Soil Moisture Effects on Bread Loaf Quality and Evaluation of Gliadins with Reversed-Phase High-Performance Liquid Chromatography[1]

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ABSTRACT

The effects of field soil moisture on hard wheat protein fractions influencing bread baking quality have not been characterized. Four spring wheat accessions (Newana, Fortuna, MT 7819, and MT 8182) were replicated four times across a controlled soil moisture gradient at Manhattan, MT, in 1986 and at Huntley, MT, in 1987. The soil moisture gradient was established with a modified line-source sprinkler irrigation system to provide four moisture regimes at Manhattan and five at Huntley. Grain samples from each plot were milled, baked, and evaluated for loaf volume and crumb score. Gliadins were extracted with 70% (v/v) ethanol and analyzed by reversed-phase high-performance liquid chromatography using a modified linear gradient. The relative area (expressed as a percentage) of a group of late-eluting gliadin peaks (referred to as the quality gliadin fraction) to the total chromatogram area increased with increased evapotranspiration (ET) for Newana and MT 8182 at both sites and for MT 7819 at Huntley. The relative ratio of the quality gliadin fraction to total chromatogram area was negatively associated with loaf volume. However, no association was found between increased ET and relative quality gliadin fraction for Fortuna at either site, although loaf volume was greatly increased over increased ET for this accession at Huntley.

Breadmaking characteristics of wheat flour are dependent on protein quality. Gliadin and glutenin endosperm storage proteins are important to the breadmaking capability of flour. Hamada et al (1982) reported that the glutenin fraction of the protein solubility classes defined by Osborne (1907) imparted toughness and strength to gluten. Finney et al (1982) utilized solubility and ultracentrifugation to separate gliadins and glutenins, which showed no loss of functionality when reconstituted into doughs. These fractions were interchanged between good and poor baking quality flours to define the functional breadmaking role of each class. Their study indicated that loaf volume potential was related to gliadin quality and mixing requirements were glutenin quality functions. They also indicated that cultivars that have good quality glutenins also commonly produce good quality gliadins.

Damidaux et al (1978), using aluminum lactate-polyacrylamide gel electrophoresis (AL-PAGE), identified a pair of allelic gliadin bands whose presence or absence was strongly correlated with durum wheat gluten strength. These components of the γ-mobility gliadins as classified by Woychik et al (1961) were designated bands 42 and 45. These conferred poor and good quality durum dough characteristics, respectively. Burnouf and Bietz (1984), using reversed-phase high-performance liquid chromatography (RP-HPLC), identified durum wheat chromatogram peaks that corresponded to PAGE bands 42 and 45. These peaks eluted in the more hydrophobic (late-eluting) region of the chromatogram, and their relative areas gave durum quality rankings similar to those based on the electrophoretic bands. Huebner and Bietz (1986) found a similar correlation between a group of late-eluting gliadin components and breadmaking quality in the bread wheats. This group of gliadin components, which they called the baking quality gliadin fraction, contained peaks with elution times similar to the quality-related durum wheat protein peaks. Furthermore, they found a high negative correlation between the area under the baking quality gliadin fraction peaks, expressed as a percentage of the total gliadin area, and baking score.

Lee and Ronalds (1967) stated that gliadins are not quantitatively affected by environment. They noted that the gliadin banding pattern of a given genotype determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) did not change when the wheat cultivar was grown under several environments. However, PAGE does not lend itself well to quantification of these components, since different proteins have very different dye-binding capacities that affect densitometric measurements of protein band content (Lawrence et al 1970). Quantification of protein components has improved significantly with the utilization of HPLC. This technique permits more uniform detection of all protein components present in the eluent by measuring absorbance at 210 nm.

The objectives of this study were to determine the effects of differential soil moisture levels on flour protein content, gliadin components, loaf volume, and crumb score of selected hard spring wheats.

MATERIALS AND METHODS

Four spring wheat accessions (Newana, Fortuna, MT 7819, and MT 8182) were randomized within four replications at each site under a line-source irrigation system. Rows were planted perpendicular to the irrigation line with 30.5-cm spacing (12-row plots at Manhattan and eight-row plots at Huntley). A 3.7-m border was planted around the experiment at each site. Plots were uniformly fertilized for maximum yield under high irrigation, based on analysis of soil samples from each site.

Four irrigation regimes (zero, low, medium, and high) were evaluated at Manhattan, and Huntley had five irrigation regimes (zero, low, medium, medium-high, and high). Irrigation main plots were 3.7 m long at Manhattan and 4.0 m long at Huntley. Decreasing field moisture regimes were superimposed perpendicular to the irrigation source with a line-source irrigation system similar to the one described by Hanks et al (1976). The system utilized model 25 sprinkler heads with 4-mm nozzles (Rain Bird Sprinkler Mfg. Co., Glendora, CA). Sprinkler pipe diameter was 5 cm at Manhattan and 7.5 cm at Huntley. Sprinkler heads on 2.5×90 cm risers were spaced 4.6 m and 6.1 m apart at Manhattan and Huntley, respectively. Irrigations were applied when wind speed was judged to be less than 2.3 m/sec to minimize drift.

Irrigations were applied 27, 40, and 80 days after planting at Manhattan, and 14, 22, 29, 60, 67, 83, and 92 days after planting at Huntley. Applied water was measured in each moisture regime using catch cans placed at plant canopy height. Growing season precipitation was measured daily at each site.

Soil moistures at planting and harvest for each location were used to calculate seasonal evapotranspiration (ET) from the water balance equation, described by Rose (1966): ET = change in soil waters precipitation + irrigation − runoff − deep drainage. Runoff was minimized by intermittent sprinkler operation, and deep drainage was negligible.

A 1.2×2.4 m area was hand-harvested from the center of each plot at Manhattan and a 1.5×3.4 m area was combine-

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harvested from each plot at Huntley for protein and bread baking quality analyses. Grain samples from each plot (1,300 g) were cleaned, weighed, tempered to 16% moisture using AACC method 26-10 (1983), and milled to approximately 70% flour extraction on a Buhler laboratory flour mill (type MLU-202) according to AACC method 26-20 (1983). Flour extraction rate was calculated for each sample as the ratio of recovered flour weight to the combined weights of all recovered milling fractions.

Flour from each plot was collected individually from the mill for test baking. A subsample of flour was taken from each mill sample for protein determination using NIR spectroscopy (Technicon InfraAnalyzer 400, Technicon Instrument Corp., Tarrytown, NY) as indicated by AACC method 39-11 (1983), and also for HPLC analysis.

Two test loaves (100 g) were made from each flour sample using the optimized straight dough method, method 10-10B (AACC 1983). Loaves were characterized by loaf volume and crumb score (rated on a scale of 1 = poor to 10 = very good). Crumb score was visually rated against loaves from "Mello Judith," a commercial baking flour. These standard loaves were assigned a crumb score value of five.

Means over field replications were used for regression analyses to determine associations among the parameters. Significance of curvilinear contributions to the regression models and of differences between regression response curves among accessions were tested using F values from the general linear test approach outlined by Neter and Wasserman (1974). A t test was used to determine slope differences (Lund 1987).

HPLC Sample Preparation

Flour samples (25 mg) from each plot were suspended in 1 ml of 70% (v/v) aqueous ethanol with a vortexer. Suspensions were allowed to stand for one hour at room temperature and centrifuged at 15,600 × g for 10 min. Supernatants were removed and pellets were discarded. Ethanol extractions were analyzed directly by HPLC.

HPLC System and Run Conditions

The HPLC system consisted of a Spectroflow 430 gradient former, a Spectroflow 400 solvent delivery system, and a Spectroflow 757 variable wavelength absorbance detector (Kratos Analytical Instruments, Ramsey, NJ). Analyses were performed using a 250 × 4.1 mm SynChropak RP-P (C_{18} analytical column. Column temperature was maintained at 70°C with an HPLC column water jacket (Alltech Associates, Inc., Deerfield, IL) and a VWR 1130 circulating water bath (VWR, Seattle, WA). All solvents were HPLC grade. Solvent A was distilled water and 0.1% trifluoroacetic acid (TFA) and solvent B was acetonitrile and 0.1% TFA. A modified linear gradient (25–50% solvent B over a 55-min period with a 15-min isocratic hold at 39% solvent B concentration) was used with a flow rate of 1.0 ml/min. An additional 5-min isocratic hold at final gradient conditions allowed for lag time between the gradient former and the detector. The gradient was returned to initial conditions over a 3-min period and allowed to equilibrate for 10 min prior to subsequent injections. Total run time between sample injections was 88 min. Random blank runs showed no protein carry-over between sample runs. The function of the 15-min isocratic hold was to achieve spatial (and temporal) separation of the late-eluting gliadin peaks from the other gliadin components. Samples were injected manually using a 20-μl sample loop, which was flushed and filled by injecting four times the loop volume.

Eluents were detected at 210 nm, and data were processed and plotted on a Chromatopac C-R3A integrator (Shimadzu Scientific Instruments, Columbia, MD) at 0.2 absorbance units full scale/8 mV. Chromatograms were automatically corrected for baseline drift at run time and stored on an IBM PC-XT computer for replotting. Replotted chromatograms were standardized to the height of the peak eluting at 38 min to facilitate visual comparisons. Total chromatogram area utilized for analyses excluded the area of the solvent peaks (first 6 min of run). Areas of individual peaks were obtained from the integrator in both relative chromatogram units and as percentages of the total chromatogram area. The area of a group of late-eluting gliadin peaks (referred to as the quality gliadin fraction, QGF) was calculated per chromatogram by summation of peak areas in the fraction. Relative QGF area was calculated as the ratio of the QGF area to total chromatogram area.

TABLE I
Mean Seasonal Evapotranspiration of Four Spring Wheat Accessions Grown Under a Controlled Soil Moisture Gradient at Manhattan and Huntley, MT

	Evapotranspiration[a] (mm)	
Irrigation Regime	Manhattan (1986)	Huntley (1987)
Dryland	331[b]	277
Low	457	317
Medium	548	378
Medium-high	... [c]	436
High	580	485

[a] Seasonal evapotranspiration = $SM_i + TWA - SM_f$, where SM_i is initial soil water content, TWA is total water applied (precipitation + irrigation), and SM_f is final soil water content.

[b] Mean values for each irrigation regime were averaged over accessions since evapotranspiration differences among accessions were not significant ($P > 0.05$).

[c] Four irrigation regimes were utilized at the Manhattan site (dryland [zero], low, medium, and high).

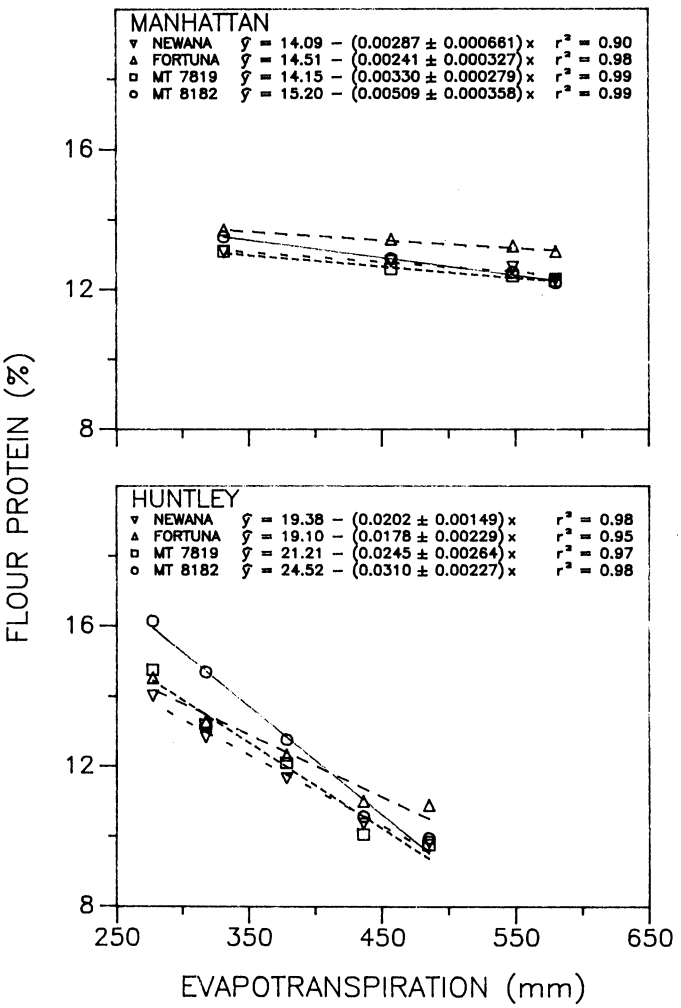

Fig. 1. Regression analyses of flour protein content (%) against seasonal evapotranspiration for four spring wheat accessions grown under a controlled soil moisture gradient at Manhattan and Huntley, MT, in 1986 and 1987, respectively.

RESULTS AND DISCUSSION

Evapotranspiration values ranged from 331 to 580 mm at Manhattan and from 277 to 485 mm at Huntley (Table I). Manhattan was the wetter of the two sites with nearly twice the initial soil water content and approximately 25% more precipitation than Huntley during the growing season.

MT 8182 had the greatest decrease ($P < 0.05$) in flour protein content as ET increased at both sites (14 to 12% and 16 to 10% at Manhattan and Huntley, respectively [Fig. 1]). Conversely, Fortuna had the smallest decrease ($P < 0.05$) in protein content at Huntley (14 to 11%). Regression slopes for flour protein content on increased ET were significantly different ($P < 0.05$) between MT 8182 and the other accessions at Manhattan and among all accessions, except between Newana and MT 7819 at Huntley.

Differences among chromatograms from samples grown under soil moisture extremes (dryland [zero] and high irrigation) for each accession at both sites are presented in Figure 2. The QGF area is shown as a group of peaks eluting between 52 and 62 min. As expected, some non-QGF peaks decreased with increased field moisture as QGF area increased. Major peak components had similar elution times among all accessions. However, corresponding individual peak areas differed among accessions.

Associations between relative QGF area and increased ET were strongest for Newana at Manhattan and MT 8182 at Huntley (Fig. 3). Slopes of relative QGF area regressed on ET were significantly different ($P < 0.05$) between Newana and the other accessions at Manhattan, and among all accessions, except between Newana and MT 7819 at Huntley. The response of relative QGF area to increased ET for Newana was similar at both sites, whereas the slope of the response for Fortuna did not differ from zero ($P > 0.05$) at either site.

Increased ET had a greater effect on loaf volume at Huntley than at Manhattan (Fig. 4). No regression slope differences for loaf volume with increased ET were found between Newana and MT 8182 or between Fortuna and MT 7819 at either site. However, significant differences ($P < 0.05$) occurred between the groups (Newana and MT 8182, Fortuna and MT 7819) at Huntley.

Decreased loaf volume and increased relative QGF area with increased ET were more evident for Newana and MT 8182 than

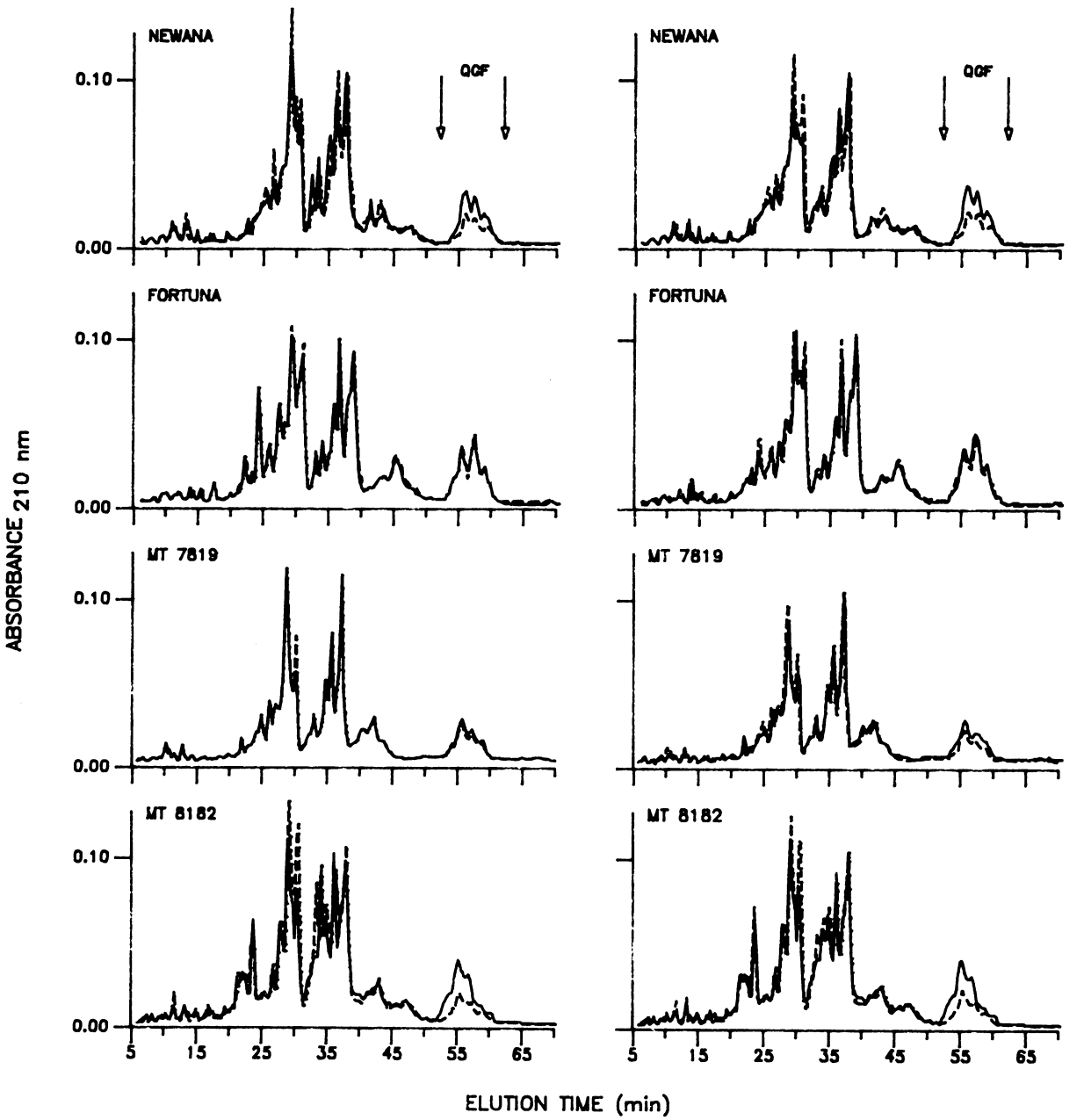

Fig. 2. Comparative chromatograms of four spring wheat accessions grown under dryland (- - -) and high irrigation (—) conditions at Manhattan (left column) and Huntley (right column), MT, in 1986 and 1987, respectively. Chromatograms were scaled to standardize the height of the peak eluting at 38 min. Quality gliadin fraction (**QGF**) area is designated between arrows at the extreme right of each chromatogram.

the other accessions at both sites. In contrast, Fortuna had very little increase in relative QGF area with increased ET at either site, but a large loaf volume decrease occurred with increased ET at Huntley.

Regression slopes for crumb score with increased ET were not significant ($P > 0.05$) for all accessions at Manhattan and significant ($P < 0.05$) for all accessions at Huntley (Fig. 5). MT 7819 regression slope for crumb score over increased ET differed

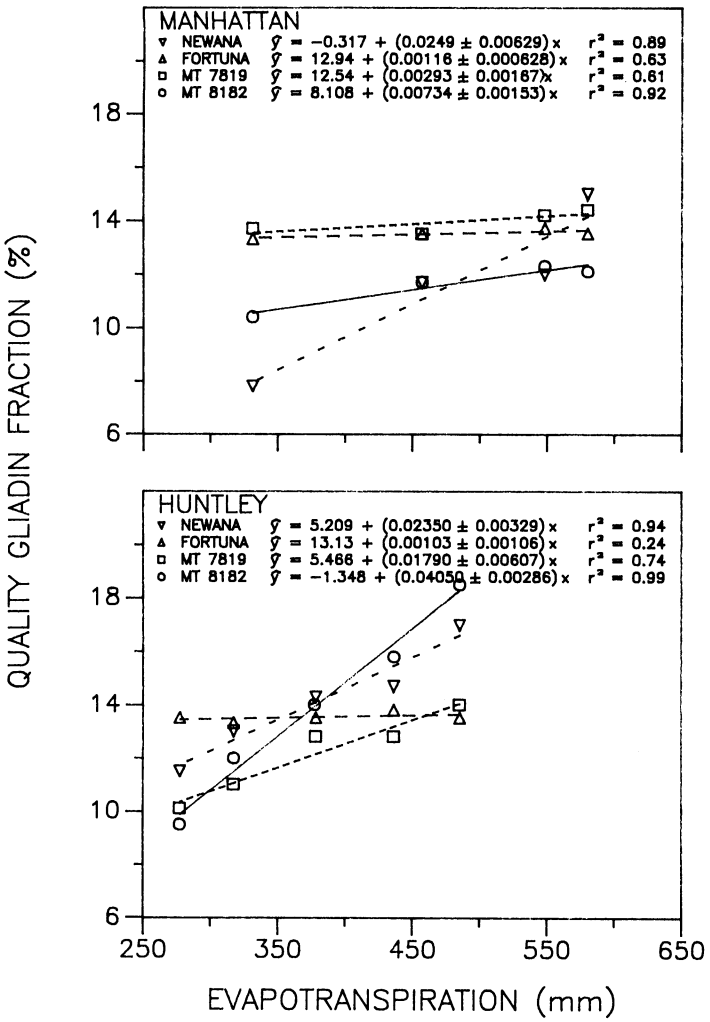

Fig. 3. Regression analyses of relative quality gliadin fraction peak area against seasonal evapotranspiration for four spring wheat accessions grown under a controlled soil moisture gradient at Manhattan and Huntley, MT, in 1986 and 1987, respectively.

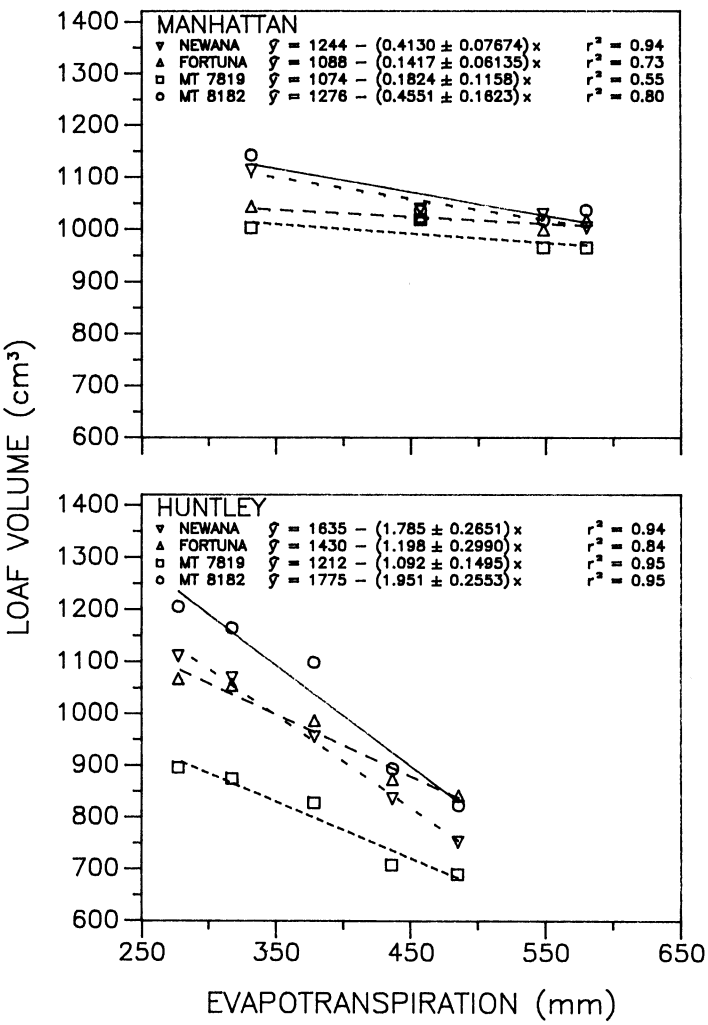

Fig. 4. Regression analyses of loaf volume against seasonal evapotranspiration for four spring wheat accessions grown under a controlled soil moisture gradient at Manhattan and Huntley, MT, in 1986 and 1987, respectively.

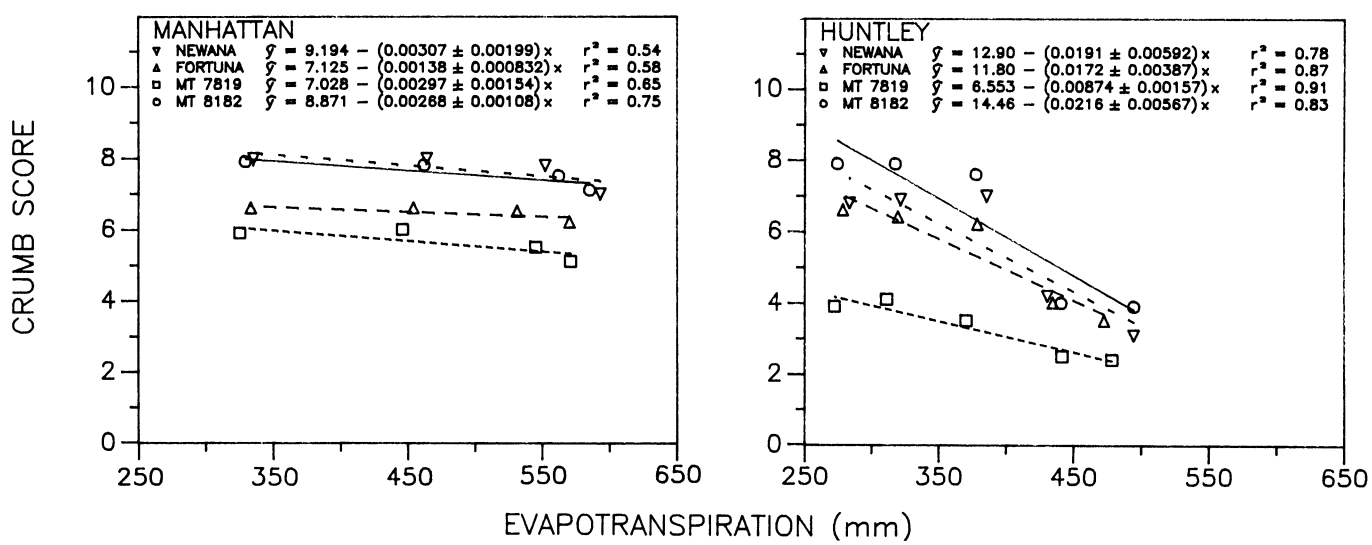

Fig. 5. Regression analyses of crumb score against seasonal evapotranspiration for four spring wheat accessions grown under a controlled soil moisture gradient at Manhattan and Huntley, MT, in 1986 and 1987, respectively.

significantly ($P < 0.05$) from the others at Huntley. Quadratic terms in the regression models made no significant contributions ($P > 0.05$) to fit of the points. Newana and MT 8182 had the highest crumb score over all moisture regimes at both sites. Additionally, crumb scores were consistently lower for MT 7819 than for the other accessions over all moisture regimes at both sites.

CONCLUSIONS

These data suggest that field soil moisture, as indicated by ET, may play a role in bread loaf volume and crumb score variability of some wheat accessions through interactions of environment with gluten components. The apparent negative association between loaf volume and relative QGF area for some wheat accessions is in agreement with results of accessions evaluated by Huebner and Bietz (1986). However, Fortuna appears to not vary the proportion of QGF with differential ET. Therefore, baking quality variation of Fortuna over environments is probably due to factors other than percent QGF.

The relationship between the late-eluting gliadins (QGF) and bread quality may not represent a cause-and-effect situation but may simply involve a correlation between gliadin quality and glutenin quality (Huebner and Bietz 1986). In either case, increased ET had a highly significant relationship with protein quality of some accessions that can be related to bread baking quality.

The apparent association between QGF and baking quality may provide a potentially useful and easily measured quality parameter for some hard wheats. However, percent QGF should be used with other quality screening parameters because there are germ plasm exceptions such as Fortuna. The apparent stability of QGF percentage in Fortuna may also represent a desirable character that, if bred into a broad array of hard wheat cultivars, might reduce the dependence of quality upon dry low-yield environments.

Comparisons of baking quality characteristics based on wheat samples grown under different environments may be somewhat biased by growing season moisture differences. These biases could be adjusted for by standardizing against a common accession or accessions at all sites. However, it is evident that soil moisture (or ET) and other environmental factors should be considered when evaluating wheat, protein, and baking quality differences.

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