EFFECTS OF TILLAGE IN FALLOW PERIOD AND SOWING METHODS ON WATER STORAGE AND GRAIN PROTEIN ACCUMULATION OF DRYLAND WHEAT

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This study aimed to investigate the effect of deep plowing (DP) and subsoiling (SS) in fallow period combined with film mulch sowing in drill (FM-DS) in growth period on dryland wheat. A field study was conducted at Wenxi experimental site of Shanxi Agricultural University. Soil water storage, grain protein content, and the activity of key enzymes in nitrogen metabolism in flag leaves were determined. Compared with no-tillage (CK), DP and SS significantly increased water storage ($P<0.05$). Similar result was observed by FM-DS when compared with drill sowing (DS) ($P<0.05$). DP combined with FM-DS worked the best. Generally, the contents of albumin, gliadin and glutenin in spikelets were also significantly increased in DP and SS, especially in DP, when compared with CK ($P<0.05$). In addition, the glutamine synthetase (GS) activity was enhanced while the glutamate dehydrogenase (GDH) activity was reduced in DP and SS ($P<0.05$), and DP worked better than SS. The contents of gliadin, glutenin, and total protein in all spikelets as well as glutenin/gliadin (glu/gli) in central spikelets and basal spikelets were higher in FM-DS than in DS ($P<0.05$). In conclusion, DP combined with FM-DS was the best strategy for water storage and the yield and quality of dryland wheat.

Keywords: Deep plowing, subsoiling, film mulch; soil moisture, protein content

INTRODUCTION

Water shortage represents the main factor which affects the yield of dryland wheat (Angus et al., 2001). Shanxi Province is located in the eastern margin of semiarid zone of Loess Plateau in China. The rainfall in this area falls mainly from July to September, little and unevenly. In summer, the water in the soil surface loses a lot through evaporation because of the high temperature. In this case, how to accumulate rainwater during the fallow period has gradually caught the growers’ attention. At present, there are some techniques for the storage and preservation of soil moisture, such as deep plowing (DP), subsoiling (SS), conservation tillage, and film mulch sowing in drill (FM-DS) technique. Researches had shown that DP (Bhatt et al., 2006), SS, and zero tillage (Wang et al., 2007) in the summer fallow period could change the soil structure, enhance the effect of water storage, and improve the water use efficiency. The use of FM-DS technique could effectively collect rainwater, reduce evaporation, maintain the soil moisture, improve the water use efficiency, and increase crop yield (Liu et al., 2009). It had been a breakthrough technology for cultivating high-yield dryland wheat (Guan et al., 2011). A previous study has shown that, mulching increased the temperature and moisture in soil, as well as the number of tillers, length of the growing period, spikelet and grain numbers per spike, and the duration from flowering to harvest (Li et al., 1999).

The content of wheat grain protein is greatly affected by environment conditions, especially by the soil moisture. It has been reported that there is an increment in the content of wheat grain protein but a decrease in protein yield when soil moisture deficiency (Xie et al., 2002); moderate drought promotes the accumulation of grain protein, while excessive drought or waterlogging reduce the wheat grain weight, yield, and protein yield (Genty et al., 1989).

Ammonia assimilation is very important for grain protein synthesis. The ability of ammonia assimilation increases with the enhancement of glutamine synthetase (GS) activity, and glutamic acid dehydrogenase (GDH) functions in recycle and transport ammonia hydrolyzed from protein (Cruz et al., 2006). In adversity condition, reduced GS activity and enhanced GDH activity help plant to reduce the toxicity of ammonia and meet the demand of nitrogen (Ireland et al., 1999). GS plays a central role in nitrogen metabolism and protein accumulation. The protein content was positively associated with GS activity, but was negatively correlated with GDH activity (Gallais et al., 2004; Miflin et al., 2002). At the early filling stage, GS activity of flag leaf was closely related with the total grain protein, glutenin, and gliadin content, while at the middle filling
stage, GS activity of flag leaf was closely related with glutenin/gliadin (gliu/gli) (Yu et al., 2009). There were many studies on wheat quality with irrigation (Abad et al., 2004; Jiang et al., 2004; Johansson et al., 2001), but little on dry-cultivation. Researches about dryland wheat focused on storage and preservation of soil moisture, or increasing yield. It remains to be further studied how to regulate the soil moisture, achieve the target of water storage through whole year, increase yield, and improve the quality of dryland wheat based on the techniques of storage and preservation of soil moisture. Thus, in this study, we combined the water storage by fallow tillage with the water conserving by FM-DS in growth period. Accumulations of grain protein in different ear axis positions were analyzed, and the effect of soil moisture on accumulation of grain protein and its physiological mechanism was elucidated. The results of this study provided the best cultivation technology of conserving water to gain high wheat yields.

MATERIALS AND METHODS

The field experiments were conducted at the Wenxi Experimental Station of Shanxi Agricultural University (35.35°N, 111.22°E), Shanxi Province, China, from 2010 to 2011. The experimental fields are hilly dryland with no irrigation conditions. The soil properties at the soil layer (0-20 cm) were tested in June 2010. The total nitrogen (TN) content was 0.65 g kg\(^{-1}\), alkali-hydrolyzable nitrogen (AN) was 32.83 mg kg\(^{-1}\), and the rapidly available phosphorus was 20.11 mg kg\(^{-1}\). About 60%-70% rainfall concentrated in July, August, and September (Table 1).

**Experimental design:** Dryland wheat used in this study was supplied by the Agricultural Committee of Wenxi County (Yunhan20410). Experiment was laid out as split-split plot arrangement and three replications. The stubble remained in fields after wheat harvest was 20-30 cm. Tillage treatment was carried out at July 15\(^{th}\). Three levels [DP (depth of 30-40 cm), SS (depth of 30-40 cm), and no-tillage (CK)] were allocated to the main plots. Shallow rotary, level soil, and techniques of storage and preservation of soil moisture were carried out at the end of August. Sub-plots were treated with two levels of sowing method [drill sowing (line spacing of 20 cm, DS) and FM-DS (line spacing of 30 cm)]. The area of experimental site was 300 m\(^2\) (50 m in length and 6 m in width). The fertilizer (150 kg hm\(^{-2}\) N, 150 kg hm\(^{-2}\) P\(_2\)O\(_5\), and 150 kg hm\(^{-2}\) K\(_2\)O) were applied before planting. The dryland wheat was sown at 225×10\(^4\) hm\(^{-2}\) on September 29\(^{th}\) with routine management.

**Determination of soil moisture:** Soil samples of each 20 cm layer were got from 0 to 300 cm at different growth stages of dryland wheat, including sowing (on September 28, 2010), pre-wintering (on November 30, 2010), elongation (on April 2, 2011), booting (on April 19, 2011), anthesis (on May 2, 2011), and maturity (on June 10, 2011) stages. The soil samples were collected into aluminium specimen boxes immediately and the fresh weight was determined. Then the samples were oven-dried at 105°C until the weight would not change and the dry weight was determined. The soil moisture was calculated.

\[
SWS = W_i \cdot D_i \cdot H_i \times 10/100 \quad \text{(SWS: soil moisture of layer i (mm); W_i: mass water content of layer i (%); D_i: soil bulk density of layer i (g cm\(^{-3}\); H_i: thickness of layer i (cm)).}
\]

**Determination of grain protein and its components in different ear axis positions:** The ears of wheat with the same growth state and flowering time were labeled during anthesis. Thirty labeled ears were picked every 5 days after anthesis. Ears with similar number of fertile spikelet were divided into three equal parts: basal spikelets, central spikelets, and apical spikelets. The separated grains were oven-dried at 80°C until the weight weren’t changed, and then ground with a high-speed grinder. The contents of grain protein and its components were determined according to the sequential extraction procedure. The nitrogen content was determined according to the semimicro Kjeldahl method (Halvorson et al. 2004).

Protein content = nitrogen content x 5.7.

**Determination of the nitrogen metabolism key enzyme activity in flag leaves:** The wheat with the same growth state and flowering time were labeled during anthesis stage. Fifteen labeled flag leaves were picked every 5 days after anthesis. The leaves were frozen rapidly in liquid nitrogen, and stored at −40°C for enzyme assay. The activities of GS and GDH were measured as Lu et al described (Lu et al., 2005).

**Determination of grain yield:** The spike number of per unit area, average kernel number and thousand kernels weight

<table>
<thead>
<tr>
<th>Year</th>
<th>Fallow period</th>
<th>Sowing Pre-wintering</th>
<th>Pre-wintering-Elongation</th>
<th>Elongation -Anthesis</th>
<th>Anthesis -Mature</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>2002-2011</td>
<td>300.6±95.6</td>
<td>42.0±17.9</td>
<td>43.2±24.2</td>
<td>31.8±11.1</td>
<td>62.1±24.8</td>
<td>479.7±106.8</td>
</tr>
<tr>
<td>2010-2011</td>
<td>401.5</td>
<td>27.1</td>
<td>19.1</td>
<td>22.2</td>
<td>64.8</td>
<td>534.7</td>
</tr>
</tbody>
</table>

Source: Meteorological station of Wenxi County, Shanxi Province, China; Values are mean±SD. Fallow period: The first 10 days of Jul. to the first 10 days of Oct.; Sowing-Pre-wintering: The first 10 days of Oct. to the last 10 days of Nov.; Pre-wintering- E elongation: The last 10 days of Nov. to the first 10 days of Apr.; Elongation – Anthesis: The first 10 days of Apr. to the first 10 days of May; Anthesis-Mature: The first 10 days of May to the middle 10 days of Jun.
were investigated. Fifty individuals of wheat from each plot were taken to measure yield, and wheat in 4 m² area was reap to calculate the economic yield. All the measurements were operated in the maturity stage.

**Data processing and statistics analysis:** Data Processing System (DPS, China) and SAS 9.0 (SAS Corp, Cary, NC, USA) were used for statistics analysis. Significance of difference was tested by ANOVA followed by LSD method with significance level of \( P=0.05 \).

**RESULTS**

**Effects of fallow tillage combined with sowing method on soil moisture in different growth stages:** Compared to CK, the soil moisture (0-300 cm soil layer) was increased by 42-70 mm in DP and SS during the sowing period. The soil moisture was increased in DP and SS in different growing stages (Table 2). Under condition of DS, the soil moisture in DP was significantly different compared with CK in sowing stage (515.65±8.14 vs. 445.80±3.76), pre-wintering stage (456.92±8.93 vs. 416.43±5.61), elongation stage (422.65±4.47 vs. 400.82±8.60), anthesis stage (322.22±0.09 vs. 309.24±5.90), and maturity stage (287.74±2.31 vs. 277.27±1.50), while in SS, it was significantly different compared with CK only in sowing (487.25±4.43 vs. 445.80±3.76) and pre-wintering (439.43±4.82 vs. 416.43±5.61) stages \( (P < 0.05) \). Under condition of FM-DS, the difference in soil moisture between DP and CK was significant in every growth stages, while the difference between SS and CK was significant only in sowing, anthesis and maturity stages \( (P < 0.05) \). These results suggest that regardless of sowing method, the effect of DP on water storage could last all growth stages compared with CK, while the effect of SS lasted only to several stages. However, the difference between DP and SS was only significant during sowing, anthesis and maturity stages \( (P < 0.05) \). Additionally, soil moisture was increased by FM-DS compared with DS from pre-wintering stage to maturity stage \( (P < 0.05) \).  

**Effects of fallow tillage combined with sowing method on the accumulation of grain protein:**

**Effect on dynamic change of grain protein content:** The grain protein content in different ear axis positions and days after anthesis varied in different treatments (Fig. 1). Generally, there was the lowest grain protein content on 15\(^{th}\) day after anthesis in every position of spikelet \( (P < 0.05) \), except that lowest on 20\(^{th}\) days after anthesis in central spikelets was observed when DP or SS combined with FM-DS \( (P < 0.05) \).

The grain protein content in apical spikelets, central spikelets, and basal spikelets increased in DP and in SS, compared with CK \( (P < 0.05) \). When compared with SS, DP could increase the grain protein content in central spikelets and basal spikelets \( (P < 0.05) \). If combined with FM-DS, the difference of grain protein content between DP and SS was remarkable during 5-20\(^{th}\) and on 35\(^{th}\) days after anthesis in central spikelets, while during 5-30\(^{th}\) days after anthesis in basal spikelets \( (P < 0.05) \). When compared with DS, FM-DS significantly increased the grain protein content in apical spikelets during 10-35\(^{th}\) days after anthesis, in central spikelets and in basal spikelets during 5-35\(^{th}\) days after anthesis \( (P < 0.05) \).

Above all, it was helpful to accumulate grain protein in different ear axis positions and in whole spikelet in DP and SS, especially when DP combined with FM-DS.

**Effects on grain protein and its components content:** Tables 3-5 show the contents of grain protein and its components in different ear axis positions. They were all highest in central spikelets, and lowest in apical spikelets \( (P < 0.05) \). Compared with CK, the contents of globulin, gliadin, glutenin, total protein, and gli/gli in basal spikelets and central spikelets were significantly increased in DP \( (P < 0.05) \). So did the contents of albumin and globulin in apical spikelets. However, when DP combined with FM-DS, the contents of glutenin, total protein, and gli/gli in apical spikelets were significantly decreased, compared with CK combined with FM-DS \( (P < 0.05) \).

**Table 2. Effects of tillage method combined with sowing method on soil water storage at the sowing stage and different growth stages (mm).**

<table>
<thead>
<tr>
<th>Tillage method</th>
<th>Sowing method</th>
<th>Sowing stage</th>
<th>Pre-wintering stage</th>
<th>Elongation stage</th>
<th>Booting stage</th>
<th>Anthesis stage</th>
<th>Maturity stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>DP</td>
<td>DS</td>
<td>515.65±8.14a</td>
<td>456.92±8.93c</td>
<td>422.65±4.47c</td>
<td>364.48±6.23c</td>
<td>322.22±0.09b</td>
<td>287.74±2.31d</td>
</tr>
<tr>
<td>DM-DS</td>
<td>517.08±1.11a</td>
<td>503.27±6.21a</td>
<td>485.60±5.59a</td>
<td>408.00±3.06a</td>
<td>348.80±0.75a</td>
<td>329.93±0.97a</td>
<td></td>
</tr>
<tr>
<td>SS</td>
<td>DS</td>
<td>487.25±4.43b</td>
<td>439.43±4.82d</td>
<td>414.07±2.76d</td>
<td>358.18±1.95a</td>
<td>313.80±4.82c</td>
<td>279.77±2.69e</td>
</tr>
<tr>
<td>DM-DS</td>
<td>489.84±0.91b</td>
<td>482.22±4.42b</td>
<td>462.68±7.42b</td>
<td>392.04±5.46b</td>
<td>341.05±3.38a</td>
<td>320.11±2.33b</td>
<td></td>
</tr>
<tr>
<td>CK</td>
<td>DS</td>
<td>445.80±3.76c</td>
<td>416.43±5.61e</td>
<td>400.82±8.06d</td>
<td>352.30±7.76c</td>
<td>309.24±5.90c</td>
<td>277.27±1.50c</td>
</tr>
<tr>
<td>DM-DS</td>
<td>446.13±4.78c</td>
<td>468.94±3.79bc</td>
<td>454.25±7.48b</td>
<td>384.43±6.69b</td>
<td>320.55±7.39b</td>
<td>301.80±2.1c</td>
<td></td>
</tr>
</tbody>
</table>

Differences were compared with LSD method, and average values followed by different letters from a to e were statistically significant \( (P<0.05) \).

DP, deep plowing; SS, subsoiling; CK, no tillage; DS, drill sowing; FM-DS, film mulch.
Figure 1. Effects of tillage method in fallow period with sowing method on grain protein content on different days after anthesis.

Table 3. Effects of tillage method combined with sowing method on grain protein and its component contents in apical spikelets.

<table>
<thead>
<tr>
<th>Tillage method</th>
<th>Sowing method</th>
<th>Albumin (%)</th>
<th>Globulin (%)</th>
<th>Gliadin (%)</th>
<th>Glutenin (%)</th>
<th>Glu/Gli</th>
</tr>
</thead>
<tbody>
<tr>
<td>DP</td>
<td>DS</td>
<td>2.18±0.04c</td>
<td>1.10±0.02a</td>
<td>3.18±0.03b</td>
<td>3.29±0.01d</td>
<td>1.03±0.01bc</td>
</tr>
<tr>
<td></td>
<td>FM-DS</td>
<td>2.35±0.05b</td>
<td>1.08±0.03a</td>
<td>3.32±0.02a</td>
<td>3.36±0.03c</td>
<td>1.01±0.00d</td>
</tr>
<tr>
<td>SS</td>
<td>DS</td>
<td>2.04±0.03d</td>
<td>1.02±0.02b</td>
<td>3.00±0.04c</td>
<td>3.02±0.05c</td>
<td>1.01±0.00d</td>
</tr>
<tr>
<td></td>
<td>FM-DS</td>
<td>2.59±0.05a</td>
<td>1.10±0.00a</td>
<td>3.38±0.03a</td>
<td>3.58±0.01a</td>
<td>1.06±0.01a</td>
</tr>
<tr>
<td>CK</td>
<td>DS</td>
<td>1.81±0.04f</td>
<td>0.98±0.02b</td>
<td>2.87±0.02d</td>
<td>2.94±0.01f</td>
<td>1.02±0.01cd</td>
</tr>
<tr>
<td></td>
<td>FM-DS</td>
<td>1.93±0.02e</td>
<td>0.93±0.02c</td>
<td>3.32±0.02a</td>
<td>3.47±0.02b</td>
<td>1.04±0.00ab</td>
</tr>
</tbody>
</table>

Differences were compared with LSD method, and average values followed by different letters from a to e were statistically significant \((P<0.05)\).

DP, deep plowing; SS, subsoiling; CK, no tillage; DS, drill sowing; FM-DS, film mulch.

Table 4. Effects of tillage method with sowing method on grain protein and its component contents in central spikelets.

<table>
<thead>
<tr>
<th>Tillage method</th>
<th>Sowing method</th>
<th>Albumin (%)</th>
<th>Globulin (%)</th>
<th>Gliadin (%)</th>
<th>Glutenin (%)</th>
<th>Glu/Gli</th>
</tr>
</thead>
<tbody>
<tr>
<td>DP</td>
<td>DS</td>
<td>2.85±0.05c</td>
<td>1.48±0.02b</td>
<td>4.63±0.05b</td>
<td>4.73±0.03c</td>
<td>1.02±0.01cd</td>
</tr>
<tr>
<td></td>
<td>FM-DS</td>
<td>3.59±0.04a</td>
<td>1.63±0.03a</td>
<td>4.98±0.02a</td>
<td>5.46±0.03a</td>
<td>1.10±0.00a</td>
</tr>
<tr>
<td>SS</td>
<td>DS</td>
<td>2.51±0.04d</td>
<td>1.36±0.02cd</td>
<td>4.53±0.04c</td>
<td>4.53±0.01d</td>
<td>1.00±0.01de</td>
</tr>
<tr>
<td></td>
<td>FM-DS</td>
<td>2.89±0.02c</td>
<td>1.49±0.04b</td>
<td>4.67±0.02b</td>
<td>4.96±0.02b</td>
<td>1.06±0.00b</td>
</tr>
<tr>
<td>CK</td>
<td>DS</td>
<td>2.35±0.04e</td>
<td>1.31±0.04d</td>
<td>4.16±0.04d</td>
<td>4.09±0.02e</td>
<td>0.98±0.00c</td>
</tr>
<tr>
<td></td>
<td>FM-DS</td>
<td>3.06±0.05b</td>
<td>1.42±0.03bc</td>
<td>4.49±0.04c</td>
<td>4.68±0.05c</td>
<td>1.04±0.02bc</td>
</tr>
</tbody>
</table>

Differences were compared with LSD method, and average values followed by different letters from a to e were statistically significant \((P<0.05)\).

DP, deep plowing; SS, subsoiling; CK, no tillage; DS, drill sowing; FM-DS, film mulch.

Table 5. Effects of tillage method with sowing method on grain protein and its component contents in basal spikelets.

...
Improvement of grain protein in wheat

<table>
<thead>
<tr>
<th>Tillage method</th>
<th>Sowing method</th>
<th>Albumin (%)</th>
<th>Globulin (%)</th>
<th>Gliadin (%)</th>
<th>Glutenin (%)</th>
<th>Glu/Gli</th>
</tr>
</thead>
<tbody>
<tr>
<td>DP</td>
<td>DS</td>
<td>2.84±0.05b</td>
<td>1.45±0.02b</td>
<td>4.16±0.05c</td>
<td>4.24±0.01d</td>
<td>1.02±0.01bc</td>
</tr>
<tr>
<td></td>
<td>FM-DS</td>
<td>3.09±0.04a</td>
<td>1.59±0.02a</td>
<td>4.48±0.03a</td>
<td>4.95±0.03a</td>
<td>1.11±0.01a</td>
</tr>
<tr>
<td>SS</td>
<td>DS</td>
<td>2.46±0.04c</td>
<td>1.47±0.04b</td>
<td>3.92±0.02d</td>
<td>3.89±0.05e</td>
<td>0.99±0.02cd</td>
</tr>
<tr>
<td></td>
<td>FM-DS</td>
<td>2.85±0.02b</td>
<td>1.46±0.02b</td>
<td>4.39±0.05ab</td>
<td>4.76±0.01b</td>
<td>1.08±0.02a</td>
</tr>
<tr>
<td>CK</td>
<td>DS</td>
<td>2.31±0.06d</td>
<td>1.33±0.04c</td>
<td>3.87±0.04d</td>
<td>3.75±0.05f</td>
<td>0.97±0.00d</td>
</tr>
<tr>
<td></td>
<td>FM-DS</td>
<td>3.05±0.03a</td>
<td>1.50±0.03b</td>
<td>4.33±0.04b</td>
<td>4.52±0.02c</td>
<td>1.04±0.00b</td>
</tr>
</tbody>
</table>

Differences were compared with LSD method, and average values followed by different letters from a to e were statistically significant (P<0.05).

DP, deep plowing; SS, subsoiling; CK, no tillage; DS, drill sowing; FM-DS, film mulch.

Table 6. Effects of tillage method with sowing method on grain protein and its component contents in whole spikelets.

<table>
<thead>
<tr>
<th>Tillage method</th>
<th>Sowing method</th>
<th>Albumin (%)</th>
<th>Globulin (%)</th>
<th>Gliadin (%)</th>
<th>Glutenin (%)</th>
<th>Glu/Gli</th>
<th>Yield (kg ha(^{-1}))</th>
<th>Protein Yield (kg ha(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>DP</td>
<td>DS</td>
<td>2.51±0.04b</td>
<td>1.45±0.02b</td>
<td>4.24±0.05c</td>
<td>0.97±0.00c</td>
<td>4794.6±39.05b</td>
<td>699.39±3.84b</td>
<td></td>
</tr>
<tr>
<td></td>
<td>FM-DS</td>
<td>2.58±0.01a</td>
<td>1.50±0.01a</td>
<td>4.78±0.01a</td>
<td>1.05±0.00a</td>
<td>5011.4±25.60a</td>
<td>755.73±3.83a</td>
<td></td>
</tr>
<tr>
<td>SS</td>
<td>DS</td>
<td>2.32±0.01d</td>
<td>1.42±0.01b</td>
<td>4.24±0.05cd</td>
<td>1.01±0.02bc</td>
<td>4588.1±94.28c</td>
<td>649.07±3.43c</td>
<td></td>
</tr>
<tr>
<td></td>
<td>FM-DS</td>
<td>2.38±0.01c</td>
<td>1.45±0.02b</td>
<td>4.45±0.03b</td>
<td>1.03±0.01ab</td>
<td>4819.5±25.88b</td>
<td>696.90±2.04b</td>
<td></td>
</tr>
<tr>
<td>CK</td>
<td>DS</td>
<td>2.26±0.03e</td>
<td>1.39±0.02c</td>
<td>4.03±0.01e</td>
<td>0.99±0.03c</td>
<td>3205.7±52.35e</td>
<td>502.95±4.65e</td>
<td></td>
</tr>
<tr>
<td></td>
<td>FM-DS</td>
<td>2.29±0.01de</td>
<td>1.49±0.01a</td>
<td>4.13±0.04e</td>
<td>1.00±0.01bc</td>
<td>3933.0±60.28b</td>
<td>550.28±2.92d</td>
<td></td>
</tr>
</tbody>
</table>

Differences were compared with LSD method, and average values followed by different letters from a to e were statistically significant (P<0.05).

DP, deep plowing; SS, subsoiling; CK, no tillage; DS, drill sowing; FM-DS, film mulch.

Compared with CK, the contents of albumin, glutenin, total protein in apical spikelets, the contents of gliadin and glutenin in central spikelets, and the contents of glutenin and total protein in basal spikelets were significantly higher in SS (P < 0.05). Compared with SS, DP significantly enhanced the grain protein content, its components contents, and glu/gli in FM-DS in central spikelets, and contents of albumin, glutenin, and total protein in basal spikelets, while significantly down-regulated the contents of albumin, glutenin, total protein, and glu/gli in FM-DS in apical spikelets (P < 0.05). Compared with DS, FM-DS significantly enhanced not only the contents of gliadin, glutenin, and total protein in different ear axis positions, but also the contents of albumin in apical spikelets, albumin, globulin, and glu/gli in central spikelets, and glu/gli in basal spikelets (P < 0.05). Thus, DP combined with FM-DS was helpful to increase the grain protein content and glu/gli in central spikelets and basal spikelets, while SS combined with FM-DS was helpful to increase the grain protein content in apical spikelets and basal spikelets, and glu/gli in basal spikelets, when compared with CK combined with FM-DS. For DP and SS, the grain yield was significantly increased (up to 886-1589 kg ha\(^{-1}\)), while the grain protein content was significantly increased by 3.4-7.8 %, compared with CK (Table 6). Compared with SS, DP significantly enhanced the contents of albumin, gliadin, and total protein, as well as the contents of globulin and glutenin when combined with FM-DS (P < 0.05). Compared with DS, FM-DS significantly increased the contents of albumin, gliadin, glutenin, and total protein (P < 0.05). Thus, fallow tillage is useful to increase grain yield, grain protein content, and its components content. When DP combined with FM-DS, the yield and the quality of grain protein increased more significantly.

**Effects of fallow tillage combined with sowing method on the key enzyme activity of nitrogen metabolism in flag leaves post anthesis stage:**

**Effects on GS and GDH in flag leaves:** The GS activity was increased in DP and SS, compared with CK. The differences in GS activity between DP combined with FM-DS and CK, and between SS combined with FM-DS and CK during 5-10\(^{th}\) and during 25-30\(^{th}\) days after anthesis were remarkable (P < 0.05). The GS activity was higher in DP than that in SS, especially when combined with FM-DS, and was higher in FM-DS than that in DS (P < 0.05). The GDH activity was significantly decreased in DP and SS, compared with CK (P < 0.05). The GDH activity was lower in DP than that in SS, especially when combined with FM-DS, and was lower in FM-DS than that in DS (P < 0.05). Therefore, it was most helpful to accumulate protein in DP combined with FM-DS, due to the increase of GS activity and decrease of GDH activity (Fig. 2).

**Relationship between the key enzyme activity of nitrogen metabolism and the accumulation of grain protein in different ear axis positions:** Table 7 shows the relationship between the key enzyme activity of nitrogen metabolism and the accumulation of grain protein in different ear axis positions in different tillage combined with different sowing
Table 7. Correlation coefficients between GS, GDH activity and soil water storage and grain protein, and its component contents.

<table>
<thead>
<tr>
<th>Activities and soil water</th>
<th>Albumin (%)</th>
<th>Globulin (%)</th>
<th>Gliadin (%)</th>
<th>Glutenin (%)</th>
<th>Glu/Gli (%)</th>
<th>Total protein (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GS activity</td>
<td>0.7558**</td>
<td>0.7272</td>
<td>0.6632</td>
<td>0.5280</td>
<td>-0.1129</td>
<td>0.3685</td>
</tr>
<tr>
<td>GDH activity</td>
<td>-0.8467*</td>
<td>-0.8553*</td>
<td>-0.6171</td>
<td>-0.5027</td>
<td>0.0603</td>
<td>-0.3707</td>
</tr>
<tr>
<td>0-300 cm Soil water</td>
<td>0.8630*</td>
<td>0.6119</td>
<td>0.8029*</td>
<td>0.7201</td>
<td>0.1928</td>
<td>0.6784</td>
</tr>
<tr>
<td>GS activity</td>
<td>0.7858*</td>
<td>0.9168**</td>
<td>0.9804**</td>
<td>0.9279**</td>
<td>0.7960*</td>
<td>0.8048**</td>
</tr>
<tr>
<td>GDH activity</td>
<td>-0.6884</td>
<td>-0.8885**</td>
<td>-0.9393**</td>
<td>-0.8844**</td>
<td>-0.7496</td>
<td>-0.7317</td>
</tr>
<tr>
<td>0-300 cm Soil water</td>
<td>0.8432*</td>
<td>0.9320**</td>
<td>0.8823*</td>
<td>0.9416**</td>
<td>0.9549**</td>
<td>0.8970**</td>
</tr>
<tr>
<td>GS activity</td>
<td>0.6952</td>
<td>0.8441*</td>
<td>0.7040</td>
<td>0.7444</td>
<td>0.7808*</td>
<td>0.9665**</td>
</tr>
<tr>
<td>GDH activity</td>
<td>-0.5883</td>
<td>-0.7214</td>
<td>-0.6531</td>
<td>-0.7075</td>
<td>-0.7583*</td>
<td>-0.9152**</td>
</tr>
<tr>
<td>0-300 cm Soil water</td>
<td>0.7378</td>
<td>0.7390</td>
<td>0.8921**</td>
<td>0.9395**</td>
<td>0.9697**</td>
<td>0.7413</td>
</tr>
<tr>
<td>GS activity</td>
<td>0.9076**</td>
<td>0.6995</td>
<td>0.9489**</td>
<td>0.9153**</td>
<td>0.5220</td>
<td>0.9899**</td>
</tr>
<tr>
<td>GDH activity</td>
<td>-0.9020**</td>
<td>-0.5622</td>
<td>-0.9505**</td>
<td>-0.9150**</td>
<td>-0.5203</td>
<td>-0.9746**</td>
</tr>
<tr>
<td>0-300 cm Soil water</td>
<td>0.7237</td>
<td>0.7120</td>
<td>0.7745*</td>
<td>0.9347**</td>
<td>0.8107*</td>
<td>0.8542*</td>
</tr>
</tbody>
</table>

* Significant at P < 0.05; ** Significant at P < 0.01.

These results indicate that the GS and GDH activity in flag leaves were the main factors to affect the protein accumulation in central spikelets and basal spikelets.

**DISCUSSION**

Researchers have found that minimal tillage or no-tillage for years could increase the volume weight of soil, affecting the absorption of nutrient and water from soil by crop roots (Birkås et al., 2004; Martinez et al., 2008). It made against to increase the yield. Baumhardt et al. (2002) found that DP removed the soil agglomerate, enhancing the ability of precipitation accepting and increasing water storage in soil. In our study, we found that it could effectively accumulate the rainfall and increase the water storage from the sowing stage to the maturity stage by DP and SS in fallow period.

method. The contents of albumin, glutenin, gliadin, and total protein in whole spikelet were significantly positively correlated with GS activity ($P < 0.01$), and were significantly negatively correlated with GDH activity ($P < 0.01$) in flag leaves.

The GS activity in flag leaves had notable correlation with grain protein content and glu/gli in central spikelets and basal spikelets, and was significantly positively correlated with albumin content in apical spikelets, protein content in central spikelets, and globulin content in basal spikelets ($P < 0.05$).

The GDH activity in flag leaves had notable correlation with grain protein content and glu/gli in basal spikelets. It also had notable correlation with the content of albumin and globulin in apical spikelets, and globulin, gliadin, and glutenin in central spikelets ($P < 0.01$).
Improvement of grain protein in wheat

This is probably the result of fallow tillage, which could loosen the soil and deepen the plough horizon. Fallow tillage is helpful to store rain in the fallow period and increase the available soil water at planting. Thus, it could provide protection for sowing in the appropriate period. In addition, our study found that the effect of water storage in DP was better than that in SS. DP could help to make the land ridged, turn the straw to the subsoil to become thoroughly decomposed, and break the plow pan formed over the years. These is more helpful to store rainfall. Mulching can reduce water loss, improve the infiltration of rainwater into the soil, enhance soil water retention, accelerate crop growth, and significantly increase crop yields (Li et al., 2012). Our study showed that the water keeping effects of soil in FM-DS could last to maturity, while the water of soil loosed quickly in DS during the growth period. It provided adequate moisture for the growth of wheat. It accounts for 75% of the annual rainfall during fallow period. Thus, DP during fallow period could help to effectively store rainfall. FM-DS could reduce evaporation, store rainfall further, and ensure water available for wheat during the whole growth period. In this study, DP combined with FM-DS could reduce the drought stress, promote the formation of grain, and achieve high yield at last. The enzyme activity of nitrogen metabolism is related to the accumulation of proteins. Sun et al. (2012) found that there was significant correlation between GS activity in flag leaves and grain protein yield. Our study found that the contents of gliadin, glutenin in central spikelets, and total protein in basal spikelets and whole spikelet were closely related to GS and GDH activity in flag leaves. Moreover, the relationships were close between the ratio of glu/gli in central spikelets and GS activity, and between the ratio of glu/gli in basal spikelets and GDH activity. It may due to the increase of GS activity which promoted the transfer of nitrogen from glutamate to other amino acids, and the transformation of inorganic nitrogen to organic nitrogen.

Grain protein content is one of the most important factors of wheat quality. Albumin and globulin are soluble with high nutritive value. Gliadin and glutenin are storage proteins which decide the visco-elasticity of dough. Also, the high ratio of glu/gli increases dough developing time which can enhance the processing quality. Wang et al. (2002) reported too much or little soil moisture content would lead to decline of nutritive value and processing quality of grain, while appropriate soil moisture could not only increase yield but also improve the quality. The position of grain in ear decided the spatial distribution characteristics of seed setting and material accumulation of wheat. In conclusion, DP in fallow period combined with FM-DS had a great effect on storage and preservation of soil moisture for dryland wheat. It could ease the drought stress in late growth period, achieve the target of storage and preservation of soil moisture in whole year, and increase the yield. Gliadin and glutenin in central spikelets, and glutenin and total protein content in basal spikelets were increased more when DP combined with FM-DS. Thus, DP combined with FM-DS was the most effective method for yield and protein quality enhancing.

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