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The effects of nitrogen and water stresses on the nitrogen-to-protein conversion factor of winter wheat

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ABSTRACT

An accurate estimation of grain protein concentration (GPC) based on the nitrogen-to-protein conversion factor (Fp) and grain nitrogen concentration is crucial for scientific research and human nutrition. The aim of this study was to evaluate the effects of nitrogen and water stresses on Fp. Field experiments consisting of 16 treatments that included the coupled stresses of nitrogen and water were conducted over 2 wheat growing seasons from 2014 to 2016. The true GPC was determined based on the total amino acid concentration, and the wheat grain nitrogen concentration was determined using the Kjeldahl method. Water stress was estimated based on the soil volumetric water content, and nitrogen stress was estimated based on the crop nitrogen concentration. Water stress, nitrogen stress, and coupled water and nitrogen stresses were used to fit dynamic Fp (Fp'), which was different from common Fp value of 5.70. The results indicated that stresses resulted in the decline of Fp. The nitrogen concentration of total amino acid had a positive relationship with the minimum of the two stresses (S_{MIN}). The observed Fp' ranged from 5.14 to 5.86, and a certain significant negative linear relationship existed between S_{MIN} and Fp' ($P < 0.001$). The relative root mean square error (RRMSE) values of Fp' and GPC based on an Fp' were 0.024 and 0.072, respectively, while the RRMSE of GPC estimated based on an Fp value of 5.70 was 0.098. GPC estimated using a variable Fp value that accounted for coupled water and nitrogen stresses was more precise than GPC estimates based on Fp value of 5.70. The new general linear model provides an improved method to calculate GPC more accurately in different environment.

1. Introduction

Protein is potentially the most important nutrient because it is the key component of cells, tissues and body fluids (Leser, 2013). Wheat is a major source of protein (Esm and Hucl, 2002; Singh and Majumdar, 2015) and the most widely grown cereal in the world because of its adaptation to different environments and broad applications for food (Shewry, 2008). An accurate estimation of grain protein concentration (GPC) is crucial for the management of human nutrition and health (Lourenço et al., 2010). The amino acid concentration is often referred to as “true protein” (Graciela et al., 2011; Heidelbaugh et al., 1975), but it is tedious and expensive to measure. Colorimetric assays are rapid and cheap, but their precision is poor (Bradford, 1976). The Kjeldahl method (Kjeldahl, 1883) is the most frequently used approach to determine the total organic nitrogen concentration of wheat, and GPC is the product of the total nitrogen concentration and the nitrogen-to-protein conversion factor (Fp). However, the selection of an appropriate Fp has been a point of controversy.

The classical assumption was that protein contains 16% nitrogen; therefore, an Fp value of 6.25 (100/6.25) was applied to estimate GPC over the years (Kjeldahl, 1883). This assumption was incorrect, and a more accurate Fp for different foods varies from 5.18 to 6.25 (Jones, 1941). The Fp of different plants, animals and foods has been fully researched, and accurate results have been obtained. However, whether Fp changes under water and nitrogen stresses remains unknown. An Fp value of 5.70 for wheat has been used to calculate GPC in all situations (Fernández and Laird, 1959; Pan et al., 2006); however, the accuracy of this value requires further verification. Tkachuk(1969) estimated Fp based on the total amino acid concentration and GNC; the resultant Fp ranged from 5.58 to 5.62 among different cultivars.

The sources of controversy for Fp include the existence of different amino acid concentrations and non-protein nitrogen (NPN). A change in

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the amino acid composition can change the Fp value (Estrella, 2008). The amino acid concentration is decisively determined by genetic type (Tanács, et al, 1997) but can be radically modified by nitrogen fertilization (Németh, 1985). The application of appropriate nitrogen fertilization affects the quality and quantity of wheat grain protein (Lv et al., 2017). In addition, irrigation can lengthen the grain-filling period, resulting in a decline in lysine, tyrosine, valine, threonine, and methionine (Daniel and Triboi, 2000). Significant interactions between nitrogen fertilization and irrigation have also been presented (Zheng et al., 2011). Theoretically, the Fp value of wheat will change under irrigation and nitrogen application. Foods contain many types of NPN, including nucleic acids, vitamins, nitrate, nitrite, alkaloids, nitrogenous glycosides, amines, polyamines, and free amino acids (Ezeagu et al., 2002). The NPN component does not contribute to the functional properties of vegetable protein flours or GPC (Fan and Sosulski, 1974). Fp based on the amino acid composition of total protein is considered the most accurate (Morr, 2010; Sosulski and Holt, 1980). Therefore, fluctuations in Fp are relatively high, and Fp does not have a fixed value.

Irrigation and nitrogen fertilization rates have significant effects on Fp, but the existence of regional features (cultivar, precipitation, temperature, and soil nutrients) makes it difficult to determine general trends. In addition, GPC is more of an individual characteristic which reflects growth status of a single tiller, while yield and grain nitrogen yield are population characteristics (Wang et al., 2015). S_N (nitrogen stress) and S_W (water stress) can be used to indicate the response of a single tiller to nitrogen and water. Consequently, S_N and S_W were used to reflect the wheat growth situation in this study. Coupled relationships (minimum, additive, and multiplicative) of S_N and S_W were used to explore the coupling effects on Fp.

Estimation of Fp and its determination methods have been studied extensively. However, there is limited information on the variation in Fp of wheat in response to S_N and S_W. In this study, we estimated GPC based on two hypotheses: (1) Fp is affected by S_N and S_W, and (2) the coupling effects of S_N and S_W have a significant linear negative correlation with Fp.

2. Materials and methods

2.1. Experimental site

The field experiment, conducted from Oct. 2014 to Jun. 2016, was located at the Ministry of Education Key Laboratory for Agricultural Soil and Water Engineering in Arid Areas at Northwest A&F University (34°14′ N, 108°3′ E, 506 m a.s.l.), where the average annual precipitation is 580 mm, the average precipitation in wheat growing season is only 203 mm, the average annual temperature is 13 °C, and the annual sunshine duration is 2196 h. Precipitation is not uniformly distributed in this area. The precipitation in Oct. 2014 to Jun. 2015 was 344 mm, and the precipitation in Oct. 2015 to Jun. 2016 was 261 mm. The Guanzhong-irrigation area belongs to semi-humid and arid regions. The soil of the experimental area has a loam texture and developed on a 3-meter-thick Loess deposit with very little groundwater recharge. The basic physical and chemical properties of the surface soil (0–20 cm) before planting were 16.01 g kg^{-1} organic matter, 1.01 g kg^{-1} total nitrogen, 17.66 mg kg^{-1} Olsen extractable P, 273.33 mg kg^{-1} Olsen exchangeable K, 0.15 cm^3 cm^{-3} wilting coefficient, and 0.45 cm^3 cm^{-3} field capacity. In the top 100 cm (0–100 cm), the average saturated water content was 31.8 g g^{-1}, pH = 8.07, soil bulk density = 1.48 g cm^{-3}, and saturated hydraulic conductivity = 279.99 cm d^{-1}.

2.2. Experimental design and treatments

The wheat cultivar grown in these experiments was "*Xiaoyan 22*" (*Triticum aestivum* L.), which is popular in Northwestern and Northern China. The experimental design was a randomized, blocked split plot with 4 main plot irrigation treatments and 4 subplot fertilization treatments, replicated 3 times. The plot dimensions were 6 m × 2.5 m with a buffer zone of 0.25 m between each plot. The irrigation treatments were I_0 (rain fed), I_1 (60 mm during wheat wintering stage), I_2 (60 mm during wheat wintering stage and 60 mm at the elongation stage), and I_3 (60 mm during wheat wintering stage, 60 mm at the elongation stage, and 60 mm at the filling stage). The fertilization treatments were N_0, N_{105}, N_{210}, and N_{315}; the subscript represents the nitrogen application rate, kg N hm^{-2}.

Soil volumetric water contents were measured during nine phenology phases (ZC_00_Sowing, ZC_10_Emergence, ZC_21_Tillering, ZC_30_Beginning of stem elongation, ZC_60_Flowering, ZC_70_First grains visible, ZC_75_Milky ripe, ZC_89_Fully ripe, ZC_99_ Harvested product). The soil volumetric water content was determined by TRIME-TDR (TRIME-PICO-IPH TDR, IMKO, Germany) and measured at seven depths (0–10, 10–20, 20–30, 30–40, 40–60, 60–80, and 80–100 cm).

Ten plant samples (aboveground portions and grains) of each plot were collected when soil volumetric water content was measured except ZC_00_Sowing, because no plant samples could be collected in ZC_00_Sowing. To keep pace with S_W or soil volumetric water content, the S_N of in ZC_00_Sowing was set as 0.00, because the nitrogen in ZC_00_Sowing was provided by the grain itself.

These plant samples were dried to a constant weight at 75 °C, ground by a steel grinder, and stored in a refrigerator. Subsamples ranging from 0.1 to 0.2 g (accurate to 0.0001 g) were digested in a microwave and alkali-distilled in Kjeldahl apparatus (JELTEC 2300, Sweden FOSS, Sweden) for the determination of nitrogen concentration. The total amino acid concentration was estimated as GPC according to the ISO 13,903–2005 method (L-8900 amino acid analysis meter, Hitachi High-Technologies Corporation). Nitrogen concentration of total aminoacids (Eq. (1)) is estimated as:

$$NC = \text{sum}(AAC_i \times AANC_i) \tag{1}$$

where NC is the nitrogen concentration of grain dry matter, g N g^{-1} dry $matter^{-1}$; AAC is a single amino acid concentration, g amino acid g^{-1} dry $matter^{-1}$; AANC is the nitrogen concentration of a single amino acid, g N g^{-1} amino $acid^{-1}$; i is 17 different amino acids contained in wheat (e.g., asparticacid, threonine, serine, …). The values of AANC are obtained from the study of Miller and Houghton (Miller and Houghton, 1945). The observed dynamic Fp (Fp') (Eq. (2)) is estimated as:

$$Fp' = \frac{TAAC}{NC} \tag{2}$$

where Fp' is dynamic Fp, dimensionless; TAAC is the total amino acid concentration of grain dry matter, g total amino acid g^{-1} dry $matter^{-1}$.

2.3. Stress factors

The estimation of stress factors was based on the entire growth period. All the stresses at different phenology would result in the change of Fp. The average nitrogen or water stress factors of nine phenology phases were used to represent the total nitrogen and water stress factors, respectively.

2.3.1. Water stress

The water stress factor (Eq. (3)) reflects expansive growth, leaf senescence, and plant development, which is determined by the soil available water capacity and crop growth rate(Hanks, 1974):

$$S_w = \frac{\sum_{i=1}^{9}\left(1 - \frac{\theta_{ai} - \theta_{wp}}{\theta_f - \theta_{wp}}\right)}{9} \tag{3}$$

where S_W is the water stress factor, dimensionless; θ_a is the soil volumetric water content, $cm^3\,cm^{-3}$; θ_{wp} is the wilting coefficient, $cm^3\,cm^{-3}$; and θ_f is the field capacity, $cm^3\,cm^{-3}$.