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Evaluation of predictors of quality for use in early generation selection

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SUMMARY – Grain protein concentration, protein quality, and colour are major quality attributes of durum wheat. Breeders and their cereal chemist colleagues use predictors of these end-use quality factors in the early generations to develop improved cultivars. Techniques used in selection must be strongly correlated with semolina or pasta quality, effective in producing genetic gains through selection, and cost-efficient. Protein concentration is relatively simple to measure with Near Infrared Reflectance or other instruments, but low heritability is a problem due to the strong influence of environment. Use of appropriate experimental designs and statistical analyses can improve heritability. Rapid tests for gluten strength, such as SDS-sedimentation volume and gluten index, provide moderately heritable predictions of gluten strength and are correlated with more time-consuming rheological tests performed on semolina. Protein markers determined by electrophoresis can be used for selection in some situations. DNA markers would provide a useful selection tool for both protein concentration and gluten strength. Pigment and other colour factors are highly heritable, and reasonably simple to measure.

Key words: Protein concentration, gluten strength, pigment content.

RESUME – *"Evaluation des prédicteurs de qualité utilisés en sélection des premières générations". La concentration en protéines des grains, la qualité des protéines et la couleur sont des attributs importants de la qualité du blé dur. Les sélectionneurs de semence et leurs collègues chimistes céréalistes utilisent des prédicteurs de ces facteurs de qualité d'utilisation finale dans les premières générations pour développer des cultivars améliorés. Les techniques utilisées dans la sélection doivent avoir une forte corrélation avec la semoule et la qualité pastière, efficaces dans la production de gains génétiques par l'intermédiaire de la sélection et rentables. La concentration en protéines est relativement simple à mesurer avec la réflexion dans le proche infrarouge ou avec d'autres instruments, mais la faible héritabilité est un problème vu la forte influence de l'environnement. L'utilisation de concepts expérimentaux appropriés et d'analyses statistiques peut améliorer l'héritabilité. Des tests rapides pour connaître la force du gluten, comme le volume de sédimentation de SDS et l'indice de gluten donnent des prévisions modérément transmissibles de la force du gluten et ils sont en corrélation avec des tests rhéologiques prenant davantage de temps effectués sur la semoule. Les marqueurs de protéines déterminés par l'électrophorèse peuvent être utilisés pour la sélection dans certaines situations. Les marqueurs d'ADN peuvent fournir un outil de sélection utile pour la concentration en protéines et la force du gluten. Le pigment et les autres facteurs de couleur sont hautement transmissibles et ils sont assez simples à mesurer.*

Mots-clés : *Concentration en protéines, force du gluten, contenu en pigment.*

Introduction

Grain protein concentration, protein quality, and colour are the major quality attributes of durum wheat *(Triticum turgidum* L. var. *durum*). Other factors such as physical condition of grain, milling yield and cooking quality are also important in final selection of genotypes for manufacture of pasta. Durum breeders strive to maintain, if not improve, all of these quality traits in development of agronomically superior cultivars (Clarke *et al.,*1998a). The challenge for breeders and their cereal chemist colleagues is to apply screening techniques for end-use quality that are strongly correlated with semolina or pasta quality, effective in producing genetic gains through selection, and cost-efficient for processing the large number of genotypes typical in the early generations.

Protein concentration

Wild emmer wheat (*T. turgidum* L. var. *dicoccoides*) has been used as a source of high grain protein concentration in durum as it has in common wheat (*T. aestivum)*. Levy and Feldman (1989) crossed several emmer accessions with the durum cultivar 'Inbar'. Grain protein concentration of the emmer accessions was about 24%, compared to about 12% for Inbar. There was little or no dominance. Grain protein concentration had a broad-sense heritability ranging from 0.29 to 0.53. Levy and Feldman (1989) did not report grain yield data, but grain protein concentration was negatively correlated with yield components such as kernel weight and number of kernels per spike.

Joppa and Cantrell (1990) studied grain protein concentration of chromosome substitution lines developed by substituting each of the *dicoccoides* chromosomes into the durum cultivar 'Langdon'. The substitution line with *dicoccoides* chromosome 6B, Langdon(DIC-6B), gave the most consistent increase in protein concentration; protein concentration was 17% for Langdon, and 18% for Langdon(DIC-6B), averaged over four experiments. Langdon(DIC-6B) also appeared to have the best end-use quality of the substitution lines (Joppa *et al.,* 1991).

We have utilized Langdon(DIC-6B) in our durum breeding program, but concluded that Langdon(DIC-6B) did not provide yield/protein genes superior to those already available in Canadian germplasm (DePauw *et al.,* 1998). Intercrossing within other Canadian germplasm, and simultaneous selection for yield and protein concentration, produced the new durum cultivar AC Avonlea (Clarke *et al.,* 1998b). In registration trials, AC Avonlea averaged 3% higher grain yield than the standard cultivar Kyle, and 0.6% units higher grain protein concentration.

Estimates of protein concentration heritability vary from low to moderate (Table 1). Selection for protein in wheat is complicated by the negative relationship with grain yield, and the influence of environmental conditions on protein concentration. Heritability may be improved by using statistical procedures to remove environmental trends. Adjustment of protein for environmental trends using the moving mean procedure tended to improved realized heritabilities for protein concentration (Clarke *et al.,* 1998c). We are investigating other data sets to devise a strategy for resource allocation for protein testing.

Table 1. Heritability of protein concentration

Parameter	Reference
Heritability 0.50 to 0.75 in two wheat crosses	McKendry *et al.,* 1988
Low heritability (0.19) at F3 (single plant) and F4 (increase row) generations	Pearson *et al.,* 1981
Protein content heritability 0.17 in F3 individual families	Gallia *et al.,* 1996
Heritability 0.35 to 0.74 for three crosses at two locations in three years	Clarke *et al.,* 1998c
F6/F4 regression heritability estimates 0.12 to 0.73 in two wheat crosses	DePauw *et al.,* 1998
Heritability estimated at 0.78 based on reciprocal crosses among six durums	Sarrafi *et al.,* 1989
Realized heritability of 0.24 (26% selection intensity) in hexaploid wheat	Fischer *et al.,* 1989
Protein influenced more by additive effects of environment than genotype	Mariani *et al.,* 1995

However, selection for protein in the early generations is costly and low heritability impedes progress. Some of the moderate heritabilities reported for protein concentration are based on replications, locations and years of testing (Clarke *et al.,* 1998c) that are impractical in the case of early generations. Molecular markers may offer a cost-effective method to improve selection for protein concentration. So far there are relatively few reports of this in the literature. Blanco *et al.* (1996) reported that markers linked to six quantitative trait loci explained 49.2 to 56.4% of the phenotypic variation for protein content in a set of 65 recombinant inbred durum lines. Gene(s) for high grain protein content located near the centromere of chromosome 6B of the Langdon(DIC-6B) substitution accounted for 66% of grain protein variation in a mapping population of 85 recombinant inbred lines (Joppa *et al.,* 1997). Eleven potential restriction fragment length polymorphism (RFLP) markers were mapped to this region. Humphreys *et al.* (1998) reported screening of a doubled haploid hexaploid wheat population with a PCR-based marker that identified lines averaging 0.3% higher protein with no loss of yield relative to lines without the marker.

Protein quality

Gluten strength predictors

Heritability of predictors of gluten strength, such as mixograph and sedimentation volume are intermediate to high (Table 2). Correlation of such tests with industrial standards for gluten strength measurement, for example the Alveograph (D'Egidio *et al.,* 1990), are an important consideration. Ruiz and Carrillo (1995) found that SDS-sedimentation volume was correlated with mixograph mixing time (r = 0.78 to 0.85) and mixograph peak height (r = 0.70 to 0.77), but not with protein concentration. Other reports show significant positive correlation of SDS-Sedimentation volume with protein concentration (e.g. Kovacs *et al.,* 1995). Our own results (unpublished) show correlations of SDS-sedimentation volume and mixograph parameters such as mixing time to be in the range of 0.60 to 0.80, and of 0.70 to 0.80 with Alveograph work input. Corresponding correlations of gluten index with mixograph properties and Alveograph work input tended to be similar to slightly larger.

Table 2. Heritability of gluten strength predictors

Parameter	Reference
Heritability of mixogram scores ranged from 0.56 to 0.64 for three crosses	Braaten *et al.,* 1962
Heritability of SDS-sedimentation volume 0.53 for reciprocal crosses involving six durum genotypes	Sarrafi *et al.,* 1989
Estimated heritability of SDS-sedimentation ranged from 0.57 to 0.68 for three crosses; realized heritability after selection ranged from 0.63 to 0.72	Clarke *et al.,* 1993
Realized heritability of gluten strength measured by micro-sedimentation ranged from 0.18 to 0.41 in two crosses	McClung and Cantrell, 1986
Realized heritability of SDS-sedimentation was 0.44 in hexaploid wheat	Fischer *et al.,* 1989

Of the prediction tests applied in early generations, SDS-sedimentation requires the least amount of sample and labour. Indeed, micro-methods using as little as 1 g of whole meal have been developed (Dick and Quick, 1983). The gluten index requires a larger sample and has a slightly larger labour requirement than the SDS-sedimentation test. The mixograph has a still greater labour requirement, which includes the need to mill flour.

We evaluated gluten index, a measurement of gluten strength (Cubadda *et al.,* 1992) used in some pasta industry laboratories, for use in early generation screening. Comparison of gluten index measurements on semolina, flour, and whole meal showed that whole meal could be used, greatly reducing cost and amount of grain required for testing. Genotype-environmental interactions were minor (Ames *et al.,* 1999). Gluten index showed similar heritability to SDS-sedimentation volume in three crosses grown at two locations for three years (Table 3). We found that gluten index showed less dependance on protein concentration than did SDS-sedimentation. That, and the greater range for gluten index than for SDS-sedimentation, made gluten index more useful for early generation screening where sufficient sample (10 g) is available.

Table 3. Heritability of SDS-sedimentation volume and gluten index in recombinant inbred lines of three durum crosses

Cross	n	SDS-sedimentation volume		Gluten index	
		Heritability	90% Confidence interval	Heritability	90% Confidence interval
8560-150D/Wascana	37	0.91	0.84-0.94	0.93	0.89-0.96
Kyle/Nile	34	0.84	0.71-0.91	0.84	0.73-0.90
8363-BB3D/STD65	39	0.90	0.83-0.94	0.88	0.80-0.92

There is potential for prediction of farinograph, extensigraph and alveograph parameters by near infrared reflectance spectroscopy (NIR) in common wheat (Williams *et al.,* 1988). Delwiche *et al.* (1998) reported good prediction of glutenin and gliadin contents, SDS-sedimentation volume and mixograph peak resistance of common wheat flour by NIR, which suggests that comparable studies should be undertaken with durum wheat.

Marker-assisted selection for gluten strength

Screening for gliadin bands γ-42 versus γ-45 (Clarke *et al.,* 1993) and low molecular weight (LMW) glutenin subunit 2 (Kovacs *et al.,* 1995) has been implemented in our breeding program. Gliadin γ-45 is associated with high gluten strength, while γ-42 tends to be associated with weaker gluten and poor viscoelastic properties in durum wheat (Kosmolak *et al.,* 1980). Genotypes carrying γ-45 can be easily and non-destructively identified using a monoclonal antibody technique (Howes *et al.,* 1989). Elimination of F_2 lines homozygous for γ-42 in four crosses reduced the population size by up to 44%, and increased ($P < 0.01$) mean sedimentation of the F_4 by 0.3 to 0.6 cm compared to unselected populations (Clarke *et al.,* 1993). Retention of lines homozygous for γ-45 further increased ($P < 0.01$) F_4 sedimentation, and was not different from selection for F_2 sedimentation at an intensity of 20%. The authors concluded that the monoclonal antibody test was faster and less costly than the SDS-sedimentation test for screening F_2 plants, and could be applied non-destructively to single seeds. Linkage of γ-42 with bronze glume color and γ-45 with white glume color (Leisle *et al.,* 1985; McClung and Cantrell,1986) can be exploited for selection, although Clarke *et al.* (1993) found recombinations.

Considerable variation in gluten strength remains within the γ-45 type, so other markers are required to differentiate strength. Because of this, Pogna *et al.* (1990) suggested that the observed association of γ-45 with strength is merely a linked genetic marker, not a functional difference. They reported that lines with glutenin subunit LMW-2 had better gluten characteristics than lines with LMW-1, and high molecular weight (HMW) subunits 7+8 gave larger SDS sedimentation values and gluten elastic recoveries than subunits 6+8 and 20. Carrillo *et al.* (1990) reported that LMW-2 occurs in two forms, LMW-2 and LMW-2^-, with the LMW-2 showing higher gluten strength. Kovacs *et al.* (1995) reported that selection for LMW-2 using a monoclonal antibody identified families with improved SDS sedimentation volumes and cooked pasta disk viscoelasticity.

However, there was still considerable overlap in the range of the strength measurements for the two LMW-2 types, indicating that there are other factors involved. Ruiz and Carrillo (1995) used random lines from four crosses to study the effects of gliadins and glutenin variants coded by the Glu-3/Gli-1, Glu-1, and Gli-A2 loci on gluten strength. They found that LMW-2, LMW-2^- and LMW-2(CB) glutenins were associated with higher SDS-sedimentation volume and improved mixograph properties relative to lines with LMW-1 and LMW-2^*. Further, they reported that only HMW glutenin subunits 20+8 were associated negatively with gluten quality, and other HMW subunits and α-gliadins had no effect. Research to identify additional protein markers for gluten strength continue, as reported by others in these proceedings.

Recently there have been attempts to utilize DNA markers for selection for gluten strength. D'Ovidio and Porceddu (1996) found a pair of oligonucleotide primers that distinguish lines with LMW-2 from those with other 1B-LMW allelic variants. A PCR assay could thus be used to test single seeds in a breeding program. We are pursuing DNA markers in a set of six random inbred populations with varying gluten strength.

Colour factors

Yellow pigment content of the endosperm is due to the presence of xanthophyll and other related compounds (Lepage and Sims 1968). Heritability is generally high (Table 4). Most reports suggest that pigment content is largely additive in inheritance, and controlled by more than one gene (Bratten *et al.,* 1962; Lee *et al.,* 1976; Johnston *et al.,* 1983). Several authors reported transgressive segregation in the crosses studied (Clark and Smith, 1928; Bratten *et al.,* 1962; Johnston *et al.,* 1983).

The literature is unclear as to the effects of environment on pigment content. Mangels (1932) reported that cool environments with ample moisture produced higher pigment content than hot, dry conditions. Other evidence suggests the reverse – pigment content was negatively associated with test weight

(Whiteside *et al.,* 1934; Worzella, 1942) and kernel weight (Worzella, 1942), both traits which may be lower in hot, dry conditions. Bratten *et al.* (1962) and Clarke *et al.* (1998d) reported high heritabilities of pigment content, with little evidence of genotype environmental interaction. However, Lee *et al.* (1976) found heritability of a 10 parent diallel to be 0.11 in one environment, and 0.79 in another. Similarly, Johnston *et al.* (1983) found heritabilities of 0.48 to 0.76 because of the influence of environment.

Table 4. Heritability and related factors for pigment content

Parameter	Reference
Heritability of carotenoid content ranges from 0.79 to 0.94 in three crosses, with little genotypic-environmental interaction	Braaten *et al.,* 1962
Heritability of pigment content ranges from 0.89 to 0.95 in three crosses	Clarke, *et al.,* 1998d
Heritability of yellow index (p.p.m.) 0.42 based on crosses involving six durum genotypes	Sarrafi *et al.,* 1989
Realized heritability of semolina colour ranged from 0.31 to 0.69 in five crosses and controlled primarily by additive gene effects	Johnston *et al.,* 1983
Colour expression (β-carotene content, yellow index and lipoxygenase activity) determined by genotype, with no environmental influence	Borrelli *et al.,* 1999
Colour exhibits transgressive segregation	Clark and Smith, 1928
Pigmentation is subject to significant regional and seasonal variation	Mangels, 1932
Environmental conditions producing higher carotene values tend to produce lower test weights and kernel weights	Whiteside *et al.,* 1934
Variability in pigment per kernel can be reduced by correcting for variations in kernel weight, as pigment (p.p.m.) correlates negatively with kernel weight	Markley, 1937

Pigment has been measured by visual comparisons with standard samples, colorimetry following solvent extraction, and light reflectance measurements (Johnston *et al.,* 1981). More recently, McCaig *et al.* (1992) used NIR to estimate pigment content. The method was highly associated with the laboratory reference (solvent extraction) method (r^2 0.94). The NIR method offers the advantages of minimal additional sampling and processing time and costs in breeding programs currently using NIR instruments which cover the visible range to measure grain protein, and elimination of the health and environmental hazards associated with organic solvent extractions.

Colour of semolina and pasta as measured by the CIE 1976 L*a*b* colour system is the factor that is ultimately important in pasta manufacture. There is some loss of pigment during milling, depending on extraction rate (Matsuo and Dexter, 1980), and there may be further losses during pasta manufacture through the activity of the lipoxygenase enzyme. Borrelli *et al.* (1999) reported that an average of approximately 8% of β-carotene was lost during milling, and a further 16% through pasta manufacture in a study of several Italian durum cultivars. Some lipoxygenase is also removed during milling and processing of semolina because of the presence of lipoxygenase in the bran, germ and aleurone layers (Taha and Sági, 1987). There is genotypic variation for lipoxygenase activity (e.g. Borrelli *et al.,* 1999) and we have observed genotypic variation in pigment loss on milling (unpublished).

Therefore, it is useful to predict semolina colour, or to measure lipoxygenase activity in early generation screening. The cost and time to produce semolina for early generation screening has led researchers to investigate use of flour or whole meal for prediction of semolina colour. Konzak *et al.* (1975) utilized a micromill capable of milling about 1000 samples (2 to 5 g) per day. Colour of the crude semolina was then scored visually or with a colour meter to distinguish between samples that were visually similar.

Johnston *et al.* (1981) found that colour of whole meal correlated well (r = 0.85 to 0.90) with semolina colour in a set of 10 durum genotypes. They suggested that colour measurement of 1 or 2-g whole meal samples offered speed and efficiency for prediction of semolina colour. We have investigated prediction

of semolina colour from b* measurements of whole meal, with similar initial results (Fig. 1). Sgrulletta and DeStefanis (1997) found that b* of whole meal could be predicted with a scanning NIR instrument. We are currently developing prediction equations for a scanning NIR instrument so that we can predict protein concentration, pigment content, and semolina b* with a single pass through the instrument. After selection, the same whole meal samples can then be used for determination of gluten index as noted above.

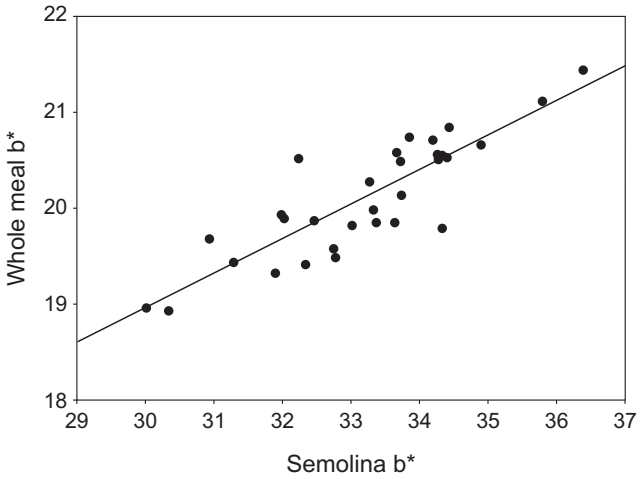

Fig. 1. Relationship of durum whole-grain meal b* with semolina b* values measured with a Minolta CR-310 Chroma Meter with D65 illumination $r^2 = 0.74$).

Relatively few breeding programs routinely attempt to measure lipoxygenase activity to eliminate lines that have potential for high colour loss during processing. This is principally due to the difficulty in measurement of lipoxygenase activity. Borrelli *et al.* (1999) reported that the two isoenzymatic forms of lipoxygenase, LOX-2 and LOX-3, were responsible for loss of colour during pasta production. The enzyme activity was highly correlated to loss of β-carotene (r = 0.84 to 0.87). Borrelli *et al.* (1999) demonstrated that lipoxygenase activity of some cultivars was more important in determination of pasta colour than was pigment content of the whole grain. Manna *et al.* (1998) used slot-blot analysis of RNA from nine durum cultivars using a Lipoxygenase-1 gene probe and found variable expression of LOX gene(s). Lipoxygenase mRNA levels showed a strong negative correlation with semolina β-carotene content and yellow index (r = -0.985 and -0.960). Matsuo *et al.* (1970) noted that pigment loss through lipoxygenase activity depended on the availability of lipid substrates, as did Barone *et al.* (1999). It would be worthwhile to develop DNA markers for lipoxygenase activity to facilitate early generation selection.

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