COORDINATED IMPROVEMENT OF GRAIN YIELD AND PROTEIN CONTENT IN DRYLAND WHEAT BY SUBSOILING AND OPTIMUM PLANTING DENSITY

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Abstract. Subsoiling during fallow period is an important agronomic practice for storing soil moisture in dryland for winter wheat. Present study was designed to evaluate the effect of different planting density and tillage condition on nitrogen metabolizing enzymes, protein content, protein yield and grain yield of dryland wheat. Field experiment was carried out in Loess Plateau, Wenxi, Shanxi province, China from 2012 to 2014, using winter wheat cultivar Yunhan 20410. Subsoiling (SS) during fallow period or no-tillage (NT) was taken as main factor and planting density (LD, 190 plants m^{-2}, MD, 260 plants m^{-2}, HD, 320 plants m^{-2}) as subplot factors. Results showed that SS has significantly increased the tiller numbers. Furthermore, SS has increased the soil water content at sowing by 83 mm and 58 mm, soil water at anthesis by 38 mm and 10 mm, and grain yield by 26%-66% and 17%-34% in 2012-2013 and 2013-2014, respectively as compared to NT, whereas, gliadin, glutenin, albumin and total protein content of grain were decreased. Under SS, the soil water storage at anthesis in 0-300 cm soil layer was highest at LD and lowest at MD. The activities of glutamine synthetase (GS) and glutamate synthase (GOGAT) in flag leaf and grain were highest under MD, whereas glutamine acid-pyruvate (GPT) was highest at HD. Under SS, the tiller numbers, grain protein and grain yield were increased with the increasing planting density but without significant difference between MD and HD. Under NT, the tiller numbers and grain yield were highest with MD, whereas, the grain total protein, albumin, glutenin, gliadin, glutenin/gliadin were highest with LD. Our study demonstrated that subsoiling during fallow period with a planting density 260 plants m^{-2} was beneficial to achieve the simultaneous improvement of yield and grain protein content of dryland wheat in Loess Plateau.
Keywords: *winter wheat, Loess Plateau, tillage, soil water storage, nitrogen metabolizing enzymes*

Introduction

The yield of winter wheat (*Triticum aestivum* L.) in dryland area is unstable and substantially lower than the average yield in other areas of China and other European countries. Stabilizing the yield of dryland wheat and improving the overall production and grain quality of dryland areas have always been the main task of research for cultivation work in the arid regions of North China (Li, 2002; Ma et al., 2005).

Soil moisture is the main limiting factor for wheat production in dryland, which affects the nutrient transport in the plant, and determines the population structure, yield and quality of wheat (Xu et al., 2003; Xie et al., 2003). A large number of studies showed that subsoiling tillage practice has obviously enhanced the water storage and moisture retention in dryland cultivation (Bhatt and Khera, 2006; Williams et al., 2006; Zhang et al., 2004). Subsoiling during summer fallow period in Loess Plateau has increased the amount of soil precipitation and the soil water storage by 76 mm in 0-200 cm (Liao et al., 2002) which increased water use efficiency by 11% and wheat grain yield increased by 8% in Weibei dryland (Bai et al., 2014). In the hilly and sloping land

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of western Henan, the soil storage was increased by 23 mm in 0-160 cm soil layer before sowing after subsoiling treatment and the average yield was 703 kg ha^{-1} higher than that of traditional farming, the yield increased by 19%, and the water use efficiency increased by 17% (Wang et al., 2004). However, the effect of subsoiling on the quality of wheat varies with the amount of precipitation. After the subsoiling during fallow period in the dryland of the Loess Plateau, the protein content of the grain was reduced in the year with less precipitation (335.0 mm) and increased in years with normal precipitation (534.7 mm) and abundant precipitation (673.1 mm) (Sun et al., 2014).

It can be seen that different management strategies play an important role in determining the quality and yield of wheat. Numerous studies have documented how planting density (Zecevic et al., 2014) and different tillage treatments affect yield and yield components of wheat (Schillinger, 2005). Optimum planting density for winter wheat varies with the planting dates, planting conditions, and planting equipment (Lloveras et al., 2004; Zecevic et al., 2014). Tillering is found beneficial for winter wheat yield formation as part of yield compensation mechanism (Gaile et al., 2017). Planting density also affect tillering and higher population density has been showed associated with the early tiller cessation resulting in fewer tillers per plant (Sparkes et al., 2006). At higher population, most of red light is absorbed and far-red light is reflected. Therefore at higher density, the cessation of axillary bud growth is due to the reduction in light quality and intensity resulting in less number of tillers. On the other hand high red to far-red delayed tillering cessation with high tillers (Sparkes et al., 2006; Xie et al., 2016).

The seeding rate might cause different ecological structure of wheat population, affecting the soil moisture and nutrients, and thus affecting the growth and development of wheat. Under the low sowing level, wheat grain yield increases with the increase of seeding rate, but after a certain threshold value increasing the seeding rate might not increase and even lead to a decrease in grain yield (Bhatta et al., 2017; Zhang et al., 2016) and adjusting seeding rate can effectively regulate the formation of grain proteins. Li et al. (2010) showed that in the medium gluten wheat ‘Lianmai 2’ the grain protein content was increased with the increase of planting density in the range of 270-360×10^4 ha^{-1}. Similarly, with the increase of planting density in the range of 240-285×10^4 ha^{-1} the grain protein and component content and the ratio of grain glutenin to gliadin increased gradually in low gluten wheat variety ‘Yangmai 12’ (Liu et al., 2006). Ma et al. (2007) showed that the activity of the glutamine synthetase and glutamate synthase increased in the flag leaf at 15 d after anthesis and the protein content of the grain was increased with the increase of planting density in the 150-300×10^4 ha^{-1} range in the high gluten wheat ‘Yumai 49-198’. In order to simultaneously improve protein content and grain yield in wheat, protein yield is an important criteria, which is the product of grain yield and relative protein content and thus corresponds to the grain protein harvested per area. It showed a highly positive correlation with grain yield, but only a small correlation with protein content (Rapp et al., 2018).

It can be seen that the increasing seeding rate has a certain promoting effect on grain protein formation, but these results are mainly concentrated under the condition of full irrigation of the water, and the effect of seeding amount on soil moisture in dryland wheat field is less than that on the plant nitrogen absorption and grain protein formation. Therefore, this study attempts to explore the effect of subsoiling during fallow period on dryland wheat based on the analysis of soil moisture, grain yield, nitrogen metabolism enzyme activity, grain protein and component under different planting densities. This

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research tries to explore the suitable sowing quantity on synchronization increase of grain yield and protein content under the subsoiling water storage condition of dryland wheat during fallow period.

Materials and Methods

Experimental site and meteorological condition

Field experiment was conducted for two winter wheat growing seasons from 2012 to 2014 at the experimental station of Shanxi Agricultural University located in Wenxi county (35°20′N, 111°17′E, and elevation 639 m), Shanxi Province, China. Experimental site is typical hilly dryland area where precipitation is a sole source of moisture and most of the precipitation (60-70%) is concentrated during the fallow period when field is left fallow. The average annual precipitation during experimental years and average from the last 12 years is presented in *Fig. 1*.

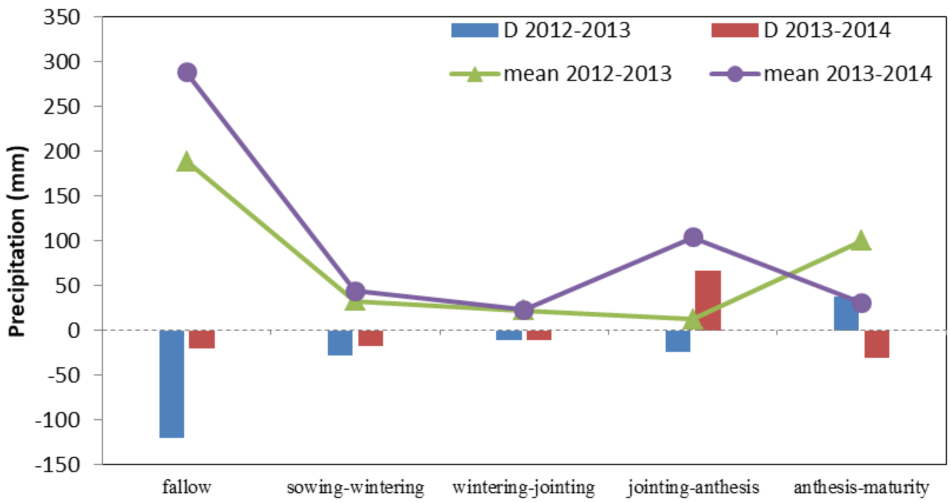

Figure 1. *Precipitation during study years (2012-2014) and difference in precipitation in respective years from average precipitation in last 30 years (D 2012-2013 and D 2013-2014) in different growth stages of wheat at the experimental site in Wenxi*

Precipitation was recorded during the fallow period (21 June - 30 September), sowing to wintering (1 October - 30 November), wintering to jointing (1 December to the following 10 April), jointing to flowering (11 April - 10 May), and from flowering to maturity (11 May - 20 June) stages. Average annual precipitation from 2002 to 2014 was 484 mm, whereas total precipitation in 2012-2013 was 355.7 mm, which is 26% lower than average precipitation, despite of higher precipitation from flowering to maturity. In 2013-2014 total precipitation was 489.7 mm which was closer to average annual precipitation. The basic nutrient properties of soil are shown in *Table 1*.

Crop management

After harvesting the previous wheat, 20-30 cm wheat stubble was left in field until mid-July. Subsoiling was performed during fallow period by subsoiler (IS-200, Xiuyuan Agricultural Machinery Co.) at the depth of 30-40 cm on 15th July in the two years,

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followed by rotary tillage to crumble large soil lumps and to level the field on 25 August, 2012 and 23 August, 2013. The seeds of winter wheat variety 'Yunhan20410' provided by the Agricultural Bureau of Wenxi, Shanxi province were sown on 1 October 2012 and 29 September 2013. Before sowing, nitrogen (N, 150 kg ha^{-1}), phosphorus (P_2O_5, 150 kg ha^{-1}), and potassium (K_2O, kg ha^{-1}) were applied to soil and no top dressing was applied during the rest of growth period. No irrigation was applied during the experiment. Wheat was harvested on 20 June.

Table 1. *Soil nutrient properties from experimental location in Shanxi (6 June 2012 and 2013)*

Soil nutrients	2012	2013
Organic matter (g kg^{-1})	11.88	10.88
Total nitrogen (g kg^{-1})	0.61	0.85
Alkali-hydrolysis nitrogen (mg kg^{-1})	38.62	39.32
Available phosphorous (mg kg^{-1})	14.61	16.62
Available potassium (mg kg^{-1})	238.16	221.56
pH	8.08	8.12

Experimental design

The experiment was conducted with two-factor split plot design, taking the tillage practice as a main factor, and planting density as a sub-plot factor. Two tillage practices were the subsoiling (SS) and no tillage (NT). Under each tillage method, three planting density rates, low (LD = 190 plants m^{-2}), medium (MD = 260 plants m^{-2}), and high (HD = 320 plants m^{-2}) were used in the experiment. Seeds were sown at the rate of 67.5, 90 and 112.5 kg ha^{-1} after which at 3-leaf stage planting density of 190, 260 and 320 plants m^{-2} were attained by thinning. Each treatment was repeated 3 times with a total of 18 plots. Each sub-plot (150 m^2) was 50 m long and 3 m wide and a line spacing of 30 cm.

Measurements

Soil water storage

Soil samples were taken before sowing and at anthesis stage after every 20 cm from 0 to 300 cm depth from each subplot with 3 replicates for each treatment. Soil samples were weighed wet, dried in oven at 105°C for 48 h, and weighed again to determine the soil water content. From each plot, gravimetric water content at a given layer was determined by averaging the values from three random sampling points placed between two plants in the row. Before the setup of plots the soil bulk density (g cm^{-3}) was measured at 20 cm increments to a depth of 300 cm from the whole soil profile (0-300 cm depth).

$$Soil\ water\ content\ (\%) = \frac{wet\ weight - dry\ weight}{dry\ weight} \times 100 \quad \text{(Eq.1)}$$

Soil water storage was determined from soil water content by using following formula:

$$SWSi(mm) = Wi \times Di \times Hi \times {}^{10}/_{100} \quad \text{(Eq.2)}$$

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where SWS_i: soil moisture of layer i (mm); W_i: soil water content of layer i (%); D_i: soil bulk density of layer i (g cm^{-3}); H_i: thickness of layer i (cm).

Protein and protein fractions in grains

At flowering, the fifteen ears with uniform flowering time and size were picked. Grains were separated in the oven where moisture was removed by heating at 105°C for 30 min and then weighted after drying at 80°C. Grains were used to measure the protein contents and protein fractions after being crushed by micro high-speed universal grinder (DE-100 g). Content of albumin, globulin, gliadin, and glutelin in grains were measured using the method of continuous extraction. Nitrogen content was determined using semi micro Kjeldahl method and wheat protein content was obtained by multiplying N content (%) by 5.7 (Halvorson et al., 2004). The protein yield was obtained in t ha^{-1} as

$$protein\ yield = grain\ yield \cdot protein\ content/100 \qquad \text{(Eq.1)}$$

Enzyme activity related to nitrogen metabolism

Plants at same growth and flowering time were labelled during anthesis. Fifteen labeled ears and flag leaves were taken at 5, 10, 15, 20, 25, and 30 d of anthesis and quickly frozen in liquid nitrogen and stored at -40°C. The activity of glutamine synthetase (GS) and glutamate synthase (GOGAT) was determined by the method described by Lin et al. (1996) and Lu et al. (2005) and glutamate pyruvate transaminase (GPT) was determined by the method described by Wu and Tao (2003).

Population tiller numbers and grain yield

At 3-leaf stage the plants from 1 m^2 were selected from each plot and number of tiller population was investigated by counting tillers at wintering, jointing, booting, anthesis and maturity stages. At maturity, plants from 1 m^2 were harvested from each plot to determine the grain yield (kg ha^{-1}).

Statistical analysis

Data were subjected to analysis of variance (ANOVA) as split-plot design using DPS and SAS 9.0. Graphs were constructed using Microsoft Excel 2007. Mean values were calculated and significance of the difference between treatments was tested by LSD (least significant difference) method at the significance level of *P*=0.05.

Results

Effects of subsoiling during fallow period on soil moisture content at sowing and anthesis stage

With the increase of soil depth, the soil water content at sowing time varied with the depth and was highest in 0-60 cm which then decreased from 60-200 cm and then again increased in 200-300 cm (*Fig. 2*). The subsoiling (SS) during fallow period has proved effective for increasing the soil moisture content at sowing time as compared to no tillage (NT) especially in 0-160 cm and in 200-240 cm soil depth during 2012-2013, and 0-200 cm and 260-300 cm in 2013-2014. At flowering stage the trend of soil moisture content varies in both years. The difference in moisture content under SS and NT was

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more significant during 2012-2013 when the soil moisture in 40-300 cm soil layer was higher in SS. In 2013-2014 the difference of soil moisture content was less prominent between SS and NT with higher soil moisture in 40-60 cm and 200-300 cm. It can be seen that SS during fallow period is conducive for the accumulation of precipitation in soil during fallow period especially during the year with less precipitation (2012-2013) and the effect of moisture storage may continue up to the flowering.

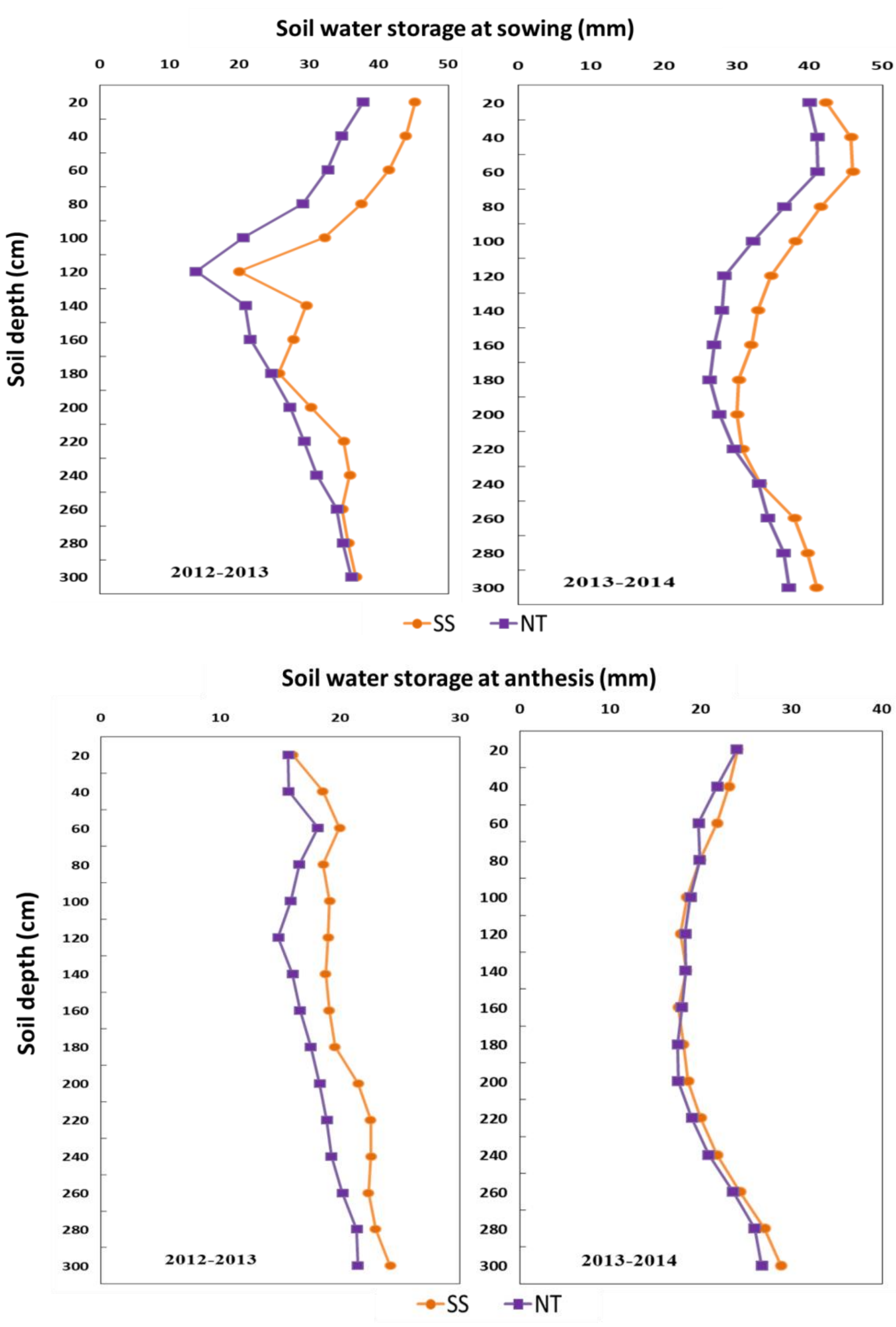

Figure 2. *Effect of subsoiling during fallow period on soil water storage at sowing and anthesis of dryland wheat (SS: subsoiling; NT: no tillage)*

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Effect of planting density on soil moisture storage at anthesis stage under subsoiling

Under subsoiling during fallow period, the soil water content was highest in LD and lowest in MD (*Fig. 3*). At lowest planting density, soil moisture in 40-200 cm and 260-300 cm soil during 2012-2013 and 0-300 cm soil depth in 2013-2014 was higher than MD and HD. In 2012-2013, soil moisture in 80-200 cm soil, and in 2013- 2014, soil moisture in 20-260 cm soil was higher in HD than MD. It can be seen that the planting density rate has certain regulatory effect on the soil moisture during flowering period, especially in 2013-2014.

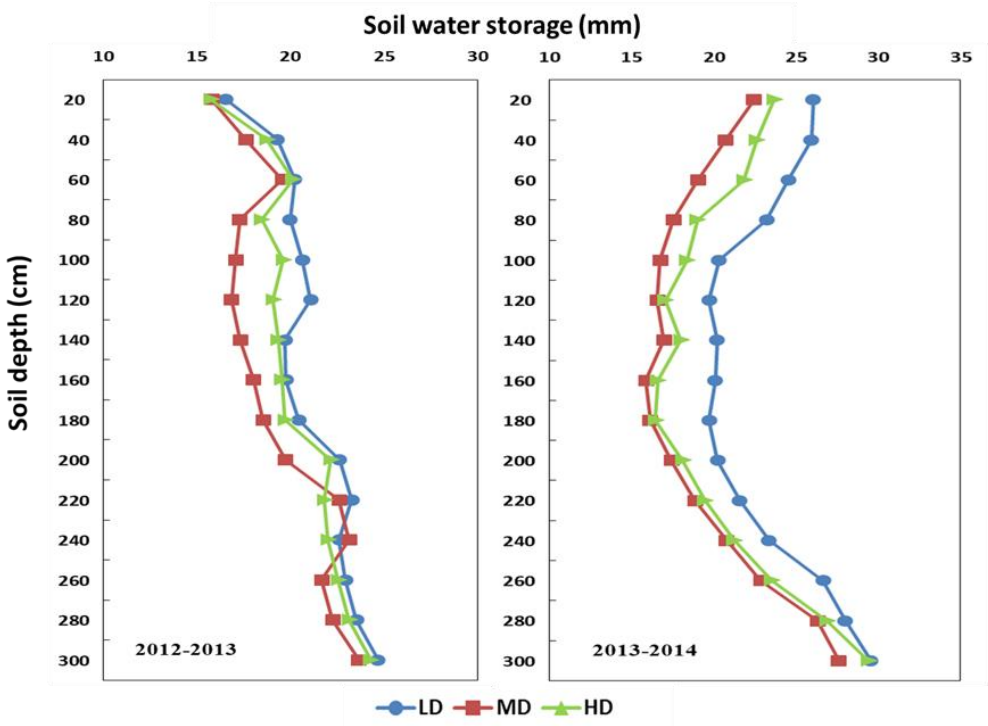

Figure 3. *Effect of different planting density under subsoiling condition on soil water storage at anthesis of dryland wheat (LD: 190 plants m^{-2}; MD: 260 plants m^{-2}; HD: 320 plants m^{-2})*

Effects of subsoiling and planting density on the number of group tillers

The numbers of tillers were significantly higher after SS conducted during fallow period than that in NT (*Table 2*). Under SS, the increasing planting density had significantly increased the tillers and the difference between three planting density was significant during wintering period. From jointing to maturity stage, increasing planting density rate from LD to MD has significantly increased the tillers whereas the difference between the MD and HD was not significant. Under NT condition, the number of tillers was highest at the medium density, and the difference between LD and MD was significant during wintering and booting stage and difference between MD and HD was significant from wintering to booting stage. Lowest tillers from jointing to mature stages were recorded at HD, while in wintering stage the lowest tillers were found at LD. It can be seen that SS during fallow period is beneficial for the formation of more tillers at high planting density which is advantageous for the formation of

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effective spike number, but the difference between medium and high sowing rate was not significant.

Table 2. *Effect of subsoiling during fallow period and seeding rates on tillers group (×10^4 ha^{-1}) at different growth stages in 2013-2014*

Treatment						
Tillage	**Seeding rate**	**Wintering**	**Jointing**	**Booting**	**Anthesis**	**Maturity**
SS	LD	1005.00±16.42d	1142.15±26.97b	580.15±16.17b	501.24±10.99b	479.00±7.00b
	MD	1204.23±17.59b	1348.50±30.06a	690.00±20.23a	540.00±10.69a	501.75±9.75a
	HD	1328.25±17.95a	1398.52±36.74a	710.25±15.21a	561.23±12.11a	510.25±3.25a
NT	LD	963.50±10.45e	1032.15±20.02cd	520.14±16.14c	465.65±11.30c	446.75±7.25c
	MD	1064.75±15.90c	1106.00±23.26bc	575.58±13.61b	486.23±13.28bc	458.50±3.00bc
	HD	985.50±8.00de	1000.32±28.13d	501.23±117.24c	456.25±11.00c	438.25±1.75c
ANOVA						
Tillage (F_T)		330.56**	128.85**	159.60**	33.21*	1295.86**
Seeding rate (F_S)		181.45**	78.99**	19.02**	8.84**	5.14*
$F_T \times F_S$		121.54**	74.70**	15.23**	11.15**	7.03*

SS: subsoiling; NT: no tillage; LD, MD and HD are planting density of 190, 260 and 320 plants m^{-2}. *, ** and *** indicate significance at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively

Effects of subsoiling and planting density on protein fractions of wheat grain in dryland

Wheat grain yield, grain protein and protein components were significantly influenced by the years, tillage treatment and planting density (*Table 3*). Average grain yield in 2012-2013 was 3044 kg ha^{-1} which was lower than in the year 2013-2014 with grain yield of 4705 kg ha^{-1}, therefore less grain yield was produced in years with less precipitation as compared to years which received high precipitation. The grain protein and protein fractions were significantly higher in year with less precipitation (2012-2013) than with more precipitation (2013-2014). The SS during fallow period had significantly increased the grain yield compared with the NT, and increase in grain yield under SS was 26%-66% in 2012-2013 and 17%-34% in 2013-2014 as compared to NT. Under SS, increasing planting density has increased the grain yield but the difference between the MD and the HD was not significant, whereas in NT, maximum yield was the achieved at MD and increasing planting density to HD has decreased grain yield.

The grain albumin, gliadin, glutenin, glutenin/gliadin ratio and total protein content were decreased by SS, in contrast to which globulin content and protein yield was increased under SS as compared with NT. Protein yield was increased significantly with SS as compared with NT. Under SS and NT the protein yield was increased by increasing planting density from LD to MD which then decreased by further increasing HD during both years.

Under SS, albumin, globulin, gliadin and glutenin were highest at MD and decreased under the condition of low and high sowing rates during both years. Under no tillage condition (NT), albumin, gliadin, glutenin, total protein content, and glutenin/gliadin ratio were highest in low sowing rate (LD) and lowest in high sowing rate (HD). It can be seen that the precipitation is favourable to increase yield of dryland wheat, but unfavourable for protein accumulation in grain.

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Table 3. *Effect of subsoiling during fallow period and seeding rates on protein and protein fractions of wheat grain*

Treatment			Albumin (%)	Globulin (%)	Gliadin (%)	Glutenin (%)	Glu/Gli	Protein content (%)	Protein yield (kg ha^{-1})	Grain yield (kg ha^{-1})
Years	**Tillage**	**Seeding rate**								
2012–2013	SS	LD	2.87 c	1.80 cd	4.42 c	4.77 c	1.08 c	14.96 c	476.5 c	3185.6 b
		MD	2.97 b	1.85 a	4.54 ab	5.20 b	1.15 ab	15.46 b	586.8 a	3795.1 a
		HD	2.93 bc	1.82 bc	4.46 bc	4.87 c	1.09 c	15.09 c	576.3 b	3819.5 a
	NT	LD	3.12 a	1.78 d	4.65 a	5.43 a	1.18 a	16.09 a	408.2 d	2537.5 d
		MD	2.99 b	1.83 ab	4.56 a	5.22 b	1.14 ab	15.61 b	409.5 d	2621.7 c
		HD	2.95 b	1.78 d	4.53 ab	5.12 b	1.13 b	15.47 b	356.5 e	2304.1 e
2013–2014	SS	LD	2.40 c	1.46 b	4.09 c	4.06 c	0.99 b	12.99 d	635.5 b	4892.9 b
		MD	2.68 b	1.49 a	4.27 ab	4.46 b	1.04 a	13.75 b	728.0 a	5292.8 a
		HD	2.49 c	1.47 b	4.15 bc	4.17 c	1.01 b	13.39 c	718.0 a	5360.7 a
	NT	LD	2.87 a	1.42 d	4.35 a	4.64 a	1.07 a	14.33 a	597.1 c	4167.6 d
		MD	2.73 b	1.44 c	4.29 a	4.49 b	1.05 a	13.87 b	626.5 b	4515.9 c
		HD	2.64 b	1.41 d	4.27 ab	4.37 b	1.02 b	13.85 b	554.0 d	4001.7 d
ANOVA	Years (Fy)		10404.0**	17089.0**	28561.0**	21707.1**	3481.0**	3230.5*	6198.6**	102828.0**
	Tillage (FT)		1881.8**	40.7*	217.8**	3741.1**	230.4**	562.1**	79201.8**	822.9**
	Seeding rate (FS)		50.82**	5.38*	76.70**	559.68**	147.2**	7.76*	501.8**	47.27**
	Fy× FT × FS		15.60**	0.03	6.48*	8.32*	10.75**	0.53	19.20**	5.10*

Values followed by different letters are significantly different at P < 0.05, SS: subsoiling; NT: no tillage; LD, MD and HD are planting density of 190, 260 and 320 plants m^{-2}. *, ** and *** indicate significance at p < 0.05, p < 0.01 and p < 0.001, respectively

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Subsoiling proved beneficial to increase the grain yield and protein yield, but the difference between the medium and the high planting density was not significant, while the SS in the fallow period decreased the content of total protein, albumin, gliadin and glutenin, but the decrease was not significant at the medium sowing rate. Therefore, the medium planting density was more favourable under SS.

Effects of planting density on activity of nitrogen metabolizing enzymes in flag leaves and grains

Nitrogen metabolizing enzymes were tested under subsoiling during fallow field at different planting density at 5 days interval after anthesis. GS and GOGAT activities in flag leaf were highest in medium planting density (MD) followed by highest planting density (HD) and minimum activities were found in lowest planting density (LD) (*Fig. 4*). The activities of GS and GOGAT reduced with time after anthesis. Therefore, under subsoiling during fallow period, the medium density was advantageous for increasing the activities of GS and GOGAT in flag leaves.

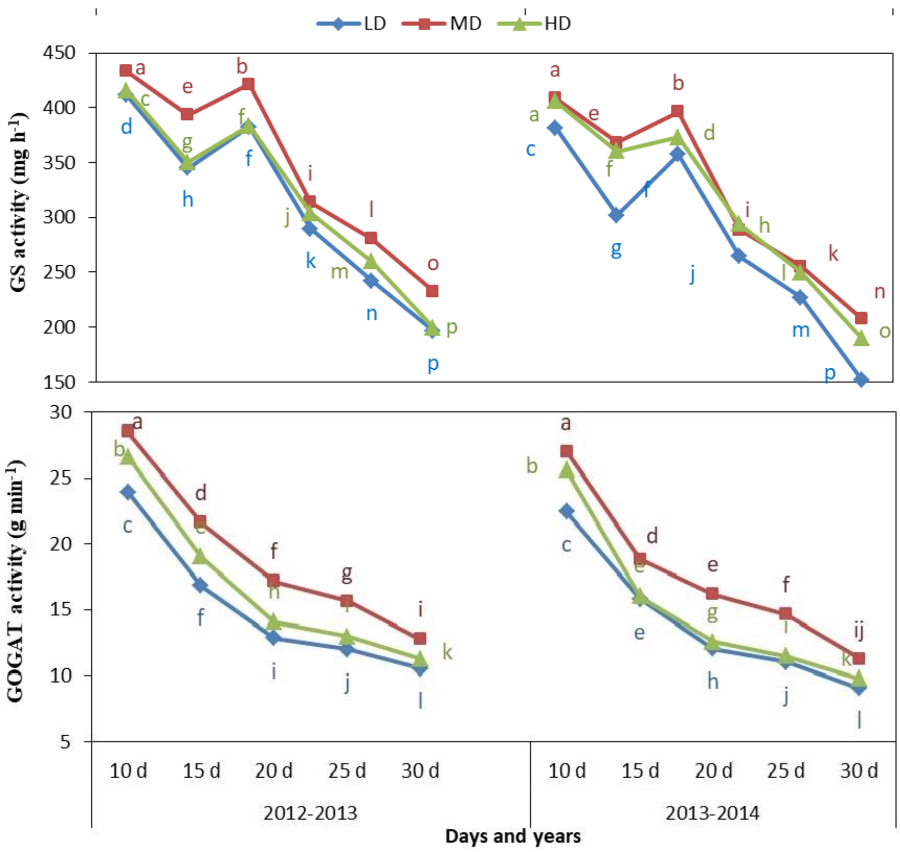

Figure 4. *Effect of different planting density under subsoiling condition on activity of glutamine synthetase (GS) and glutamate synthase (GOGAT) in flag leaves after anthsis of dryland wheat (LD: 190 plants m^{-2}; MD: 260 plants m^{-2}; HD: 320 plants m^{-2})*

In grains GS activity was highest at MD and lowest in LD (*Fig. 5*). GOGAT activity was also highest in MD except 20 d after anthesis during 2013^{-2}014 and 30 d after anthesis during both years. The GPT activity was highest at HD during 10, 15, 25 and 30 d after anthesis with only exception of 20 d after anthesis during both years, whereas

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lowest GPT activity was recorded at LD (*Fig. 5*). It can be seen that the medium and high planting density were more favourable to improve the GS, GOGAT and GPT activities in grain; especially the medium density has a greater effect on the grain GS and the GOGAT activities during early stages of grain filling.

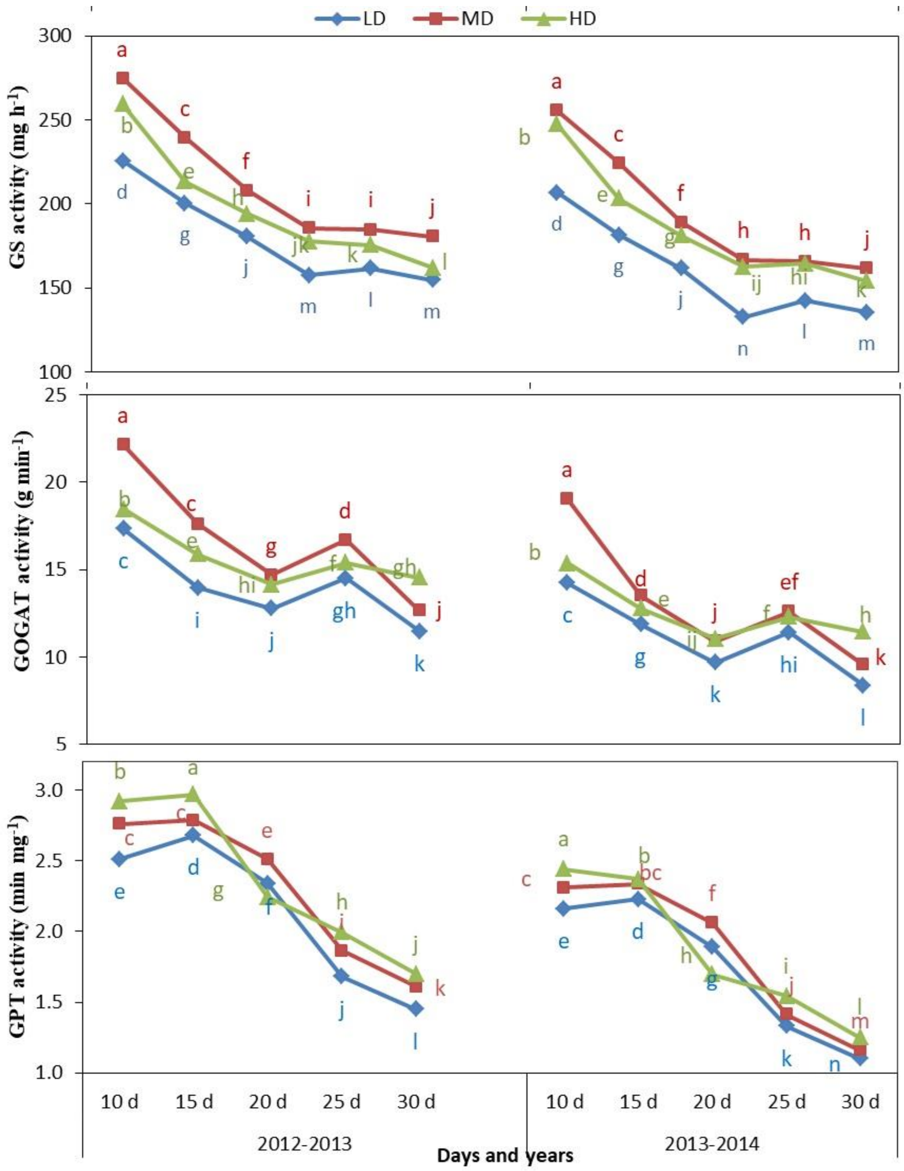

Figure 5. *Effect of different planting density under subsoiling condition on activity of glutamine synthetase (GS), glutamate synthase (GOGAT) and glutamate pyruvate transaminase (GPT) in grains after anthesis of dryland wheat (LD: 190 plants m^{-2}; MD: 260 plants m^{-2}; HD: 320 plants m^{-2})*

Relationship between soil moisture and grain protein at anthesis, and soil moisture content and protein yield and grain yield

Correlation coefficients indicated that albumin, globulin, gliadin and protein content were negatively related with the soil moisture content at anthesis (*Table 4*). In 2012-2013, albumin content was significantly related with moisture in 120-220 cm, while

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gliadin, glutenin and protein contents showed negative significant relationship with soil moisture in 120-200 cm and then 260-300 cm soil layer. Protein yield showed significant correlation with soil moisture at 240 and 300 cm soil layer. In 2013-2014, during which the precipitation was more, the soil moisture in upper and lowest layer showed significant negative relationship with protein contents. Albumin, gliadin, glutenin and protein contents were negatively affected with soil moisture in 20-100 cm and 300 cm soil layer.

Table 4. *Correlation coefficient between soil water storage at anthesis and grain protein accumulation*

Years	Soil depth (cm)	Albumin	Globulin	Gliadin	Glutenin	Glu/Gli	Protein content	Protein yield
2012-2013	20	0.594	-0.179	0.274	0.351	0.372	0.459	0.059
	40	-0.136	0.149	-0.465	-0.382	-0.353	-0.320	0.633
	60	0.104	0.072	-0.255	-0.170	-0.142	-0.091	0.538
	80	-0.237	0.122	-0.483	-0.448	-0.439	-0.355	0.276
	100	-0.453	-0.049	-0.770*	-0.717	-0.699	-0.616	0.463
	120	-0.822*	0.288	-0.939**	-0.914**	-0.901**	-0.904**	0.580
	140	-0.831*	0.421	-0.905**	-0.903**	-0.899**	-0.912**	0.614
	160	-0.871*	0.388	-0.942**	-0.932**	-0.924**	-0.951**	0.657
	180	-0.914**	0.469	-0.862*	-0.885**	-0.889**	-0.926**	0.473
	200	-0.924**	0.394	-0.928**	-0.943**	-0.943**	-0.968**	0.532
	220	-0.784*	0.668	-0.748	-0.721	-0.705	-0.812*	0.745
	240	-0.650	0.646	-0.662	-0.607	-0.580	-0.709	0.888**
	260	-0.750	0.465	-0.862*	-0.832*	-0.817*	-0.852*	0.742
	280	-0.652	0.289	-0.854*	-0.808*	-0.789*	-0.786*	0.693
	300	-0.661	0.243	-0.828*	-0.765*	-0.737	-0.778*	0.760*
2013-2014	20	-0.935**	0.675	-0.933**	-0.911**	-0.878**	-0.944**	0.524
	40	-0.904**	0.689	-0.925**	-0.882**	-0.835*	-0.966**	0.530
	60	-0.919**	0.642	-0.912**	-0.895**	-0.865*	-0.964**	0.493
	80	-0.861*	0.558	-0.880**	-0.843*	-0.801*	-0.939**	0.372
	100	-0.763*	0.668	-0.790*	-0.741	-0.689	-0.857*	0.538
	120	0.033	-0.414	-0.077	-0.021	0.009	-0.006	-0.499
	140	-0.147	-0.226	-0.273	-0.203	-0.159	0.159	-0.295
	160	-0.173	-0.389	-0.261	-0.223	-0.202	-0.215	-0.527
	180	0.102	-0.378	-0.017	0.051	0.090	0.046	-0.455
	200	-0.403	0.201	-0.533	-0.434	-0.361	-0.468	0.096
	220	-0.301	0.233	-0.445	-0.330	-0.246	-0.402	0.140
	240	-0.445	0.298	-0.569	-0.465	-0.385	-0.558	0.161
	260	-0.323	-0.057	-0.432	-0.363	-0.316	-0.354	-0.177
	280	-0.495	0.247	-0.593	-0.522	-0.466	-0.497	0.165
	300	-0.804*	0.505	-0.866*	-0.820*	-0.775*	-0.779*	0.456

*, ** indicate significance at $p < 0.05$, $p < 0.01$, respectively

Relationship between soil moisture at anthesis and protein content and grain yield

The relationship between the soil water content in 0-300 cm soil at anthesis and grain yield and protein content was determined with two polynomial (*Fig. 6*), and the equation of soil water content and protein yield was $y_1 = -0.0003x^2 + 0.1699x - 6.9076$ ($R^2 = 0.7200$), the equation of soil water content and grain yield was $y_2 = -1.0762x^2 + 648.02x - 93902$ ($R^2 = 0.8199$) in 2012-2013. The polynomial equation of soil moisture storage and protein yield in the 2013-2014 was $y_3 = 2e\text{-}05x^2 - 0.0306x + 20.892$ ($R^2 = 0.7640$), the equation of soil water storage and grain yield was $y_4 =.-0.9216x^2 + 595.64x - 90716$ ($R^2 = 0.7782$). With the increase of soil moisture at anthesis, the grain yield was increased first and then decreased, whereas, grain protein content was decreased.

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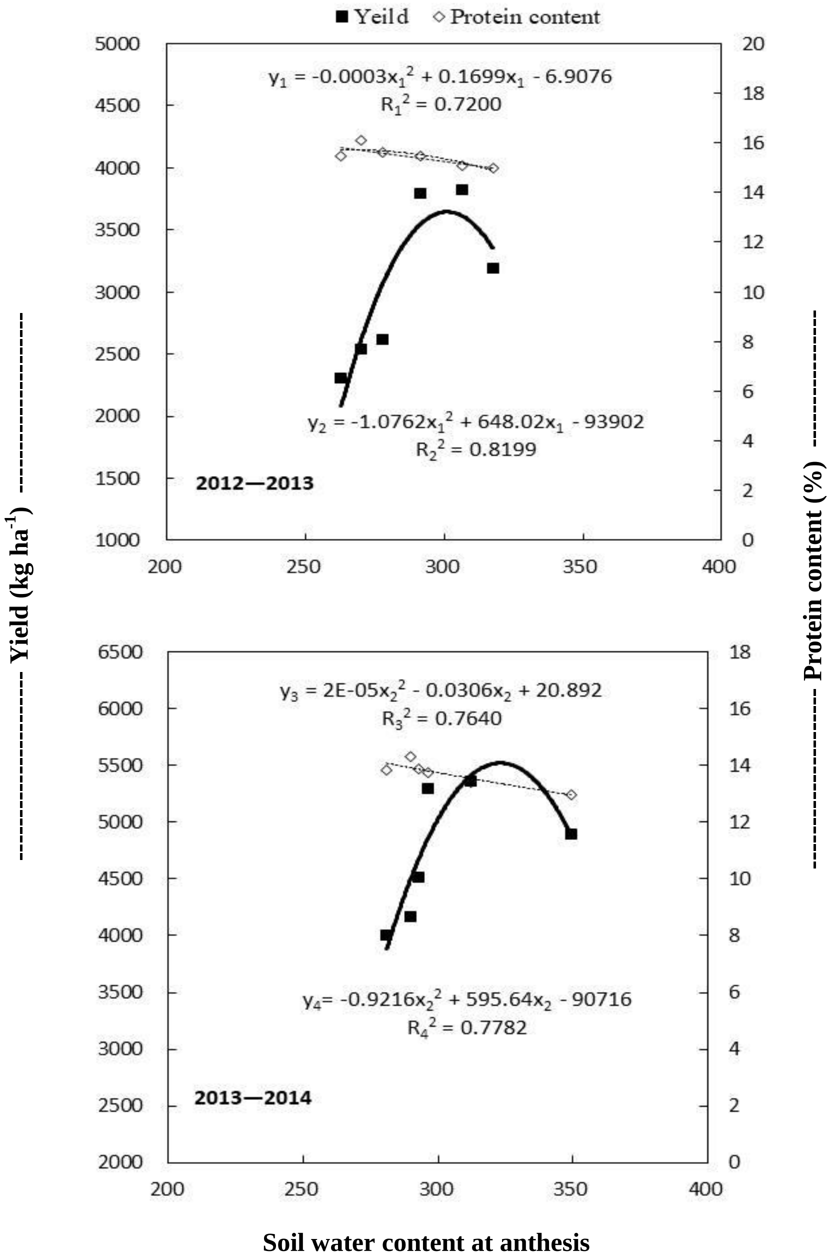

Figure 6. *Correlation between soil water content and protein content (y_1 and y_3), and soil water content and grain yield (y_2 and y_4)*

Discussion

Effect of soil water storage, and yield and quality improvement under subsoiling during fallow period in dryland winter wheat

Accumulating of precipitation during the fallow period and coordinating the relationship between planting density and seeding rate is important for dryland wheat production. Present study showed that subsoiling (SS) during fallow period is conducive for the accumulation of precipitation in soil during fallow period especially during the year with less precipitation (2012-2013) and the effect of moisture storage may continue up to the flowering. Previous research also demonstrated that fallow subsoiling can

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effectively accumulate summer rainfall, and improve the soil storage capacity of winter wheat before sowing (Hou et al., 2009; Williams et al., 2006). Subsoiling increased rooting depth and also played a role in decelerating the yield stagnation under long term no tillage cultivation of wheat (Izumi et al., 2009). The subsoiling treatment and high stubble during summer fallow period in Luoyang dry zone increased soil water availability and increased yield by 5% (Su et al., 2007). Subsoiling during fallow period in the Weibei dryland increased soil water storage capacity by 21 mm in 0-300 cm soil layer before sowing, and increased yield by 5% as compared with tillage (Mao et al., 2010).

Subsoiling was proved beneficial in the Loess Plateau for the accumulation of precipitation in deep soil, with higher grain yield in years with different precipitation, and more obvious effect of water storage on increase in yield was observed in the year with less precipitation (Sun et al., 2013; Sun et al, 2018). Subsoiling not only improved the soil water storage at different growth stages but also increased the water utilization and ultimately increased yield of dryland wheat (Sun et al., 2015). In contrast, no increase in yield was observed by rotary subsoiling in winter wheat field even though the soil moisture was increased and runoff and soil erosion was reduced by rotary subsoiling (Pikul Jr. and Aase, 1999; Williams et al., 2006). Wang et al. (2009) showed that the use of subsoiling during the fallow period can increase the grain protein content and improve the quality. Zhao et al. (2012) showed that subsoiling during the fallow period reduced the proline content of the flag leaf and grains on the 5-15 d after anthesis, and the grain GS activity was increased, while the effects of grain protein content was differed with years with different precipitation, as the grain protein content was reduced in year with less precipitation by using the subsoiling during fallow period. Whereas, the use of subsoiling during fallow period is beneficial to improve grain protein content in the year with more precipitation. In summary, grain protein formation is closely related to soil moisture.

Regulatory effect of planting density on yield formation of wheat

Constructing a reasonable group structure and forming a strong seedling before winter is important for wheat production in dryland. Plant density significantly impacts the competition among plants and consequently affects the utilization of available resources including light, water, and nitrogen (Olsen et al., 2006). At high planting density, the water consumption is high at the early stage of growth which intensified the drought at the later stage and yield is reduced. On the other hand, when the seeding amount is too less, the population is less and the number of panicles is insufficient resulting in reduced yield (Zhang et al., 2016; Shi et al., 2017; Lei et al., 2017). Appropriate seeding rate can optimize the yield component and increase production. Hai et al. (2002) in the dryland wheat field in Shaanxi Province showed that when seeding rate was increased from 75 kg ha^{-1} to 165 kg ha^{-1} then the highest yield was achieved at 105 kg ha^{-1}. The water consumption is high at the high seeding rate, although the yield output is reduced, and the medium planting density consumes less water with the high yield output. It can be seen that increasing the seeding rate under the condition of sufficient water can increase the yield, but increasing the seeding amount under the condition of insufficient water will reduce the production to a certain extent.

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Regulatory effect of planting density on protein content and protein fractions of wheat grains

In the present study, the total protein, albumin, gliadin, glutenin content and the ratio of glutenin to gliadin were highest at planting density 190 plants m^{-2} and gradually decreased by increasing the planting density. After subsoiling during the fallow period, with the increase of planting density, the grain protein and component content, and the ratio of glutenin to gliadin were increased first and then decreased, and maximum protein content and protein fractions were found at the planting density of 260 plants m^{-2}. It may be due to the reason that at high plant population tillers group were reduced whereas nitrogen metabolism is higher in the early stage of grain filling. These observations are consistent with the findings from other researchers. Wang et al. (2014) conducted trial in the dryland of central Guanzhong area and showed that the grain protein content was first increased and then decreased with increasing the planting density in the range of 245×10^4 ha^{-1} to 330×10^4 ha^{-1}, and was the highest at 285×10^4 ha^{-1}. Present results indicated that nitrogen metabolizing enzymes were highest under the medium planting density. Nitrogen metabolizing enzymes [glutamate synthase (GS), glutamate synthase (GOGAT) and glutamate pyruvate transaminase (GPT)] are involved in the primary assimilation off ammonium into glutamate and glutamine, which can be used to translocate nitrogen from source to sink (Maclaux-Daubresse et al., 2006; Nigro et al., 2013). GPT is responsible for the biosynthesis of other amino acids from the major nitrogen-carrier glutamine and thus is very important for the formation of storage protein in grain and regulation of nitrogen metabolism (Zhou et al., 2006). Higher activities of GS, GOGAT and GPT leading to higher grain protein content (Kaur et al., 2015). Nitrogen assimilation and reutilization is very important and related to grain nitrogen and nitrogen content in the aboveground part of the plant has direct effect on nitrogen yield (Nikolic et al., 2012).

In present study, the amount of nitrogen translocation and the contribution of nitrogen translocation from leaves and spike to grain were increased by increasing planting density, but the difference between planting density of 260 plants m^{-2} and 320 plants m^{-2} was not significant. In short, the adjustment of seeding rate affects the accumulation of soil moisture and plant nitrogen, thus affecting the formation of grain protein. Desai et al. (1978) showed that the grain protein content of wheat was mainly regulated by the amount of nitrogen accumulated in various organs before flowering. Xue et al. (2017) showed that under the condition of subsoiling by increasing seeding rate, the accumulation of nitrogen in various organs before anthesis was increased.

Relationship between soil moisture and yield and protein accumulation in wheat

Under the conditions of this experiment, the soil water storage during anthesis in 300 cm soil layer was in the range of 260-350 mm. With the increase of soil moisture, the grain yield showed a trend of first increasing and then decreased. In 2012-2013, soil storage capacity of 0-300 cm at anthesis was in the range of 263-318 mm, and grain yield was increased first and then decreased with the planting density. The soil water storage at the highest planting density (320 plants m^{-2}) was 306 mm, but was 291 mm at the planting density of 260 plants m^{-2} but the difference of yield was not significant, while yield was decreased at planting density 190 plants m^{-2} when soil water storage was 318 mm. This may be due to the low population density and number of spikes, and

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the insufficient number of spikes leads to a decrease in yield. At the same time, with the increase of soil moisture, the grain protein content showed a gradual decline.

Zhou et al. (2006) showed that the relative water content of soil during grain filling stage of wheat was in the range of 50%-80%. With the increase of soil moisture, the yield was increased first and then decreased and highest yield was attained at 70% soil moisture content, whereas, the grain protein content showed a gradual decline. Zhang et al. (2006) carried out the pot experiment in which soil moisture content in the whole growth period was in the range of 50%-80%. With the increase of soil moisture, the yield was increased first and then decreased whereas grain protein content showed a gradual decline. It can be seen that adjusting soil moisture can obtain the optimal soil moisture content with simultaneous increase in yield and grain protein content. It is shown that the optimum soil moisture content can be obtained by adjusting the soil moisture and synchronous increment of grain protein content.

In present study, in 2012-2013, under the no-tillage, the grain protein content was highest when the soil water storage was 263 mm (planting density 190 plants m^{-2}), whereas under the subsoiling, grain protein content and yield was the highest at the planting density of 260 plants m^{-2} when the soil water storage was 291 mm. In the same way, in 2013-2014 the soil water storage capacity was maximum (296 mm) at the planting density of 260 plants m^{-2} Thus, the soil moisture content was improved at planting density of 260 plants m^{-2} which could achieve simultaneous increase in yield and protein content.

Conclusion

The different planting density significantly affected the grain yield and protein content of dryland wheat. Under the conditions of this experiment, gliadin, glutenin, total protein content and the ratio of glutenin to gliadin were closely related with the soil water storage in 120-200 cm and 260-280 cm soil layer in the 2012-2013 and 20-80 cm in the 2013-2014 at anthesis stage. The relationship between soil water storage at anthesis with grain yield and protein content indicated that albumin, globulin, gliadin and protein content were negatively related with the soil moisture content at anthesis, whereas, the grain yield was increased first and then decreased, whereas, grain protein content was decreased. The grain yield was increased by increasing the planting density from 190 to 320 plants m^{-2}, whereas the grain protein content was maximum at 260 plants m^{-2} and protein content in no tillage was higher than that under subsoiling. Furthermore, the activities of glutamine synthetase (GS), glutamate synthase (GOGAT) in grain and flag leaves were also highest at 260 plants m^{-2}. Therefore, subsoiling and optimum planting density (260 plants m^{-2}) simultaneously improved the yield and protein content. The further studies might be conducted to elucidate how GS, GOGAT and GPT activities are related with the soil water content and nitrogen use efficiency at different planting density of dryland winter wheat.

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