experiments in each of three environments. A location/year was considered an environment: experiments were grown at two locations (Corvallis and Madras, Oregon) in 1987 and at one location (Madras) in 1988. Fertility, weed control, and irrigation were in accordance with recommended practice for each location (data are available on request). Unbordered hill plots, spaced 45 cm apart and evaluated at four seeding rates - 10, 20, 30, and 40 seeds/hill - were planted by jab planter in a 4 x 24 factorial arrangement using a randomized complete block design with eight replications. Row plots consisting of six, 6 m-long rows at a 30 cm row spacing were evaluated in a randomized complete block design with four replications. The seeding rate was 15 g/row. Emergence data were not recorded.

Plant height, biological yield, grain yield, and grain physical quality data were measured in each experimental unit in all environments. Yield components - number of spikes per unit area, number of seeds per spike, and 100 seed weight - were measured in each hill plot and on a one linear m of row at Corvallis and Madras in 1987. Percent plump seeds, calculated as the percentage of a 100 g sample of seed remaining on a 2.38 mm X 1.91 cm slotted sieve after 30 seconds of

shaking on a Niagra sample grader, were measured in hill and row plots at Madras in 1987 and 1988.

Chemical malt analyses were performed on a 75 g sample of seed bulked from all replicates of each treatment within each environment. Samples were analyzed by the USDA/ARS Cereal Crops Research Unit, Madison, WI. Malting procedures and chemical analyses of malt were generally those given in "Methods of Analysis of the American Society of Brewing Chemists (ASBC)". A complete protocol is available upon request.

Data were first analyzed separately for each of the row and hill plot experiments in each location. The combined analyses of variance of agronomic traits in hill plots (Table 2) were used as a guide for construction of Spearman's rank correlations of hill and row plot performance. For all traits except number of spikes per hill, genotype mean squares were approximately 10 times greater than genotype x environment mean squares. Thus, rank correlations for all agronomic traits, except number of spikes per unit area, were computed on a phenotypic mean basis using combined analysis data. Rank correlations of number of spikes per unit area were computed separately within each environment.

Genetic components of variance were estimated from the combined analyses of variance and were used to compute heritabilities. "Genotypic" correlations between the two plot types were computed as follows (Frey 1965):

$$COV(XY)/\sqrt{\sigma^2_x \sigma^2_y}$$

where X and Y are the mean values in hill and row plots, respectively, and $\sigma 2_x$ $\sigma 2_y$ are measures of genetic variance for trait X and Y determined by the variance component approach. Walsh et al. (1976) provide justification for use of the statistic.

Results and Discussion

Agronomic Traits

The combined analyses of variance of agronomic traits revealed significant genotype effects for all agronomic characters in both plot types (Tables I.1 and I.2). In row plots, genotype x environment interaction was significant for all traits, except plant height and number of spikes per meter (Table I.1).

As shown in Table 2, genotypes in hill plots responded differentially to seeding rate in terms of seeds per head, 100 seed weight, and percent plump seeds. Genotype response to seeding rate was consistent for other agronomic traits. Seeding rate had a significant effect on all traits except plant height and 100 seed weight. Grain yield increased in a linear fashion with seeding rate, confirming the results of Walsh et al. (1976). The number of spikes per hill also increased with seeding rate, but reached a maximum at 30 seeds per hill. Harvest index was relatively constant across seeding rates. With increasing seeding rate, the percent plump seeds declined.

When the genotype sums of squares in hill plot analyses of variance were partitioned by spike morphology (two-rowed vs. six-rowed), highly significant differences were detected for all agronomic traits. Two-rowed genotypes had more spikes per unit area, higher seed weight, and a higher percentage of plump seed than six-rowed genotypes. On a mean basis, six-rowed genotypes were taller, higher yielding, and had more seeds per head than two-rowed lines. Harvest index was comparable for two-rowed and six-rowed lines. Within each spike class, there was significant variation in trait expression. Two-rowed and six-rowed

genotypes did not respond differentially to seeding rate, indicating the two classes of germplasm can be evaluated in the same experiment at the same seeding rate.

Performance in hill and row plots was compared in terms of criteria reported in the literature. The coefficients of variation for yield components, harvest index, and percent plump seed were similar between hill and row plots (Table I.3). However, C.V.s for plant height and grain yield were higher in hill than in row plots. Similar results have been reported for oats (Frey 1965), barley (Walsh et al. 1976) and wheat (Baker and Leisle 1970). High C.V. values may discourage breeders from using hill plots, but as pointed out by Walsh et al. (1976) other criteria merit consideration.

Ranges of trait expression (expressed as a percentage of experiment mean) in the two plot types have been used to assess the effectiveness of hill plots to predict row plot performance. Interpretation of the statistic varies. Frey (1965) in oats and Khadr et al.(1970) in wheat interpreted comparable ranges of performance in hill and row plots as evidence for insignificant competitive effects between adjacent hills. Walsh et al. (1976), in doubled haploid barley, observed a greater range of variation for yield in hill plots than in row plots, but speculated that if large germplasm arrays are evaluated in experiments with 10 or more replicates, competitive effects would be cancelled.

As shown in Table I.3, ranges of expression were comparable for grain yield and 100 seed weight. Ranges of expression for percent plump seed and number of seeds per head were somewhat lower in hill plots than

in row plots, while the reverse was true for plant height and harvest index. Overall, ranges of trait expression in these data do not follow a consistent pattern that would allow for assessment of hill plot merit.

From a breeding standpoint, the percentage of lines in common at a given selection intensity is a useful comparison of plot types. We concur with Walsh et al. (1976) in considering this the "ultimate test" of genotype performance in the two plot types. The percentage of genotypes in common at 25 and 50% selection intensities in hill and row plots (Table I.4) was highest for plant height (100%) and lowest for grain yield at all seeding rates. At lower selection intensities (50 vs. 25%) more than 90% of lines were in common for percent plump seeds in the two plot types at all seeding rates. At a 25% selection intensity selection for grain yield in hill plots, with the objective of maximum row plot performance, would be ineffective. Even at a 50% selection intensity, only 50% of lines were in common. Percentages of lines in common for number of seeds per spike, harvest index, and 100 seed weight at a 25% selection intensity were 100, 82,and 67, respectively. At a 50% selection intensity, the percentage of lines in common of the three traits were 100%.

Because of the resources required for measurement of yield components, these are not routine selection criteria in most malting barley improvement programs. While comparable expression of seed weight and seeds per spike in hill and row plots is of limited practical value, these data are useful in interpreting the poor relationship of grain yield expression in the two plot types. If merited, hill plot selection for harvest index should be effective. Plant height and percent plump

seeds are characters of primary importance amenable to routine screening. Selection for plant height in irrigated hill plots of spring habit malting barley germplasm would be effective at strict selection intensities. Effective selection for percent plump seeds would require more relaxed intensities.

As noted in the Materials and Methods, Spearman's rank correlations were computed on a phenotypic mean basis from multiple environment data, except for the correlation of number of spikes per hill (hill plots) with number of spikes per meter (row plots). These correlations were computed separately for each environment and seeding rate and ranged from 0.31 to 0.65. These modest correlations may reflect differences in the tillering response of genotypes in hill and row plots. Because emergence data were not recorded, differences in spikes per unit area can only be assumed to be a reflection of tillering. The effect of seeding rate, seed size, plant survival, and other factors contributing to stand establishment cannot be assessed. In both plot types, two-rowed genotypes tillered more profusely than six-rowed genotypes. However, the response of genotypes within the two spike classes varied substantially. That is, genotypes responded differentially to competition relationships in hill plots, and the response did not reflect row plot performance.

Spearman's rank correlation coefficients between hill and row plots for agronomic traits (Table I.5) were lowest for grain yield and highest for plant height. In general, seeding rate had little effect on rank correlations. All correlation coefficients were significant ($p=0.05$) except those for grain yield, which were not of biological

significance. Expression of plant height, number of seeds per spike, harvest index, and hundred seed weight was comparable in the two plot types, as measured by Spearman's rank correlations and selection for these characteristics in hill plots, with the objective of row plot performance, should be effective.

The correlations for percent plump seeds were modest and although significant, we do not consider them of biological significance. However, multiple evaluation criteria are needed to effectively compare performance in the two plot types. Based on modest rank correlations and the number of lines in common at a 50% selection intensity, selection for percent plump seeds in hill plots should be effective at low selection intensities.

The lack of biologically significant correlations for grain yield may be due to competitive effects in hill plots (Smith et al.1970), unfair ranking of cultivars using mean yields (Baker and Leisle 1970), or insufficient replication (Walsh et al. 1976). The latter authors reported that 10 replications were required to achieve comparable measures of grain yield performance in hill and row plots of barley.

If comparable measures of grain yield in hill and row plots require 10 or more replications of hill plots, then the potential efficiencies of the technique are less attractive. Inconsistent expression of grain yield in hill and row plots of irrigated spring malting barley may be due to the impact of hill plot competitive effects on the expression of tillering. Grain yield is a function of spikes per unit area, seeds per spike and seed weight. Expression of the latter two components was consistent in the two plot types while spikes per

unit area was not. This finding is in contrast to the results of Baker and Leisle (1970) in wheat, who reported that competition effects were manifested in yield per head rather than differential tillering. Smith et al. (1970) assessed competitive effects in oat hill plots and concluded that competitive effects were more pronounced for yield than for any of the yield components. However, these authors also observed that number of panicles per hill was subject to competitive effects initiated early in crop development.

Baker and Leisle (1970) argued that comparison of trait expression in hill and row plots based on ranking or phenotypic correlations is a biased estimate that can be overcome by correcting for the attenuation caused by environmental influences through the use of "genetic" correlations. The "genetic" correlations of Baker and Leisle (1970) appear to be identical to the "genotypic" correlations used by Frey (1965) and Walsh et al. (1976). As is apparent in the computational formula presented in Materials and Methods, this statistic requires estimation of genetic parameters also used in computing heritabilities.

Grain yield heritability estimates in irrigated hill and row plots of spring malting barley are comparable to those reported by Walsh et al. (1976) for six-rowed feed barley. For example, heritabilities (H^2) of grain yield in hill plots were generally higher than in row plots. For grain yield, H^2 on a plot basis was .20 in row plots and ranged from 0.20 - 0.33, depending on seeding rate, in hill plots. Heritability on a line mean basis was 0.75 in row plots and ranged from 0.79 -0.88, depending on seeding rate, in hill plots. Heritabilities of other agronomic traits were comparable in the two plot types.

Walsh et al. (1976) interpreted similar heritabilities in hill and row plots to mean trait expression in the two plot types is comparable. Our heritability data indicate that there is ample genetic variance for traits such as grain yield in hill plots of irrigated spring malting barley. However, plant population dynamics, as manifested in tillering capacity, allow genotypes to excel in hill plots that were are not outstanding in row plots. Yield differences among genotypes in hill plots are real, but are not related to row plot performance.

Genotypic correlations were consistently higher than phenotypic and rank correlations but did not illustrate relationships distinct from other measures of comparable performance (CV, selection intensity, and rank correlation) in the two plot types. For example, at a seeding rate of 20 seeds per hill, genotypic correlations for plant height and grain yield were 1.06 and -0.23, respectively. Traits showing high rank correlation and high numbers of lines in common at routine selection intensities also showed high genotypic correlations. We found phenotypic rank correlations and selection intensities most meaningful in interpreting our data, as these measures are the most likely to be used in a routine breeding context.

Quality Traits

Malting quality is the net result of a number of interacting physical and chemical properties. A comprehensive discussion of barley malting quality is provided by Burger and LaBerge (1985). Physical properties important in malting quality - seed weight and percent plump seed - were discussed in the preceding section. Key chemical properties

of malting barley are total grain protein, fine grind extract, diastatic power, and alpha amylase. Elite lines in a malting barley breeding program must meet minimum standards for these characteristics. To our knowledge this is the first comprehensive comparison of malting quality trait expression in row and hill plots.

Malting quality parameters - grain protein, fine grind extract, diastatic power, and alpha amylase - were measured on bulk 75 g samples of seed of all treatment replications in each environment. Replicated analyses of treatments within environments are precluded by the high cost of analysis. Multiple environment mean data were used to compare hill and row plots in terms of range, selection intensity, and Spearman's rank correlations. Lacking estimates of within-environment experimental errors, CVs, heritabilities, and genotypic correlations were not computed. However, as noted in the previous section, these latter statistics did not assist in interpreting agronomic trait data.

Means, standard deviations, and range of expression (expressed as a percent of experiment mean) of selected quality traits in hill and row plots are compared in Table I.6. Overall, genotypes had higher grain protein and diastatic power in hill plots than in row plots, while malt extracts in hill plots were lower than in row plots. Total protein was negatively correlated with malt extract and positively correlated with diastatic power, as reported by Ulmer et al.(1985). Thus, with the overall higher protein in hill plots, lower malt extract and higher diastatic power values were expected. The mean expression of alpha amylase was similar in the two plot types. Within - environment standard deviations were comparable in the two plot experiments.

Ranges of expression in hill plots were higher than in row plots for protein, diastatic power, and alpha amylase. Ranges of malt extracts were comparable in the two plot experiments.

The Spearman's rank correlation coefficients between hill and row plot experiments of quality data (Table I.7) show little effect of seeding rate on protein, diastatic power, and alpha amylase. Malt extract percentage declined somewhat with increasing seeding rates. Overall, the expression of malting quality traits was comparable in the two plot types, although we consider correlations for malt extracts of marginal biological significance. All correlations were significant ($p \leq 0.05$).

The percentage of lines in common in hill and row plot experiments for quality traits at 25 and 50% selection intensities (Table I.4) was high for diastatic power and alpha amylase. At a 25% selection intensity, little progress from hill plot selection for malt extract would be expected. However, at a 50% selection intensity, at seeding rates of 10 to 30 seed per hill, over 75% of the high extract lines were in common. Likewise, selection for protein at a 50% selection intensity would be effective, with more than 80% of lines in common. The high number of lines in common for diastatic power and alpha amylase supported by high phenotypic rank correlations, indicates that even stringent selection for these characteristics in hill plots would be effective. Selection for malt extract and total protein would be effective at lower selection intensities.

Conclusions

Hill plots should be effective for screening large spring malting barley germplasm arrays when primary traits of interest are plant height, kernel plumpness, and enzymatically related malting quality parameters. Selection for malt extract and total protein should be effective at relaxed intensities (50%). Selection for grain yield is best to postponed until numbers of lines have been sufficiently reduced to allow row plot evaluation. A seeding rate of 10 seed per hill should be effective for the described agronomic and quality traits.

Table I.1. Pertinent significant F ratios ($p \leq 0.05$) from the combined analyses of variance of agronomic traits measured in row plot evaluations of spring malting barley at Corvallis and Madras, Oregon.

Source of Variation	Pertinent F ratios						
	Plant+ height	Grain+ yield	Harvest+ index	No. of++ seeds/spike	No. of++ spikes/m	100 seed weight++	%plump++ seeds
Genotype(G)	40.46	9.47	4.13	199.34	11.62	16.15	23.05
G x ENV	ns	2.33	1.52	2.35	ns	2.29	4.49

ns = non-significant
\+ = three environment analysis
++ = two environment analysis

Table I.2. Pertinent significant F ratios ($p \leq 0.05$) from the combined analyses of variance of agronomic traits measured in hill plot evaluations of spring malting barley at Corvallis and Madras, Oregon.

Source of Variation	Pertinent F ratios						
	Plant+ height	Grain+ yield	Harvest+ index	No. of++ seeds/spike	No. of++ spikes/hill	100 seed weight++	%plump++ seeds
Genotype(G)	26.5	56.5	44.6	401.1	17.7	34.1	36.3
Type(two vs six)	71.6	174.5	111.4	8586.5	296.7	348.6	141.1
Among two-row	24.7	47.6	49.6	17.5	97.9	19.6	24.7
Among six-row	23.8	63.3	20.4	68.2	2.6	20.5	54.9
Seeding rate(SR)	ns	126.7	3.1	3.6	87.1	ns	14.0
G x SR	ns	ns	ns	1.4	ns	1.6	1.5
Type x SR	ns	ns	ns	ns	ns	ns	ns
G x ENV	ns	6.4	4.1	2.7	4.1	3.3	2.7
SR x ENV	2.3	3.6	ns	ns	ns	ns	ns

ns = non significant.
+ = three environment analysis
++ = two environment analysis

Table I.3. Coefficients of variation and ranges (as a percentage of experiment mean) for agronomic traits measured in multiple environment hill and row plot evaluations of spring malting barley.

Statistic	Plant height	Harvest index	Grain yield	%plump seeds	No. of seeds/spike	No. of spikes/area	100 seed weight
Row plots							
C.V.	7.6	14.7	10.8	8.1	8.7	17.0	6.6
Range (% of mean)	79-121	83-113	68-113	63-111	62-203	53-113	81-115
Hill plots							
C.V.	20.0	14.3	21.0	9.7	11.7	24.5	10.0
Range (% of mean)	81-136	78-118	64-114	79-114	57-189	65-131	81-116

Table I.4. Percentage of lines in common in multiple environment hill and row plot evaluations of agronomic and malting quality traits in spring malting barley at 25 and 50% selection intensities.+

Hill plot seeding rates	Percentage of lines in common						
	Plant height	Grain yield	%plump seeds	Grain protein	%malt extract	Diastatic power	Alpha amylase
10	100(100)	17(50)	50(92)	83(83)	67(75)	83(83)	83(75)
20	100(100)	0(50)	50(100)	67(83)	67(83)	83(83)	67(92)
30	83(92)	0(42)	50(92)	50(83)	67(75)	67(92)	83(75)
40	100(100)	17(50)	33(92)	67(75)	33(58)	83(83)	67(92)

+ values not in parentheses = % of lines in common at 25% selection intensity
values in parentheses = % of lines in common at 50% selection intensity

Table I.5. Spearman's rank correlation coefficients for agronomic traits measured multiple environment hill and row plot evaluations of spring malting barley.

Seeding rate	Plant+ Height	Harvest+ index	Grain+ yield	No. of++ spikes/head	100 seed++ weight	%plump++ seeds
10	0.97**	0.81**	-0.05	0.89**	0.81**	0.60**
20	0.96**	0.81**	-0.14	0.87**	0.82**	0.50*
30	0.75**	0.79**	-0.21	0.93**	0.81**	0.50*
40	0.97**	0.74**	0.03	0.85**	0.81**	0.42*

*,** = significant at the 0.05 and 0.01 levels, respectively
+ = based on three environment means
++ = based on two environment means

Table I.6. Means, standard deviations, and ranges (as a percentage of experiment mean) of malting quality traits measured in multiple environment hill and row plot evaluations of spring malting barley.

Statistic	Environment	Grain protein (%)	Malt extract (%)	Diastatic power (Deg)	Alpha amylase (20 Deg units)
Row plots					
Mean	COR87*	11.6 ±2.3	77.8 ±1.4	102 ±25.3	39.7 ±10.6
	MAD87	11.7 ±0.8	79.5 ±2.5	102 ±24.2	37.8 ± 9.6
	MAD88	12.0 ±1.1	78.1 ±1.9	122 ±29.2	38.4 ± 8.9
Range(%	COR87	87-123	94-103	44-146	41-129
of mean)	MAD87	86-114	95-107	52-144	38-137
	MAD88	83-116	93-103	50-153	38-138
Hill plots					
Mean	COR87	14.4 ±1.4	75.5 ±1.4	111 ±28.8	36.3 ± 7.3
	MAD87	15.0 ±1.2	75.5 ±1.6	140 ±32.1	38.9 ± 8.6
	MAD88	12.3 ±1.2	77.2 ±1.7	114 ±24.8	35.2 ± 8.9
Range(%	COR87	72-116	94-103	41-158	34-136
of mean)	MAD87	77-116	94-103	39-149	36-151
	MAD88	79-121	93-104	41-154	30-167

* COR = Corvallis
MAD = Madras

Table I.7. Spearman's rank correlations for malting quality traits measured in multiple environment hill and row plot evaluations of spring malting barley.

Seeding rate	Grain protein	Malt extract	Diastatic power	Alpha amylase
10	0.71**	0.61**	0.90**	0.87**
20	0.71**	0.57**	0.87**	0.88**
30	0.68**	0.52**	0.88**	0.85**
40	0.72**	0.43*	0.93**	0.85**

*,** = significant at 0.05 and 0.05 level, respectively.

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MANUSCRIPT II

Near-Infrared Reflectance Estimates of Grain Protein and Malt Extract in Hill and Row Plot Evaluations of Spring Malting Barley

Abstract

Expensive, time-consuming analyses can limit selection responses for grain protein and malt extract in a malting barley improvement program. Alternative breeding strategies, such as doubled haploid recurrent selection, rapidly produce more genotypes than can be evaluated in conventional plots. Prior to implementing a doubled haploid recurrent selection program for malting quality we sought to test the utility of hill plot evaluation and near infrared reflectance (NIR) prediction for grain protein and malt extract. Five and six-wavelength calibration equations were generated for prediction of grain protein and malt extract, respectively. The multiple correlation coefficient of the protein equation (0.96) was higher than that of the malt extract equation (0.88). Calibration equations for both traits based on separate locations and spike classes (two-row vs. six-row) were less robust than the multiple environment, combined equations. The grain protein and malt extract equations had acceptable predictive power for both row and hill plot samples. However, in view of differential trait expression in hill and row plots, NIR prediction based on hill plot evaluation is appropriate for grain protein. NIR prediction of malt extract is best deferred until genotypes are evaluated in row plots.

Introduction

Grain protein and malt extract are key selection criteria in a malting barley breeding program. The time required for chemical malt analysis, as well as cost and seed requirement, limit selection progress for these traits. A number of screening techniques for grain protein and malt extract have been proposed that would be amenable to routine use in plant breeding programs. Alternative procedures for malt extract determination include sedimentation (Palmer, 1975), viscosity of barley extract (Bendelow, 1977), and Near Infrared Spectroscopy (NIR) (Morgan, 1979; McGuire, 1982). NIR analysis has been used successfully to predict quality characters in a number of crops, including grain protein in wheat (Tkachuk, 1981), oats, corn, and soybean (Hymowitz et al., 1974). In barley, NIR has been used to predict grain protein (Pomeranz et al., 1977), lysine content (Gill et al., 1979), β-glucan content (Greenberg, 1974) and malt extract (Morgan, 1979 and McGuire, 1982). Of the various screening techniques for barley grain protein and malt extract, NIR appears the most promising.

In conjunction with hill plot evaluation, NIR screening allows for rapid cycles of recurrent selection in oats (Frey et al., 1988). Theoretical considerations favor doubled haploid recurrent selection (Patel et al., 1985), based on hill plot evaluation and NIR screening for protein and extract, in malting barley improvement. Prior to initiating such a program, we sought to test the effectiveness of NIR prediction of malt extract and grain protein in row and hill plot evaluations of spring malting barley. Agronomic and quality trait

expression in the two plot types is described elsewhere (Tragoonrung et al., submitted).

The specific objectives of this investigation were to (i) compare chemical and NIR measures of grain protein and malt extract, (ii) investigate the effect of plot type on NIR prediction of grain protein and malt extract, and (iii) consider the feasibility of doubled haploid recurrent selection for malting quality based on hill plot evaluation and NIR screening.

Materials and Methods

Barley samples used in this study were of spring growth habit from two advanced breeding nurseries - the Malting Elite Line Trial (MELT) and the Western Regional Spring Barley Nursery (WRSBN). Both nurseries were grown at two locations (Madras and Corvallis, OR) in 1987 and at one location (Madras) in 1988. The MELT contained 24 elite lines and cultivars, all with some degree of malting quality, except the feed barley check, cultivar 'Steptoe'. There were 18 two-row and 6 six-row genotypes in the nursery. The MELT was grown in separate, but adjacent, hill and row plot experiments at each location. Of the four seeding rates evaluated in the hill plot experiments, 10 seeds per hill was identified as the most appropriate for quality trait evaluation (Tragoonrung et al., submitted), and only treatments at this seeding rate were used for evaluation of NIR. The WRSBN consisted of 19 malting quality (all two-row) and 11 feed lines and cultivars (all six-row). The WRSBN was grown only in row plots. Management practices (irrigation, fertility, and weed control) were in accordance with recommended practice for each location (data available on request).

Chemical malt analyses were performed on a 75 g sample of bulked seed from all replications of each treatment in each environment. Samples were analyzed by the USDA/ARS Cereal Crops Research Unit, Madison, WI. Malting procedures and chemical analyses of malt were generally those given in "Methods of Analysis of the American Society of Brewing Chemists (ASBC)". A complete protocol is available upon request.

Fifteen g samples of the bulked seed used for chemical malt analyses were milled with a UDY cyclone mill fitted with 0.5 mm sieve at approximately the same date that chemical malt analyses were initiated. The choice of sieve size was based on preliminary comparisons of 0.5 mm and 1 mm sieves, in which the former gave higher r values for grain protein calibrations than the latter (data not shown). NIR analyses were conducted on the day of milling, after flour had cooled to room temperature, using a Technicon InfraAlyser 400. The 19 fixed-filter InfraAnalyser was interfaced to a Leading Edge PC computer equipped with Technicon IDAS software (Bran and Luebbe Technicon Industrial Systems).

Forty three samples from the MELT and WRSBN row plots grown at Corvallis and Madras in 1987 were used for protein calibration. Samples were equally distributed over a range of 8.9 to 14.8% protein. Fifty four equally distributed samples from the MELT and WRSBN row plots grown at Corvallis and Madras in 1987 were used for the malt extract calibration. Chemical malt extracts (fine grind) ranged from 71.3 to 85.1%. Three NIR readings, Log (1/reflectance), were taken on each sample, per manufacturer's instructions, by re-packing three sub-samples (approx. 4 g) of bulk flour. Multiple linear regression models of three to six wavelengths vs. chemical values were generated by IDAS for the entire calibration set, and for two subsets: i) Corvallis vs. Madras (both two-rowed and six-rowed spike types), and ii) two-rowed vs. six-rowed spike types (from both locations).

Calibration equations for grain protein and malt extract were tested using three sets of validation samples, subsequently referred to as "unknowns". Unknowns were samples not used in calibration, but whose

chemical grain protein and malt extract values were known. The objectives of these comparisons were to i) test the fit of equations using unknowns from the same source as the samples used in calibration (MELT and WRSBN row plots grown at Corvallis and Madras in ; ii) test the effect of year on prediction using unknowns from MELT row plots grown at Madras in 1988, and iii) test the effect of plot type, and year an location effects on plot type, using unknowns from MELT hill plots at Corvallis and Madras in 1987 and at Madras in 1988. Root mean square difference (RMSD) and standard error of a difference (SED) between chemical and NIR-predicted values were used to test the fit of equations (Williams, 1975). The percentages of lines in common at 25 and 50% selection intensities , as measured by chemical and NIR analysis, were used to test the effectiveness of NIR as a screening tool for grain protein and malt extract.

Results and Discussion

Calibration

Calibration equations for grain protein and malt extract were selected based on multiple correlation coefficient (R), standard error of estimation (SEE), standard error of prediction (SEP), index of systematic error (ISE), index of random error (IRE), and error mean square (MSE). SEE and SEP are measures of how well the instrument matches calibration samples and how well the instrument predicts the value of unknown samples, respectively (Rotolo, 1979). The utility and computation of SEE, ISE (a measure of the sensitivity of the calibration to a uniform shift in the Log(1/R) readings) and IRE (a measure of the sensitivity of the calibration to the random (electronic) noise of the data are discussed in IDAS (Bran and Luebbe Technicon Industrial System, 1987).

Four equations, based on three to six wavelengths, were generated for grain protein (Table II.1). The best five-wavelength equation was chosen for grain protein based on statistics listed in Table 2. Two of the five wavelengths (2190 and 2100 nm) agree with Technicon recommendations for protein analysis (Bran and Luebbe Technicon Industrial System, 1987). The R value (0.96) of this calibration (Table 2) was slightly lower than reported values for protein in wheat, 0.988 (Tkachuk, 1981), oats, 0.98, and corn, 0.99 (Hymowitz et al., 1974).

To test the effects of location on prediction equation fit, distinct five filter equations were generated based on samples from Corvallis and Madras, 1987. The R values for the separate location calibration equations were 0.98 for Corvallis and 0.86 for Madras.

Corvallis, not an important spring malting barley area, is considered a test environment. Grain protein at Corvallis ranged from 8.9 - 14.8%. Madras is a target environment considered representative of irrigated spring malting barley production in central Oregon. The narrower range of chemical grain protein values obtained at Madras - 9.0 to 12.5 % - may, in part, account for the lower R value of the calibration equation obtained at this location. A combination of samples from various locations is expected to provide a more robust prediction equation.

Four equations, based on three to six wavelengths, were generated for malt extract (Table II.1). Based on the statistics presented in Table II.2, a six-wavelength equation was chosen for malt extract prediction. The R value of this malt extract regression was lower than that reported by McGuire (1982) but higher than that reported by Morgan (1979).

To test the effects of location on prediction equation fit, distinct six-filter prediction equations were generated, based on samples from Corvallis and Madras, 1987. The R values for Corvallis and Madras in 1987 were 0.92 and 0.84, respectively. Ranges of chemical malt extract were 71.3-81.1 at Corvallis and 74.7-85.1 at Madras. As with the protein calibration, the test environment provided a better fit of NIR predicted values to known chemical values. While differences in fit for the protein equation may be attributable to range of chemical values, ranges for malt extract were comparable at the two location. Overall, malt extract was higher at Madras than at Corvallis. As with grain protein prediction, a broad array of samples provided a more robust prediction equation.

Two-rowed and six-rowed malting barleys have distinct malting quality profiles and more uniform seed size is expected in two-row genotypes. The effect of spike morphology on NIR prediction of grain protein and malt extract was tested by running separate calibrations for each spike type and comparing these equations with the overall equation involving both two-row and six-row classes (Table 3). The best five-wavelength equation was chosen for each separate calibration and compared with the optimum five-wavelength calibrations for protein and the best six-wavelength equation for malt extract. The number of samples used in the separate two-row and six-row calibrations do not sum to the number used in generating the combined calibration, because of random selection of samples to generate equal distributions of chemical values.

While the somewhat smaller sample sizes used for separate two-row and six-row equations would be expected to generate less robust equations, it is of note that prediction equations for the two spike classes were, in general, comparable (Table II.4). Six-row types had a better fit for protein than two-row genotypes, but the separate calibration had little advantage over the combined prediction equation. Separate calibration by spike morphology appears to have little advantage for NIR prediction of grain protein and malt extract.

Tests of calibration equations

The calibration equations for protein and malt extract based on both spike classes evaluated at both locations were used for prediction. The general equations should be more robust and have better predictive

ability than separate calibration equations based on i) location, or ii) spike morphology.

Three sets of unknowns - MELT and WRSBN row plots grown at Corvallis and Madras in 1987; MELT row plots grown at Madras in 1988; and MELT hill plots grown at Corvallis and Madras in 1987 and at Madras in 1988 - were used to test the calibration equations developed for grain protein and malt extract. Calibration equations were evaluated in terms of RMSD and SED, calculated as described by William (1975). These statistics are presented, along with lab means and NIR means in Table 5.

Prediction of malt extract and grain protein using unknowns from the same environments used to generate calibration equations was highly successful. The RMSD and SEE values for grain protein were 0.73 and 0.69, while the same statistics for malt extract prediction were 0.98 and 0.98 (Table II.5).

Bias adjustment was required for NIR prediction of grain protein and malt extract in row plot unknowns from an environment not included in the calibration set (Madras, 1988). RMSD and SEE values are therefore equal. The RMSD and SEE of this set of unknowns was somewhat higher than for unknowns within the calibration set. NIR screening for grain protein and malt extract in row plots should be effective. Bias adjustment for year and location effects may be required.

The grain protein prediction equation based on row plot chemical values was effective in predicting hill plot protein in all three environments. Correction for bias was not required. With the exception of the Madras, 1987 hill plots, RMSD values were comparable to those for row plots. NIR malt extract predictions were higher than chemical

values, consequently bias adjustment was required for all environments. RSMDs for hill plots were somewhat higher than for row plots (Table II.5). Statistics presented support NIR prediction of grain protein and malt extract in hill plots, based on row plot calibrations.

Considering NIR as a breeding tool, the numbers of lines in common at 25 and 50 % selection intensities, as measured by chemical analysis and NIR prediction, are useful statistics. Selection for malt extract in lines from the calibration set, based on NIR prediction, would be effective at the two selection intensities. Effective selection for grain protein would require less stringent selection. When row plots outside the calibration set (Madras, 1988) are considered, selection for either grain protein or malt extract would only be effective at relaxed intensities (Table II.6).

The foregoing discussion assumed comparable trait expression in the two plot types. A comparison of chemical grain protein and malt extract values in the hill and row plots, however, revealed differential trait expression. Based on chemical values, at a 50% selection intensity, 83% of lines were in common for grain protein and 75% of lines were in common for malt extract (Tragoonrung et al., submitted). Thus, progress from selection based on NIR prediction of spring malting barley evaluated in hill plots would be complicated by a) differential trait expression in row and hill plots, and b) the discrepancy between NIR-predicted and laboratory values for protein and extract.

Considering both trait expression in hill and row plots and NIR prediction, hill plot evaluation and NIR screening should be effective for grain protein selection. Selection for malt extract would likely be

less effective than selection for grain protein, given differential expression in hill and row plots and errors associated with NIR prediction.

Table II.1. Wavelengths and constants of the best three to six-wavelength models for grain protein and malt extract based on row plot samples from Corvallis and Madras, OR (1987).

Statistic	Wavelength(nm)				Constant			
	Equation1	Equation2	Equation3	Equation4	Equation1	Equation2	Equation3	Equation4
Protein					13.106	7.282	7.184	9.637
	1734	1445	1940	1940	1527.095	-357.571	623.477	710.142
	1778	1734	1982	1982	-1719.151	1483.362	-881.763	-998.224
	1982	1778	2100	2100	147.191	-1289.146	-780.996	-823.204
		1940	2190	2190		141.205	1577.678	2502.352
			2230	2208			-570.118	-1253.912
				2336				-165.046
Malt extract								
					48.886	45.881	27.169	43.774
	1445	1445	1445	1445	-894.384	-774.233	-727.771	-839.932
	1778	1722	1722	1680	5117.610	-3410.890	-2842.914	-1399.112
	1818	1759	1759	1778	-4245.481	6671.008	3294.584	2157.641
		1818	2310	1940		-2515.971	2240.024	159.834
			2336	2310			-2010.139	2418.385
				2336				-2509.227

Table II.2. Multiple correlation coefficient(R), standard error of estimation(SEE), standard error of prediction(SEP), index of systematic error(ISE), index of random error(IRE), and error mean square(MSE) of the best three to six-wavelength models for grain protein and malt extract based on row plot samples from Corvallis and Madras (1987).

Statistic	Protein				Malt extract			
	Equation1	Equation2	Equation3	Equation4	Equation1	Equation2	Equation3	Equation4
R	0.93	0.95	0.96	0.96	0.83	0.86	0.87	0.88
SEE	0.52	0.46	0.42	0.96	1.49	1.38	1.33	1.30
SEP	0.76	0.69	0.64	0.64	1.50	1.40	1.36	1.33
ISE	-44.87	-22.15	-31.72	-27.89	-22.26	-30.09	-46.22	-12.41
IRE	2304.16	2002.51	2142.49	3168.55	6709.24	7941.41	5340.83	4414.61
MSE	0.27	0.21	0.18	0.18	2.21	1.89	1.78	1.69

Table II.3. Wavelengths and constants of protein (five wavelength) and malt extract (six wavelength) equations for two-row and six-row genotypes evaluated in row plots at Corvallis and Madras in 1987.

Trait	Wavelength(nm)			Constant		
	Two-row	Six-row	Mixture	Two-row	Six-row	Mixture
Protein				6.450	15.414	7.184
	1940	1680	1940	118.393	715.233	623.477
	2139	1778	1982	-914.512	977.208	-811.763
	2208	1818	2100	1397.150	-1857.624	-780.996
	2230	2100	2190	-1245.141	-521.001	1577.678
	2270	2180	2230	629.591	665.070	-570.118
Malt extract				84.814	43.143	43.774
	1445	1445	1445	-1004.225	-521.924	-839.932
	1778	1680	1680	4677.914	-1206.348	-1399.112
	1818	1778	1778	-3378.136	1579.433	2157.641
	2180	1982	1940	-711.092	322.130	159.834
	2336	2310	2310	4034.846	2304.581	2418.385
	2348	2336	2336	-3682.097	-2461.126	-2509.227

Table II.4. Multiple correlation coefficient(R), standard error of estimation(SEE), standard error of prediction(SEP), index of systematic error(ISE), index of random error(IRE), error mean square(MSE), and range of the best five-wavelength(protein) and six-wavelength(malt extract) calibration equations for the separate analysis of two-row and six-row genotypes evaluated in row plots at Corvallis and Madras in 1987.

Statistic	Protein			Malt extract		
	Two-row	Six-row	Mixture	Two-row	Six-row	Mixture
R	0.96	0.98	0.96	0.82	0.84	0.88
SEE	0.41	0.21	0.42	1.03	1.44	1.30
SEP	0.42	0.22	0.64	1.07	1.49	1.33
ISE	-14.52	-21.11	-31.72	-62.78	16.75	-12.41
IRE	2179.25	2372.98	2142.49	8040.76	3961.67	4414.41
MSE	0.18	0.04	0.18	1.07	2.07	1.69
Range	8.9-14.8	9.0-12.3	8.9-14.8	76.3-85.1	71.3-82.0	71.3-85.1
N†	34	22	43	36	33	54

† N = numbers of samples used for calibration.

Table II.5. Root mean square difference (RMSD), standard error of difference (SED), Lab and NIR means (LABM and NIRM) for grain protein and malt extract using three distinct sets of validation samples.

Environments	Protein				Malt extract			
	RMSD	SED	LABM	NIRM	RMSD	SED	LABM	NIRM
Row plots								
COR87+MAD87	0.73	0.69	11.1(30)	11.3	0.98	0.98	78.7(28)	78.8
MAD88	0.99	0.99	11.9(18)	10.4	1.26	1.26	77.0(18)	82.8
Hill plots								
COR87	0.95	0.82	14.6(18)	14.1	1.59	1.59	74.9(18)	79.6
MAD87	1.26	1.13	15.0(20)	14.5	1.26	1.26	75.1(18)	80.5
MAD88	0.70	0.43	12.6(20)	12.9	1.89	1.89	76.7(18)	82.1

COR87 = Corvallis, 1987; MAD87 = Madras, 1987; MAD88 = Madras, 1988.
Numbers in parentheses = number of validation samples.

Table II.6. Percentage of lines in common between Lab and NIR values at 25 and 50% selection intensities in hill and row plot validation samples.

Environment	Protein		Malt extract	
	Top 25%	Top 50%	Top 25%	Top 50%
Row plots				
COR87+MAD87	57	93	86	100
MAD88	50	78	50	89
Hill plots				
COR87	50	89	75	67
MAD87	80	70	75	78
MAD88	80	90	50	78

COR87 = Corvallis,1987; MAD87 = Madras, 1987; MAD88 = Madras, 1988.

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CONCLUSION

This research has provided information required for analysis of feasibility of hill plot evaluation and NIR screening as tools for malting barley improvement.

When primary traits of interest are plant height, kernel plumpness, grain protein, alpha amylase and diastatic power, hill plots should be a highly effective breeding tool. The use of hill plots reduces the number of years required for cultivar development or recurrent selection because large numbers of lines can be evaluated without prior seed increase. The appropriate seeding rate for hill plot evaluation is 10 seeds per hill.

NIR prediction of grain protein in hill plots, based on row-plot chemical data, should be effective. The prediction of malt extract based on a row-plot-calibrated equation would be useful only for samples from row plots.

Selection for grain protein and agronomic traits other than grain yield, based on hill plot evaluation and NIR prediction, should be effective.

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APPENDICES

APPENDIX 1 Wavelengths and constants of protein (five wavelength) and malt extract (six wavelength) equations evaluated in row plots at Corvallis and Madras in 1987.

Trait	Wavelength(nm)		Constant	
	Corvallis	Madras	Corvallis	Madras
Protein:			10.832	10.532
	1734	2139	1602.991	-365.328
	1759	2180	-1882.721	1618.034
	2208	2230	1521.843	-939.117
	2230	2270	-1004.850	-596.751
	2348	2348	-252.432	287.919
Malt extract:			74.022	45.699
	1445	1445	-1023.710	-550.064
	1722	1722	-1587.769	-4320.090
	1778	1759	6701.450	8717.706
	1818	1818	-3988.207	-3538.911
	1982	2139	141.535	-947.026
	2230	2270	-307.075	651.493

APPENDIX 2 Multiple correlation coefficients(R), standard error of estimation(SEE), standard error of prediction(SEP), index of systematic error(ISE), index of random error (IRE), error mean square(MSE), and range from regression analyses for protein (five wavelength) and malt extract (six wavelength) evaluated in row plots at Corvallis and Madras in 1987.

Statistic	Protein		Malt extract	
	Corvallis	Madras	Corvallis	Madras
r	0.98	0.86	0.92	0.84
SEE	0.23	0.55	1.04	1.23
SEP	0.25	0.89	1.06	1.43
ISE	-15.18	4.79	-63.76	13.11
IRE	3082.80	2018.02	8031.10	10431.17
MSE	0.06	0.31	1.08	1.52
Range	8.9-14.8	9.0-12.5	71.3-81.1	74.7-85.1
N†	30	29	40	37

† N = numbers of samples used for calibration.

APPENDIX 3 Mean squares from the combined analyses of variance of agronomic traits measured in eight replications(REP) in hill plot evaluations of spring malting barley at Corvallis(1987) and Madras(1987 and 1988), Oregon.

Source of variation	df	Plant height	Harvest index	Grain yield
ENV	2	15630.99	4.721	842635.37
REP(ENV)	21	917.03	0.116	14014.11
Genotype(G)	23	5525.04	0.110	18476.98
Type	1	14905.49	0.267	57032.41
Two-row	17	5141.65	0.119	15560.26
Six-row	5	4952.50	0.049	20682.75
Seed rate(SR)	3	356.82	0.007	41409.28
G x SR	69	177.04	0.003	362.73
Type x SR	3	66.27	0.002	428.22
Two x SR	51	227.18	0.003	281.40
Six x SR	15	28.73	0.004	626.16
ENV x G	46	483.26	0.010	2083.98
ENV x SR	6	253.45	0.005	1153.06
ENV x G x SR	138	214.31	0.002	270.63
Error	1995	208.28	0.002	326.81

ENV = environment.

APPENDIX 4 Mean squares from the combined analyses of variance of agronomic traits measured in four replications(REP) in hill plot evaluations of spring malting barley at Corvallis and Madras, in 1987 Oregon.

Source of variation	df	%plump seed	No. of seeds/ head	No. of heads/ hill	100 seed weight
ENV	1	331.78	453.35	211307.19	10.75
REP(ENV)	6	1132.23	12.53	3397.46	3.48
Genotype(G)	23	2384.55	5149.57	7929.94	6.81
Type	1	9264.25	110251.67	132721.12	69.72
Two-row	17	1619.80	224.01	43843.28	3.91
Six-row	5	3608.79	876.07	1164.86	4.10
Seed rate(SR)	3	919.62	46.64	38985.12	0.27
G x SR	69	99.67	18.25	521.21	0.33
Type x SR	3	62.83	9.79	36.26	0.36
Two x SR	51	84.33	9.61	554.58	0.24
Six x SR	15	159.18	41.39	313.31	0.17
ENV x G	23	176.23	34.33	1845.31	0.65
ENV x SR	3	134.88	12.48	460.90	0.32
ENV x G x SR	69	55.68	23.12	497.15	0.26
Error	570	65.64	12.84	447.39	0.20

ENV = environment.

APPENDIX 5 Mean squares from the combined analyses of variance of agronomic traits measured in four replications(REP) in row plot evaluations of spring malting barley at Corvallis(1987) and Madras(1987 and 1988), Oregon.

Source of variation	df	Plant height	%plump seeds	Harvest index	Grain yield
ENV	2	3775.09	2766.830	0.682	130181308.00
REP(ENV)	9	138.05	54.00	0.013	654105.10
Genotype(G)	23	1243.84	1016.48	0.017	1542985.00
ENV x G	46	60.68	198.15	0.006	379663.60
Error	207	6363.80	44.09	0.004	162901.70

ENV = environment.

APPENDIX 6 Mean squares from the combined analyses of variance of agronomic traits measured in four replications(REP) in row plot evaluations of spring malting barley at Corvallis and Madras, in 1987 Oregon.

Source of variation	df	No. of heads/ meter	No. of seeds/ head	100 seed weight
ENV	1	17654.79	170.63	0.04
REP(ENV)	6	686.59	3.63	0.09
Genotype(G)	23	5810.74	1352.02	1.52
ENV x G	23	755.61	77.18	0.22
Error	138	499.92	6.78	0.09

ENV = environment.