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ORIGINAL PAPER

Grain quality variations from year to year among the Chinese genotypes

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Abstract
Under the random effect of year, the correct evaluation of varieties is the basis for the producing of high-quality wheat. In this study, 11 winter wheat varieties mainly cultivated in the North Yellow and Huai Valley of China were grown from 2011 to 2014 to investigate the effects of genotype, year, and their interaction on 15 major bread-making quality traits as well as the relationships between quality parameters, reliability, and suitability of the genotypes. We found that protein content, wet gluten content, sedimentation volume, test weight, and falling number were mostly influenced by the year. Annual variations in the relationships between quality traits differed considerably. Correlation coefficients between gluten index and both maximum resistance and dough stability ($r=0.68$ to 0.88) were significant over the three growing seasons. A high intra-class correlation coefficient for gluten index (0.74) was observed. Gluten index is a reliable early-generation predictor of gluten strength. Bidimensional clustering analysis and heatmap are useful for suitability analysis of high-quality wheat. Safety-first indices can be useful to plant breeders for reliability analyses of high-quality genotypes, and only one genotype was screened out. The considerable effects of year were demonstrated, suggesting that the reliability of quality genotypes should be improved in the North Yellow and Huai Valley of China.

Keywords Winter wheat · Quality characteristics · Correlation analysis · Reliability analysis · Suitability analysis

Abbreviations

Area	Area under the extensograph curve
DS	Degree of dough softening
DT	Dough development time
EU	Extensograph units
EX	Dough extensibility
FN	Falling number
FU	Farinograph units
GI	Gluten index
HI	Hardness index
NYC	North Yellow and Huai Valley of China
PC	Protein content
R/E	The ratio of RM to EX
RM	Maximum resistance of dough
SFI	Safety-first index
ST	Dough stability
SV	Sedimentation volume
TW	Test weight
WAS	Flour water absorption
WG	Wet gluten

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Introduction

High-quality wheat is required for breeders and farmers, and the milling and baking industries. The North Yellow and Huai Valley of China (NYC) is one of the most important wheat producing areas in China. Therefore, understanding wheat grain quality traits is crucial for breeding and production of high-quality wheat in the NYC.

Grain quality is known to be influenced by genotype, the environment, and their interaction (Vázquez et al. 2012;

Kaya and Akcura 2014; Tomić et al. 2015). Grain hardness index (HI) is predominantly determined by a single major gene (Ha) (Pasha et al. 2010). Therefore, the heritability of the HI is high, and the environment has little influence on it (Surma et al. 2012). Test weight (TW) is mainly affected by the environment (Kaya and Akcura 2014; Khazratkulova et al. 2015), although it is moderately influenced by the genotype (Surma et al. 2012). Falling number (FN) is influenced by both genotype and environment significantly (Gooding et al. 1997; Wang et al. 2008), but (Barnard and Smith 2012) speculated that environmental effects are the major source of variation in FN. Gluten index (GI), which is an indicator of gluten strength (Vida et al. 2014), is predominantly determined by the genotype (Tomić et al. 2015), although the environmental effects are significant (Eljak et al. 2018). The main factors that influence the other traits of wheat quality are not clear. Generally, protein content (PC) and wet gluten content (WG) are mainly affected by the environment (Surma et al. 2012; Kaya and Akcura 2014; Khazratkulova et al. 2015); however, JianWei et al. (2011) demonstrated that the variation due to genotype and genotype-by-environment interaction was larger than the variation due to the environment. Surma et al. (2012) and Kaya et al. (2014) found that Sedimentation volume (SV) was mostly influenced by the environment, although Oelofse et al. (2010) showed that the most important sources of variation were the genotypes followed by interactions. In general, farinograph parameters, dough development (DT), and stability time (ST) in particular, are mainly influenced by the environment, while extensograph parameters, especially for area under the curve (Area) and extensibility (EX), are mostly affected by the genotype (Grausgruber et al. 2000; Ma et al. 2002; Vázquez et al. 2012). However, Zhao et al. (2011) and Li et al. (2016) demonstrated that DT and ST, degree of softening (DS), and water absorption (WAS) were affected by genotype, and Caffe-Treml et al. (2011) found that maximum resistance (RM), Area, and EX were determined by the environment.

The complexity of relationships between quality parameters in wheat is well known (Eljak et al. 2018). Some of the relationships between quality parameters have been confirmed in previous studies. TW has no correlation with all other quality traits (Mutwali et al. 2015). HI shows significant positive correlations with some other quality traits, such as WAS, PC, WG, SV, RM, and Area, and shows a significant negative correlation with DS (Surma et al. 2012; Yin et al. 2014; Zhang et al. 2014). Although it has no significant correlation with RM (Færgestad et al.2000), PC shows significant positive correlations with DT, EX, and Area (Caffe-Treml et al. 2011; Horvat et al. 2012). GI positively interacts with ST, RM, EX, Area, and the ratio of RM to EX (R/E) (Horvat et al. 2012). Significant correlations among dough properties have been found (Marchylo et al. 2001). ST was positively correlated with DT, but was negatively correlated with DS (Liu et al. 2005). RM had a positive association with both Area and R/E, but was negatively associated with EX (Caffe-Treml et al. 2011). Some of these previous results were not consistent since the sites, materials, and methods differed between different studies. It has been demonstrated that FN has a significantly positive association with both PC and GI (Wang et al. 2008; Oelofse et al. 2010; Kaur et al. 2013; Eljak et al. 2018), but is significantly negatively correlated with both DT and EX (Yin et al. 2014). However, JianWei et al. (2011) reported that FN showed few correlations with any other quality traits. Wet gluten content (WG) has a significant association with all of the farinograph and extensograph parameters (Yin et al. 2014). However, (Horvat et al. 2012) showed that the correlations between WG and most of the dough rheological properties are not significant, even though WG is positively correlated with WAS and negatively correlated with DS. Correlations between protein quantity and quality have been reported by a number of authors. In general, PC has a significant positive correlation with WG (Kaya and Akcura 2014); they are significantly positively correlated with SV (Oelofse et al. 2010), but not with GI (Clarke et al. 2010). However, Vázquez et al. (2012) demonstrated a close correlation between PC and GI, and Kaya et al. (2014) reported a nonsignificant relationship between PC and SV. In addition, the genomic and environmental effects revealed by some studies have complicated the relationships between quality traits (Mehmet Ali et al. 2011; Horvat et al. 2012; Tomić et al. 2015). The correlation between PC and EX is not stable and varies with year (Caffe-Treml et al. 2011). The SV is related to all parameters of the dough rheological properties, but the environmental effects on these correlations are unclear (Oelofse et al. 2010).

When evaluating the quality of individual wheat genotypes, three characteristics of the main quality traits should be considered: exceeding the thresholds, stability, and suitability. Many stability analysis methods ranging between univariate and multivariate, parametric and nonparametric have been described (Flores et al. 1998; Mohammadi and Amri 2008; Knapp et al. 2016). However, few of them are ideal for the analysis of quality trait stability. The processing industry requires a constant supply of high-quality raw materials. At the same time, the specified traits should meet the market criteria. For the screening of high-quality wheat genotypes, therefore, a method combining "Shukla stability variance," "environmental variance," and the "safety-first rule" is more reasonable (Eskridge 1990; Grausgruber et al. 2000). Because of the shortage of analytical methods, bidimensional clustering analysis combined with heatmapping is a useful approach for the classification and suitability analysis of quality genotypes (Cavanagh et al. 2010; Mutwali et al. 2015). However, studies on this method have not been reported in the NYC.

The objectives of this study were: (1) to investigate the influence of genotype, environment, and their interaction on winter wheat grain quality parameters; (2) to study the relationships between quality characteristics and the early-generation predictor of strong gluten; (3) to evaluate the reliability and suitability of quality wheat genotypes.

Materials and methods

Wheat genotypes and field trials

In this study, we used 11 wheat genotypes representing a wide range of yield and quality from the NYC (Table S1). The field experiments were performed at the experimental farm of the Xin-Xiang Academy of Agricultural Sciences in Hui-Xian City, Henan Province, P.R. China (35º 26′ N, 113º 45′ E). This site is located on the NYC, which is characterized by a temperate, semi-humid climate. The average precipitation in the growing season is 160.3 mm, and the annual effective accumulated temperature is 2171.3 °C. The soil is classified as sandy loam with a mean bulk density of 1.35 g/cm^3. Based on the analysis of samples taken from the plowed layer (0–20 cm depth), the soil organic matter content was 1.417%, N, P, and K contents were 0.108%, 11.39 mg/kg and 111.2 mg/kg, respectively, and the pH was 8.16.

The experiments were set up in a randomized complete block design with three replications for three consecutive seasons (from 2011 to 2014). The plots were 1.4 m wide by 9.5 m long. Total phosphorus (P_2O_5; 150 kg/ha) and potassium (K_2O; 112.5 kg/ha) and 65% nitrogen (N; 195 kg/ha) was broadcast prior to plowing, and 35% nitrogen (105 kg/ha) was applied at the early jointing stage. Sowing was performed with a plot seed drill (Wintersteiger, Austria) during the second 10 days of October, and in all experiments the seedling density was 2.4 M/ha; the rows were spaced 0.2 m apart. The plots were harvested at full maturity with a plot combine (Wintersteiger, Austria) during the first ten days of June.

Quality testing

Since many of the tests for quality traits evaluation are time-consuming and expensive, the number of samples is reduced by compositing samples over replications. HI was measured by HI apparatus. Weight 25 g of wheat, transfer it to HI apparatus, grind, and sieve for 50 s. Record the weight of throughs (W). $\text{HI} = 100 - 4 \times W$. TW was reported in g/L. FN, SV, and PC were determined according to AACC Method 56-81B, AACC Method 56-61A, and AACC Method 46-11A, respectively. WG and GI were determined on the basis of the ICC158 standard (ICC 1995) using a Perten Glutomatic 2200 instrument (Perten, Sweden). The rheological properties of the wheat dough were determined using the Brabender farinograph (Brabender, Germany) according to AACC Method 54-21 and the Brabender Extensograph (Brabender, Germany) according to AACC Method 54-10.

Statistical analyses

The R 3.2.3 software (R Foundation for Statistical Computing, Vienna, Austria) was used for data analysis and plotting. The package "lme4" (version 1.1-12) was used to estimate variance components, which were reported as a proportion of total variance. Heatmaps were produced using the package "gplots." The standard deviation of random effect was estimated using the restricted maximum likelihood method, and the likelihood ratio method was used to test significance.

The following linear mixed model was used:

$$Y_{ij} = \mu + g_i + \text{year}_j + e_{ij}$$

where Y_{ij} is the response variable, μ is the grand mean that was assumed to be fixed, g_i is the effect of genotype, year_j is the effect of year, e_{ij} is the residual error that contains the genotype-by-environment interaction.

The intra-class correlation coefficient (ICCC) was defined as the ratio of the genotypic variance to genotypic plus residual variances.

$$\text{ICCC} = \sigma_{\text{G}}^2 / \left(\sigma_{\text{G}}^2 + \sigma_{\text{e}}^2\right)$$

where σ_{G}^2 is the genotypic variance component and σ_{e}^2 is the residual variance component that contains genotype-by-environment interaction.

The stability and reliability of each genotype were calculated as Shukla's stability variance and the safety-first index (SFI) (Eskridge 1990). In this study, a value of $\alpha = 0.25$, which implied that a genotype exceeded the limits for trading in three out of 4 years, was used in the calculation of SFI. Pearson correlation coefficients among all quality traits were calculated using the software package "psych." The means of quality traits are standardized by column, and singular value decomposition is then performed. The fitted values of quality traits were evaluated using the top four principal components (accumulative variance contribution rate accounted for 87.8%). The accumulative variance contribution rate of first two principal components accounted for 64.6%. Bidimensional clustering analyses were performed using the hierarchical clustering method with the Euclidean distance of the fitted values (using negative values of DS).

Results

Effects of genotype and year and their interaction on quality traits

Table S2 presents the estimates of variance components and ICCC. Genotype represented the main source of variation for HI, GI, WAS, ST, DS, and for extensograph parameters (RM, EX, Area, and R/E). Environment represented the main source of variation for PC, WG, SV, and TW. Genotype-by-year interaction represented the main source of variation for DT. However, FN was influenced by both the main effects of genotype and environment and their interaction. ICCC of extensograph parameters was higher than for farinograph parameters. ICCC of HI and GI was relatively high. However, it was low for TW, SV, and FN.

Relationships between quality parameters and the influence of year

The relationships between quality parameters are presented in Table S3. Correlation coefficients were computed using genotype means in each year. Correlation coefficients between quality traits can be categorized in three groups: (1) those that are insignificant in all 3 years; (2) those that are significant in only one or two out of 3 years; and (3) those coefficients that are significant in all 3 years. Correlations between TW and each of the other quality traits, and between HI and each of the other quality parameters (except for WAS) were included in group (1). Correlations between SV and each Rheological parameter, PC, and WG, and between GI and WG were included in group (2). Relationships between GD and both RM and Area, and between HI and WAS were included in group (3). RM had no correlation with EX in any of the three growing seasons.

Suitability analyses of quality wheat genotypes

The heatmap visually illustrates the processing suitability of the wheat genotypes (Figure S1). Genotypes with higher PC, RM, EX, and Area are suitable for making bread (Caffe-Treml et al. 2011). The genotypes X26 and S21 can be categorized into the bread wheat group. Genotypes with medium RM, Area, and EX, lower PC, and higher TW are suitable for making steamed bread (Zhang et al. 2016). The genotypes J20 and X979 can be categorized into the steamed bread wheat group. Genotypes with higher SV, ET, and FN are suitable for making Chinese dried noodles. Genotype Z366 may be an ideal choice for Chinese dried noodles.

Reliability analyses of quality wheat genotypes

Quality wheats must outperform the limits of certain traits in grain trading. According to the Chinese National Standards (GB/T 17892-1999) and Zhengzhou Commodity Exchange Standards (Q/ZSJ 001-2003), the limits for top-grade strong gluten wheat for TW, FN, PC, WG, ST, and Area are 770 g/L, 300 s, 15%, 35%, 12 min, and 90 cm^2, respectively. The means of the quality traits for genotypes S21, X26, X979, and Z366 exceeded all six limits (Table S4). Considering the safety-first indices (Table S5), only genotype S21 outperformed in all six limits. However, the ranks of Shukla's stability variance of ST and Area for S21 were both in the bottom third (Table S6).

Discussion

The HI, WAS, GI, and extensograph parameters were predominantly influenced by the genotype, and their ICCC was relatively high. Previous studies have shown that the Ha locus on chromosome 5DS and some additional modifying genes make the distinction among different hardness genotypes (Eagles et al. 2002; Surma et al. 2012). Wheat with higher HI has a capacity to absorb water because of the broken starch granules that are produced in the flour mill (Pasha et al. 2010). Ma et al. (2002) found that RM, R/E, and Area are mainly affected by the genotype, which was similar with our results. However, Eagles et al. (2002) reported that the genotypic variance was lower than the environmental variance for EX, and the ICCC was lower as well. The genotypic variance for RM may be explained by glutenin genes. GI is an indicator of gluten strength, and Vida et al. (2014) indicated that gluten strength with higher heritability was determined by genotype. Our results confirmed the previous observation. However, GI is also significantly influenced by environment (i.e., nitrogen fertilization, irrigation, and climatic conditions) (Oikonomou et al. 2015). The environmental variance for gluten strength may be explained by changes in the molecular weight distribution of wheat proteins (Southan and Macritchie 1999).

Environmental effects are the predominant factors in determining TW, FN, PC, WG, and SV. TW is predominantly influenced by year, thus confirming the results of (Kaya and Akcura 2014). Several studies indicated that FN was affected by year, genotype, and their interaction (Wang et al. 2008; Rakita et al. 2015). Our study revealed that variance components due to the environment and residual variances were greater than those due to genotype for FN. Therefore, FN is determined by environment and the interaction with genotype. Variance components due to the environment were greater than those due to genotypes for PC, which agreed with the findings of (Gooding et al. 1997).

Variance from the environment was higher than that from the genotype for WG, which was confirmed by the results of (Kaya and Akcura 2014). Oelofse et al. (2010) showed that the genotypic variance component contributed 85.96% of the total variation for SV. However, our results indicated that year contributed up to 81.5%. Therefore, SV is predominantly influenced by environment, in agreement with the results of (Surma et al. 2012).

Breeders should make every effort to enhance RM through genetic improvement under the conditions that prevail in the NYC. Considering the limits for quality wheat trading, TW, FN, PC, WG, ST, Area, and Bread scores must be improved simultaneously. In our study, variance components due to years were greater than those due to genotypes for TW, FN, PC, and WG. Additionally, ICCC for ST was relatively low, which illustrates its low heritability. Therefore, it is difficult to enhance these traits through genetic improvement. The Area is mainly determined by RM. In accordance with the results of (Caffe-Treml et al. 2011), our studies suggest that correlation coefficients ($r = 0.87$ to 0.94) between RM and Area were significant in all 3 years of the experiment. Area is positively significantly correlated with Bread volume (Kieffer et al. 1998; Jinfu et al. 2017). Therefore, Bread volume could be improved by increasing RM. RM could be greatly enhanced through genetic improvement because of the higher genetic variance and heritability shown by the present study.

GI is an early-generation predictor of RM and ST in multi-year field trails in the NYC. A high correlation coefficient between two quality traits, which unusually suggests a strong heritable association and possibly a narrow gene base, is useful for breeders (Gaines 1991). Correlation coefficients between GI and both RM and ST ($r = 0.68$ to 0.88) were significant and relatively stable over all three growing seasons in this study. The study of (Ames et al. 2003) indicated that GI appears to be relatively independent of protein concentration. Correlation coefficients between GI and PC ($r = 0.31$ to 0.50) were not significant, in our study. Therefore, GI is a reliable selection criterion for RM and ST where there is variation in protein concentration.

The suitability of a genotype for a particular purpose or use could be represented on the Heatmap by comparing the fitted values of traits. If the cumulative variance contribution rate of the first two principal components is low, a biplot is not ideal for describing the patterns in the data (Yan and Frégeaureid 2008). In this study, the Heatmap represented the first four principal components, for which the cumulative variance contribution rate was 87.8%. In comparison, the cumulative variance contribution rate of the first two principal components was 64.6% in the biplot. The relative magnitude of the quality trait values is also provided, as they are shown in color on the heatmap. Therefore, heatmaps can be used for quality variety comparison tests.

Safety-first indices can be useful to plant breeders for reliability analyses of quality genotypes. The instability of raw materials as defined by the grain processing industry is due to the effects of environment and genotype-by-environment interactions (Grausgruber et al. 2000). The safety-first index combines Shukla's population variance for a certain genotype and the environmental variance component with acceptable probability (Eskridge 1990) and indicates whether a certain trait will exceed the market threshold. Therefore, it can be simply applied to quality genotype selection. In our study, a value of $\alpha = 0.25$ was used, which translated into a willingness to accept a quarter of chance of a lower value than the trading limit in a particular season. Unfortunately, there was only one genotype (S21), with instable ST, that outperformed all six trading limits. Therefore, outstanding quality genotypes are very scarce at present in the NYC.

Glu-1 quality scores alone were not sufficient to explain the variability in wheat protein quality. The quality score of Glu-B1 7 + 8 was greater than that of Glu-B1 7 + 9 (Payne et al. 1987). Subunit GluD1 5 + 10 was associated with a larger sedimentation volume and higher dough strength in genotypes compared to those with subunit GluD1 2 + 12 (Saint Pierre et al. 2008). In our study, the protein quality (i.e., SV and RM) of J17 and Z366 was inferior to that of X26 and S21, although the Glu-1 quality scores of J17 and Z366 were greater than those of X26 and S21. The same results were found in J17 versus X979 and X19 versus ZM24. This could be because low molecular weight glutenin subunits and gliadins are incorporated into the gluten protein polymer. Therefore, a better understanding of the biochemical basis of protein quality is extremely important to improve processing quality of wheat grain.

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Compliance with ethical standards

Conflicts of Interest The authors declare no conflicts of interest.

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