INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
16–0340/2017/19–5–1193–1200
DOI: 10.17957/IJAB/15.0412
http://www.fspublishers.org

Full Length Article

Subsoiling Combined with Film Mulch Reduces Post-anthesis Proline Accumulation and Improves Water Storage and Grain Quality in Dryland Wheat Crop

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Abstract

This study aimed to investigate the effects of fallow tillage in fallow period combined with sowing methods in growth period on water storage post-anthesis proline accumulation and grain quality of dryland wheat. The field study was conducted at Wenxi experimental site of Shanxi Agricultural University. Tillage treatments included deep tillage (DT), subsoiling (SS) and sowing methods included drilling (DS), furrowing (FS) and film mulch (FM). Soil water storage, post-anthesis proline content, glutamine synthetase (GS) and glutamic acid dehydrogenase (GDH) activities grain protein content and wheat processing quality under different combinations of tillage and sowing treatments were determined. Soil water storage at the depth of 0-300 cm was improved by tillage in fallow period. Compared with other treatments combination of SS and FM significantly improved soil water storage and decreased proline content in flag leaf and grain. This combination also significantly increased GS activity and decreased GDH activity after anthesis. Additionally, grain protein content and processing quality including sedimentation value and wet/dry gluten contents were improved. Correlation analysis indicated that there were significant correlations between proline content and the activities of GS and GDH in grains between protein content and the activity of GS in flag leaves, as well as between protein content and sedimentation value and gluten content. Combination of SS with FM is the best integrated cultivation techniques in dryland wheat crop in terms of reducing post-anthesis proline accumulation and improving water storage and grain quality. © 2017 Friends Science Publishers

Keywords: Dryland wheat; Tillage mode; Sowing method; Proline accumulation; Grain quality

Introduction

Shanxi province is located in the Loess Plateau eastern margin in China. The climate in this area is mostly semiarid with annual precipitation ranging from 200 mm to 600 mm, mainly from July to September (Shengxiu and Ling, 1992; Sun *et al.*, 2015). Water deficiency has great impact on agricultural practices in this area (Zhang *et al.*, 2011). Dryland farming in this area is dominated by monoculture cropping systems, and dryland wheat is one of single crops (Zhang *et al.*, 2011). In this area, precipitation is not adequate to meet the requirement of evapotranspiration during the growing season, which would limit crop yields to a large extent (Li *et al.*, 2013). Additionally, under drought stress, many plants accumulate compatible solutes to raise osmotic pressure to maintain turgor and drive gradient for water uptake (Rhodes and Samaras, 1994). Proline is one of the most important compatible solutes, which serves as an available source of carbon, nitrogen and reduction equivalent during recovery from stress (Blum and Ebercon, 1976; Ahmad and Hellebust, 1988). Higher proline content in wheat after drought stress has been reported by many studies (Patel and Vora, 1985; Vendruscolo *et al.*, 2007).

Furthermore, Xie *et al*. (2003) have found that environmental conditions especially the soil moisture can affect the content of wheat grain protein (Xie *et al.*, 2003). Moderate drought promotes grain protein accumulation, while excessive drought reduces the protein yield, wheat grain weight and yield (Genty *et al.*, 1989). Specially, grain protein content could affect the processing quality of wheat kernels such as sedimentation value and gluten content (Sun *et al.*, 2007; Shi *et al.*, 2009). In addition, ammonia assimilation plays an important role in grain protein synthesis. Glutamine synthetase (GS) and glutamic acid dehydrogenase (GDH) play key roles in recycle and transport of ammonia hydrolyzed from protein (Cruz *et al.*, 2006).

In view of the critical role of soil moisture in wheat grain yield, protein yield and processing quality, exploring a method to accumulate soil water has gradually caught the attentions of more and more farmers. Some techniques, such

To cite this paper: Zhao, H., J. Zhang, Y. Yang, H. Li, H. Han, A. Ji and Z. Gao, 2017. Subsoiling combined with film mulch reduces post-anthesis proline accumulation and improves water storage and grain quality in dryland wheat crop. *Int. J. Agric. Biol*. 19: 1193–1200

as conservation tillage, deep tillage (DT) and subsoiling (SS) have be applied to preserve the soil moisture in fallow period (Wang *et al.*, 2007). Summer fallowing is a basic method for conserving precipitation in the soil of dryland areas, which can satisfy the moisture demand of crop (Unger and Jones, 1981). Moreover, the additional soil water can be conserved not only during the fallow periods prior to planting but also during the growing season (Greb, 1966; Adams *et al.*, 1976). For instance, applying film mulch (FM) sowing in growing season has been reported to improve water storage and use efficiency (Sun *et al.*, 2015). However, most of the present studies focus on the effect of single technique on moisture conservation.

Therefore, the present study combined two tillage modes (DT and SS) in fallow period with three sowing methods (drilling sowing (DS), furrowing sowing (FS) and FM) in growing period to investigate the effects of different technology combinations on soil moisture post-anthesis proline accumulation GS and GDH activities and grain protein content and processing quality in dryland wheat crop. This study aimed to reveal the optimal combination of tillage mode with sowing method and to provide theoretical basis for improvement of the quality and yields of dryland wheat.

Materials and Methods

Study Area

The field study was conducted from 2011 to 2012 in the summer fallow land of Wenxi Experimental Station (35°93′-35°34′ N and 110°59′-111°37′ E) of Shanxi Agricultural University, Shanxi Province China, with wheat as the test crop. This district belongs to warm temperate continental climate with distinctive seasons. The average annual temperature is 12.6°C. Annual precipitation averages 490mm with larger rainfall in July, August and September. During 2011 to 2012 rainfall was abundant higher than in previous years (Table 1). The frost-free period is 185 days. The soils are mainly clay loam and silty clay loam. Soil organic matter, alkali-hydrolyzable nitrogen and the rapidly available phosphorus at 0-20 cm depth were 611.88 $g{\cdot}kg^1$, 38.62 $mg{\cdot}kg^1$ and 14.61 $mg{\cdot}kg^1$ respectively.

Experimental Design

A total area of 150 m^2 was mapped out for the experiment. Dryland wheat (YunHan 20410) supplied by the Agricultural Committee of Wenxi County was used in this study. The experiment was arranged in two factors split plot design with tillage modes as the main-plot treatments and sowing methods as the sub-plot treatments using three replications. Tillage treatments included were deep tillage (DT, depth of 30–35 cm), subsoiling (SS, depth of 35–40 cm) and no-tillage (NT). Sowing treatments included drill sowing (DS, line spacing of 20 cm), furrowing sowing (FS, furrow depth of 10 cm wheat was sowed at the furrow bottom with two lines per furrow and line spacing of 19 cm) and film mulch sowing in drill (FM-DS, film width of 40 cm wheat was sowed on both sides of film with line spacing of 30 cm). The fertilizers (150 kg/hm^2 N, 150 kg/hm^2 P_2O_5, and 75 kg/hm^2 K_2O) were applied before planting. Wheat was sown at 225 × $10^4/hm^2$ on October 1st with routine management.

Determination of Soil Moisture

The soil moisture was measured gravimetrically (drying method) at different growth stages of dryland wheat, including before-sowing stage (112 days after preceding wheat harvesting), wintering stage (185 days after preceding wheat harvesting), elongation stage (301 days after preceding wheat harvesting), heading stage (321 days after preceding wheat harvesting), anthesis stage (327 days after preceding wheat harvesting) and mature stage (365 days after preceding wheat harvesting). Soil samples were obtained by earth auger at 20 cm increments (up to 300 cm) and collected into the aluminum specimen boxes immediately. After fresh weight dry weight was determined by oven-drying at 105°C until the constant weight. The soil moisture was calculated as following:

$$SM = [(FW - DW)/DW \times 100] \times T \times BD \quad (1)$$

Where SM represents soil moisture (mm); FW represents fresh weight of soil; DW dry weight of soil; T represents thickness of soil layer (mm); BD represents bulk density of soil layer (g/cm^3).

Determination of free Proline Content

During anthesis the ears of wheat with the same growth state and flowering time were labeled. Five labeled ears were picked every 5 days after anthesis. The free proline content in flag leaves and grains were determined according to the previous study (Bates *et al.*, 1973). Approximately 0.5 g plant material was ground in 10 mL 3% aqueous sulfosalicylic acid. After filtration 2 mL filtrate was reacted with 2 mL glacial acetic acid and 2 mL acid ninhydrin for 1 h at 100°C and then terminated in an ice bath. The reaction mixture was then extracted with 4 mL toluene and mixed vigorously in a test tube stirrer for 15–20 sec. The chromophore containing toluene was aspirated from the aqueous phase and the absorbance at 520 nm was recorded. The proline concentration was determined from a standard curve and calculated on a fresh weight basis.

Determination of Nitrogen Metabolism Key Enzyme Activity

Ten labeled ears were picked every 5 days after anthesis. The flag leaves and grains were frozen by liquid nitrogen rapidly and stored at -40°C which were used for the determination of the enzymatic activities of GS and GDH as a previous study described (Lu *et al.*, 2005).

Briefly, frozen flag leaves and grains were respectively ground for GS extraction in the extraction buffer. After centrifugation at 19,000 × g for 30 min at 4°C, GS activity in extract was detected in a reaction mixture containing imidazole buffer (Rhodes *et al.*, 1975). One unit of GS activity was the amount of enzyme catalyzing the formation of 1 μmol γ-glutamylhydroxamate per minute at 37°C.

For GDH extraction, frozen flag leaves and grains were also ground in the extraction buffer. After centrifugation at 19,000 × g twice (30 min each time), GDH activity was detected in both the aminating and the deaminating directions by following the absorption change at 340 nm (Loulakakis and Roubelakis-Angelakis, 1990). One unit of GDH activity was defined as the reduction or oxidation of 1 μmol of coenzyme (NAD or NADH respectively) per minute at 30°C.

Determination of Grain Protein and its Components

Ten labeled ears were picked every 5 days after anthesis. Their grains were separated and oven-dried at 80°C until the weight were not changed and then ground with a high-speed grinder. The nitrogen content was determined according to the semimicro Kjeldahl method (Halvorson *et al.*, 2004).

Protein content = nitrogen content × 5.7 (2)

The components of grain protein were determined according to the sequential extraction procedure.

Determination of Protein Yield

In the maturity stage the spike number of per unit area, average kernel number and thousand kernels weight were investigated. A total of twenty individuals of wheat were obtained from each plot to measure yield randomly. Additionally, wheat in 4 m^2 area was reaped to calculate the economic yield and protein yield.

Determination of Sedimentation Value and Gluten Content

A total of 1 kg wheat grains from each plot were ground using test flour miller (LRMM8040-3-D) and the flour yield was 62%. The sedimentation value was detected by standard method of American Association of Cereal Chemists (AACC) (56-61A). The wet gluten content was determined using hand-washing method, while the dry gluten content was determined from wet gluten by an oven drying method.

Data Processing and Statistics Analysis

Statistical analyses were performed using Data processing System (DPS and China) and SAS 9.0 (SAS Corp, Cary, NC and USA). Significance of the difference was tested by one-way analysis of variance (ANOVA) followed by LSD method. Correlation between variables was assessed using Pearson's correlation coefficient within SAS. Comparisons with P<0.05 was considered statistically significant.

Results

Effect of Fallow Tillage Combined with Sowing Method on Soil Moisture

During before-sowing stage, the soil moisture in 0–300 cm soil layer showed a variation trend of "low-high-low" with the increase of soil depth. The highest soil moisture appeared in 180 cm soil layer. Compared to NT the soil moisture in 0–300 cm soil layer increased by 19.18 and 26.93 mm in DT and SS respectively; in 100–200 cm soil layer increased by 28.14 and 37.76 mm; in 200–300 cm soil layer increased by 6.7 and 9.22 mm; in 0–300 cm increased by 54.02 and 73.91 mm (Fig. 1). The result indicated that fallow tillage could hold water storage so as to provide adequate soil moisture for wheat sowing.

Additionally, the soil moisture (0–300 cm soil layer) in growing stages showed a declined trend with the highest moisture in wintering stage (Table 2). Compared to DT and SS significantly increased the soil moisture (0–300 cm soil layer) from wintering to heading stage and decreased the soil moisture from anthesis stage to mature stage (P < 0.05). Under the same tillage mode the soil moisture (0–300 cm soil layer) in FM group was significantly higher than in FS and DS groups from wintering stage to heading stage (P<0.05). While from anthesis stage to mature stage, the soil moisture in FM group was significantly lower than in FM and DS groups (P < 0.05). Under conditions of tillage combined with sowing methods SS + FM significantly increased the soil moisture (0–300 cm soil layer) compared with SS + DS/FS during wintering stage to heading stage (P < 0.05). Due to the increase of water consumption at late growth stages of wheat the soil moisture in anthesis stage and mature stage decreased significantly (P< 0.05) (Table 2). This result suggested that SS combined with FM could hold water storage at all growth stages.

Effect of Fallow Tillage Combined with Sowing Method on free Proline Content

As shown in Fig. 2A, the free proline content in flag leaves presented bimodal curve and the peaks appeared on the 15 and 25 d after anthesis. The proline content was lower in SS than that in DT, especially when combined with FM. In addition, combination of SS and FM significantly decreased proline content in flag leaves after anthesis from 5 to 25 d (P < 0.05).

The proline content in grains presented unimodalcurve and the peak appeared on 15 d after anthesis (Fig. 2B). In fallow period the proline content in SS treatment was significantly lower than in DT. Based on the same tillage mode the proline content in grains in FM treatment was lower than in DS and FS treatments on 5 to 15 d post-anthesis (P < 0.05).

Table 1: Precipitation (mm) at the experimental site in Wenxi, Shanxi Province, China

Fallow period	Sowing-pre-wintering	Pre-wintering-elongation	Elongation-anthesis	Anthesis-mature	Total
256.57±76.05	43.60±11.95	32.77±13.27	32.08±4.84	62.00±5.45	427.02±63.44
459.90	137.40	28.20	20.50	27.10	673.10

Data source: Meteorological station in Wenxi, Shanxi Province, China.
Fallow period: Middle of June to early October; Sowing-pre-wintering: Early October to late November; Pre-wintering-elongation:Late November and early April; Elongation-anthesis: Early April to early May; Anthesis-mature: Early May to mid-June

Table 2: Effects of sowing methodscombined with tillage modes in fallow on soil water storage at the depth of 0-300

Tillage mode	Sowing method	WS	TGS	ES	HS	AS	MS
DT	DS	642.30±2.46 f	607.74±0.08 f	550.17±0.53 f	448.20±1.52 e	418.90±1.26 a	321.54±0.70 a
	FS	678.29±1.15 d	635.31±2.83 d	579.65±1.16 d	472.80±2.33 c	399.74±3.02 c	299.46±0.43 c
	FM	701.25±0.12 c	650.07±2.23 c	598.62±1.18 c	497.24±1.03 b	390.26±1.38 d	289.79±2.03 e
SS	DS	667.48±0.86 e	629.62±1.52 e	563.76±0.57 e	462.40±1.11 d	405.75±1.00 b	315.45±0.06 b
	FS	705.21±1.93 b	656.89±0.04 b	602.75±0.98 b	496.34±1.97 b	387.86±1.95 d	295.21±1.24 d
	FM	734.02±1.83 a	670.98±1.94 a	626.19±1.28 a	516.23±2.56 a	379.10±1.76 e	285.95±0.84 f

The different lowercase letters within the same column mean significant difference (P < 0.05) between treatments
WS: wintering stage; TGS: turning green stage; ES: elongation stage; HS: heading stage; AS: anthesis stage; MS: mature stage
DT: deep tillage; DS: drilling sowing; FS: furrowing sowing; FM: film mulch; SS: subsoiling

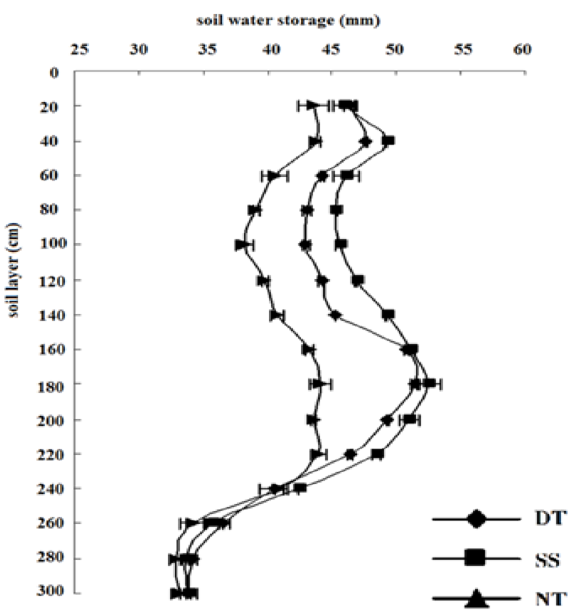

Fig. 1: Effects of three advance technology patterns on soil water storage at the depth of 0-300 cm. DT: deep tillage; SS: subsoiling; NT: no-tillage

Moreover, SS combined with FM, significantly decreased grain proline content from 5 to 15 d and improved grain proline content from 20 to 30 d on post-anthesis ($P < 0.05$).

Effect of Fallow Tillage Combined with Sowing Method on GS Activity

The effects of tillage combined with sowing methods on GS activity were consistent in flag leaves and grains showed declining trend after anthesis (Fig. 3A and B). Compared with DT and SS treatment could significantly increase GS activities in flag leaves on post-anthesis and in grains from 5 to 10 d on post-anthesis ($P < 0.05$). Additionally, under the same tillage mode FM could increase the GS activities in flag leaves and grains compared to DS and FS ($P < 0.05$). Among the different combinations of tillage and sowing methods GS had the highest activities in SS + FM treatment in both flag leaves and grains.

Effect of Fallow Tillage Combined with sowing Method on GDH Activity

The GDH activity in flag leaves showed "M" type with peaks on 10 d and 25 d after anthesis (Fig. 3C) while in grains the GDH activity presented rising trend after anthesis (Fig. 3D). Compared with DT and SS treatment significantly decreased the GDH activity in flag leaves and grains on 5 to 15 d after anthesis ($P < 0.05$). When SS combined with FM the GDH activity was significantly lower than in combination with DS and FS after anthesis (except for 30 d in flag leaves and 15 d in grains) ($P< 0.05$).

Effect of Fallow Tillage Combined with Sowing Method on Grain Protein and its Components Content

Compared with DT, the contents of albumin, gliadin, glutenin, total protein and protein yield were significantly increased in SS ($P<0.05$). When SS combined with FM, the contents of all protein components, total protein, glu/gli and protein yield were significantly higher than the other combination treatments ($P < 0.05$) (Table 3).

Effect of Fallow Tillage Combined with Sowing Method on Sedimentation Value and Gluten Content

Based on the same sowing method, the sedimentation values and gluten contents in SS treatments were significantly higher than in DT groups ($P < 0.05$) (Table 4).

Table 3: Effects of sowing methods combined with tillage modes in fallow on grain protein and its component contents at maturity stage

Tillage mode	Sowing method	Albumin (%)	Globulin (%)	Gliadin (%)	Glutenin (%)	Glu/Gli	Protein (%)	Proteinyield (kg/hm^2)
DT	DS	2.44±0.02 d	1.44±0.01 b	4.07±0.01 d	4.13±0.05 e	1.01±0.01 c	13.33±0.02 de	721.21±1.78 f
	FS	2.21±0.02 e	1.35±0.02 e	4.05±0.04 d	3.99±0.02 f	0.99±0.01 d	13.14±0.06 e	764.16±3.67 d
	FM	2.61±0.03 c	1.32±0.01 f	4.38±0.04 b	4.48±0.02 c	1.02±0.01 c	14.09±0.07 c	840.36±1.22 c
SS	DS	2.48±0.01 d	1.38±0.01 d	4.21±0.04 c	4.31±0.02 d	1.02±0.01 c	13.49±0.08 d	756.91±2.08 e
	FS	2.75±0.03 b	1.41±0.02 c	4.42±0.03 b	4.62±0.03 b	1.05±0.01 b	14.46±0.16 b	895.62±1.09 b
	FM	2.81±0.03 a	1.49±0.01 a	4.52±0.01 a	4.82±0.04 a	1.07±0.01 a	15.12±0.07 a	945.75±0.21 a

The different lowercase letters within the same column mean significant difference ($P < 0.05$) between treatments
DT: deep tillage; DS: drilling sowing; FS: furrowing sowing; FM: film mulch; SS: subsoiling

Table 4: Effects of sowing methods combined with tillage mode in fallow on gluten characters, sedimentation value

Tillage mode	Sowing Method	Sedimentation value (mL)	Wet gluten content (%)	Dry gluten content (%)
DT	DS	30.50±0.71 d	28.53±0.01 d	9.12±0.05 e
	FS	30.00±0.61 d	27.43±0.09 e	8.72±0.04 f
	FM	34.00±0.01 b	32.33±059 c	10.14±0.03 c
SS	DS	32.00±0.02 c	28.78±0.04 d	9.30±0.01 d
	FS	34.50±0.71 b	34.23±0.15 b	10.69±0.01 b
	FM	36.00±0.02 a	35.23±0.39 a	11.04±0.03 a

The different lowercase letters within the same column mean significant difference ($P < 0.05$) between treatments
DT: deep tillage; DS: drilling sowing; FS: furrowing sowing; FM: film mulch; SS: subsoiling

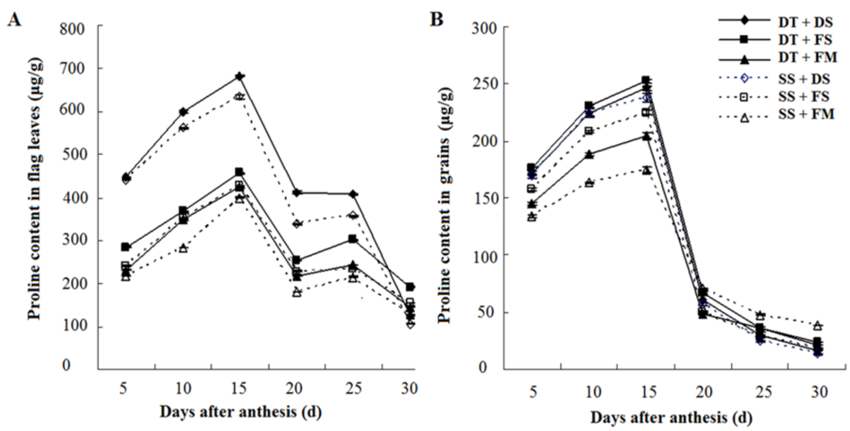

Fig. 2: Effects of sowing methodscombined with tillage modes in fallow on free proline content in flag leaves (A) and grains (B). DT: deep tillage; DS: drilling sowing; FS: furrowing sowing; FM: film mulch; SS: subsoiling

Moreover, based on the same tillage mode the sedimentation values and gluten contents in FM groups were significantly higher than in the other two group ($P< 0.05$). Specially, group of SS + FM had the highest sedimentation value and gluten content.

Correlation Analysis between Proline and Nitrogen Metabolism Key Enzyme Activity and Grain Protein

The proline content in flag leaves was negatively correlated with GS activity (in flag leaves and grains) and grain protein content ($P > 0.05$) and significantly positively correlated with GDH activity in flag leaves and grains ($P < 0.05$) (Table 5). Similarly the proline content in grains was negatively correlated with GS activity (in flag leaves and grains) and grain protein content ($P > 0.05$), and positively correlated with GDH activity in flag leaves ($P< 0.05$) and grains ($P > 0.05$).

Correlation Analysis between Grain Protein and Nitrogen Metabolism Key Enzyme Activity

The contents of albumin, globulin, gliadin, glutenin and total protein in grains were positively correlated with GS activity and negatively correlated with GDH activity in flag leaves and grains. Specially, the correlations between albumin/gliadin/glutenin/total protein and GS activity in flag leaves and between gliadin/total protein and GSactivityin grains were significant or extremely significant ($P < 0.05$ or $P < 0.01$) (Table 6).

Correlation Analysis between Grain Protein and Sedimentation Value and Gluten Content

Except for globulin, the contents of albumin, gliadin, glutenin and glu/gli were significantly positively correlated with sedimentation value and wet/dry gluten contents ($P<0.01$). Besides, the correlation between sedimentation value and glutenin content was higher than other proteins. The correlation between wet gluten content and gliadin content was higher than other proteins, while correlation between dry gluten content and glutenin content was higher than other proteins (Table 7).

Discussion

Field management practices have an influence on soil surface conditions and soil water which play an important role in crop development and growth in dryland farming (Zhang *et al.*, 2011). Studies have suggested that no-tillage for years could increase the volume weight of soil affecting the nutrient and water absorption by crop roots. Fallow tillage during spring could remove soil agglomerate and create a soil mulch to reduce evaporation and increase water storage in soil (Baumhardt and Jones, 2002; Martínez *et al.*, 2008). Moreover, fallow tillage disrupts compacted soil layers and increases the volume of soil explored by roots which results in increased water use and subsequently crop yields (Doty and Reicosky, 1978). In accordance with previous studies, our study also found that fallow tillage could hold water storage suggesting that fallow tillage was helpful to store rain in pre-sowing period and increase the available soil water at planting. Conserving soil moisture combined with reasonable sowing methods would benefit to the water storage as well. Soil surface mulch is a widely employed water management practice, which effectively reduces water loss by evaporation, enhances soil water retention, accelerate crop growth and increase crop yields (Pabin *et al.*, 2003; Unger *et al.*, 2012). The present study further confirmed the effect of SS combined with FM on water storage.

Under water stress, proline increases proportionately faster than other amino acids in plants (Bates *et al.*, 1973). Proline is a compatible solute that have a major role in osmotic adjustment (Voetberg and Sharp, 1991) and suggested for selecting drought-resistant varieties. In this study the free proline contents in flag leaves and grains (before pustulation period) were decreased when SS combined with FM suggesting the good effect of moisture conservation for SS combined with FM. It is well known that proline is synthesized from glutamate thereby having close relationship with ammonia assimilation. As a result, proline plays an importantly role in both carbon-nitrogen metabolism and drought stress (Verslues and Bray, 2006). Interestingly, the enzyme activity of nitrogen metabolism is physically associated with the formations of proline and grain protein (Verslues and Bray, 2006). Studies have suggested that the protein content is positively correlated with GS activity, but negatively associated with GDH activity (Miflin and Habash, 2002; Gallais and Hirel, 2004). In early reports, Skopelitis *et al.* (2006) have demonstrated that GS plays a main role in synthesis of proline under drought and GDH activity plays a key role in the production of glutamicacid for proline synthesis.

Table 5: Correlation coefficients between proline content and grains protein, activities of the relevant enzymes for nitrogen metabolism

Proline content	Activity of enzymes in flag leaves		Activity of enzymes in grains		Protein content
	GS	GDH	GS	GDH	
Flag leaves	-0.7624	0.8534*	-0.9073	0.8201*	-0.7458
Grains	-0.7397	0.8069*	-0.7736	0.7263	-0.8973

Correlation between variables was assessed using Pearson's correlation coefficient within SAS. Comparisons with P<0.05 were considered significantly different. *P<0.05; **P<0.01

Table 6: Correlation coefficients between grains protein, protein ingredient and activities of the enzymes for nitrogen metabolism

Protein ingredient	Activity of enzymes in flag leaves		Activity of enzymes in grains	
	GS	GDH	GS	GDH
Albumin content	0.8455*	-0.6184	0.6035	-0.6427
Globulin content	0.4987	-0.0107	0.1943	-0.3628
Gliadin content	0.9071**	-0.8370	0.8026*	-0.8101
Glutenin content	0.9192**	-0.7663	0.7496	-0.7931
Protein content	0.9385**	-0.7708	0.8181*	-0.8446

Correlation between variables was assessed using Pearson's correlation coefficient within SAS. Comparisons with P<0.05 were considered significantly different *P<0.05; **P<0.01

Table 7: The correlation between protein content, protein ingredient contents and sedimentation value, gluten content

Protein ingredient	Sedimentation value	Wet gluten content	Dry gluten content (%)
Protein content	0.9652**	0.9770**	0.9841**
Albumin content	0.9382**	0.9609**	0.9663**
Globulin content	-0.0644	-0.0508	-0.0064
Gliadin content	0.9664**	0.9665**	0.9594**
Glutenin content	0.9753**	0.9582**	0.9687**
Glu/Gli	0.9391**	0.8937**	0.9301**

Correlation between variables was assessed using Pearson's correlation coefficient within SAS. Comparisons with P<0.05 were considered significantly different *P<0.05; **P<0.01

Sun *et al*. (Sun *et al*., 2012) have found that there was significant correlation between GS activity in flag leaves and grain protein yield. Our study also found that the free proline contents in flag leaves and grains and grain protein contents were closely related to GS and GDH activities in flag leaves and grains.

Mature wheat grains contain 9-15% protein and half of the protein is deposited in the starchy endosperm cells (Shewry *et al.*, 2003). Grain protein content is an important quality index for cereal crops (Zhao *et al*., 2005). Specially, water is an important factor that influences the accumulation of grain protein (Dupont and Altenbach, 2003). Suitable water can not only increase production but also improve grain quality (Zhen-Wen and Song-Lie, 2002). In this study, SS combined with FM increased the grain protein content more effectively

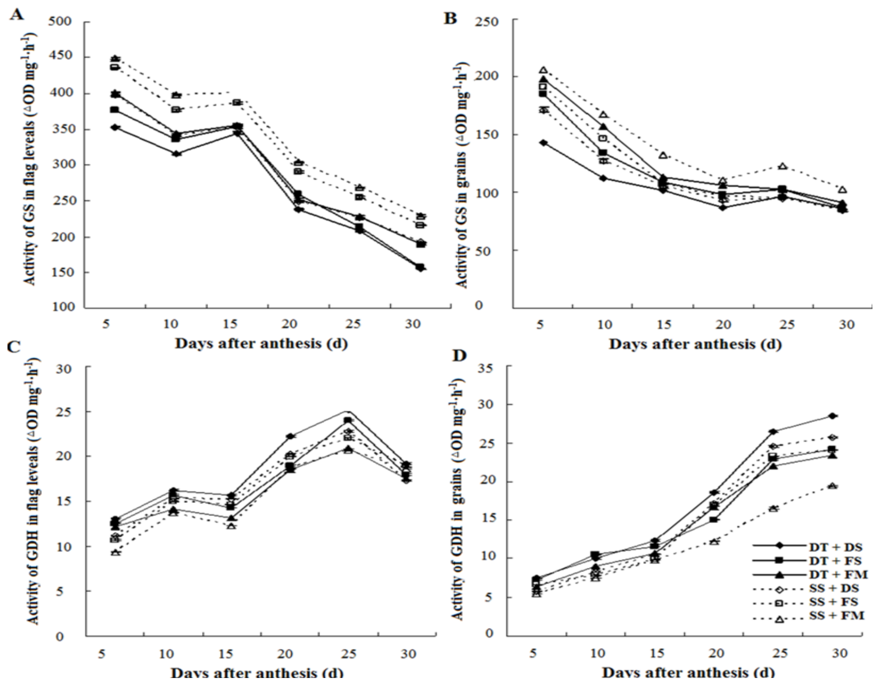

Fig. 3: Effect of sowing methods combined with tillage modes in fallow on GS (A, B) and GDH (C, D) activities in flag leaves (A, C) and grains (B, D). DT: deep tillage; DS: drilling sowing; FS: furrowing sowing; FM: film mulch; SS: subsoiling

than other combinations, which may be due to its better effects of storing and conserving water.

Previous study has suggested that wheat grain protein content directly determines the wheat processing quality, because wet gluten content mainly represents the protein content level (Shewry *et al.*, 2003). Protein content above 12.5% in wheat provides sufficient gluten to form good dough for bread making (Zhao *et al.*, 2005). Furthermore, sedimentation value is a comprehensive index reflecting the quantity quality of gluten (Huangwen *et al.*, 2004). In this study, we found that the gluten content and sedimentation value in SS + FM group were higher than in other groups besides grain proteins contents were significantly positively correlated with sedimentation value and wet/dry gluten contents, which further indicated the influence of wheat grain protein content on wheat processing quality.

Specially, approximately 80% of the total proteins in a typical wheat flour were glutenin and gliadin (Bietz and Wall, 1975; Tatham and Shewry, 1995). In general, the gliadin contributes to dough viscosity and glutenin contributes to dough elasticity. Dough viscosity and elasticity comprise the functional properties of dough (Khatkar and Schofield, 1997). Insufficient or excessive soil moisture is not conducive to the accumulation of glutenin and gliadin. In accordance with the findings above, the present study showed that glutenin and gliadin contents were higher than other proteins besides their contents in SS + FM group were higher than in other groups. Moreover, the correlation between wet gluten content/sedimentation value and gliadin/glutenin contents was higher than other proteins. Taken together, we speculate that improvement of glutenin and gliadin contents should be served as main direction to improve the dryland wheat grain quality.

In conclusion, the present study indicates that SS combined with FM has a great effect on the conservation of soil moisture in dryland wheat easing the drought stress in late growth period. Additionally SS combined with FM can decrease the free proline contents in flag leaves (post-anthesis stage) and grains (before filling period) slowing down the drought stress on post-anthesis stage of wheat. Moreover, the free proline contents are correlated with nitrogen metabolism enzyme activities that have an influence on the formation of grain proteins. Furthermore, grain protein content ultimately determines the wheat processing quality. Thus, SS combined with FM is the most effective method for raising yield and quality of dryland wheat.

Acknowledgements

This study was supported by Special fund for Modern Agriculture Industry Technology System Construction (No.CARS-03-01-24); NSFC (Natural Science Foundation of China) (No.31101112); the public welfare industry special scientific research funds (No.201303104); Youth Fund of Shanxi Province (No.2010021028-3).

References

Adams, J., G. Arkin and J. Ritchie, 1976. Influence of row spacing and straw mulch on first stage drying. *Soil Sci. Soc. Amer. J*., 40: 436–442

Ahmad, I. and J.A. Hellebust, 1988. The relationship between inorganic nitrogen metabolism and proline accumulation in osmoregulatory responses of two euryhaline microalgae. *Plant Physiol*., 88: 348–354

Bates, L., R. Waldren and I. Teare, 1973. Rapid determination of free proline for water-stress studies. *Plant Soil*, 39: 205–207

Baumhardt, R. and O. Jones, 2002. Residue management and tillage effects on soil-water storage and grain yield of dryland wheat and sorghum for a clay loam in texas. *Soil Till. Res*., 68: 71–82

Bietz, J. and J. Wall, 1975. The effect of various extractants on the subunit composition and association of wheat glutenin. *Cereal Chem*., 52: 145–155

Blum, A. and A. Ebercon, 1976. Genotypic responses in sorghum to drought stress. Iii. Free proline accumulation and drought resistance1. *Crop Sci*., 16: 428–431

Cruz, C., A. Bio, M. Dominguez-Valdivia, P. Aparicio-Tejo, C. Lamsfus and M. Martins-Loucao, 2006. How does glutamine synthetase activity determine plant tolerance to ammonium? *Planta*, 223: 1068–1080

Doty, C.W. and D.C. Reicosky, 1978. Chiseling to minimize the effects of drought. *Trans. Asae. Am. Soc. Agric. Eng*., 21: 0495–0499

Dupont, F. and S. Altenbach, 2003. Molecular and biochemical impacts of environmental factors on wheat grain development and protein synthesis. *J. Cereal Sci*., 38: 133–146

Gallais, A. and B. Hirel, 2004. An approach to the genetics of nitrogen use efficiency in maize. *J. Exp. Bot*., 55: 295–306

Genty, B., J.M. Briantais and N.R. Baker, 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochim. Biophys. Acta*, 990: 87–92

Greb, B., 1966. Effect of surface-applied wheat straw on soil water losses by solar distillation. *Soil Sci. Soc. Am. J*., 30: 786–788

Halvorson, A.D., D.C. Nielsen and C.A. Reule, 2004. Nitrogen fertilization and rotation effects on no-till dryland wheat production. *Agron. J*., 96: 1196–1201

Huangwen, Z., T. Jichun and L. Yanling, 2004. Characteristic and correlation analysis of grain quality among wheat varieties. *Shandong Agric. Sci*., 6: 1–4

Khatkar, B.S. and J. Schofield, 1997. Molecular and physico-chemical basis of breadmaking: Properties of wheat gluten proteins: A critical appraisal. *J. Food Sci. Technol*., 34: 85–102

Li, S., Z. Wang, S. Li, Y. Gao and X. Tian, 2013. Effect of plastic sheet mulch, wheat straw mulch, and maize growth on water loss by evaporation in dryland areas of china. *Agric. Water Manag*., 116: 39–49

Loulakakis, C.A. and K. Roubelakis-Angelakis, 1990. Intracellular localization and properties of nadh-glutamate dehydrogenase from vitis vinifera l: Purification and characterization of the major leaf isoenzyme. *J. Exp. Bot*., 41: 1223–1230

Lu, B., Y. Yuan, C. Zhang, J. Ou, W. Zhou and Q. Lin, 2005. Modulation of key enzymes involved in ammonium assimilation and carbon metabolism by low temperature in rice (*oryza sativa* L.) roots. *Plant Sci*., 169: 295–302

Martínez, E., J.P. Fuentes, P. Silva, S. Valle and E. Acevedo, 2008. Soil physical properties and wheat root growth as affected by no-tillage and conventional tillage systems in a mediterranean environment of chile. *Soil Tillage Res*., 99: 232–244

Miflin, B.J. and D.Z. Habash, 2002. Carbon and nitrogen relationships and signalling. The role of glutamine synthetase and glutamate dehydrogenase in nitrogen assimilation and possibilities for improvement in the nitrogen utilization of crops. *J. Exp. Bot*., 53: 979–987

Pabin, J., J. Lipiec, S. Wlodek and A. Biskupski, 2003. Effect of different tillage systems and straw management on some physical properties of soil and on the yield of winter rye in monoculture. *Int. Agrophys*., 17: 175–182

Patel, J.A. and A.B. Vora, 1985. Free proline accumulation in drought-stressed plants. *Plant Soil*, 84: 427–429

Rhodes, D., G.A. Rendon and G.R. Stewart, 1975. The control of glutamine synthetase level in *lemna minor* L. *Planta*, 125: 201–211

Rhodes, D. and Y. Samaras, 1994. *Genetic Control of Osmoregulation in Plants*, pp: 347–361. Cellular and molecular physiology of cell volume regulation

Shengxiu, L. and X. Ling, 1992. Distribution and management of drylands in the people's republic of china. *In*: *Advances in Soil Science*, pp: 147–302. *Springer*

Shewry, P.R., N.G. Halford, A.S. Tatham, Y. Popineau, D. Lafiandra and P.S. Belton, 2003. The high molecular weight subunits of wheat glutenin and their role in determining wheat processing properties. *Adv. Food Nutr. Res*., 45: 219–302

Shi, Y., Y.L. Zhang, Zhen-Wen and Yu, 2009. Contents of grain protein components and their relationships to processing quality in wheat. *Acta Agron. Sinica*, 35: 1306–1312

Skopelitis, D.S., N.V. Paranychianakis, K.A. Paschalidis, E.D. Pliakonis, I.D. Delis, D.I. Yakoumakis, A. Kouvarakis, A.K. Papadakis, E.G. Stephanou and K.A. Roubelakis-Angelakis, 2006. Abiotic stress generates ros that signal expression of anionic glutamate dehydrogenases to form glutamate for proline synthesis in tobacco and grapevine. *Plant Cell*, 18: 2767–2781

Sun, M., Y. Deng, Z. Gao, H. Zhao, A. Ren, G. Li, Z. Yang, X. Hao and Y. Zong, 2015. Effects of tillage in fallow period and sowing methods on water storage and grain protein accumulation of dryland wheat. *Pak. J. Agric. Sci*., 52: 1–8

Sun, X.Y., M.A. Chuan-Xi, S.I. Hong-Qi, W. Wang and C. Feng, 2007. Effects on gluten quality of high molecular weight glutenin subunits coded by two alleles and its interactions at glu-a1 and glu-b1 loci in common wheat. *Acta Laser Biol. Sin.*, 16: 432

Sun, Y., J. Ma, Y. Sun, H. Xu, Z. Yang, S. Liu, X. Jia and H. Zheng, 2012. The effects of different water and nitrogen managements on yield and nitrogen use efficiency in hybrid rice of china. *Field Crops Res*., 127: 85–98

Tatham, A.S. and P.R. Shewry, 1995. The s-poor prolamins of wheat, barley and rye. *J. Cereal Sci*., 22: 1–16

Unger, P., R. Baumhardt and F. Arriaga, 2012. Mulch tillage for conserving water. *Adv. Soil Sci*., 3: 427–453

Unger, P.W. and O.R. Jones, 1981. Effect of soil water content and a growing season straw mulch on grain sorghum. *Soil Sci. Soc. Am. J*., 45: 129–134

Vendruscolo, E.C.G., I. Schuster, M. Pileggi, C.A. Scapim, H.B.C. Molinari, C.J. Marur and L.G.E. Vieira, 2007. Stress-induced synthesis of proline confers tolerance to water deficit in transgenic wheat. *J. Plant Physiol*., 164: 1367–1376

Verslues, P.E. and E.A. Bray, 2006. Role of abscisic acid (aba) and arabidopsis thaliana aba-insensitive loci in low water potential-induced aba and proline accumulation. *J. Exp. Bot*., 57: 201–212

Voetberg, G.S. and R.E. Sharp, 1991. Growth of the maize primary root at low water potentials iii. Role of increased proline deposition in osmotic adjustment. *Plant Physiol*., 96: 1125–1130

Wang, X.B., D.X. Cai, W.B. Hoogmoed, O. Oenema and U.D. Perdok, 2007. Developments in conservation tillage in rainfed regions of north china. *Soil Till. Res*., 93: 239–250

Xie, Z.J., D. Jiang, W.X. Cao, T.B. Dai and Q. Jing, 2003. Effects of post-anthesis soil water status on the activities of key regulatory enzymes of starch and protein accumulation in wheat grains. *Acta Phytophysiol. Sin*., 29: 309–316

Zhang, S., P. Li, X. Yang, Z. Wang and X. Chen, 2011. Effects of tillage and plastic mulch on soil water, growth and yield of spring-sown maize. *Soil Tillage Res*., 112: 92–97

Zhao, C., L. Liu, J. Wang, W. Huang, X. Song and C. Li, 2005. Predicting grain protein content of winter wheat using remote sensing data based on nitrogen status and water stress. *Int. J. Appl. Earth Obs. Geoinf*., 7: 1–9

Zhen-Wen, W.Y.F.Y. and L.S.X.Y. Song-Lie, 2002. Effect of nitrogen nutrition on the change of key enzyme activity during the nitrogen metabolism and kernel protein content in winter wheat. *Acta Agron. Sin*., 6: 4

(Received 11 July 2016; Accepted 22 May 2017)