Effect of Two Different Low Molecular Weight Glutenin Subunits on Durum Wheat Pasta Quality Parameters[1]

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ABSTRACT

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F2-derived F_4 and F_5 durum wheat families from the cross DT471/DT624, homozygous for either of two low molecular weight glutenin subunits (LMWGS 2 and LMWGS 2^-) were used in a study to determine the effect of these subunits on protein content (PROT), cooked pasta disk viscoelasticity (PDV), cooked gluten viscoelasticity (CGV), sodium dodecyl sulfate sedimentation volume (SV), mixograph mixing development time (MDT), mixograph peak height (MPH), and mixograph total energy (MTE). These subunits were identified using a monoclonal antibody (MAb45/8) specific for the LMWGS 2 allele. Both parents and progeny were homozygous for HMWGS 6+8 and gliadin band 45. Families homozygous for LMWGS 2 had higher PDV (t = 2.4, $P < 0.01$) and SV values ($t = 4.9$, $P = 0.001$) than families homozygous for LMWGS 2^-; CGV differed in one year, and MDT and PROT were similar for the two alleles. PDV and SV values were positively correlated with each other ($r = 0.52$, $P < 0.001$) as well as with PROT values ($r = 0.59$, $P < 0.001$, $r = 0.46$, $P < 0.001$, respectively). Neither PDV or SV were correlated with MDT, while PDV showed only a low correlation with CGV ($r = 0.30$, $P < 0.05$). CGV was not correlated with MDT nor consistently with SV. The major effect of LMWGS 2 type was on PDV and SV rather than on measures of gluten strength (MDT, CGV). PDV and SV values were not greatly influenced by either MDT or CGV values, indicating that the former two are being influenced by additional quality components other than gluten strength. This study showed that LMWGS 2 allele is beneficial to improve pasta cooking quality of future cultivars and that selection can be readily accomplished using monoclonal antibody MAb45/8.

Cooked pasta made from superior quality durum wheat cultivars retains good rheological characteristics, such as firmness and elasticity, and it is resistant to surface disintegration and stickiness. It has been shown that gluten composition is the major factor that determines these quality characteristics (Autran and Feillet 1987; du Cros 1987; Feillet et al 1989; Kovacs et al 1991, 1993a). Among high molecular weight glutenin subunits (HMWGS), HMWGS 6+8 or 7+8 give better quality than HMWGS 20, while among low molecular weight glutenin subunits (LMWGS), LMWGS 2 gives better quality than LMWGS 1. Carrillo et al (1990a,b) showed that there are two types of LMWGS 2 (2 and 2^-), both of which are usually associated with γ-gliadin band 45. Among 35 durum wheat cultivars and 139 Spanish land races, they found that those having LMWGS 2 had higher sodium dodecyl sulfate sedimentation volume (SV) than ones having LMWGS 2^- or LMWGS 1.

In durum wheat breeding programs, early generation selection for quality is limited by the small sample size, especially in head-row selection breeding schemes, where less than 2 g of seed is available for testing. Traditionally, protein content and sedimentation volume were the only tests available for quality evaluation using such small samples. More recently monoclonal antibody tests, which can be performed with as little as one half kernel, have been applied to select superior quality durum lines (Howes et al 1989, Kovacs et al 1993a). Other simple physical tests are also used to select lines with superior pasta quality, including cooked pasta disk viscoelasticity (PDV), cooked gluten viscoelasticity (CGV), mixograph mixing development time (MDT), mixograph peak height (MPH), mixograph total energy (MTE), and sodium dodecyl sulfate sedimentation volume (SV).

In this study, we examined the effect of LMWGS 2 and LMWGS 2^- alleles upon quality parameters generally examined in the Canadian durum wheat breeding programs (SV, CGV, MDT, MTE, MPH, PDV, and protein). The effectiveness of selecting for the LMWGS 2 allele based on differences in these parameters or by a monoclonal antibody test was determined. To eliminate the effect of other HMWGS types, we examined progenies from a cross where both parents had HMWGS 6+8, the predominant HMWGS in Canadian durum cultivars. A monoclonal antibody test (Howes et al, *in press*) was used to identify progenies homozygous for LMWGS 2 or LMWGS 2^-.

MATERIALS AND METHODS

A selection of 140 F_2-derived F4 families from the cross DT471/DT624 were grown at Portage la Prairie, Manitoba in 1992. Of these, 67 homozygous families (33 homozygous for LMWGS 2 and 34 homozygous for LMWGS 2^-) were identified for quality evaluation. In 1993, 64 of the F_2-derived F_5 homozygous families (34 homozygous for LMWGS 2 and 30 homozygous for LMWGS 2^-) were grown at Glenlea, Manitoba for quality evaluation.

Monoclonal Antibody Test

To identify the LMWGS types, enzyme-linked immunosorbent assay (ELISA) was performed on 10 half kernels from each family, extracting kernel proteins including LMWGS with 50% propan-2-ol (16 hr, 40°C) and diluting the protein extract (1:50) with 70% ethanol before binding on microtiter plates (1 hr, 20°C) as described previously (Howes et al 1989). MAb 45/8 was diluted (1:100) to give optimal specificity between parents, so that DT624 kernels gave a fourfold higher absorbency than DT471 kernels.

Quality Evaluation

A subsample of seed was milled into whole meal flour using a Udy cyclone grinder. Using whole meal flour, protein content (14% moisture content) was determined by near-infrared reflectance spectroscopy (model 860-01, Dickey-John Insta Lab, Auburn, IL) and SV was obtained according to Kovacs (1985). Semolina was milled by the method of Black and Bushuk (1967). Using flour milled from semolina, mixograph values (MDT, MPH, and MTE) and gluten viscoelasticity were determined according to Pon et al (1988) and Kovacs et al (1994), respectively. Semolina PDV was measured by the method of Feillet et al (1977). Statistical analysis was performed according to standard procedures (SAS 1985).

RESULTS

The mean values, t-test and range of values for LMWGS 2 and 2^- progenies from the cross DT624/DT471 are shown in Table I. In 1992, lines homozygous for LMWGS 2 had significantly higher mean values for PDV, SV, MPH, and MTE than

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TABLE I
Means, *t*-Test, and Range Values for Two Low Molecular Weight Glutenin Subunit (LMWGS 2 and 2-) Lines of Cross DT471/DT624 Grown in Two Crop Years

Samples[a]	Means			Range	
	LMWGS 2	LMWGS 2-	*t*-TEST[b]	LMWGS 2	LMWGS 2-
1992	($n = 33$)	($n = 34$)		($n = 33$)	($n = 34$)
PDV	43.3	41.5	**	35.9–49.6	31.8–47.0
SV	37.3	32.8	***	29.0–42.0	26.0–46.0
CGV	49.8	49.0	NS	40.6–58.4	40.6–54.9
MDT	2.2	2.2	NS	1.8–2.8	1.8–2.9
MPH	16.3	15.4	**	13.4–19.9	12.0–17.7
MTE	31.1	29.3	**	26.1–37.4	23.6–33.9
PROT	13.4	13.4	NS	11.6–15.0	11.0–15.3
1993	($n = 34$)	($n = 30$)		($n = 34$)	$n = 30$)
PDV	40.8	39.3	**	36.6–43.9	35.2–43.0
SV	38.9	35.3	***	26.0–47.0	30.0–42.0
CGV	65.2	56.5	***	53.1–74.7	30.4–73.4
MDT	2.1	2.1	NS	1.7–2.9	1.7–2.7
MPH	16.9	16.1	**	15.3–19.9	14.0–18.1
MTE	33.6	32.4	*	29.7–39.0	28.6–36.3
PROT	16.0	16.0	NS	14.8–17.2	15.3–17.1

[a] PDV = cooked pasta disk viscoelasticity; SV = sodium dodecyl sulfate sedimentation volume; CGV = cooked gluten viscoelasticity; MDT = mixograph mixing development time; MPH = mixograph peak height; MTE = mixograph total energy; PROT = protein content.
[b] *, **, *** means are significantly different at the 0.05, 0.01, and 0.001 level of probability, respectively. NS = not significant.

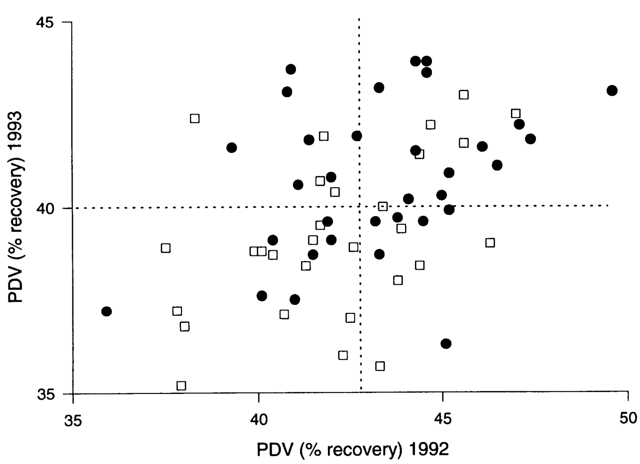

Fig. 1. Influence of two low molecular weight glutenin subunits (LMWGS 2 = ● and LMWGS 2^- = □) on cooked pasta disk viscoelasticity (PDV) in samples grown in 1992 and 1993.

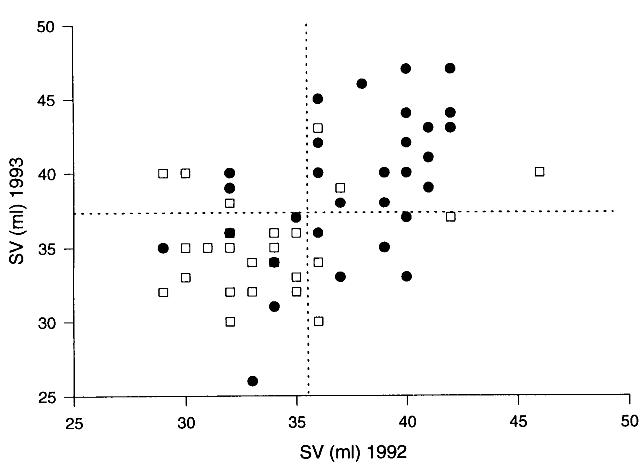

Fig. 2. Influence of two low molecular weight glutenin subunits (LMWGS 2 = ● and LMWGS 2^- = □) on sodium dodecyl sulfate sedimentation volume (SV) in samples grown in 1992 and 1993.

lines having LMWGS 2^-, whereas CGV, MDT, and PROT values were similar in both LMWGS types. In 1993, lines homozygous for LMWGS 2 had significantly higher mean values for PDV, SV, CGV, MPH, and MTE than lines having LMWGS 2^-. Only MDT and PROT values were similar for both alleles.

Of lines having PDV values above the mean in 1992 and 1993, 20 and 19 respectively were homozygous for LMWGS 2 (Fig. 1). Similarly, of lines having SV values above the mean in the two years, most (19 and 22, respectively) were homozygous for LMWGS 2 (Fig. 2). In fact, lines that were high in both PDV and SV over the two years in general had LMWGS 2.

Pearson correlation coefficients were determined between quality characteristics of durum wheat lines (Table IIA and B). PDV was correlated in both years with SV and CGV, but with MPH, MTE and protein only in 1992. SV correlated with MPH, MTE, and PROT in both years, but with CGV only in 1992. CGV did not correlate with any of the mixograph parameters. There was a significant association between PROT and MPH and MTE in 1992 but not in 1993. MDT correlated only with MTE in 1993. The ELISA test to identify the LMWGS 2 type was significantly associated with PDV, SV, MPH, and MTE in both years but only with CGV in the 1993 crop year.

Comparing the influence of the environment on the same lines grown in the two crop years, there was no significant correlation between years in protein content and MDT, but there was a significant correlation between years in SV, PDV, CGV, MPH, and MTE using parametric or Pearson and Spearman rank

TABLE II
Correlations Among Determinations of Quality Characteristics[a,b]

Samples	SV	CGV	MDT	MPH	MTE	PROT
1992 ($n = 67$)						
PDV	0.52***	0.30*	−0.14	0.47***	0.52***	0.59***
SV		0.22	−0.02	0.60***	0.57***	0.46***
CGV			0.23	0.15	0.14	−0.08
MDT				0.18	0.15	−0.18
MPH					0.98***	0.59***
MTE						0.59***
PROT						
1993 ($n = 64$)						
PDV	0.30**	0.53**	−0.21	0.20	0.07	−0.01
SV		0.39***	0.06	0.55***	0.55***	0.35**
CGV			0.13	0.23	0.19	−0.12
MDT				0.13	0.30**	0.17
MPH					0.94***	0.06
MTE						0.13
PROT						

[a] PDV = cooked pasta disk viscoelasticity; SV = sodium dodecyl sulfate sedimentation volume; CGV = cooked gluten viscoelasticity; MDT = mixograph mixing development time; MPH = mixograph peak height; MTE = mixograph total energy; PROT = protein content.
[b] *, **, *** correlation significantly different from zero at the 0.05, 0.01, and 0.001 level of probability, respectively.

TABLE III
Correlations of Quality Characteristics[a] Between Two Crop Years (1992 and 1993)[b]

	Pearson Correlation	Spearman Rank
PDV	0.44***	0.43***
SV	0.55***	0.56***
CGV	0.37**	0.38**
MDT	−0.01	−0.00
MPH	0.40***	0.41***
MTE	0.40***	0.41***
PROT	0.22	0.16

[a] PDV = cooked pasta disk viscoelasticity; SV = sodium dodecyl sulfate sedimentation volume; CGV = cooked gluten viscoelasticity; MDT = mixograph mixing development time; MPH = mixograph peak height; MTE = mixograph total energy; PROT = protein content.
[b] **, *** correlation significantly different from zero at the 0.01 and 0.001 level of probability, respectively.

correlations (Table III). The differences between the two types of correlations were small. Data showed normal frequency distributions except for MDT in 1992 and CGV for 1993 (data not shown).

DISCUSSION

Rheological properties of durum wheat pasta are among the most important parameters related to cooking quality. It has been shown that SV, MDT, and CGV measurements, used in durum wheat breeding programs, are good predictors of pasta rheological properties. It is also well documented that protein composition has an important role in pasta cooking quality, but the importance of protein quantity is less clear (Feillet 1984).

Although there was considerable overlapping of values, showing the influence of environment, we found that lines homozygous for LMWGS 2, in both years, had higher mean values for PDV, SV, MPH, and MTE than lines having LMWGS 2^{-}. The effect on SV supports the results of Carrillo et al (1990a,b). Values of MDT and protein were not significantly different between the two LMWGS 2 types. The difference of the effect of LMWGS 2 types on the CGV between the two years data shows the environment of the ripening grain can also have a major influence upon some of these quality tests. Of the 13 lines having PDV and SV above the mean in both years, 12 were LMWGS 2. Thus selecting for LMWGS type, which is not influenced by environment, would retain almost all of the lines that consistently had higher PDV and SV in two different years. Conversely, discarding lines that have LMWGS 2^{-} would eliminate most of the lines which had PDV and SV below the means in both years.

Correlation among quality characteristics were not consistent between the two years. This may be due in part to differences in samples due to environment. In 1993, the plants matured very late and slowly due to cooler temperatures and shorter day length. In addition, stress due to diseases resulted in some kernel shriveling and significantly higher protein in 1993. PDV showed significant correlations with SV and CGV in both years, but only in 1992 with MPH, MPE, and protein, while SV was consistently correlated with the latter characteristics. Although SV was correlated with protein, lines having LMWGS 2 in general had SV values above the mean, irrespective of protein content. MDT showed no association with any of the other quality characteristics, which is contrary to other studies (DuCros 1987, Kovacs et al 1993a). The high correlation between MPH and MTE is expected since peak height is a major determinant of the area under the curve.

The pattern of correlations were similar for lines homozygous for either LMWGS 2 or LMWGS 2^{-} (data not shown). The reliability of correlations of quality characteristics between the two crop years is assumed because of small differences between Spearman rank and Pearson correlations.

A large proportion of durum wheat cultivars grown worldwide have HMWGS 6+8 and LMWGS 2^{-}. Some of the germ plasm being used as a source for other desirable characteristics, such as smut resistance, salt tolerance, and high pigment content, also has LMWGS 2. Selecting for progeny having the LMWGS 2 allele would be beneficial in further improving pasta cooking quality of future cultivars. Unlike the other quality test, selecting for the LMWGS2 allele can be performed at very early generations (seed from F2 plants or single head selections) where there is insufficient seed for any other quality tests. More importantly, the LMWGS2 allele can be selected from lines grown in growth cabinets, greenhouses, or winter nurseries, since the test is independent of the growing environment. Furthermore, since the LMWGS2 allele is independent of protein content, selection is based upon a different criteria than used for SV, PDV, MPH, or MTE tests, as all of these other tests were correlated with protein content. Selection for this allele can be achieved efficiently using the monoclonal antibody MAb45/8, although further improvements in the test to give discrimination between single half kernels, either homozygous for LMWGS2 or segregating for both LMWGS2 and LMWGS2^{-}, would be desirable.

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