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Genotype and Environment Effects on Gliadin Content and Polyphenol Oxidase Activity in Wheat

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The environment in which a given genotype is grown may influence its grain quality characteristics. When varieties are × evaluated over numerous environments, a variety environment interaction usually is observed, but the relative magnitude of environmental(E), genetic(G), and G × E effects on quality is unclear. In order to determine relative contribution of genotype, environment, and G × E interaction to the variations observed in grain quality characteristics, 18 Korean wheat cultivars and experimental lines were evaluated in two environments in 1998 and 1999. Correlation coefficients between grain quality and agronomic characteristics were also estimated. The analysis of variance for the optical density obtained by reaction bet- ween gliadin and anti-gliadin polyclonal antibody (AGPab) indicated that gliadin content measured by Enzyme-Linked Immunosorbent Assay (ELISA) was significantly in- fluenced by environment and cultivar differences. The significant differences of year and year × location were also found. The ratio of the variances associated with environmental effects to the variances associated with genetic effect gave relatively greater influence of environmental factor on gliadin content. The different protein content from same genotype grown in different environment might be associated with degree of storage protein accumulations. Significant relationships between ELISA and protein content, yield, ten spike weight, and ten spike number were detected. Polyphenol oxidase (PPO) activity was significantly influenced by year, location, cultivar and year × location. The variance in grain PPO activities among growing years appeared larger than the variation produced by the cultivar examined. This suggested that the growing environment contributed more to variability in grain PPO concentration.

Key words: wheat, gliadin, polyphenol oxidase (PPO), environment effect.

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Wheat end-use quality requirements are diverse and depend on the type of wheat and expected uses. Diversity of wheat quality are subjected to the effects of wheat's genotype (G) and growing environment (E). Selection of the best performers from among hundreds of experimental lines would be simplified if all cultivars achieved the same relative rankings when grown under a range of environmental conditions. Therefore, evaluation of wheat quality is essential for the production of suitable wheats that satisfy the consumers' needs. Developing, improving, and modifying test methods for the precise and accurate evaluation of wheat quality, are also is very important.

If cultivar by environmental interactions were not significant, researchers could reduce time and expense of obtaining quality data by evaluation of samples.

Understanding genetic and environmental influences responsible for variation in grain quality of wheat is important for the production and marketing of a consistent and high quality product. However, breeders have little information on quality variation attributable to differential responses of genotypes to environmental conditions.

Since breeders had difficulty in predicting the potential quality of new varieties because of the change in relative ranking of cultivars across environments, they continue to evaluate new strains in different environments (Baenziger *et al.*, 1985; McGuire *et al.*, 1974; Busch *et al.*, 1969). In general, multiple environments were needed to properly assess genotypic difference in quality; seasonal effects were usually greater than site effects. Genotypes varied in stability of quality criteria, with some cultivars more responsive than others to environments favorable for wheat quality. Estimated components of variance have also been used to assess locations and seasonal contributions (Campbell *et al.*, 1977; Ghaderi *et al.*, 1971).

Grain protein percentage is an important factor in the determination of the quality and nutrition of wheat. Part of the variation in grain protein percentage is genetic, but most of it is due to environmental conditions (Stoddard Marshall, 1990). Generally, increased grain protein content was accom-

panied by decreased yield (Terman *et al.*, 1969).

High levels of grain protein concentration and high grain yield are the primary objectives of bread wheat breeding programs. Achieving these objectives, however, has been difficult due to the large environmental components of variation in grain protein concentration and grain yield and because of the well documented negative correlation between these two economically important traits (Terman *et al.*, 1969). Gliadins are monomeric proteins, readily solubilized in aqueous-alcoholic solutions, and contain only intramolecular disulfide bonds. Gliadin, as a second important seed storage protein to gluten, is important in end-use qualities such as dough-making, loaf volume and viscosity (Fido *et al.*, 1997; Peleg, 1994). Therefore, ELISA enable to detect presence or absence of specific proteins with its specificity, convenience, and high level of sensitivity. It is also able to measure quality and quantity of specific proteins in which we are interested (Seo *et al.*, 1995). Development of wheat with high protein quality has been one of the important aim of wheat breeders. In this respect, employment of ELISA using antibodies specific to quality related proteins might be particularly useful to plant breeders for screening of high grain quality in wheats.

Color is one of the most important considerations in assessment of flour quality, particularly in regard to the quality of the end products such as noodles and bread. The presence of polyphenol oxidase (PPO) in wheat was verified (Hatcher Kruger, 1993; Kruger *et al.*, 1976; Abrol Uprely, 1970) and may be related to an undesirable brown discoloration of wheat-based end products during processing or storage (Baik *et al.*, 1995; Kruger *et al.*, 1994).

PPO activity and distribution within the wheat grain has been reported to differ with variety (Lamkin *et al.*, 1981) and stage of development (Kruger et al., 1976). Researchers (Lamkin *et al.*, 1981; Hatcher and Kruger, 1993) have measured PPO activities of wheat or wheat products using spectrophotometric or oxygen consumption monitoring methods. However, these assays are time-consuming and not suited to a plant-breeding and screening program. Kruger *et al.* (1994) have developed the quantitative assay for grain without the requirement for seed grinding. The assay on the whole seed gave a relationship that was equally as good as that of total ground grain PPO (Kruger *et al.* 1992). The outer layer, rather than the inner part of the bran, likely contains the bulk of the seed PPO because permeability and consequent reaction residual whole seed PPO with substrate occurred rapidly.

In most cases, this whole kernel assay using steep water will be the most convenient and sensitive because a better approximation of total PPO will be measured. Ninety six samples at a time can be run simultaneously by the microplate reader and reaction rates are calculated automatically. This method should be particularly useful to plant breeders for screening of PPO levels in wheats.

The objectives of this study were : I) to determine relative contribution of genotype, environment, and genotype and environment interaction to the variation observed in grain quality characteristics of 18 Korean wheat cultivars and experimental lines genotypes tested in two environments in 1998 and 1999; and II) to estimate correlation coefficient among grain quality and agronomic traits.

MATERIALS AND METHODS

Wheat samples

Grain samples were obtained from 18 Korean wheat cultivars and experimental lines grown at two locations [Deokso (Korea University Research Farm) and Suwon (National Agriculture Experimental Station)] in 1998 and 1999.

The seed was sawn in Deokso (Oct. 16, 1997 and Oct. 7, 1998) and Suwon (Oct. 15, 1997 and Oct. 22, 1998). The experimental design was a randomized complete block with three replications planted in each of two environments. The plots of both Deokso (5 m-long row) and Suwon (8 m-long row) were consisted of 5 rows spaced 30 cm apart. The plot area was 7.5 m^2 in Deokso and 12 m^2 in Suwon. Seeding rate of 7080 g/10 a was applied in both Deokso and Suwon. The names of tested Wheat cultivars and experimental lines are listed in Table 1.

Enzyme-linked immunosorbent assay (ELISA)

Methods used for the production and characterization of anti-gliadin polyclonal antybody (AGPab) were described elsewhere (Chang *et al.*, 1999).

Gliadins were extracted from 25 mg of flour with 1 ml of 70% ethanol by vortexing at 4°C for 1 hour. After centrifugation at 20,000 × g, supernatants were transferred for use in ELISA.

Each sample extract was diluted to 1 : 625 for use as antigen and 30 μl was applied to each well in the microtitrate plates. The plates were covered with polyethylene film to prevent evaporation and were incubated overnight at 37°C. After the plates were dried, the plates were blocked with 200 μg/well of 2% (w/v) BSA in phosphate-buffered saline solution (PBS, 136.9 mM NaCl, 2.7 mM KCl, 10.1 mM Na_2HPO_4, pH 7.4) for 90 minutes. After blocking, the solution was removed and the plates were washed three times with 0.2% (v/v) PBS-Tween solution. After washing, 50 μl of anti-gliadin polyclonal antibody solution, diluted to 1 : 400 in PBS, was added to each well. The plates were incubated at 37°C for 90 minutes, and then the anti-gliadin polyclonal antibody solution was removed. The plates were

washed as described above, and were incubated with 50 μl alkaline-phosphatase labelled goat anti-rabbit IgG (sigma) diluted with 1 : 2,500 in PBS for 90 minutes at 37°C. After unbound enzyme-labelled antibodies were removed by washing, as described above, 200 μl of 0.05% (w/v) p-nitrophenylphosphate in substrate buffer (2M diethanolamine, 2mM $MgCl_2$, pH 9.7 diluted with H_2O) was added to each well to allow alkaline-phosphatase and substrate reaction. After 30 minute incubation using a benchmark microplate reader (Bio-Rad), optical densities (OD) of the alkaline phosphatase reaction products were determined by the microplate reader fitted with a 405 nm filter.

Polyphenol oxidase (PPO) assay

Assay was performed by the whole grain PPO analysis of Kruger *et al.* (1994) with some modifications. Wheat grain of 2.5 g was steeped by 5 ml of distilled water by gentle rocking for 16 hrs at 23°C. Each well of microtiter plate was applied by 100 μl of steep solution with 3 replications. Each well was added by 150 μl of freshly prepared reaction buffer (1 g $C_6H_6O_2$, 200 mM Na_2HPO_4, 100 mM $C_6H_8O_7H_2O$). Reaction product was measured by a kinetic microplate reader (405 nm) at 30 minutes after incubation.

A mushroom PPO (84,000 units/mg solid, Sigma Chemical Company, St. Louis, MO) was used to provide standard curve with which the measured optical densities were converted into PPO activities. In order to keep accuracy of the procedure, all samples including standard mushroom PPO were replicated with three times within a plate. Two replica of each sample were also applied in different plates. The standard solution was made of 1 mg/ml in sterile water and stored at -80°C. It was diluted to 1/10 before each use. Three control cultivars ("Eltan", "Vista", and "Platte") were also used in this assay. Generally, Eltan and Platte had a known low PPO activity, and Vista had a known high PPO activity.

1D SDS-PAGE

The procedures for preparation and running gel were followed as described by Seo at al (1998). Flour samples used for protein extraction were provided by bulked grain samples obtained from each plot. Unreduced protein samples were obtained by omission of the dithiorythritol in the extraction solution. Treated samples of 8 μl were loaded in 12% SDS-polyacrylamide gel and ran for 3 hours at 40 watts.

Statistical analysis

Statistical analysis of the data obtained was ran with SAS/STAT programs (1995).

RESULTS AND DISCUSSION

I) Gliadin quantity

Anti-gliadin polyclonal antibody (AGPab) and antigen reaction

Different dilutions of AGPab reacted to antigens prepared by mechanical mixtures were observed to evaluate specific binding of different concentrations of antibody to different ranges of antigen concentrations, and to find optimum concentrations of antibody and antigen for further applications (Fig. 1).

Reactions of AGPab to gliadin were linearly decreased as AGPab and antigen were diluted. Reactions from different concentrations of antibodies and antigens gave higher coefficient of determination than 0.96 from the antigen ranges between $1/5^6$ to $1/5^3$. Therefore, AGPab could be applied in quantification of gliadin which was known to affect wheat end-use quality. There was a high coefficient of determination (r^2=0.975) for antibody dilution of 1/400 between antigen concentrations from $1/5^6$ to $1/5^3$. Antigen concentration between $1/5^6$ to $1/5^3$ generally gave high coefficient of determination. This result showed choose of antibody dilution ranges of near 1/400 for further use in gliadin quantification.

Variance analysis

Optical densities obtained from the reaction between AGPab and gliadins dilution (1/400) from per 25 mg flour sample (OD) were averaged over environments. The mean optical densities of the 18 wheat lines varied among cultivars and ranged from 0.741 for 'Olgeurumil' to 2.056 for 'Geurumil'. 'Gobunmil' had the highest two-year mean OD (1.487), whereas 'Alchanmil' had the lowest (1.169) (Table 1).

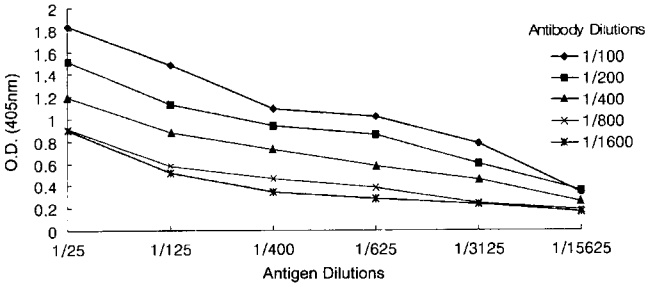

Fig. 1. Reaction of different concentrations of anti-gliadin polyclonal antibody on different concentrations of gliadin solutions. Coefficient of determination ranges between antigen dilutions from 1/15625 to 1/125 were r^2=0.970 r^2=0.969, r^2=0.975, r^2=0.919, and r^2=0.829, for antibody dilutions 1/100, 1/400, 1/800, and 1/600, respectively.

Table 1. Values for ELISA optical density(OD) and Polyphenol oxidase(PPO) activity of 18 Korean cultivars and experimental lines in two locations in 1998 and 1999.

Cultivars or lines	ELISA OD†	Cultivars or lines	PPO Activity (units/g)
	Mean		Mean
Gobunmil	1.487	Suwon 274	399.00
Suwon 277	1.449	Tapdongmil	391.85
Suwon 276	1.422	Suwon 275	357.00
Geurumil	1.389	Gobunmil	351.38
Keumkangmil	1.380	Eunpamil	343.94
Eunpamil	1.378	Keumkangmil	339.72
Suwon 258	1.361	Geurumil	314.76
Suwon 265	1.356	Suwon 258	300.24
Suwon 279	1.349	Olgeurumil	298.93
Suwon 261	1.338	Suwon 261	291.96
Suwon 280	1.328	Suwon 279	281.33
Tapdongmil	1.328	Alchanmil	275.46
Suwon 278	1.326	Suwon 280	270.43
Urimil	1.326	Suwon 265	269.18
Suwon 274	1.319	Suwon 276	254.85
Olgeurumil	1.272	Suwon 277	251.55
Suwon 275	1.234	Suwon 278	244.52
Alchanmil	1.169	Urimil	243.50
L.S.D.‡ (0.05)	0.137		50.80

†OD=optical density obtained from reaction between AGPab (1/400) and diluted gliadin (1/625) from 25 mg flour samples.
‡Least significant difference value for comparison of means.

Table 2. Mean squares for ELISA optical densities (OD) and polyphenol oxidase (OD) activity in grain of 18 Korean wheat cultivars and experimental lines.

Source	df	Mea square	
		ELISA OD	PPO (units/g)
Year	1	10.106**	644334**
Location	1	0.096	39077**
Cultivar	17	0.063**	28906**
Year×Location	1	0.283**	696402**
Cultivar×Year	17	0.025	7191*
Cultivar×Location	17	0.013	5129
Cultivar×Year×Location	17	0.035	3624

*,**significant at the 0.5, and 0.01 levels of probability, respectively.

Table 3. Ratios of variances estimated for environment and genotype main effects and their interaction for ELISA optical densities (OD) and polyphenol oxidase (PPO) activities from 18 wheat cultivars and experimental lines over 1998 and 1999.

Source	ELISA OD	PPO activity
Environment/Genotype	29.30	7.18
Environment/(Genotype×Environment)	44.39	66.17
Genotype/(Genotype×Environment)	1.52	9.21

The analysis of variance indicated that OD was significantly influenced by environment and cultivar differences (Table 2). The effects of year and year × location were also significantly influenced at the 1% level of probability.

Variance ratios of genotype, environment, and their interaction on ELISA optical densities were shown in Table 3. The very large influence of environment × relative to genotype for gliadin content as measured by ELISA was found. The ratio of genotype environment effect was found to be lower magnitude than individual genotype and environment effect (Table 3). Simple correlations were used to test for relationships of ELISA absorbancies with protein content and some agronomic traits (Table 4). Results showed numerous significant relationships between ELISA and protein content, yield, ten spike weight and ten spike number. The component traits of grain yield (number of spikes per plant, kernel number per spike, and kernel weight) were correlated negatively with OD, however protein content showed significant positive correlation. Numerous negative correlations between grain yield and grain protein percentage of cultivars across environments in this study are typical of wheat.

ELISA could be used to analyze influence of genotype, environment and genotype × environment interaction. In this study, optical density of ELISA was affected by genotype and environment. Since early generation screening for breeding programs requires methodologies that can accomodate the large number of samples in short period of time, use of ELISA can provide rapid screening breeding materials.

In this study, optical density of 18 Korean wheat cultivars and experimental lines were significantly affected by environment, and had also showed variable expression. Therefore, AGPab might be usefully applied to study genetics and

Table 4. Correlation coefficients between ELISA optical densities (OD) and polyphenol oxidase (PPO) activities and some agronomic traits in 18 Korean wheat cultivars and experimental lines (n=72).

	Protein content	Yield	Test weight	1000 Kernel weight	Kernel weight per ten spikes	Kernel no. per 10 spike
ELISA OD	0.634**	-0.401**	0.023	-0.117	-0.483**	-0.555**
PPO	0.048	-0.133	0.103	0.492**	0.45**	-

** significant at the 0.01 levels of probability

biochemistry of seed storage proteins and useful for measuring wheat quality characteristics.

For example, several highly significant correlations between ELISA variables and quality characteristics such as flour protein content and loaf volume, were found with other experiments (Seo *et al.*, 1995; Howes *et al*, 1989).

The end-use quality variation is derived from genetic and environmental factors, and their interactions (Peterson *et al.*, 1992). End-use quality is not only influenced by the compositions of protein subunits, but also by other factors including grain hardness, lipid composition, and starch granule distribution. For this reasons, the use of ELISA technology combined with other quality tests would be expected.

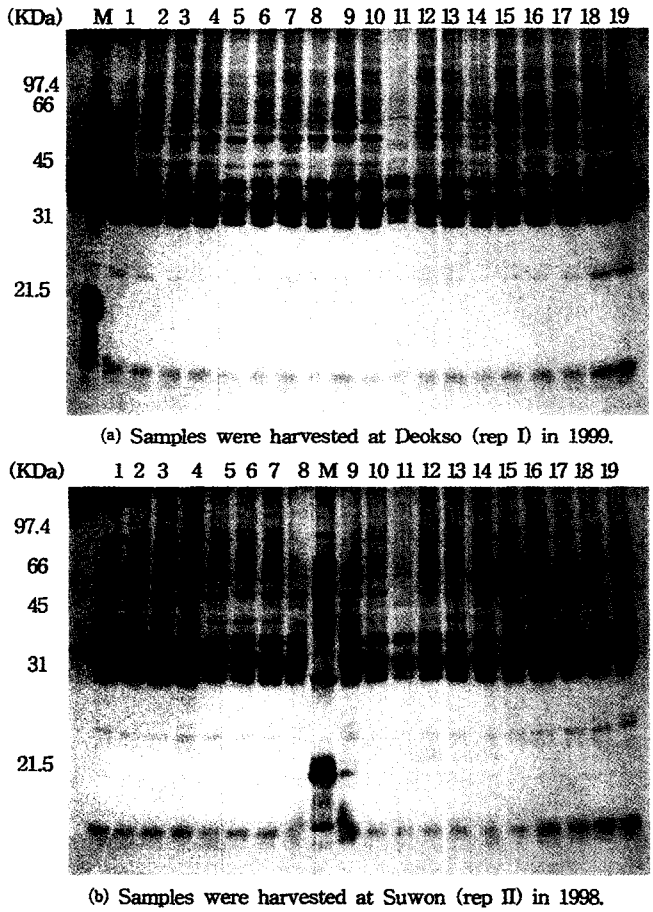

Fig. 2. Ond dimensional SDS-PAGE patterns of gliadin from 18 Korea wheat cultivars and experimental line. (a) samples were harvested at Deokso (rep I) in 1999 and (b) samples were harvested at Suwon (rep II) in 1998.
Lane 1: Urimil; 2: Tapdongmil; 3: Eunpamil; 4: Geurumil; 5: Alchanmil; 6: Olgeurumil; 7: Gobunmil; 8: Suwon 265; 9: Keumkangmil; 10: Suwon 274; 11: Suwon 275; 12: Suwon 276; 13: Suwon 277; 14: Suwon 278; 15: Suwon 279; 16: Suwon 280; 17: Suwon 261; 18: Suwon 258 19: suwon 258. M: molecular size marker.

SDS-PAGE Analysis

Gliadins extracted from 18 Korean wheats cultivars and experimental lines at each location in 1998 and 1999 were loaded in gels run in same conditions. One-dimensional SDS-PAGE pattern of the 18 Korean wheat samples was shown in Fig. 2(a, b). Since the subunit patterns of each line were identical to the plant grown in different locations and years, the expression of seed storage protein subunits was genetically fixed. Therefore, the different protein content of same genotype grown in different environment might be associated not with the presence and absence of specific subunits but with degree of storage protein accumulations. This results agreed with the result found by Stoddard and Marshall (1990).

II) Polyphenol Oxidase (PPO) Activity

Variance in polyphenol oxidase activity

Polyphenol oxidase (PPO) activity among Korean wheat cultivars and experimental lines ranged from 100 units/g for 'Suwon 276' in 1999 at Suwon to 613 units/g for 'Tapdongmil' in 1999 at Suwon. The means of PPO activity averaged environments were presented in Table 1. 'Keumkangmil' had the highest two-year mean PPO activity, 399 units/g, whereas 'Urimil' and 'Suwon 277' had the lower mean PPO activity, 243.50 units/g and 244.52 units/g, respectively.

The analysis of variance showed that PPO activity was significantly influenced by year, location, cultivar and year × location (Table 2). Relative influence of genotype and environment and their interaction were provided in Table 3. The greater influence of environment than effect of genotype on polyphenol oxidase activity was found. The variance in grain PPO activities among growing environment appeared larger than the variance produced by the cultivar examined. This suggest that the growing environment contributed more to variability in grain PPO concentration. Similar results were found by other researchers (Shelton *et al.*, 1997; Baik *et al.*, 1994). Baik *et al.* (1994) found differences in grain PPO activity between growing locations, when comparing grains grown in Australia with those from the United States. Shelton *et al.* (1997) also found there were genotypic differences or location effects on flour PPO activity in hard red wheat samples.

Correlations of PPO activity in grain with quality characteristics

Simple correlations were used to test for relationship of PPO activity with some agronomic traits and protein content (Table 4). Results showed numerous significant relationships between PPO activity and thousand kernel weight, ten spike weight and yield. No significant relationship of PPO activity with test weight and protein content was found.

Increasing one thousand kernel weight was related to increased levels of grain PPO activity in Korean wheat samples, which was a contradict to the result of Baik *et al.* (1994), in that, a negative correlation between one thousand kernel weight and PPO activity existed. Although posible differences of PPO concentration throughout the wheat kernel (Shelton *et al.*, 1997), the PPO enzyme that was leached out of the wheat kernel, or was near the surface of the bran and accessible to substrate, provided for a convenient quantitative assay that correlated well with the standard assay (Kruger *et al.*, 1994). Therefore, performing the assay on the whole seed, with the steep water gave a relationship that was equally as good as that of total ground grain PPO.

In this study, the environment contributed more to variability in grain PPO concentration among Korean wheat sample. This may indicate that enhancement of wheat-based end product quality is possible through the wheat breeding and selection strategies.

The PPO activities of Korean wheat cultivar also varied, generally showed low PPO activity compared with control variety, but the most important finding for the Korean wheat cultivars was that, at least of the limited number of samples examined, activities agreed with each other within a fairly narrow. Therefore, whole grain assay used in this study is rapid, convenient, and quantitative assay that correlates well with the standard assay. It is particularly relevant for plant-breeding purposes, where throughput is important.

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REFERENCES

Abrol, Y. P. and D. C. Uprety. 1970. Studies on darkening of whole wheat meal dough. *Curr. Sci.* 40:421.

Baenziger, P. S., R. L. Clements, M. S. McIntosh, W. T. Yamazaki, T. M. Starling, D. J. Sammons, and J. W. Johtson. 1985. Effect of cultivar, environment and their interaction and stability analyses on milling and baking quality of soft red winter wheat. *Crop Sci.* 22:5-8.

Baik, B. K., Z. Czuchajowska, and Y. Pomeranz, 1994. Comparison of polyphenol oxidse activities in wheats and flours from Australian and U.S. cultivars. *J. Cereal Sci.* 19:291.

Baik, B. K., Z. Czuchajowska, and Y. Pomeranz, 1995. Discoloration of dough for oriental noodles. *Cereal Chem.* 72:198.

Busch, R. H., W. C. Shuey, and R. C. Frohberg. 1969. Response of hard red spring wheat (*Triticum aestivum* L.) to environments in relation to six quality characteristics. *Crop Sci.* 9:813-817.

Campbell, L. G., and H. N. Lefever. 1977. Cultivar × environment interactions in soft red winter wheat yield tests. *Crop Sci.* 17: 604-608.

Chang, S. J., B. H. Hong, and Y. W. Seo. 1999. Development and characterization of anti-gliadin polyclonal antibody in wheat. *Korean J Crop Sci.* 44(4):339-344.

Fido, R. J., F. Bekes, and A. S. Tatham. 1997. Effects of α-, β-, γ- and ω-gliadins on the dough mixing properites of wheat flour. *J. Cereal Sci.* 26:271-277.

Ghaderi, A. and E. H. Everson, 1971. Genotype-environment studies of test weight and its components in soft winter wheat. *Crop Sci.* 11:617-619

Hatcher, D. W. and J. E. Kruger. 1993. Distribution of polyphenoloxidase in flour mill streams of Canadian common wheat classes milled to three extraction rates. *Cereal Chem.* 70:51.

Howes, N. K., M. I. Kovacs, and D. Leisle. 1989. Screening of durum wheats for past-making quality with monoclonal antibodies for gliadin 45. *Genome,* 32:1096-1099.

Kruger, J. E., 1976. Changes in the polyphenol oxidases of wheat during kernel growth and maturation. *Cereal Chem.* 53:201.

Kruger, J. E., B. Morgan, R. R. Matsuo, and K. R. Preston. 1992. A comparison of methods for the prediction of Cantonese noodle color. *Can. J. Plant Sci.* 72:1021-1029.

Kruger, J. E., D. W. Hatcher, and R. DePauw. 1994. A whole seed assay for polyphenol oxidase in Canadian Prairie spring wheats and it's usefulness as a measure of noodle darkening. *Cereal Chem.* 71:324.

Lamkin, W. M., B. S. Miller, S. W. Nelson, D. D. Traylor, and M. S. Lee. 1981. Polyphenol oxidase activities of hard red winter, soft red winter, hard red spring, white common, club, and durum wheat cultivars. *Cereal Chem.* 58:27.

McGuire, C. F. and F. H. McNeal. 1974. Quality response of 10 hard red spring wheat cultivars to 25 environments. *Crop Sci.* 14:175-180.

Peleg, M. 1994. Mathematical characterization and graphical pentation of the stiffness-temperature-moisture relationship of gliadin. *Biotechnol. prog.* 10:625-654.

Peterson, C. J., R. A. Graybosch, P. S. Baenziger, and A. W. Grombacher. 1992. Genotype and environment effects on quality characteristics of hard red winter wheat. *Crop Sci.* 32:98-103.

SAS. 1995. SAS users guide. SAS Institute Cary, NC.

Seo, Y. W., R. A. Graybosch, C. J. Peterson, and D. R. Shelton. 1995. Assessment of enzyme-linked immunoassay of rye secalins as a tool in the perdiction of 1RS wheat quality. *Cereal Chem.* 72(3):252-254.

Seo, Y. W. and B. H. Hong. 1998. Electrophoretic and immunological evaluation of secalin in rye, triticale, and wheat-rye translocation wheat. *Korean J Crop Sci.* 43:228-233.

Shelton, D. R., W. J. Park, C. J. Peterson, T. J. Martin, S. D. Kachman, and R. L. Wehling. 1997. Variation in polyphenol oxidase activity and quality characteristics among hard white wheat and hard red winter wheat samples. *Cereal Chem.* 74(1):7-11.

Stoddard, F. L. and D. R. Marshall. 1990. Variability in grain protein in australian hexaploid wheats. *Aust. J. Agric. Res.* 41:277-288.

Terman, G. L., R. E. Ramig, A. F. Dreier, and Olson. 1969. Yield-protein relationships in wheat grain, as affected by nitrogen and water. *Agron J.* 61:755-759.