Predicting the Protein Content of Grain in Winter Wheat with Meteorological and Genotypic Factors

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Abstract: Meteorological conditions including temperature, sunshine and precipitation during grain growth are the primary factors determining the variation of the protein content of grain (PC) in wheat. On the basis of field experiments, a simplified regression model was developed for predicting the PC in winter wheat. From stepwise regression analysis, it was found that the PC of high-protein cultivars was correlated with the difference between daily maximum and minimum temperatures ($\Delta T$) when $\Delta T$ variation under the environment was significant, but with the interaction of mean temperature ($T_{mean}$) × total sunshine hours from anthesis to maturity (TSUN) under the environment with $\Delta T$ variation less than 5%. In medium-protein cultivars, the PC was correlated with TSUN, and in low-protein cultivars, with the combination of $T_{mean}$, total rainfall from anthesis to maturity (TR) and TSUN. The climatic factors influencing PC were further quantified incorporating five genetic parameters. The $\Delta T$ and TSUN were linearly correlated with PC, and $T_{mean}$ was quadratically correlated with PC. The precipitation was linearly correlated with PC if it was less than 50mm, otherwise quadratically. The average root mean square error (RMSE) values of the estimated PC relative to the observed value were less than 7 percent, indicating a good fit between the estimated and observed PC. Thus, it is concluded that the present model can predict the PC of different winter wheat cultivars under various climate environments.

Key words: Genotypic parameter, Precipitation, Protein content of grain, Sunshine hours, Temperature, Winter wheat.
wheat cultivars and certain range of conditions so that errant predictions would be given with extrapolated conditions. It is assumed that identification and incorporation of key factors influencing the PC under different environments and genotypes into the model would greatly aid in developing a simplified model with high predictability.

In this paper, a new method for predicting PC is introduced on the basis of the quantitative relationships between PC in winter wheat and meteorological parameters. We seek to determine: (i) how meteorological parameters affect PC over the main winter wheat growing areas of China; and (ii) whether these relationships can be used for predicting PC for various winter wheat cultivar types under different environments. Some genotypic parameters are incorporated into the model to discriminate the difference of wheat cultivars in sensitivity to the climates. The new routine is tested across a wide range of field observations and wheat genotypes.

**Materials and Methods**

1. **Experiment design and data collection**

   A total of four field experiments, involving different eco-sites, cultivars and sowing dates were conducted from 1999 to 2002 in main winter wheat-growing areas of China. These different field experiments were designed in the present study to support model development or model validation.

   In order to determine the influence of meteorological parameters on PC, the main field experiment (Exp.1) was conducted with six wheat cultivars and two or three sowing dates at four eco-sites in 2001. We chose four eco-sites Nanjing, Xuzhou, Tai’an and Baoding, whose latitudes are different in about two degrees from each other within the major wheat growing regions in China as shown in Table 1. Three sowing dates were implemented to represent early, optimal and late sowing situations at Nanjing and Xuzhou, while only optimal and late sowing treatments were performed in Tai’an and Baoding to avoid the high risk of exposing early seedlings to low winter temperatures. Optimal sowing dates were 6 Nov. at Nanjing, 15 Oct. at Xuzhou, 10 Oct. at Tai’an and 1 Oct. at Baoding. Early and late sowing dates were 15 days earlier or later than the optimal sowing date at each site. Six cultivars, Xuzhou 26, Yumai 34, Wanmai 38, Yangmai 10, Xuzhou 25 and Ningmai 9 were planted at Nanjing, Xuzhou and Tai’an, while the weak-vernalization cultivars, Yangmai 10 and Ningmai 9, were not used at Baoding since they could not survive through the cold winter. The plots of 15m$^2$ were arranged in a randomized complete block design with three replications of cultivars × sowing dates at each site. The soil was loam or loamy sand in the experiment sites with similar fertilities (Table 1). In all experiments, N, P and K were applied at the same fertilization rates at each site (120 kg ha$^{-1}$ N, P, K; by broadcasting into the soil as urea, potassium chloride and superphosphate calcium 3 days before sowing, and additional 120 kg ha$^{-1}$ N was input into soil as urea at jointing day of each site with each sowing date), all fields were not irrigated except under strong stress. Other field managements followed local practices for high yield in wheat.

   In addition, three supplemental field experiments were conducted in 1999, 2000 and 2002. In 1999 (Exp.2), 40 wheat cultivars, (coded 1 to 40 in Table 2; cultivars number 41 and 42 were used in the following Exp.3), were planted in Nanjing and Xuzhou at optimal sowing date. In 2000 (Exp.3), eight wheat cultivars, Jinan 17, Xuzhou 26, Yangmai 10, Huaimai 18, Wanmai 38, Yangmai 10, Xuzhou 25 and Ningmai 9 were selected to conduct the field experiments at six sites of Jiangyan (32º30’N), Hai’an (32º30’N), Baoding (33º12’N), Yandu (33º18’N), Xuzhou (34º54’N) and Ganyu (34º54’N). In 2002 (Exp.4), three wheat cultivars including Xuzhou 26, Yanmai 10 and Ningmai 9, were planted on three sowing dates (early, optimal and late) at Nanjing. At each eco-site of the

| Site      | Longitude, Latitude | Soil type | Organic matter (g · kg$^{-1}$) | Total N (g · kg$^{-1}$) | *Available N (mg · kg$^{-1}$) | Total P$_2$O$_5$ (mg · kg$^{-1}$) | Total K$_2$O (mg · kg$^{-1}$) |
|-----------|---------------------|-----------|-------------------------------|------------------------|-------------------------------|---------------------------------|-------------------------------|
| Nanjing   | 118º42’E, 32º03’N   | Loam      | 12.92                         | 1.28                   | 101.5                         | 54.8                            | 65.3                           |
| Xuzhou    | 117º06’E, 34º18’N   | Loamy sand| 14.58                         | 1.29                   | 99.2                          | 56.1                            | 87.5                           |
| Tai’an    | 117º12’E, 36º12’N   | Loamy sand| 12.80                         | 1.06                   | 102.6                         | 64.0                            | 50.2                           |
| Baoding   | 115º24’E, 38º48’N   | Loam      | 10.21                         | 0.91                   | 86.3                          | 56.5                            | 90.0                           |

* Available N is the inorganic nitrogen readily available for plant uptake, mainly including nitrate and ammonium in soil, which were determined by separate measurement methods as the normal protocol.
three field experiments, management practices were according to the high yield standards under local conditions.

In all experiments, the main phenological stages such as jointing, booting, heading, anthesis, filling and maturity were recorded in each plot. At maturity, the plants of 1m$^2$ in each plot were harvested and grains were assayed for quality traits a month later. PC was determined by measuring N using the semi-micro Kjeldahl method. A multiplier of 5.7 was used to convert the N content to protein content (Tkachuk, 1969). Meteorological data, including daily precipitation, temperature and sunshine hours, were obtained from local weather stations of the experiment sites. The data from the main field experiment were used for model development and the data from the three supplemental experiments for model validation.

2. Statistical methods

Regression analysis was conducted using the SPSS statistical package. In model testing, the predicted results were compared with the field measurements to evaluate reliability and accuracy of the model output under practical conditions. The field data sets were from a large range of growing conditions, including different years, eco-sites, cultivars and sowing dates. The following statistic method, root mean square error (RMSE, % value) against the observed mean, was used to calculate the fitness between the estimated results and observed data (Michele et al., 2003):

$$\text{RMSE} = \sqrt{\frac{\sum_{i=1}^{n} (P_i - O)^2}{n}} \times \frac{100}{\bar{O}} \quad (1)$$

Where $P_i$ and $O_i$ are predicted and observed values, respectively; $\bar{O}$ is the observed mean value. RMSE (%) shows the relative difference between the simulated and observed data. The prediction is considered excellent with the RMSE < 10%, good if 10-20%, fair if 20-30%, poor if > 30% (Jamieson et al., 1991).

Results

1. Model development

Under non-limitation of water and nutrient supply, and optimal meteorological and soil fertility conditions, PC in wheat may increase up to its

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Table 2. The characteristic protein content of grain (Pc), physiological vernalization time (PVT), temperature sensitivity (TS), photoperiod sensitivity (PS) and rainfall sensitivity (RS) of different wheat cultivars.

| Code | Cultivar      | Pc (%) | PVT (d) | PS | Code | Cultivar      | Pc (%) | PS | TS | RS |
|------|---------------|--------|---------|----|------|---------------|--------|----|----|----|
| 1    | Shan 253 $^\dagger$ $^\ddagger$ | 16.9   | 23.3    | –  | 22  | Su 9356 $^\ddagger$ | 14.6   | 0.65 | –  | –  |
| 2    | Shan 89150 $^w$    | 16.2   | 23.6    | –  | 23  | Zhoumai13 $^w$   | 12.0   | 0.68 | –  | –  |
| 3    | Yanzhong 188 $^w$   | 15.3   | 24.6    | –  | 24  | Anmoung 92484 $^s$| 13.8   | 0.87 | –  | –  |
| 4    | Lankao 906 $^w$     | 17.9   | 25.7    | –  | 25  | Chongqing bread wheat $^s$ | 14.0   | 0.82 | –  | –  |
| 5    | 62014 $^w$        | 16.5   | 21.7    | –  | 26  | Huaimai9417 $^w$ | 14.5   | 0.89 | –  | –  |
| 6    | 97207 $^w$        | 16.8   | 21.7    | –  | 27  | Huaimai 17 $^s$  | 12.8   | 0.85 | –  | –  |
| 7    | 910331 $^w$       | 15.8   | 23.1    | –  | 28  | Ningmai 18 $^s$  | 12.0   | 0.95 | –  | –  |
| 8    | Yannong 15 $^w$    | 15.4   | 25.2    | –  | 29  | Shan 229 $^s$    | 14.2   | 0.68 | –  | –  |
| 9    | PH85–16 $^w$      | 15.7   | 25.2    | –  | 30  | Yangmai158 $^s$  | 12.6   | 0.76 | –  | –  |
| 10   | Xuzhou 26 $^w$    | 15.3   | 20.0    | –  | 31  | Wen mai 8 $^s$   | 11.8   | 0.80 | –  | –  |
| 11   | Yuanfeng 898 $^w$ | 16.5   | 25.5    | –  | 32  | Yangmai 10 $^s$  | 14.9   | 0.72 | –  | –  |
| 12   | PH82–22 $^w$      | 16.9   | 20.2    | –  | 33  | Huaimai 18 $^w$  | 13.3   | 0.80 | –  | –  |
| 13   | Huaimai 894 $^s$  | 15.6   | 18.9    | –  | 34  | Yumai21 $^w$     | 11.2   | 0.99 | 0.17 | 0.025 |
| 14   | Gaoyou 505 $^w$   | 16.5   | 21.8    | –  | 35  | Xuzhou 25 $^w$   | 11.4   | 0.90 | 0.24 | 0.158 |
| 15   | Neixiang 188 $^w$ | 15.9   | 24.0    | –  | 36  | Ningmai 8 $^s$   | 11.3   | 0.95 | 0.21 | 0.030 |
| 16   | Youmai 3 $^w$     | 15.6   | 23.9    | –  | 37  | Wannai 18 $^w$   | 11.4   | 0.92 | 0.25 | 0.175 |
| 17   | Yumai 34 $^s$     | 15.4   | 17.8    | –  | 38  | Ningmai 9 $^s$   | 10.6   | 0.92 | 0.23 | 0.145 |
| 18   | Jinan 17 $^w$     | 15.1   | 24.0    | –  | 39  | Fengyou 5 $^w$   | 10.9   | 0.92 | 0.22 | 0.115 |
| 19   | Wanmai 38 $^w$    | 14.8   | 0.68    | –  | 40  | Zhengzhou 8998 $^w$ | 10.5   | 0.92 | 0.23 | 0.300 |
| 20   | Fengyou 3 $^w$    | 13.8   | 0.79    | –  | 41  | Jianmai 1 $^w$   | 11.5   | 0.85 | 0.22 | 0.158 |
| 21   | 9189 $^w$      | 12.3   | 0.80    | –  | 42  | Yangmai 9 $^s$   | 9.8    | 0.83 | 0.25 | 0.118 |

$^\dagger$Forty-two genotypes from different experiments in total.
$^\ddagger$W, winter-type cultivars; S, spring-type cultivars.
The values of these parameters were obtained from the general analysis of each wheat cultivar and special experiments.
threshold (Jin, 1992; Evans and Fischer, 1999). The threshold value was considered as potential PC of common wheat (PCp, %). According to the surveys of PC of 33,797 wheat cultivars (Jin, 1992; Jing et al., 2003), PC of winter wheat scarcely exceeds 20%, PCp value was set as 20% in our model.

The practical PC is significantly less than PCp, being affected by some impact factors. The model is of the following form:

$$PC = PC_p \times (c + r_1 \times m_1 + r_2 \times m_2 + \ldots)$$  \hspace{1cm} (2)

Where PC is the practical PC (%), c is the constant, m_1 and m_2 are influencing factors, r_1 and r_2 are the estimated weight of the effect of m_1 and m_2, respectively.

The model is established based on the data collected from Exp.1, which was conducted using different cultivars and sowing dates at four eco-sites, but on the soil with similar fertility, the same fertilization rate and well-irrigated conditions. Possible effects of soil moisture and fertility, water and nitrogen management on the PC are not included in the model. Cultivar effects as genotypic parameters are considered in the regression analysis against various meteorological factors for different wheat quality types.

### 1) Determination of parameter Pc and quality type

Different quality types of wheat showed great differences in sensitivity to meteorological factors. Higher-protein wheat is proposed to be more sensitive to the daily variation of temperature, but lower-protein wheat showed significant variation with different temperature, sunshine and precipitation conditions (Benzian and Lane, 1986; Jin, 1992; Jing et al., 2003). Thus, it is necessary to adapt the model for different types of wheat quality.

The parameter Pc (%), which refers to characteristic PC of a given wheat cultivar under feasible growing conditions, is supposed to vary with the genotype. The Pc values of the 42 wheat cultivars used in the present study (Table 2) were determined from the average PC of each cultivar from the multiple field trials under different environments and cultural conditions at feasible growing sites before and after the release of each cultivar (Jing et al., 2003).

According to the national criteria GB/T17892-1999 and GB/T17893-1999 in China, wheat with crude protein higher than 15% on a dry base is classified into strong-gluten wheat, and that with lower than 11.5% on a dry base into weak-gluten wheat. Consequently, the wheat cultivar with Pc > 15% was considered as high-protein cultivar, and Pc < 11.5% and 11.5% < Pc < 15% were considered as low-protein and medium-protein cultivars, respectively. The different quality types of wheat would exhibit differential responses to environmental conditions, as proposed by Jacques et al. (2000).

### 2) Determination of key meteorological factors

Meteorological factors, such as daily mean, maximum and minimum temperatures, ∆T (difference between daily maximum and minimum temperatures, °C), growing degree-days from anthesis to maturity (GDD, °C·d), total rainfall from anthesis to maturity (TR, mm), daily mean sunshine and total sunshine hours from anthesis to maturity (TSUN, h) and the number of days from heading to maturity, were supposed to influence the PC in wheat (Spiertz et al., 1977; Rao et al., 1993; Daniel and Tribou, 2002). Using the data from the main experiment, linear and quadratic relationships between meteorological factors and PC were tested. As a result, five main factors were identified, which were daily mean temperature (Tmean, °C), ∆T from anthesis to maturity, GDD, TR and TSUN (Table 3). Although TR showed a negative correlation, the other four factors showed a significantly positive correlation with PC in linear or quadratic forms.

Interaction of meteorological variables could strengthen or weaken the effects on PC in wheat (Bassett et al., 1989; Cooper et al., 2001). Thus, the stepwise regression analysis was further performed to determine the key meteorological factors influencing PC of different wheat types using the field experiment data collected in 2001. As shown in Table 4, the factor showing the minimum F value in each regression step was deleted until all factors were significantly correlated with PC. In the high-protein wheat cultivars such as Xuzhou 26 and Yumai 34, ∆T was the only limiting meteorological factor through five regression steps. TSUN was correlated with PC in medium-protein cultivars such as Yangmai 10 and Wannmai 38 through five regression steps. In the low-protein cultivars including Xuzhou 25 and Ningmai 9, Tmean, TR and TSUN were identified as key meteorological factors.

Coefficient of variation (CV, %) of the average ∆T within four eco-sites was a significant variation level (18.4%), and the PC of high-protein cultivars was

| Tmean ($^\circ$C) | ∆T ($^\circ$C) | TR (mm) | TSUN (h) | GDD (°C·d) | Mean sunshine (h) | Tmean ($^\circ$C) | Tmax ($^\circ$C) | Tmin ($^\circ$C) | Duration from heading to maturity (d) |
|----------------|--------------|----------|-----------|-------------|-------------------|----------------|----------------|----------------|-------------------------------------|
| Linear         | 0.41**       | 0.44**   | -0.36**   | 0.48**      | 0.35*             | 0.16           | 0.27           | 0.23           | -0.03                               |
| Quadratic      | 0.56**       | 0.46**   | 0.39**    | 0.48**      | 0.37*             | 0.20           | 0.31           | 0.26           | 0.13                                |

* Difference at significant at P< 0.05 and P<0.01, respectively.
closely associated with the average \( \Delta T \) from anthesis to maturity. On the other hand, the CV of average \( \Delta T \) under different sowing dates within each eco-site was less than 5%, although the PC of high-protein wheat still showed significant variation. Re-analysis by stepwise regression on the correlation between other climate parameters except for \( \Delta T \) and PC under different sowing dates at the same eco-site indicated that the PC of high-protein wheat was mainly determined by the interaction of Tmean × TSUN. Thus, for quantifying the PC of high-protein wheat the climate parameter of \( \Delta T \) should be considered first, and if \( \Delta T \) was little changed under a given environment, PC should be determined by the interaction of Tmean × TSUN. In medium-protein wheat, when TSUN at a given site insignificantly fluctuated, namely CV was less than 5%, PC was also invariable on the basis of stepwise regression analysis. In low-protein cultivars, PC was affected by the daily mean temperature, total rainfall and sunshine hours from anthesis to maturity. Of these three factors, if one was unchangeable, the other two factors basically determined the variation of protein content.

Combining the above analyses, the model can be refined by using the factors Tmean, \( \Delta T \), TSUN and TR. In high-protein wheat, \( \Delta T \) is the only influencing meteorological factor under the condition with significant variation of \( \Delta T \). If mean \( \Delta T \) is relatively unchangeable or CV is less than 5%, PC of high-protein wheat cultivars is determined by the interaction of \( F(T) \times F(TSUN) \):

\[
PC = PC_p \times F(\Delta T) \quad \text{CV} \geq 5\%
\]

\[
PC = PC_p \times F(TSUN) \times F(T) \times (0.015 \times PC) \quad \text{CV} < 5\% \quad (3)
\]

where 0.015 is the constant obtained from our experiments.

For medium-protein wheat:

\[
PC = PC_p \times F(TSUN) \quad (4)
\]

For low-protein wheat:

\[
PC = PC_p \times [r_T \times F(T) + r_{TR} \times F(TR) + r_{TSUN} \times F(TSUN)] \quad (5)
\]

where, the \( F(T) \), \( F(\Delta T) \), and \( F(TSUN) \), and \( F(TR) \) are the functions influencing the effects of Tmean, \( \Delta T \), TSUN and TR on PC, respectively, within the range of 0-1.0; the \( r_T \), \( r_{TSUN} \) and \( r_{TR} \) refer to the corresponding weights or weight factors of Tmean, TSUN and TR. The \( r_T \), \( r_{TSUN} \) and \( r_{TR} \) can be calculated using the coefficients of partial correlation with Tmean, TSUN and TR (Table 4):

| Regression step | Cultivar | Determination coefficient (\( R^2 \)) | F value | Tmean (ºC) | \( \Delta T \) (ºC) | TR (mm) | TSUN (h) | GDD (ºC·d) |
|-----------------|---------|----------------------------------|---------|-------------|---------------|--------|--------|------------|
| First step      | H †     | 0.71*                            | T        | 0.02        | 1.42          | 0.62   | 0.50   | 0.06        |
|                 | M       | 0.84**                           | T        | 6.46*       | 3.65          | 5.00*  | 14.22**| 5.45*       |
|                 | L       | 0.82**                           | T        | 7.12*       | 0.47          | 6.50*  | 8.35*  | 4.07        |
| Second step     | H       | 0.71*                            | T        | 2.85        |               | 0.65   | 0.97   | 0.23        |
|                 | M       | 0.78*                            | T        | 2.43        |               | 5.36*  | 12.29**| 3.12        |
|                 | L       | 0.82**                           | T        | 10.83**     |               | 13.11**| 13.73**| 4.16        |
| Third step      | H       | 0.70*                            | T        | 3.58        |               | 1.45   | 0.86   |            |
|                 | M       | 0.73*                            | T        | 2.76        |               | 9.22** | 1.21   |            |
|                 | L       | 0.75**                           | T        | 5.49*       |               | 9.33** | 10.53**|            |
| Fourth step     | H       | 0.68**                           | T        | 5.70*       |               | 0.69   |        |            |
|                 | M       | 0.70**                           | T        | 1.59        |               | 13.74**|        |            |
| Fifth step      | H       | 0.66**                           | T        | 12.56**     |               |        |        |            |
|                 | M       | 0.67**                           | T        | 12.74**     |               |        |        |            |

Partial correlation coefficient ‡

| Weight | 0.25 | 0.36 | 0.39 |

† H, M, L refer to the high, medium and low protein cultivars, respectively.
‡ Partial correlation coefficient of mean temperature, total rainfall and sunshine in low-protein cultivars.
* * Difference at significant levels of 0.05 and 0.01, respectively.

For the regression analyses of H, M and L, Exp.1 was conducted in six wheat cultivars with two or three sowing dates at four eco-sites in China in 2001, and each treatment contained three repeated observations.
\[ r_i = \frac{R_i^2}{R_{T^2} + R_{TR}^2 + R_{TSUN}^2} \quad i = T, TR, TSUN \quad (6) \]

where, \(R_T\), \(R_{TR}\) and \(R_{TSUN}\) refer to coefficients of partial correlations with Tmean, TR and TSUN, respectively, with the corresponding weight values of 0.25, 0.36 and 0.39, respectively (Table 4).

(3) **Estimation of functional parameters**

PC of high-protein wheat is linearly correlated with \(\Delta T\) from anthesis to maturity (Fig. 1A). Winter-type cultivar, which requires a longer vernalization for its phasic development (Jin, 1992; Cao and Moss, 1997), was more sensitive to the \(\Delta T\) than half-winter-type (medium requirement for vernalization days) and spring-type cultivars (less requirement for vernalization days). These relations can be quantified as follows:

\[ F(\Delta T) = 0.0335 \times \Delta T + 0.025 \times PC \times \left(1 + \frac{PVT - 20}{40 - PVT}\right) \quad (7) \]

where \(F(\Delta T)\) is function influencing the effect of \(\Delta T\) on PC; 0.0335 and 0.025 are the constants determined from our experiments; PVT is genotypic parameter, referring to physiological vernalization time (Table 2). The PVT is proposed to characterize the sensitivity of different wheat cultivars to low temperature or vernalization requirement, with values between 0 (strong spring type) and 60 (strong winter type) days (Cao and Moss, 1997).

The total sunshine hours (TSUN) is the key meteorological factor for the PC of medium and low-protein wheat. With increasing TSUN, PC was linearly increasing (Fig. 1B). The impact of TSUN could be also linked to photoperiod sensitivity (PS) in different wheat cultivars:

\[ F(TSUN) = FV \times TSUN + PS \times \frac{PC}{PC_p} \quad (8) \]

where, \(F(TSUN)\) is the factor influencing the effect of total sunshine hours on PC as shown in the equation (3)-(5); \(FV\) is the slope of the linear relationship varying with wheat types, which are 0.0003, 0.0004 and 0.0005 (h⁻¹) for low-protein, medium-protein and high-protein cultivars, respectively; PS, another genotypic parameter (valued between 0 and 1), refers to photoperiod sensitivity which reveals differential responses of wheat genotypes to the same photoperiod.
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Daily mean temperature (T\text{mean}) is significantly correlated with PC in quadratic shown in Fig. 1C:

\[
F(T) = 1 - 0.0057 \times (T\text{mean} - T\text{meano})^2 + TS \times \frac{PC_p}{Pc}
\]

where, 0.0057 is the constant obtained from our experiments; \( F(T) \) is the factor influencing the effect of daily mean temperature on PC of low-protein cultivar as introduced into the equation (5); \( T\text{meano} \) (ºC) is the optimum mean temperature for wheat grain nitrogen accumulation, as determined by Schimper (1903) and Klages (1934), and is equal to the average mean temperature that gives outstanding PC and low annual variation of PC, and is regarded as 22º C in the model; TS is a genotypic parameter referring to sensitivity of different wheat genotypes to temperature.

Little precipitation during grain filling may limit wheat growth and affect grain number, yield and PC (Semenov and Porter, 1995). In this survey, when total rainfall from anthesis to maturity is less than 50 mm, PC of low-protein cultivar is negatively and linearly related to the total rainfall. When TR is more than 50 mm, PC of low-protein wheat is expressed as a quadratic function of the TR (Fig.1D).

\[
F(TR) = \begin{cases} 
-0.0018 \times TR + 0.36 \times \frac{PC_p}{Pc} & \text{TR} \leq 5\% \\
1 - RS \times 10^4 \times (TR - TRo)^2 + 0.2 \times \frac{PC_p}{Pc} & \text{TR} > 5\%
\end{cases}
\]

where, 0.0018, 0.36 and 0.2 are the constants obtained from our experiments; \( F(TR) \) is the factor influencing the effect of total rainfall on PC of low-protein cultivar as shown in equation (5); TRo (mm) is the optimum precipitation for N accumulation in grain during grain growth, which is considered as the average TR that give outstanding grain yield and protein content with low annual variation, and is regarded as 150 mm in our study. Precipitation increases soil moisture to affect wheat growth, but genetic differences are great even if under the same condition of soil types, moisture and precipitation. Therefore, the genotypic parameter, RS, refers to the sensitivity of a given wheat cultivar to precipitation variation as incorporated in Equation (10).

Five genotypic parameters, i.e. Pc, PVT, TS, PS and RS, were incorporated in the model. Pc and PVT reflect intrinsic characteristics of a given cultivar, and TS, PS and RS the genetic sensitivity to the environment or adaptability to environment. Pc is needed for all wheat cultivars, while other genotypic parameters are used for different quality types of winter wheat. In high-protein wheat, PVT is needed under significant variation of post-anthesis mean \( \Delta T \), while the TS and PS are needed if the CV (%) of post-anthesis mean \( \Delta T \) is less than 5%. In medium-protein wheat, only PS is needed. In low-protein wheat, PS and RS are needed (Table 2).

A total of 42 wheat cultivars from the different experiments were characterized for genotypic parameters as shown in Table 2. On the basis of analysis of the performance of the six cultivars under different growing conditions in Exp.1, the parameters TS, PS and RS were determined by adjusting the simulated values to the observed data. The results showed that PS valued between 0.65 and 1.0, with greater value for low-protein wheat than for medium-protein wheat, and TS varied from 0.15 to 0.3 and RS from 0.01 to 0.3 for low-protein type. Similarly, the parameters of other 36 cultivars were determined using the experiment data collected at Xuzhou in 1999 (Exp.2) and in 2000 (Exp.3).

### 2. Model testing

The model validation was conducted using the data sets of three supplemental experiments. Firstly, the model was tested using the experimental data including three sowing dates and three cultivars in 2002. The mean \( \Delta T \) from anthesis to maturity under the three sowing dates was 7.66, 8.05 and 8.41, respectively, with the CV of 4.76% (less than 5%) so that the PC of high-protein cultivar Xuzhou 26 was determined by interaction of \( F(T) \times F(T\text{SN}) \) as described in Equation (3). The genotypic parameters, TS and PS, which were 0.25 and 0.74, respectively, were determined during the construction of the model.

#### Table 5. Prediction errors of PC (%) in three wheat cultivars with three sowing dates at Nanjing in 2002.

| Cultivar   | Sowing date (day, month) | RMSE (%) |
|------------|--------------------------|----------|
|            | 22, Oct.                 | 1.43     |
| Xuzhou 26  | −0.01                    | 0.72     | 7.23     |
| Yangmai 10 | 1.04                     | −0.59    | 0.07     | 5.74     |
| Ningmai 9  | −0.80                    | 0.45     | −0.04    | 4.89     |
|            |                          | 3.38     | 3.45     | 5.99     |

(Slafer and Rawson, 1994; Cao and Moss, 1997).
For medium and low-protein cultivars Yangmai 10 and Ningmai 9, their PCs were calculated by $F(T)$, $F(TSUN)$ and $F(TR)$, and the genotypic parameters listed in Table 2. Comparison of the predicted with the observed PCs indicated that the RMSE values of three wheat cultivars and three sowing dates were less than 10%, averaging 5.99% (Table 5). Especially, the prediction of PC under optimal and late sowing dates was more accurate than with early sowing. Also, better prediction was made for medium (Yangmai 10) and low-protein (Ningmai 9) cultivars. When the observed PC was plotted against the estimated data for all treatments (Fig. 2A), the model performed reliably with different cultivar types and sowing dates.

Then, the model was validated by the field experiment conducted in 2000, including eight wheat cultivars and six eco-sites. The CV of $\Delta T$ among six sites were all greater than 5% (averaging 9.76%), so PCs of two high-protein cultivars, Xuzhou 26 and Jinan 17, were determined by the mean $\Delta T$ from anthesis to maturity at each eco-site. The results showed that the RMSEs of all treatments were less than 10%, averaging 6.91% (Table 6). When the observed PCs were plotted against the estimated data (Fig. 2B), all points were close to the bisecting line with high coefficient of determination ($R^2=0.65$). This indicates that the model could well predict the PCs of winter wheat cultivars under different growing environments.

Finally, the model was tested by comparing with the experimental data obtained on 40 wheat cultivars examined at Nanjing in 1999. Table 2 shows the genetic parameters of 40 cultivars, mostly determined by the data from Xuzhou experiments (Exp.2 and Exp.3). Comparison of the estimated with the observed PC indicated that the RMSE was 2.47%, 2.40% and 6.35% in high, medium and low-protein cultivars, respectively, averaging 3.34% for 40 cultivars. When the observed PC values were plotted against the estimated data for all treatments (Fig. 2C), there was a high goodness of fit with excellent $R^2$ of 0.94. Thus, the model performed well in predicting the PC of different winter wheat genotypes.

**Discussion and Conclusion**

In this paper, we presented a simplified method for quantifying the PC in winter wheat based on the meteorological and genetic factors. Four meteorological factors, daily mean temperature, mean $\Delta T$, total sunshine hours and precipitation from anthesis to maturity, were identified by stepwise regression analysis to determine PC in different quality types. This supports the previous suggestions by Benzian and Lane (1986) and Asseng et al. (2002). However, another relevant factor, growing degree-days (GDD), which has been reported to have positive impact on PC (Benzian and Lane, 1986; Gooding et al., 1997), was eliminated through stepwise regression selection. This may be because the mean temperature plays a key role in determining the PC, while GDD varies with the daily temperature (Wheeler et al., 1996).

Five genotypic parameters $Pc$, $PVT$, $TS$, $PS$ and $RS$, were incorporated to make the present model applicable to different cultivar types of winter wheat.
Pc and RS were parameters newly introduced in the present study, and PVT, TS and PS were introduced into the process-based simulation model for wheat development by Cao and Moss (1997). Thus, certain genotypic parameters in wheat could be shared in the process-based models and statistical models. Precise calibration of these parameters should be of significant importance for improving the model performance under diverse circumstances. In particular, validation of the present model showed significant genetic differences in photoperiod sensitivity (PS), as reported by Slafer and Rawson (1994) and Cao and Moss (1997).

In process-based simulation models, the accuracy of PC prediction is determined by the process components such as the uptake, distribution and redistribution of N. Simulated errors in these components may double the error in prediction of final PC in wheat (Asseng et al., 2002). Thus, a simplified statistical model for PC, to a certain extent, could be more accurate and feasible than the process-based model. Evaluation of the present model by field experiments conducted in different years, with different eco-sites, sowing dates and cultivars proved that the model could be well used for prediction of PC under a large range of climate conditions. However, the present results (Table 5) also indicated that prediction of PC under optimal and late sowing (15 days later than optimal sowing) was more reliable than under early sowing (15 days earlier than optimal sowing). Thus, the model performance under early sowing remains to be improved, although such early sowing is not a normal production practice in China.

Benzian and Lane (1986) and Smith and Gooding (1999) reported that including the effects of soil wetness and soil texture could improve climate-based prediction of the PC in wheat. In the present study, similar soil types were selected and soil fertilization and wetness at sowing were controlled to similar levels to minimize the soil impact on PC. Yet, the soil properties are so complicated and the soil water and nutrients during wheat growth were not so strictly controlled, which may have an impact on accuracy of model prediction. Therefore, further study should be conducted to carefully expand the climate-based model for including the impact of major soil factors.

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* In Chinese.
** In Chinese with English abstract.
Appendix: The abbreviated variables and their description used in the present paper.

| Variable | Description | Unit |
|----------|-------------|------|
| CV       | coefficient of variation | %    |
| F(T)     | the function influencing the effect of Tmean on PC | -    |
| F(ΔT)    | the function influencing the effect of ΔT on PC | -    |
| F(TSUN)  | the function influencing the effect of TSUN on PC | -    |
| F(TR)    | the function influencing the effect of TR on PC | -    |
| FV       | the slope of the linear relationship between F(TSUN) and TSUN | h⁻¹ |
| GDD      | growing degree-days from anthesis to maturity | °C ⋅d |
| PC       | protein content of grain | %    |
| Pc       | characteristic protein content of grain for wheat cultivar | % |
| PCp      | potential protein content of grain for common wheat | % |
| PS       | photoperiod sensitivity | -    |
| PVT      | physiological vernalization time | d    |
| rT       | the corresponding weight or weight factor of Tmean | -    |
| rTSUN    | the corresponding weight or weight factor of TSUN | -    |
| rTR      | the corresponding weight or weight factor of TR | -    |
| RT       | coefficient of partial correlation with Tmean | -    |
| RTR      | coefficient of partial correlation with TR | -    |
| RTSUN    | coefficient of partial correlation with TSUN | -    |
| RMSE     | root mean square error | %    |
| RS       | sensitivity of a given wheat cultivar to precipitation variation | -   |
| Tmean    | daily mean temperature | °C  |
| Tmeano   | the optimum mean temperature for grain nitrogen accumulation | °C  |
| TR       | total rainfall from anthesis to maturity | mm  |
| TRo      | optimum precipitation for grain nitrogen accumulation | mm  |
| TS       | sensitivity of different wheat genotypes to temperature | -   |
| TSUN     | total sunshine hours from anthesis to maturity | h   |
| ΔT       | difference between daily maximum and minimum temperatures | °C  |