

# Transgenic Enhancement of High-Molecular-Weight Glutenin Subunit 1Dy10 Concentration: Effects in Wheat Flour Blends and Sponge and Dough Baking<sup>1</sup>

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## ABSTRACT

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Dough strength is needed for efficient breadmaking quality. This property is strongly influenced in wheat (*Triticum aestivum* L.) by gluten seed storage proteins and, in particular, by high-molecular-weight (HMW) glutenin subunit composition. Experiments were designed to elevate expression of a key native HMW glutenin subunit (1Dy10) via genetic engineering and to determine whether resultant flours can be used in sponge and dough applications, the most common commercial bread-baking procedure. Both unblended and blended samples from transgenic and nontransgenic sister lines were tested, with blended samples being formed by addition to a control sample. Dough properties, as determined by farinograph evaluation, were improved by the transgene-encoded increases in 1Dy10 in both undiluted and blended flours. Mean farinograph stability of transgenic samples was twice that of the control, and blends with transgenic samples demonstrated increases in stabilities proportional

to the amount of transgenic flour included. Mean farinograph quality numbers of transgenic samples, and of all blends containing transgenic flour, were significantly higher than both the control and all nontransgenic treatments. In the sponge and dough bake procedure, undiluted transgenic samples induced lower scores, relative to both control and undiluted nontransgenic samples, for water absorption, crumb body firmness, and loaf volume. In blends, however, the transgenic samples resulted in improvements in some sponge and dough loaf attributes, including loaf symmetry and crumb color score, without any concomitant loss of loaf volume in transgenic blends. These improved variables relate to finished product appearance and to consumer selection in markets. The use of transgenic flours with increased 1Dy10 glutenin content in commercial blends could provide advantages in sponge and dough bake applications.

High-molecular-weight (HMW) glutenin subunits are the most important determinants of breadmaking quality, and subunit composition explains a large percentage of the variability observed between genotypes (Shewry et al 1994; Weegels et al 1996). Genetic engineering has been used to elevate the expression of these important proteins (Altpeter et al 1996; Blechl and Anderson 1996; Barro et al 1997; Blechl et al 2007; Rakszegi et al 2008; Leon et al 2009), often resulting in the production of over- or super-strong doughs, especially when the overexpressed subunit is 1Dx5 in backgrounds already containing the *Glul-D1-b* allele encoding HMW glutenin subunits 1Dx5 and 1Dy10 (Rooke et al 1999; Alvarez et al 2001; Popineau et al 2001; Barro et al 2003; Darlington et al 2003; Blechl et al 2007). Based on mixing properties in 2 g mixographs, Rooke et al (1999) were the first to suggest that such wheats might be suitable sources of flours that could be blended to improve the gluten strength of weaker flours. This hypothesis was tested on field-grown samples of one of the transgenic wheats made by Rooke et al (1999) that overexpressed 1Dx5 in a background that contained subunits 1Ax1, 1Dx5, 1Dy10, 1Bx17, and 1By18 (Darlington et al 2003). Blends containing between 10 and 50% of the transgenic wheat were made with flours from Hereward, a cultivar classified as good for breadmaking, and subjected to the Chorleywood bread process (Darlington et al 2003). Loaf volumes declined proportional to the levels of the transgenic flour added. No other quality characteristics of those blends were reported (Darlington et al 2003).

Most previous investigations on the effects of transgene-encoded HMW glutenins on mixing and baking have evaluated derived lines in one-step straight-dough applications (Vasil et al 2001;

Graybosch et al 2011). However, such applications rarely are used in commercial settings (Ross and Bettge 2009; Cavanagh et al 2010), especially in the United States, where sponge and dough procedures predominate. (See Ross and Bettge [2009] for a complete description and comparison of the procedures.) However, the straight-dough method is still widely used in breeding and genetics programs, as it requires less grain for complete and replicated analyses, is more sensitive to flour quality, and thus can more readily differentiate genotypes early in the breeding process. For the present investigation, we generated sufficient seed to evaluate transgenic and nontransgenic sister lines with the sponge and dough procedure. Our goals were to determine how transgenic flours characterized previously as overly strong in straight-dough applications performed in sponge and dough applications and whether such wheats can be used as blending wheats.

## MATERIALS AND METHODS

Plant materials and transformation events were described by Graybosch et al (2011). The present investigation, however, used only lines derived from transgenic event B52a-6. This event is characterized by transgene-driven overexpression of native HMW glutenin subunit 1Dy10 and by the presence of two novel proteins that migrate in sodium dodecyl sulfate polyacrylamide gel electrophoresis in the zone between native wheat HMW glutenin subunits 1Dx5 and 1Bx7 (Graybosch et al 2011). This event was selected because its effects on straight-dough mixing, although dramatic, were more benign than those observed for the other investigated events (Graybosch et al 2011).

Three breeding lines (MD2069, MD2070, and MD2072) carrying the transgenes and three nontransgenic sister lines (MD2078, MD2083, and MD2087) were selected for analysis. Transgenic and nontransgenic sister lines were derived from the pedigree B52a-6/Jagger//Heyne (Graybosch et al 2011). In addition, two hard white wheat controls, Trego (medium dough strength) and NW99L7068 (weak dough strength), were utilized. Trego is a hard white winter wheat cultivar developed by Kansas State University. NW99L7068 is a hard white wheat experimental breeding line developed by USDA-ARS with the following pedigree: KS84HW1968 \* RioBlanco/HBY762A/4/Sumner/CO820026/2/PI372129/3/TAM-107. To obtain adequate flour for sponge and dough applications, composite samples were used. All lines and

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controls were grown in three-replication studies at the University of Nebraska Agricultural Research and Development Center, Ithaca, NE, U.S.A. (41.176°N, 96.470°W) during harvest years 2005, 2006, and 2007. Composite samples were made from each transgenic and nontransgenic sister line by combining flour samples from the three harvest years. Grain yields differed over the three harvest years (Graybosch et al 2011). Consequently, composites contained different amounts of flour from each harvest year; equal amounts of flour, however, were used from each experimental line or cultivar within each harvest year. Quality characteristics and proximate analyses of wheat samples were reported earlier (Graybosch et al 2011). A control flour sample was developed by combining all flour samples of Trego and NW99L7068. Individual flour blends were produced for each transgenic and nontransgenic line by addition to the control flour sample. Blends were produced at addition rates of 50% transgenic (or nontransgenic) to 50% control and 25% transgenic (or nontransgenic) to 75% control.

The amount of HMW glutenin subunit 1Dy10 in each blend was monitored to determine the efficiency of blending. Glutenin proteins were extracted and separated with an Agilent 2100 bio-analyzer (Agilent Technologies, Palo Alto, CA, U.S.A.) as described by Uthayakumaran et al (2005). Mean values for the amount of subunit 1Dy10 present in each sample were calculated from four independent runs. Values were expressed as percentage of total HMW glutenin protein. Protein concentration of flour samples was determined by nitrogen combustion (AACC International Approved Method 46-30.01), with the assay completed in triplicate. Farinograph evaluations (AACCI Approved Method 54-21.02) were conducted in duplicate.

Straight-dough (pup) loaves were produced from 100 g flour samples following AACCI Approved Method 10-10.03. The following variables were recorded: bake absorption (%), bake mix time (min), dough weight (g), loaf weight (g), crumb score (0–6), and loaf volume (mL). The sponge and dough procedure utilized a sponge formula of 490 g of flour, 5.6 g of instant dry yeast, 3.5 g of yeast food, 294 mL of H<sub>2</sub>O, and 35 mL of malt solution. The sponge was mixed in a Hobart A-120 mixer (Troy, OH, U.S.A.) with a McDuffy bowl and fork agitator. Ingredients were mixed for 1 min at speed setting 1 and mixed again for 1 min at speed setting 2. Subsequently, the sponge was allowed to ferment for 4 hr at 28.9°C in a covered cabinet. Dough ingredients were 210 g of flour, 49 g of granulated sucrose, 21 g of all-purpose shortening, 14 g of NaCl, 0.98 g of calcium propionate, and 119 mL of H<sub>2</sub>O. Doughs were mixed for 30 sec at speed setting 1; subsequently, the sponge was added and mixed for 30 sec, again at setting 1, followed by mixing at speed setting 2 until optimal dough development was attained, as judged by the bake techni-

cian. The fully mixed dough was allowed to rest for 20 min at 28.9°C in a covered cabinet. The dough was divided into two 534 g pieces, rested 10 min, and molded with a Moline molder (Duluth, MN, U.S.A.). Loaves were proofed to height (20 mm above pan, 90 mm total height) at 43.3°C and baked for 20 min at 215.6°C. During and after the sponge and dough process, the following variables were recorded: sponge score (0–5), dough score (0–30), loaf symmetry score (0–10), crust color (0–10), break and shred score (0–10), external score (0–30), grain score (0–10), crumb body firmness (0–10), crumb color score (0–10), internal score (0–40), total baking score (0–100), bake absorption (%), bake mix time (min), loaf volume (mL), specific volume (mL/g), and dough proof time (min). Both straight-dough and sponge and dough procedures were conducted in triplicate. Higher values for all scored variables indicated more desirable characteristics.

SAS version 9.1 for PC (Cary, NC, U.S.A.) was used for all computations. Analysis of variance (Proc GLM) was used to test for differences among treatments and among lines within treatments. Treatments were as follows: control, 100% transgenic, 100% nontransgenic, 50% transgenic (or nontransgenic) plus 50% control, and 25% transgenic (or nontransgenic) plus 75% control. "Lines" designated the three transgenic (or nontransgenic) lines used within each treatment. Duncan's multiple range test was used to compare treatment means. ANOVA and mean comparisons are presented only for those variables with significant mean squares. SigmaPlot version 11.0 (Systat Software, San Jose, CA, U.S.A.) was used to correlate bake mix times and loaf volumes of the two bake methods, using the mean values of each line and blend.

## RESULTS AND DISCUSSION

Mean flour protein concentration (Table I) of the undiluted transgenic samples was slightly higher than that of the nontransgenic sister lines; both classes had protein contents significantly higher than the control. The flour protein content advantage of the transgenics was maintained in the blends. Although significantly higher statistically, the advantage was slight and likely of little practical consequence. The mean concentration of 1Dy10 in the transgenic samples was 51.45%, nearly twice that of the mean of the nontransgenic sister lines (Table I). The control sample, formed from a blend of Trego (1Dx5+1Dy10) and NW99L7068 (1Dx2+1Dy12), contained only 8.57% subunit 1Dy10. Observed 1Dy10 concentrations in all flour blends were intermediate between their respective originating flours.

No differences in mean farinograph absorptions were detected among the treatments. However, farinograph characterization revealed stark differences between transgenic and nontransgenic

TABLE I  
Mean Squares from Analysis of Variance and Treatment Mean Comparisons for Flour Protein and Farinograph Characteristics

Flour Protein and Farinograph Variables	Flour Protein (%)	1Dy10 <sup>a</sup> (%)	Farinograph				
			Peak Time (min)	Stability (min)	Breakdown (min)	Tolerance Index (BU)	Quality Number (score)
Source of variation <sup>y</sup>							
Treatment	1.81*	0.19*	1,137.10*	1,472.56*	1,532.12*	74.03*	167.78*
Line (treatment)	0.11*	0.002	12.97*	14.12*	16.16	19.12	1.77
Mean comparisons <sup>z</sup>							
Control	10.87g	8.57e	12.73d	29.03d	25.50d	11.33ab	255.00d
Transgenic	12.37a	51.45a	42.12a	58.94a	58.32a	7.89abc	583.22a
Nontransgenic	12.23b	26.15c	14.08d	26.26e	24.30d	7.00cd	243.00d
50% transgenic, 50% control	11.78c	34.35b	30.14b	49.97b	47.97b	12.11a	479.67b
50% nontransgenic, 50% control	11.63d	19.11d	11.72d	26.89de	26.19d	4.44d	261.89d
25% transgenic, 75% control	11.40e	24.69c	20.71c	38.82c	39.04c	4.33d	390.44c
25% nontransgenic, 75% control	11.32f	17.09d	12.49d	27.41de	25.92d	9.44abc	259.22d

<sup>a</sup> 1Dy10 (%) = amount of HMW glutenin subunit 1Dy10 as a percentage of total HMW glutenin protein.

<sup>y</sup> \* indicates significant differences at *P* = 0.05.

<sup>z</sup> Means followed by the same letter did not differ significantly.

samples and blends in other variables (Table I). The three most obvious effects of the transgenic samples were increases in peak time, stability, and time to breakdown. The mean peak time of the transgenic samples was more than three times as long as that of the control; mean peak times of the blends with either 50 or 25% transgenic flour were approximately 2 and 1.5 times, respectively, longer to peak than the control. Mean peak times of all nontransgenic treatments did not differ from the control. Mean farinograph stability of transgenic samples was twice that of the control, and blends with transgenic samples demonstrated increases in stabilities proportional to the amount of transgenic flour included. Mean times to breakdown of all transgenic treatments were significantly higher than the control and higher than all nontransgenic treatments at similar levels of addition to the control.

In the straight-dough procedure, the most obvious effect of overexpression of 1Dy10 was the dramatic increase in bake mix times (Table II). Mean bake mix times of transgenic samples exceeded 28 min; the control sample had a bake mix time of 6.13 min, not significantly different from the mean of any nontransgenic treatments. Both 50/50 and 25/75 transgenic blends displayed mean bake mix times significantly greater than that of the control. Bake mix times of transgenic blends were directly proportional to the amount of transgenic flour present in the blend (Table II). Even in samples with only 25% transgenic flour, bake mix times still exceeded that of the control sample by approximately 4 min.

No additional improving effects of the transgenic flours were obvious in the straight-dough procedure (Table II). Mean bake absorption was higher in the undiluted transgenic treatment than in the control, but similar results were observed with the nontransgenic treatments. Thus, one is unable to conclude that this

effect resulted from the presence of the overexpressed 1Dy10 or from some other factor in this particular genetic background. Loaf volumes of all transgenic treatments, both undiluted and in blends, all were significantly lower than that of both the control and the nontransgenic sister lines. The majority of nontransgenic treatments had loaf volumes not significantly different from the control. Thus, the presence of overexpressed 1Dy10, even in the 25% blends, still depressed loaf volumes in the straight-dough procedure. No doubt the extra-strong gluten, as evidenced by the increased bake mix times, did not allow proper expansion of loaves during proofing or baking. These results were in agreement with those of Darlington et al (2003), who used the Chorleywood bread process to show that even flour blends containing 10% of a transgenic flour with increased levels of subunit 1Dx5 had reduced loaf volumes. The rheological properties of undiluted flours of that transgenic event suggested the presence of a highly cross-linked protein network (Popineau et al 2001). In contrast, transgene-encoded 1Ax1 resulted in only moderate increases in elasticity and viscosity, as measured by rheology (Popineau et al 2001). Consistent with Popineau et al (2001), Vasil et al (2001) found that most of their flours with transgene-encoded HMW glutenin subunit 1Ax1, when subjected to the straight-dough process, made breads with equal or better loaf volumes than their nontransformed parent.

In the sponge and dough procedure (Table III), undiluted transgenic samples induced lower scores, relative to both control and undiluted nontransgenic samples, for bake absorption, crumb body firmness, and loaf volume. Bake mix times, however, were markedly increased by transgene-encoded increases in 1Dy10. The undiluted transgenic treatment demonstrated a bake mix time in this method four times longer than that of the control and three

TABLE II  
Mean Squares from Analysis of Variance and Treatment Mean Comparisons for Straight-Dough (Pup) Loaf Characteristics

Straight-Dough Characteristics	Bake Absorption (%)	Bake Mix Time (min)	Dough Weight (g)	Loaf Weight (g)	Crumb Score (0-6)	Loaf Volume (mL)
Source of variation <sup>y</sup>						
Treatment	4.69*	474.40*	25.81*	10.56*	37.05*	29,849.71*
Line (treatment)	4.60*	1.94	4.29*	2.91	1.13*	2,333.33
Mean comparisons <sup>z</sup>						
Control	61.67c	6.13d	169.87b	148.67a	3.83bc	891.67a
Transgenic	63.80a	28.86a	167.04c	145.96c	2.22d	728.89e
Nontransgenic	63.14ab	6.74d	171.57a	148.89a	4.70a	897.22a
50% transgenic, 50% control	62.78bc	14.37b	169.08b	146.70c	3.89bc	792.22d
50% nontransgenic, 50% control	63.01ab	6.51d	171.43a	148.14ab	4.17b	877.78ab
25% transgenic, 75% control	61.77c	10.06c	169.17b	147.10bc	4.28b	828.89cd
25% nontransgenic, 75% control	63.24ab	6.56d	171.56a	148.56a	3.63c	843.89bc

<sup>y</sup> \* indicates significant differences at  $P = 0.05$ .

<sup>z</sup> Means followed by the same letter did not differ significantly.

TABLE III  
Mean Squares from Analysis of Variance and Treatment Mean Comparisons for Sponge and Dough Characteristics

Sponge and Dough Loaf Variables	Bake Absorption (%)	Bake Mix Time (min)	Dough Score (0-30)	Loaf Symmetry Score (0-10)	Crust Color (0-10)	External Score (0-30)	Grain Score (0-10)	Crumb Body Firmness (0-10)	Crumb Color Score (0-10)	Total Baking Score (0-100)	Loaf Volume (mL)
Source of variation <sup>y</sup>											
Treatment	2.93*	348.37*	4.48*	1.15*	1.13*	3.09	2.19*	4.27*	4.51*	21.87*	37,786.03*
Line (treatment)	2.44*	14.50*	1.19	1.88*	1.19*	12.22*	1.96*	0.65	0.54*	44.81*	24,027.14*
Mean comparisons <sup>z</sup>											
Control (C)	58.00a	6.00e	24.67b	8.17c	8.33ab	24.50b	7.33c	10.00a	8.00d	83.33c	2,391.67a
Transgenic (T)	56.89cd	23.33a	27.72a	8.89ab	8.11b	24.83ab	8.67a	7.78c	10.00a	87.33ab	2,212.50b
Nontransgenic (N)	58.22a	8.33c	27.17a	8.56bc	8.44ab	25.56ab	8.61a	9.11ab	9.33b	89.11a	2,398.61a
50% T, 50% C	56.67d	13.00b	27.44a	9.22a	8.67ab	26.44a	8.89a	8.89b	9.17b	89.61a	2,354.44a
50% N, 50% C	57.33b	5.67f	27.22a	8.61abc	9.11a	26.00ab	8.44a	9.67ab	8.50c	88.72ab	2,383.89a
25% T, 75% C	56.67d	8.00d	27.11a	8.61abc	8.89ab	25.83ab	8.22ab	9.11ab	8.44c	87.61ab	2,370.94a
25% N, 75% C	57.11bc	6.00e	26.39a	8.11c	9.00a	25.22ab	7.56bc	9.78ab	7.94d	86.01bc	2,370.94a

<sup>y</sup> \* indicates significant differences at  $P = 0.05$ .

<sup>z</sup> Means followed by the same letter did not differ significantly.

times longer than the undiluted nontransgenic treatment. Mean bake mix times of the transgenic blended treatments were intermediate between those of the undiluted transgenic treatment and the control. The mix times of individual samples observed in the two bake methods were significantly and positively correlated by the following equation:

$$y = 1.36 + 0.77x; r^2 = 0.96,$$

where  $y$  = sponge and dough mix time,  $x$  = straight-dough mix time, and  $n = 19$ .

Mean loaf volumes of the 50 and 25% blended transgenic treatments (Table III) did not differ significantly from that of the control or from any nontransgenic treatments. Loaf volumes produced by the two bake methods were significantly correlated and predicted by the following equation:

$$y = 1,758 + 0.72x; r^2 = 0.46,$$

where  $y$  = sponge and dough loaf volume,  $x$  = straight-dough loaf volume, and  $n = 19$ .

Comparison of the two methods suggests that the straight-dough procedure was actually more sensitive to differences induced by the overexpression of 1Dy10. In all transgenic samples, both undiluted and blended, straight-dough loaf volumes were significantly depressed. In the sponge and dough application, loaf volume was depressed only in the undiluted transgenic treatment. This observation suggests that before future transgenic events are declared useless, they should be tested with methods that more closely resemble those used in commercial settings.

The total baking scores of all transgenic treatments, either undiluted or in blends, were significantly higher than that of the control. However, nontransgenic treatments had identical effects: improved total score without any statistically significant loss of loaf volume. Again, one is unable to conclude that the improved total baking score of the transgenic sample arose from the increased concentration of 1Dy10 or merely resulted from the presence of the transgene in this particular genetic background. The transgenic samples did, however, provide obvious advantages in specific attributes, including loaf symmetry score in blended samples and crumb color scores in all treatments. The undiluted and 50% transgenic treatments had mean scores for these variables that were statistically greater than those observed for the control. Similar trends were observed for the nontransgenic treatments; however, the observed effects were greater in the transgenic samples. For example, the loaf symmetry score of the 25% transgenic treatment was significantly greater than that of the 25% nontransgenic sample and was equal in magnitude to that of the 50% nontransgenic treatment. These two variables, loaf symmetry score and crumb color score, no doubt relate to finished product appearance and perhaps to consumer selection in markets. The transgenic samples, therefore, resulted in improvements in some sponge and dough attributes, often without any concomitant loss of loaf volume.

Although blending with transgenic flours improved some attributes of the control flour, the control sample also improved or corrected defects of transgenic flours. Straight-dough crumb scores and loaf volumes and sponge and dough crumb firmness improved when transgenic blends were compared with undiluted transgenic flours. Thus, transgenic flours may be combined with typical wheat flours to develop commercial flours with superior attributes for many different traits, allowing one to truly consider the creation of "designer flours." One trait that did not appear to respond to blending was bake absorption in the sponge and dough procedure using either the transgenic or nontransgenic flours.

In the present investigation, three different transgenic lines of the same pedigree were used. Significant effects of lines within treatments (Tables I-III) were observed for many traits, including

flour protein content, farinograph peak time and stability, and several straight-dough and sponge and dough characteristics. Such observations indicate transgenic lines with the same transgene locus vary in some attributes, and they suggest interactions between transgenes and additional biochemical and genetic attributes. Similar interactions long have been known to occur with native HMW glutenin subunits (Kostler et al 1991). Future evaluations of transgenic events, therefore, should be conducted in multiple genetic backgrounds.

## CONCLUSIONS

The sponge and dough procedure is widely used in the United States; indeed, the majority of mass-produced bread arises from this method (Kulp and Ponte 2000). It also is a demanding procedure, especially with regard to gluten strength requirements (Ross and Bettge 2009). The present investigation indicated transgenic wheat lines overexpressing subunit 1Dy10 could provide additional gluten strength in these applications without sacrificing final product appearance and volume. In addition, such lines would allow millers to offer bakers flour samples with defined mix time requirements for automated systems, especially in crop years in which mix times might be depressed by adverse environmental conditions. Finally, bakers could avoid the need to add gluten during bakery operations, if preblended flours fulfilling the same needs could be developed by ingredient suppliers. The effects on dough strength of adding flour from transgenic event B52a-6 were clearly proportional to the amount added and were easily monitored and predicted with the farinograph.

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