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西北农林科技大学

专业技术职务任职资格评审佐证材料

（教师岗位系列）

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岗位类型：　　教学科研型教师

申报时间：　　2020 年 4 月

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具备 高等学校

教师资格。

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2018 年 7 月 20 日

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检索报告

根据委托人王瑞委托，通过网络检索，王瑞发表的 3 篇论文被《科学引文索引》扩展版（SCI-Expanded）数据库收录。数据库具体检索结果如下：

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作者:Zhang, YH (Zhang, Yuanhong)[1,2] ; Wang, R (Wang, Rui)[1,2] ; Wang, SL (Wang, Shulan)[1,2] ; Ning, F (Ning, Fang)[1,2] ; Wang, H (Wang, Hao)[1,2] ; Wen, PF (Wen, Pengfei)[1,2] ; Li, A (Li, Ao)[1,2] ; Dong, ZY (Dong, Zhaoyang)[1,2] ; Xu, ZG (Xu, Zonggui)[3] ; Zhang, YJ (Zhang, Yujiao)[1,2] ; Li, J (Li, Jun)[1,2]

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小类	WATER RESOURCES 水资源	**2**	**-**
大类	农林科学	**1**	**是**

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作者:Zhang, YJ (Zhang, Yujiao)[1,2] ; Wang, R (Wang, Rui)[1] ; Wang, H (Wang, Hao)[1] ; Wang, SL (Wang, Shulan)[1] ; Wang, XL (Wang, Xiaoli)[1] ; Li, J (Li, Jun)[1]

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3.标题: Carbon allocation, osmotic adjustment, antioxidant capacity and growth in cotton under long-term soil drought during flowering and boll-forming period

作者:Wang, R (Wang, Rui)[1] ; Gao, M (Gao, Min)[1] ; Ji, S (Ji, Shu)[1] ; Wang, SS (Wang, Shanshan)[1] ; Meng, YL (Meng, Yali)[1] ; Zhou, ZG (Zhou, Zhiguo)[1]

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Effect of planting density on deep soil water and maize yield on the Loess Plateau of China

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ABSTRACT

Dryland farmers tend to increase maize plant density with drought and density stress tolerance hybrids to achieve higher grain yield in recent years. However, could this strategy improve yield or water use efficiency (WUE) and be sustainable without decreasing deep soil water in drought-prone environments is not clear. A 4-year of successive field study was carried out with three different drought and density stress tolerance maize hybrids and four plant density arrange from 52,500 to 97,500 plants ha^{-1}. To quantify the responses of grain yield formation and WUE to increasing plant density under various rainfall condition and evaluate the effect on deep soil water balance. Results showed that using of drought and density stress tolerance hybrids could achieve higher grain yield and WUE with higher plant density in normal years, which was associated with an increase in kernels number per square meter. But in dry year, as fewer water was available during reproductive growth stage in higher plant density, grain yield and WUE was gradually decreased with increasing plant density, especially in density stress sensitive hybrid. Soil water balance at 0 to 200 cm depth was not broken by high plant density from the perspective of same water availability at sowing in each year, despite of the lower soil water content during maize growth stage. However, high plant density tended to consume more deep soil water which was hardly been replenished by precipitation, especially in high density tolerance hybrids. Hence, higher density that exceed 60000 plants ha^{-1} couple with drought and density stress tolerance hybrids is a potential way to improve maize production in dryland, but it increases the risk of deep soil desiccation.

1. Introduction

The increase in plant density has been one of the main managements contributed to maize grain yield improvements over the decades, which remarkably increase resources use efficiency when combination with high density tolerance hybrids (Tollenaar and Wu, 1999; Tokatlidis and Koutroubas, 2004. Jia et al., 2018). Density stress tolerance is an important trait that breeders intend to improve (Fasoula and Fasoula, 2000). Seed companies are also promoting high-density maize cultivation for the purpose of commercial profit. Even on the Loess Plateau of China where soil fertility has improved but water is still a major limiting factor for dryland maize production, farmers tend to increase maize plant density with drought and density stress tolerance hybrids to achieve higher grain yield. Considering the insufficient and fluctuating precipitation in semiarid area, higher maize plant density probably increases the risk of grain yield loss and soil water over-consumption.

Previous studies have indicated that rainfed maize density should be lower than that in irrigated maize due to water deficiency (Liu et al., 2014). Drought coupled with high density stress would cause more serious barrenness and grain yield loss (Karlen and Camp, 1985). Currently, the recommended maize plant density is about 60000 plants ha^{-1} in dryland (Ren et al., 2016). However, the optimum density for rainfed maize is variable and highly depend on soil water availability during growth stage. Ning et al. (2019) indicated maize grain yield is highly correlation with precipitation from fallow to tasseling on the semiarid Loess Plateau. Solomon et al (2017) suggested that choice of maize plant densities should be based on seasonal weather forecasts to maximize opportunities for higher yields. However, the forecast of precipitation is not accurate enough so far. Density-neutral maize

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hybrids has been considered as another way to reduce the risk of grain yield loss cause by various rainfed condition in dryland farming system (Tokatlidis et al., 2011). But its development is slow and most commercial hybrids are highly density-dependent. It seems that the trend of increasing maize plant density is dominant in no mater irrigated or rainfed condition.

It is reported that improper vegetation utilization, such as using of wrong vegetation types, excessive community density and productivity, leads to soil desiccation in semiarid and semi-humid regions (Wang et al., 2004). Although excessive depletion of deep soil water was usually observed in artificial forest and alfalfa land, and maize production was evaluated without causing soil desiccation by model simulation in the last decade. The continuous increase of hybrid drought tolerance, plant density and the climate change are increasing the risk of soil desiccation in maize production system. Therefor we need to reassess its impact on deep soil water balance, especially in water scarce arid regions.

Thus, we carried out a 4-year of successive field study with three different drought and density stress tolerance maize hybrids and four plant density arrange from 52,500 to 97,500 plants ha^{-1} on the Loess Plateau, and the plant growth, yield formation and soil water consumption were assessed. The primary objectives of the present study were to 1) quantify the responses of grain yield formation and WUE to increasing plant density under various rainfall condition 2) and evaluate the effect on deep soil water balance.

2. Materials and methods

2.1. Site description

The field experiment was conducted on the Heyang Dryland Agricultural Research Station of Northwest A & F University, which was in Ganjing Town (35°19′ N, 110°4′ E, and 877 m altitude), Heyang County, Shaanxi Province, China, over four growing seasons from 2015 to 2018. The site was in the southeast of the Loess Plateau, which is represented by a temperate semi-arid continental monsoon climate, with an average annual temperature of 11.5 °C, a potential evaporation of 1832.8 mm and an annual mean precipitation of 498.4 mm over the last 15 years. The total yearly frost-free period was 190 days. Before the experiment, continuous spring maize was planted. The field experiment was carried out in a flat field on level terrain with dark loessial soil that was a typical soil type on the Loess Plateau, with a mean soil bulk density of 1.31 g cm^{-3} and classified as middle loam soil based on the FAO/UNESCO Soil Classification (1993). The dynamic of temperature and precipitation during the experimental periods are shown in Fig. 1. The climate data were obtained from the weather station at the experimental station.

2.2. Experimental design and treatments

The experiment initiated in 2015 was a split plots design with three replications and planting density as the main plot treatment, and variety as the sub-plot treatment. Each plot was 3.5 m wide and 13.0 m long. Four planting densities were evaluated in the experiment: D1 (52,500 plant ha^{-1}), D2 (67,500 plant ha^{-1}), D3 (82,500 plant ha^{-1}) and D4 (97,500 plant ha^{-1}), with row spacing of 50 cm and plants spacing controlled by the planting population, which was 38.1, 29.6, 24.3 and 20.5 cm, respectively. The three varieties that with similar growth period applied in the experiment were YY22, ZD958 and XY335. High density tolerance of the three varieties is increased in turn and drought tolerance of ZD958 and XY335 was great than that in YY22 (Xue et al., 2010).

Maize was sown in late April and plots were hand-planted at two seeds per hole at a depth of 4–5 cm. Only one seedling remained at the three-leaf stage. The one-time application of the full amount of fertilizer was conducted by broadcast before sowing with rates of 225 kg N ha^{-1},120 kg P_2O_5 ha^{-1} and 90 kg K_2O ha^{-1}. The fertilizer types for N, P_2O_5 and K_2O were urea (N 46.4%), ammonium phosphate (P_2O_5 45%, N 16%) and potassium sulfate (K_2O 50%), respectively. The harvest of maize was performed in mid-September. After the harvest, the field was kept fallow until the sowing of the next maize season the next April and with no-tillage management.

For all treatments, weeds were controlled by spraying chemical herbicides before crop emergence, as well as by hand once or twice in the growing season based on visual estimates of weed populations. Irrigation and plastic film mulch were not applied throughout the entire year in all experimental years.

2.3. Measurements

2.3.1. Soil sampling and analysis

Soil water content of all treatments was determined by the gravimetric method at seedling, jointing, tasseling, filling and maturity stage (Table 1). Development progress of the three hybrids was similar and sampling time are the same. Soil samples were taken in 20 cm increments from 0 to 200 cm layer at each growth stage and 0 to 500 cm layer only at maturity stage in 2018. The soil samples were obtained randomly using a hand soil drill at two locations in the middle of a row for each treatment. After obtaining the wet weight, the soil samples were then dried for 8 h in an oven at 105℃ to constant weight and reweighed. The soil moisture was then calculated. Soil water storage was calculated by summing the soil water storage in different layers, and both of the index parameters were calculated by the following equations (Zhang et al., 2017).

$$SM\ (\%) = (M - M2)/M2 \times 100\%$$

where SM is the soil moisture, M1 (g) is the weight of wet soil and M2 (g) is the weight of dry soil. In this study, the soil water content refers to the soil moisture.

$$SWS\ (mm) = \Sigma\ Wi \cdot Di \cdot Hi \cdot 10/100$$

where SWS is the soil water storage, Wi (mm) is the gravimetric soil water (%) in the i layer, Di (g cm^{-3}) is the soil bulk density (g cm^{-3}) in the i layer, with soil bulk density determined using the ring-cutting method (Liu et al., 2010), Hi (cm) is the thickness of the i layer.

ET was calculated by the following soil water balance equation:

$$ET = P + I + C + \Delta W - D - R$$

where ET (mm) is the evapotranspiration in a specific growth stage; P is the precipitation in the corresponding growth stage; I (mm) is the total irrigation, with I = 0 for the rain-fed conditions; C is the upward flow into the root zone; ΔW (mm) is the difference in the soil water storage between two growth stages; D is the amount of water discharged outside the root; and R is the surface runoff. In the experiment, the groundwater level was below 40 m in depth, so the groundwater flow to the roots could be ignored. Runoff was never observed, as the experimental field was flat, and the drainage was assumed to be insignificant over a 200 cm depth.

2.3.2. Aboveground dry matter

Five plants were randomly sampled from each plot at the seedling, jointing, tasseling, filling and maturity stages in each experimental year, and the leaf area of these plants was measured. The plants from each treatment were used to measure aboveground dry matter after measuring leaf area at the aforementioned growth stages. Aboveground dry matter was determined after plants were fixated at 105 °C for 0.5 h and then oven-dried at 85 °C for a minimum of 48 h to constant weight. In this study, the aboveground dry matter refers to the biomass.

2.3.3. Grain yield and harvest index

In each treatment, excluding edge effects, three random quadrats

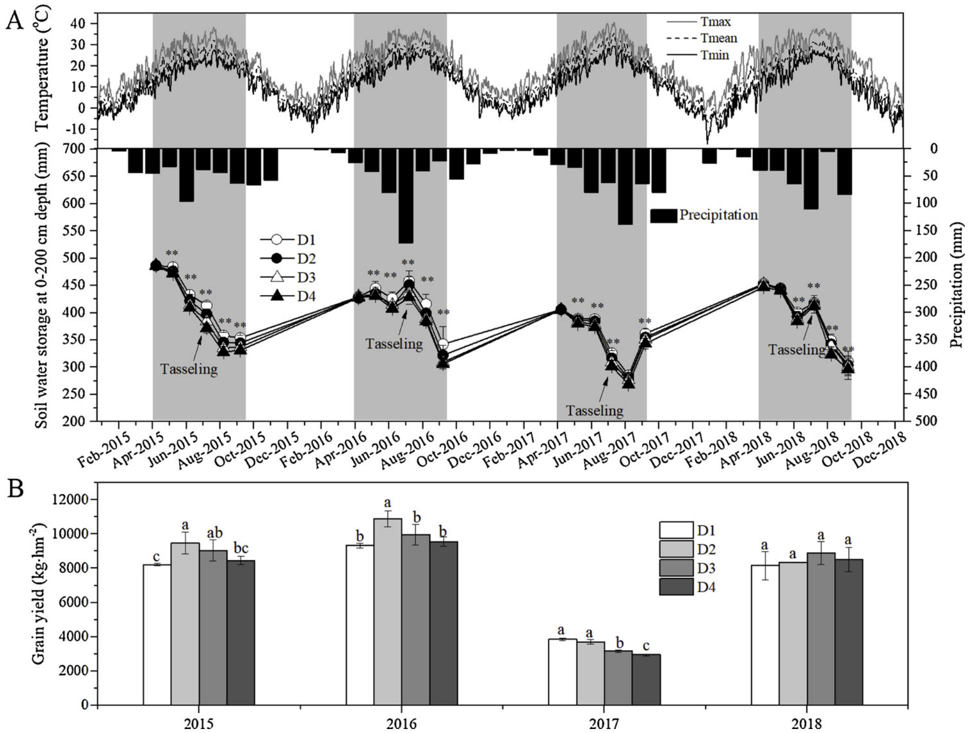

Fig. 1. Dynamics of temperature, precipitation, soil water storage (A) and grain yield (B) from 2015 to 2018. Data of soil water storage and grain yield are the average value across three hybrids under different plant density. "**" above dots state significantly different according to LSD (0.01).
The same letter above bars in a group state not significantly different according to LSD (0.05).

Table 1
Maize growth period and sampling date from 2015 to 2018.

Years	Sowing	Seedling	Jointing	Tasseling	Filling	Maturity
2015	28-Apr	31-May	19-Jun	20-Jul	26-Aug	25-Sep
2016	26-Apr	1-Jun	27-Jun	25-Jul	25-Aug	17-Sep
2017	27-Apr	25-May	26-Jun	13-Jul	14-Aug	22-Sep
2018	27-Apr	26-May	28-Jun	18-Jul	17-Aug	10-Sep

covering 9.0 m^2 were selected to measure maize grain yield, including yield components (kernel number per ear, ear number per square and 100-kernel weight). Maize grain yield was measured by adjusting grain moisture content to 14%. In this study, the crop yield refers to the grain yield. Harvest index was calculated as the ratio of grain yield to aboveground biomass.

2.4. Statistical analysis

The significance of main effects, sub-plot effects, and interaction effects and the differences between treatments were assessed by split plot analysis with two-way ANOVA. All of the data were analyzed using the IBM SPSS statistical software package (version 20.0, SPSS Inc., Chicago, IL, USA), followed by the least significant difference test (LSD). Treatment differences were considered statistically significant at $p < 0.05$, and the figures were generated using Origin 2015.

The optimum plant density was estimated according to Tollenaar (1992): ln(Yp) = a − bD. Where Yp is the yield per plant in grams and D is the density in plants/m^2, with optimal density being equal to 1/b. The source to sink ratio (SSR) was estimated according to Chen et al. (2016) and defined as the ratio of post-silking dry matter accumulation to kernel number per square meter.

3. Results

3.1. Overview of maize growth conditions and yield performance

Temperature was higher in 2017 than other years, especially around tasseling there were 12 days whose maximum temperature exceeded 38oC in 2017 while not observed in other years (Fig. 1A). 2016 and 2017 received more precipitation during maize growth period than that in 2015 and 2018, but soil water storage was dramatically lower in 2017. Thus, we defined 2017 as a dry year in the present study. Soil water content decreased with increasing plant density at most of plant growing stage (Fig. 1A). Grain yield showed a parabolic relationship with plant density expect in 2017 whose yield were lowest and decreased linearly with increasing density (Fig. 1B).

3.2. Yield components

Maize grain yield components were notably altered by plant density (Fig. 2). Kernel number per meter showed an upward trend with increasing plant density except in the dry 2017. It fluctuated dramatically between treatments and growth seasons and ranged from 1210 to 4290 kernels per meter. On the contrary, kernel weight was relatively stable and decreased progressively with increasing density ranged from 31.7 to 21.3 g. Grain yield showed a parabolic relationship with plant density expect in 2017, which primarily associated with the fluctuation in kernel number. Coefficient of Variance (CV) of grain yield through the four grown seasons were increased with the increasing plant density ranged from 32.0% to 41.2%.

Average grain yield across the three varieties of D2 and D3 were 9.7% and 5.1% great than that in D1 through the four years, whereas yield of D4 was lowest (Table 2). The differences in yield components were also significant between varieties and years (Table 2). Average grain yield of XY335 and ZD958 was greater than YY22, as well as kernel number per meter. But the highest kernel weight was observed in YY22. Average grain yield in 2017 was dramatic lower than other years, which primarily associated with reduction of the kernel number. There were significant interactions between density and variety, density and year, variety and year on yield components, which indicated that density determination was variety and year specific.

Fig. 3 shows the optimal plant density that estimated on the basis of the four densities in 2015–2018. XY335 has the highest optimal plant density, then ZD958, and lowest in YY22. For high XY335 and ZD958, optimal plant densities excessed 70,000 plant ha^{-1} in normal years, but less than 70000 plant hm-2 for XY335 YY22. The optimal plant

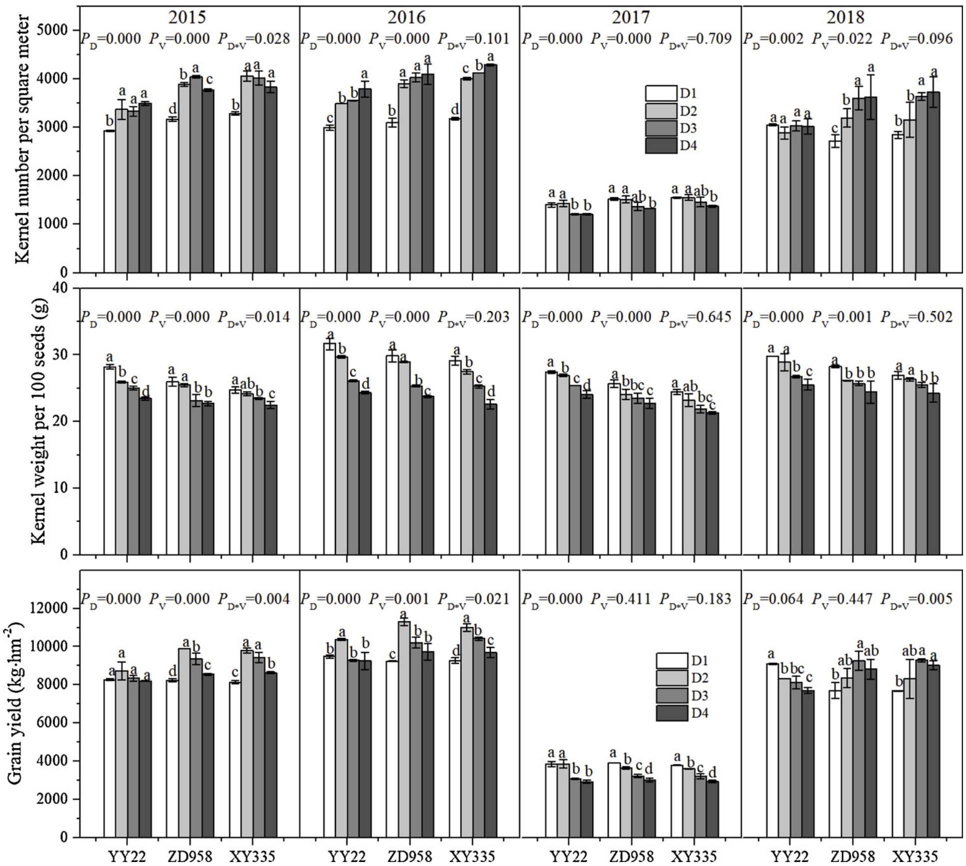

Fig. 2. Yield components of different maize variety under various plant density from 2015 to 2018. Data are the mean (± SD) of three replications. The same letter above bars in a group state not significantly different according to LSD (0.05).

Table 2
Analysis of variance of kernel number, kernel weight, and grain yield.

Factor		Kernel number per meter	Kernel weight (g 100 seed^{-1})	Grain yield (kg hm^{-2})
Density	D1	2647c	27.69a	7389c
	D2	3038b	26.44b	8103a
	D3	3118a	24.76c	7766c
	D4	3131a	23.48d	7374c
Variety	YY22	2764c	26.83a	7431b
	ZD958	3054b	25.36b	7777a
	XY335	3133a	24.58c	7765a
Year	2015	3600b	24.57c	8800b
	2016	3714a	27.02a	9938a
	2017	1412d	24.57c	3420d
	2018	3209c	26.56b	8474c
Source of variation				
Density		***	***	***
Variety		***	***	***
Year		***	***	***
D*V		**	*	***
D*Y		***	***	***
V*Y		*	*	*
D*V*Y		ns	ns	**

Note: D1: 52500 plant ha^{-1}, D2: 67500 plant ha^{-1}, D3: 82500 plant ha^{-1}, D4: 97500 plant ha^{-1}.
Different letters following means in the same treatment represent significant at the 5% level (LSD). ns, nonsignificant; * Significant at the $P =$ 0.05 level; ** Significant at the $P =$ 0.01 level; *** Significant at the $P =$ 0.001 level.

densities in the dry year (2017) varied from 48,000 to 51000 plant hm^{-1}, whereas that in other years varied from 58,000 to 103,000 plant ha^{-1}.

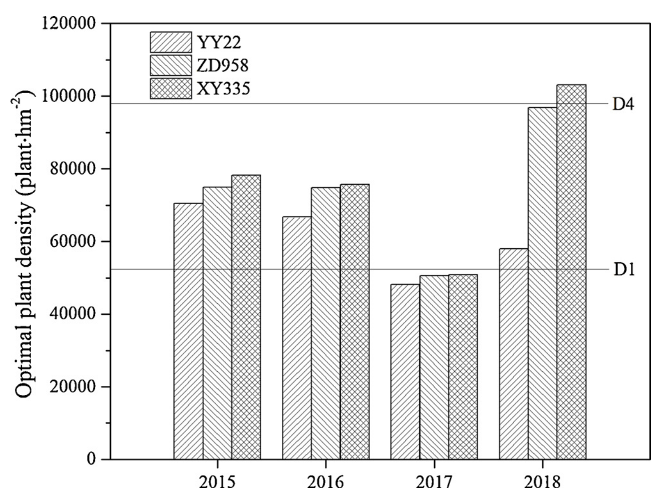

Fig. 3. Optimum plant densities of the three varieties from 2015 to 2018.

3.3. Source to sink relationship

Plant biomass was increased with increasing plant density, whereas harvest index with a downward tendency (Fig. 4). Plant biomass and harvest index both were lower in the dry year (2017) than other years. Harvest index usually was less than 0.5 expect under lower density in 2016 who received most precipitation during the growth period. The ration of pre-tasseling plant biomass (PB) to total plant biomass (TB) tended to decrease with increasing plant density range from 0.68 to 0.44, and observed dramatic higher value in 2017 than other year.

SSR tended to decrease with increasing plant density in 2015 and

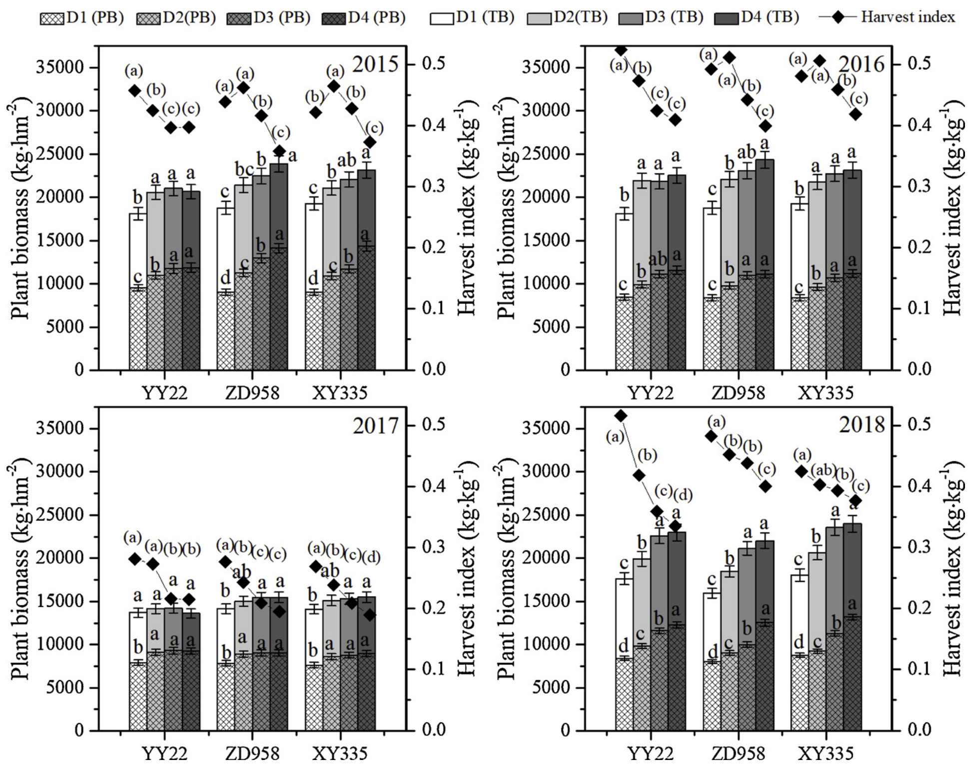

Fig. 4. Plant biomass and harvest index under different plant density from 2015 to 2018. PB stands for pre-tasseling biomass and TB stands for total biomass. Data are the mean (± SD) of three replications. The same letter above bars/dots in a group state not significantly different according to LSD (0.05).

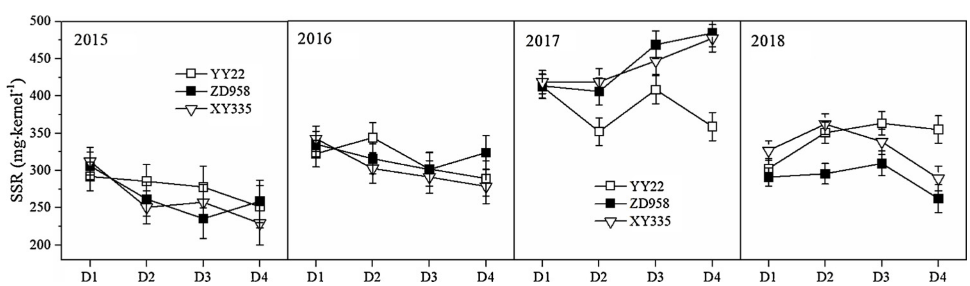

Fig. 5. Changes of source to sink ratio (SSR) under different plant density from 2015 to 2018. Data are the mean (± SD) of three replications.

2016, whereas increased in the dry 2017. And in 2018, SSR showed a parabolic relationship with plant density (Fig. 5). The value of SSR was high in 2017 than other years. These results indicated high plant density weaken source to sink strength and limited yield increase in normal years. But source to sink strength was enhanced by high plant density in dry year, which probably due to the dramatically decreased kernel number.

3.4. Water consumption and water use efficiency

High plant density enhanced the total water consumption (or evapotranspiration) during maize growth period (Fig. 6). At the first three growth periods (S-V3, V3-V9, V9-VT), water consumption was increased with increasing plant density, whereas tended to decrease at the later growth periods. Most water was consumed at V9-VT period in 2015 and 2016, whereas at VT-R3 and V3-V9 in 2017 and 2018.

WUE of YY22 showed a downward trend with increasing plant density in the four experience years (Fig. 7). But WUE of ZD958 and XY335 only linearly decreased with increasing plant density in the dry 2017 but increased first and then decreased in other years (Fig. 7).

Soil water storage at sowing was similar under different density and variety. While at maturity, soil water storage was lower under higher density and in XY335 than other varieties. On the contrary, ET was higher under higher density and in XY335. The highest average WUE was observed at D2 treatment, and the lowest at D4. ZD958 had a higher WUE than YY22 and XY335. WUE was sharply decreased in 2017, which was less than the half of that in the other years (Table 3).

3.5. Dynamic of soil water content

As shown in Fig. 8, soil water profile dynamic at 0 to 200 cm layer was similar among the three varieties, while soil water content was decreased with increasing plant density, especially post tasseling stage. Soil water content usually was less than 12% at filling and maturity period. Soil water at depth from 0 to 200 cm all could be utilized by maize production. Precipitation was hardly to replenish soil moisture that in > 200 cm depth.

As shown in Fig. 9, the CV of soil water content was decreased with soil depth. High plant density increased CV of soil water content, especially at 0–100 depth. CV of soil water content in XY335 was slightly higher than that in ZD958 and YY22. Plant density affected CV of soil water content at deeper soil depth (exceed 100 cm) in XY335 and ZD958, while no difference for that was found in YY22.

Variation in deep soil water (0–500 cm) during the maturity generally ranged from 8.7 to 21.2% for all treatments in 2018 (Fig. 10), the vertical change tendencies in soil water content were similar for all

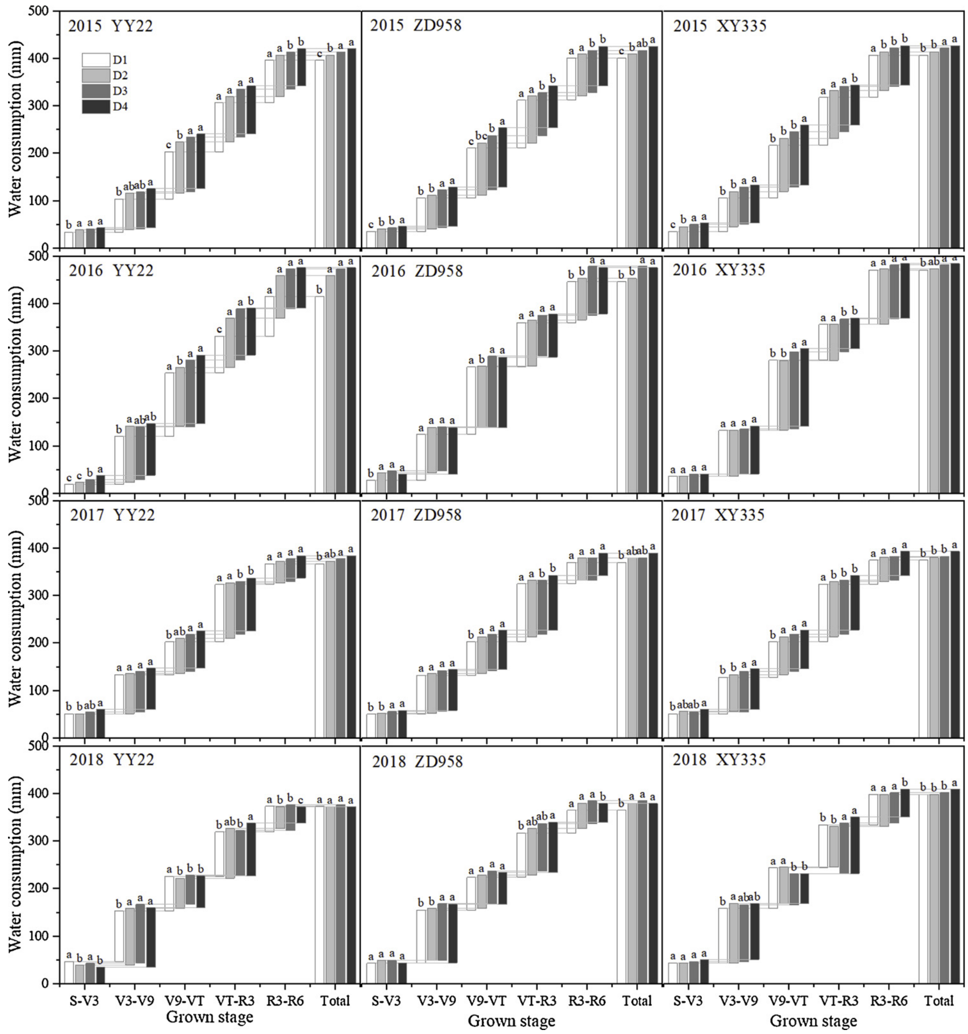

Fig. 6. Changes of water consumption during different growth period under various plant density from 2015 to 2018. Data are the mean of three replications. S, V3, V9, VT, R3, R6 stand for sowing stage, 3-leaf stage, 9-leaf stage, tasseling, milk stage and maturity, respectively. The same letter above bars in a group state not significantly different according to LSD (0.05).

cultivars across all plant density. Soil water content at depth exceed 200 cm was higher and more stable than that in 0–200 cm depth. However, similarly to 0–200 cm depth soil layer, soil water content at depth excess 200 cm was also slightly declined by high plant density, especially in XY335.

4. Discussion

4.1. Grain yield formation

Previous studies recommended maize plant density be about 60000 plants ha^{-1} and should not exceed 70000 plants ha^{-1} in dryland considering the limited water availability (Liu et al., 2014; Ren et al., 2016). However, in the present study we found the optimal density usually exceed 70000 plants per hectare for modern high density and

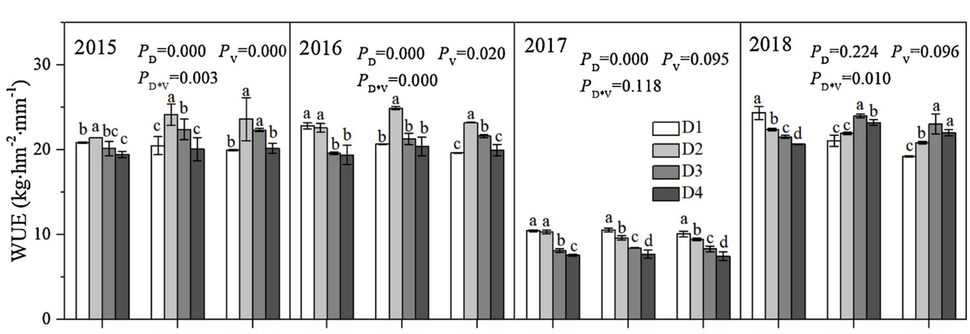

Fig. 7. Changes of WUE under various plant density from 2015 to 2018. Data are the mean of three replications. The same letter above bars in a group state not significantly different according to LSD (0.05).

Table 3
Analysis of variance of soil water storage, evapotranspiration (ET) and water use efficiency (WUE).

Factor		SWSs (mm)	SWSm (mm)	P (mm)	ET (mm)	WUE (kg $hm^{-2} mm^{-1}$)
Density	D1	445a	348a	297	399c	18.36b
	D2	443a	336b	297	408b	19.55a
	D3	445a	328c	297	416a	18.42b
	D4	442a	319d	297	420a	17.37c
Variety	YY22	444a	338a	297	404b	18.24b
	ZD958	445a	334a	297	409b	18.83a
	XY335	442a	327b	297	420a	18.20b
Year	2015	487a	343a	269	414b	21.27b
	2016	430c	325b	358	466a	21.36b
	2017	407d	355a	327	380c	9.03c
	2018	452b	308c	237	384c	22.05a
Source of variation						
Density		ns	**	–	**	***
Variety		ns	*	–	*	**
Year		***	***	–	***	***
D*V		ns	ns	–	ns	**
D*Y		ns	ns	–	ns	**
V*Y		ns	*	–	*	**
D*V*Y		ns	ns	–	ns	*

Note: D1: 52500 plant ha^{-1}, D2: 67500 plant ha^{-1}, D3: 82500 plant ha^{-1}, D4: 97500 plant ha^{-1}. SWSs stand for 0–200 cm soil water storage at sowing; SWSm stand for 0–200 cm soil water storage at maturity; P stand for precipitation during maize growth period; ET state evapotranspiration.
Different letters following means in the same treatment represent significant at the 5% level (LSD).
ns, nonsignificant; * Significant at the $P = 0.05$ level; ** Significant at the $P = 0.01$ level; *** Significant at the $P = 0.001$ level.

drought tolerance hybrids (Fig. 3). This indicates that increasing plant density couple with high density and drought tolerance hybrid is a potential way to increasing grain yield in dryland maize production system.

Kernel number and weight are the two interinhibitive factors that determine grain yield under different plant density. High density usually increased kernel number per unit area but decreased kernel weight (Echarte et al., 2000). But the evaluation of which factor dominant yield dynamic was difficult and different in various studies (Echarte et al., 2000; Hashemi et al., 2005; Chen et al., 2016). In the present study, the fluctuation of yield among years mainly attributed to the dynamic of kernel number as it was dramatically decreased in the dry year. Kernel weight declined with the increasing plant density which suggested source supply was limited for grain filling. The analysis of SSR conforms that the insufficient source was usually the main factor that limited yield improved with increasing plant density. But source to sink strength was enhanced by high plant density in dry year, which probably due to the dramatically decreased kernel number (Fig. 2). Just like kernel number and weight, plant biomass and harvest index were interinhibitive. The even-increased plant biomass but sharply decreased harvest index could not achieve continuous increase in grain yield with increasing plant density. Thus, optimizing canopy photosynthetic capacity and improving photosynthate distribution efficiency meanwhile increasing kernel number would be the key to improve maize yield by increasing plant density. What's more, attention needs to be paid to occasionally kernel number decreasing caused by high plant density and drought stress around tussling in dryland maize.

4.2. Water use and water use efficiency

Water is the major limiting for primary production in most dryland farming system (Deng et al., 2006). Improving crops yield by increasing water use and water use efficiency (WUE) had been the main goal for crop managements (Angus and Van Herwaarden, 2001). But water use and WUE usually cannot be obtained simultaneously under water stress condition. WUE, defined as the ratio of grain yield to crop water use, is often considered an important determinant of yield under stress. Blum (2009) argued that effective use of water, which implies maximal soil moisture capture for transpiration, but not WUE is a major target for yield improvement in water-limited environment, as high WUE often coupled with reduced yield and reduced drought resistance. In the present study, water use or evapotranspiration were progressively increased with increasing plant density, while WUE showed a parabolic relation with plant density. The density of 97500 kg hm^{-2} decreased grain yield and WUE compared with density of 52500 kg hm^{-2}, meanwhile increased the risk of excessive water use. The evaluation of water consumption at different maize grown stage showed higher plant density consumed more water at vegetable growth stage while consumed less water at reproductive stage. This suggest that the limited water could not afford maize grain filling under excessive plant density in the dryland and resulted in a low WUE. In terms of the three variety, although ZD958 and XY335 both achieved higher grain yield than YY22, the water use strategy differed. ZD958 increased WUE while XY335 use more water. Considering the scarce water resources and fragile ecosystems, varieties like ZD958 were recommended in the semiarid area. As it is complicated to evaluate the efficiency of water use which involves stomatal and non-stomatal transpiration and soil evaporation (Blum, 2009), we consider high WUE coupled with improve yield as a main target for crop management and breeding.

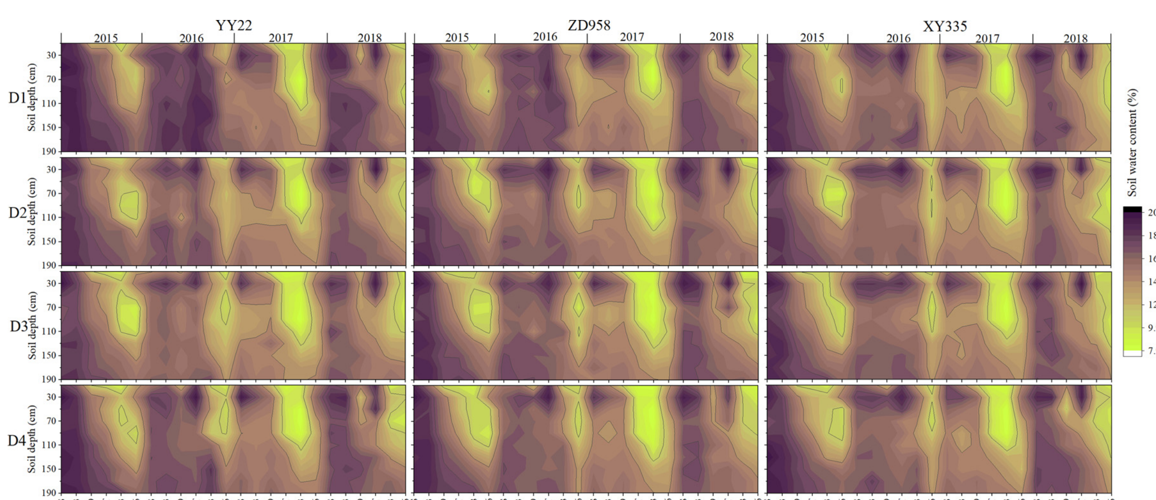

Fig. 8. Dynamic of soil water profile in 0–200 cm soil layer from 2015 to 2018.

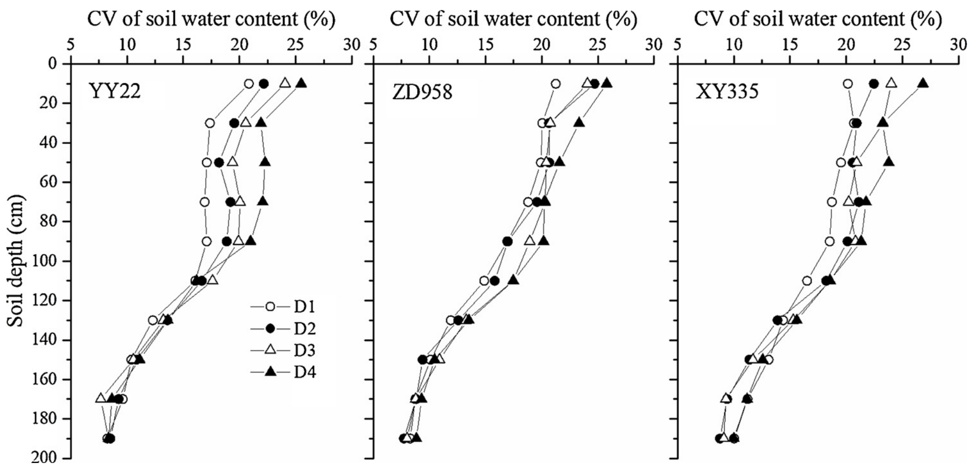

Fig. 9. The coefficient of variation (CV) of soil water content in 0–200 cm soil layers through the four growth seasons. Data are the CV of 24 values.

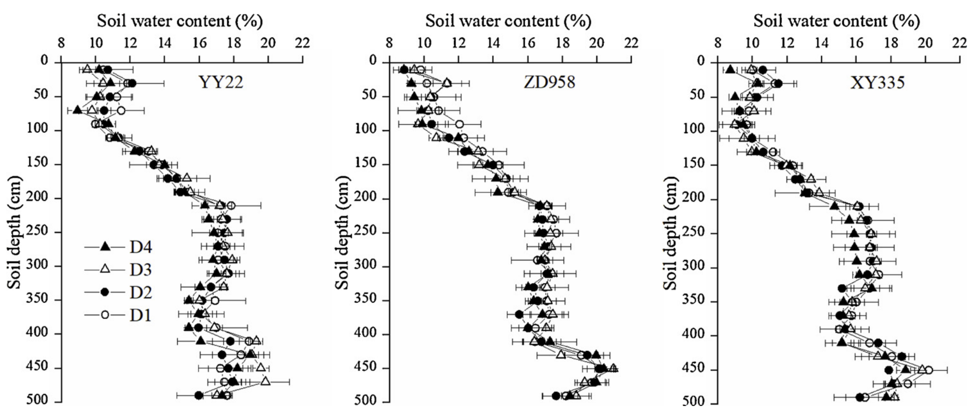

Fig. 10. Soil water profile in 0–500 cm depth at harvest in 2018. Data are the mean (± SD) of three replications.

4.3. Soil water balance

Soil moisture has declined due to orchard production and afforestation across the Loess Plateau (Jia et al., 2017; Peng et al., 2017). But previous studies on maize showed no soil desiccation occurs in dryland maize production system (Li et al., 2007; Wang et al., 2008). In our study, soil water storage at 0–200 cm depth declined with the increasing plant density during maize grown period while recovered after the fallow, which suggests soil water balance was not disturbed. However, soil water content at depth excess 200 cm which was hardly resupplied by rainfall was also consumed by excessive density, especially in XY335 who consumed more and deeper water than other varieties. Therefore, the risk of deep soil desiccation was increased by high plant density, which would cause adverse effects on the resistance of the dryland maize production system. Crop rotation and fallow should performed occasionally depend on water availability to improve system resistance. It is agreed that the period of the present study was limited. Additional work is required to investigate the effect of high plant density coupled with drought tolerance hybrids on soil water balance in a long run.

5. Conclusions

In this work, we have evaluated the effects of high plant density on maize grain yield formation and soil water balance in dryland. Grain yield and WUE both showed parabolic relations with plant density. The optimal plant density usually exceeded 70000 plant hm^{-2} for drought and density stress tolerance hybrids expect in extreme drought in this specific site. High plant density did not disturb the soil water balance at 0–200 cm layer but increased the risk of deep soil desiccation. In conclusion, higher density couple with drought and density stress tolerance hybrids is a potential way to improve maize yield in dryland. Simultaneously, attention need pay to deep soil desiccation and dryland farming system resistance.

Acknowledgement

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Soil water use and crop yield increase under different long-term fertilization practices incorporated with two-year tillage rotations

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ABSTRACT

Water shortage and soil nutritionally depleted are the most limiting factors of crop production on the Loess Plateau. Conservation tillage rotation, conserved soil water and enhanced soil fertility effectively, produced high and stable crop yields in a spring maize-winter wheat-fallow rotation over a two-year period (hereafter called maize-wheat-fallow rotation). Corresponding and appropriate fertilization practices should enable the full potential of tillage rotation. Therefore, a long-term two-factor split-plot experiment (2007–2016) was established in Heyang County, Shaanxi Province, China, a typical rainfed dryland agricultural region. Beginning in 2007, three fertilization practices (balanced fertilization (BF), low fertilization (LF) and conventional fertilization (CF)) and four tillage systems (no tillage rotated with subsoiling annually (NS), subsoiling rotated with conventional tillage annually (SC), conventional tillage rotated with no tillage annually (CN), and continuous conventional tillage (CT)) were conducted to assess crop yield and soil water variation. The results showed that CCN (CF + CN, 5440 kg ha^{-1}) and BNS (BF + NS, 8415 kg ha^{-1}) produced the highest crop yield for winter wheat and spring maize, respectively. LNS (LF + NS, 419 mm) had a better soil water condition in fallow periods. NS and CN rotations had a better soil water condition (reserved more soil water for crop growth) than SC and CT during the wheat and maize growth season. However, both the average ET of winter wheat and spring maize under all of the treatments had no significant differences ($P > 0.05$) in ten years. In ten years, NS and CN rotations had higher water use efficiency (WUEs) than the SC rotation and CT, BNS had the highest WUE (19.0 kg ha^{-1} mm^{-1}) for the spring maize – winter wheat rotation. Tillage rotation and fertilization had an interactive effect on soil water use and crop growth, and the results might clarify the response of crop yield and soil water variation to fertilization and tillage rotations. For further and sustainable development of crop production, BNS (5133 and 8415 kg ha^{-1} for wheat and maize yield, respectively; soil water in fallow: 386 mm) and BCN (5221 and 8116 kg ha^{-1} for wheat and maize yield, respectively; soil water in fallow: 388 mm) are recommended as the optimal fertilization and tillage rotations for rainfed crop production on the Loess Plateau.

1. Introduction

As an important rainfed crop production area, water shortage and soil nutrients depletion are considered to be the most limiting factors in the Loess Plateau, China (Wang et al., 2009). Sustained crop productivity relies on constant renewal of soil nutrients and water supply (Wang and Shangguan, 2015; Yousaf et al., 2017). Hence, application of chemical fertilizers and increased water capture are necessary for enhancing crop yields and sustaining soil fertility. To pursue high crop yield, excessive fertilizers have been applied to fields by farmers in recent years (Liu et al., 2013). However, inappropriate or excessive fertilizer application does not guarantee constantly increasing yields, potentially results in low nutrients efficiency, and causes environmental problems in agro-ecosystems (Wang et al., 2012a,b). With excessive fertilization, more soil water is consumed, a situation exacerbated by water shortage in fields (Zhang et al., 2015b).

Limited by climate and geographical conditions, annual winter wheat-summer fallow or annual spring maize-winter fallow is the prevailing crop system in this area. There is a fallow period each year. However, the prevailing conventional tillage applied after crop harvest aggravates the water deficit during fallow periods (Zhao et al., 2009). In this area, conventional tillage means plowing and turning the surface soil at deep levels. The bare soil surface brings high evaporation in fallow periods and causes a dry soil water condition for crop growth in

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subsequent years (Feng et al., 2011; He et al., 2006; Huang et al., 2006). The prevailing conventional tillage and excessive fertilization practices create a severe water deficit.

Conservation tillage including crop residue mulching, no tillage, subsoiling and rotary tillage etc. has been recommended as an effective practice to conserve soil water and enhance soil fertility, alleviating soil water shortage under rainfed conditions in numerous areas (Hou et al., 2012; Xie et al., 2016). However, continuous mono-one tillage practice applied annually would bring some defects to crop growth and soil in field, such as long time no tillage would lead to compact soil surface and more weeds in fields, long time conventional tillage would result in high soil water evaporation and soil erosion. Tillage rotation has been reported as one of the most recommended tillage practices for conserving soil water in this area (Zhang et al., 2015a). Rotation of two or three practices, including no tillage, subsoiling and conventional tillage in a specific sequence can offset some defects brought about by annual one-tillage practices. Enhanced the soil structure and incorporated crop residue to field, tillage rotation can capture precipitation effectively, conserve soil water in fallow periods and enhance the soil nutrient conditions in fields (Hou et al., 2012; Sun et al., 2010; Zhu et al., 2015).

Although tillage rotation has a significant effect on soil fertility, it cannot entirely replace the benefits of fertilizers. Farmers without access to mineral fertilizer cannot compensate for N deficiencies and will suffer yield reductions as a direct result (Giller et al., 2009). Moreover, straw mulching (straw returned to field and covered on soil surface) with fertilizers promotes higher crop yields and fertilizer efficiency (Fan et al., 2004). As a major component of NT, straw mulching has been proven as one of the most effective measures for conserving soil water (Feng et al., 2011). Thus, proper fertilization with tillage rotation has significant importance in rainfed dryland agriculture.

However, the effects of tillage rotations on crop yield and soil conditions have primarily focused on short-term experiments (2–5 years) in this area, and the results were not consistent with previous studies (Hou et al., 2012; Nie et al., 2015; Zhu et al., 2015). Relevant long-term experiments on tillage rotation with fertilization are limited. With proper fertilization, tillage rotation may couple soil water and nutrients rationally and function more effectively and economically. In addition, the effects of conservation tillage rotation and fertilization on soil water and crop yield is a long and sustainable process, and these effects are site- and climate-dependent (Xie et al., 2016). Therefore, determination of an appropriate tillage rotation system with fertilization is essential for crop production on the Loess Plateau. Hence, a long-term experiment (ten years) conducted in Heyang, Shaanxi Province, China was established to study soil water use and crop yield in a spring maize-winter wheat-fallow rotation field over a two-year period. Our objectives were to study the soil water conservation effect in fallow periods, soil water use during growth periods and crop yield variation in rainfed fields and to select an optimal tillage rotation with fertilization for crop production on the Loess Plateau.

2. Methods

2.1. Study site description

The field experiment has been conducted since 2007 at the Dryland Agricultural Research Station of Northwest A&F University, which is located in Ganjing (35°19′ N, 106°4′ E), Heyang County, Shaanxi Province, China. The research station was located on the Loess Plateau, which is characterized by a temperate semi-arid continental monsoon climate at an altitude of 877 m. The annual mean temperature and evaporation were 11.5 °C and 1833 mm, respectively. During the last 30 years, the annual mean precipitation was 526 mm (the data derived from Heyang Weather Station). The annual precipitation displayed an uneven characteristic, 60% of which mainly occurred in the summer season (July, August, and September) that deviated from the winter wheat growth season. The climate details for this experiment are shown in Fig. 1.

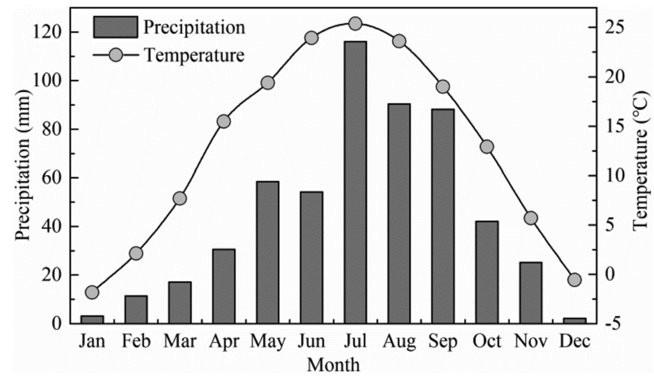

Fig. 1. Average monthly precipitation and temperature in Heyang County.

Before the experiment, continuous spring maize was planted, and conventional tillage with crop residue removal or burning was applied every year after crop harvest. The field experiment was conducted on level terrain with dark loessial soil, which is a typical soil type in the Loess Plateau, and it is classified as middle loam soil based on the FAO/UNESCO Soil Classification (1993). The soil properties and nutrient conditions at a soil depth of 0–60 cm before this experiment started in 2007 are shown in Table 1.

2.2. Experimental design and treatments

The field experiment was designed with three fertilizations (balanced fertilization (BF), low fertilization (LF) and conventional fertilization (CF)) as the main treatments and was spilt into four tillage practices (no tillage rotated with subsoiling annually (NS), subsoiling rotated with conventional tillage annually (SC), conventional tillage rotated with no tillage annually (CN) and continuous conventional tillage (CT)) as sub-treatments. Each sub-plot was 5 m wide and 22.5 m long (112.5 m^2) with a 60 cm interval between plots. These three fertilization and four tillage practices were combined into twelve fertilization with tillage rotation systems (Table 2): BNS (BF + NS), BSC (BF + SC), BCN (BF + CN), BCT (BF + CT), LNS (LF + NS), LSC (LF + SC), LCN (LF + CN), LCT (LF + CT), CNS (CF + NS), CSC (CF + SC), CCN (CF + CN), CCT (CF + CT).

The fertilizers were spread evenly on the soil surface before sowing. The fertilizers for N were urea and diammonium phosphate, the fertilizer for P_2O_5 was diammonium phosphate, the fertilizer for P_2O_5 was potassium chloride. For the balanced fertilization (BF), 150 kg N ha^{-1}, 120 kg P_2O_5 ha^{-1}, and 90 kg K_2O ha^{-1} were applied to the soil before sowing each year. For the low fertilization (LF), 75 kg N ha^{-1}, 60 kg P_2O_5 ha^{-1}, and 45 kg K_2O ha^{-1} were applied to the soil before sowing. For the conventional fertilization (CF), 255 kg N ha^{-1} and 180 kg P_2O_5 ha^{-1} were applied to the soil before sowing each year. The soil in the Loess Plateau is rich in potassium (K), and the soil total K is relatively high, so farmers generally apply fertilizers without potassium. Conventional fertilization (CF) was determined according to the mean fertilization level of the local farmers. Balanced fertilization (BF) was applied at the recommended rate determined by the local soil nutrient conditions and adequate nutrient ratios of crop production. The fertilizer rate of low fertilization (LF) was one-half that of balanced fertilization.

NS treatment means no tillage applied in the first year and rotated with subsoiling in the second year, and the following years would replicate the rotation in the first and second year by sequence. SC treatment means subsoiling applied in the first year and rotated with conventional tillage in the second year, and the following years would replicate the rotation in the first and second year by sequence. CN treatment means conventional tillage applied in the first year and

Table 1
Basic physical and chemical properties of the soil in 2007.

Soil depth (cm)	Bulk density ($g\,cm^{-3}$)	Organic matter ($g\,kg^{-1}$)	Total N ($g\,kg^{-1}$)	Total P ($g\,kg^{-1}$)	Total K ($g\,kg^{-1}$)	Alkali-hydrolyzable N ($mg\,kg^{-1}$)
0-60	1.42	7.78	0.51	0.28	5.75	22.54

Table 2
Abbreviation of different treatments.

Abbreviation	Illustration
BF	Balanced fertilization
LF	Low fertilization
CF	Conventional fertilization
NS	No tillage rotated with subsoiling each year
SC	Subsoiling rotated with conventional tillage each year
CN	Conventional tillage rotated with no tillage each year
CT	Conventional tillage annually
BNS	No tillage with subsoiling rotation under balanced fertilization
BSC	Subsoiling with conventional tillage rotation under balanced fertilization
BCN	Conventional tillage with no tillage rotation under balanced fertilization
BCT	Conventional tillage under balanced fertilization
LNS	No tillage with subsoiling rotation under low fertilization
LSC	Subsoiling with conventional tillage rotation under low fertilization
LCN	Conventional tillage with no tillage rotation under low fertilization
LCT	Conventional tillage under low fertilization
CNS	No tillage with subsoiling rotation under conventional fertilization
CSC	Subsoiling with conventional tillage rotation under conventional fertilization
CCN	Conventional tillage with no tillage rotation under conventional fertilization
CCT	Conventional tillage under conventional fertilization

rotated with no tillage in the second year, and the following years would replicate the rotation in the first and second year by sequence. CT treatment means conventional tillage applied each year. For the no tillage practice, the crop straw was chopped and spread evenly on the surface of the experimental plots after the harvest of the previous crop using a combine harvester. For the subsoiling practice, the crop straw was left on the soil surface as mulch, and the soil was then subsoiled to a depth of 30–35 cm with an interval of 60 cm by a subsoiler with adjustable wings. The surface soil had little disturbance with the subsoiling. All tillage practices were applied after the crop harvests. For the conventional tillage practice, the crop straw was incorporated into the soil layer and plowed to a depth of 22–25 cm. For all tillage practices, there was a one-time herbicide spray in the fallow period, and no other soil disturbance or management was applied before sowing. To produce a good soil condition for crop emergence and improve the effect of fertilizers, rotary tillage was applied at crop sowing one time each year.

The cultivated varieties of winter wheat were Jinmai 47 in 2007–2008, 2009–2010, 2011–2012 and 2013–2014 and Chang 6359 in 2015–2016 (Table 3). The cultivated varieties of spring maize were Yuyu 22 in 2009, 2011 and 2013 and Zhengdan 958 in 2015. Winter wheat and spring maize were sown at a density of 3.3 million plants ha^{-1} and 60 thousand plants ha^{-1}, respectively. The row spaces for winter wheat and spring maize were 0.2 m and 0.6 m, respectively. The operation of tillage practices and crop planting details are shown in Fig. 2 and Table 3.

2.3. Measurements

The gravimetric water content was multiplied by the soil bulk density which measured every year after crop harvest to obtain the volumetric water content. Soil water storage was calculated for a 2.0 m profile by multiplying the mean soil volumetric water content by soil profile depth. The soil moisture storage was measured using a 5 cm soil auger at 20 cm increments to a depth of 200 cm with three replications in all experimental plots every month. The soil water content was determined using the oven-drying method. The crop yield was determined by manual harvesting, threshing, and air-drying grain from three 3 m^2 and 9 m^2 areas selected at random in each plot for winter wheat and spring maize, respectively.

The requisite indices were calculated according to the following formulae.

$$\text{SWC (\%)} = (\text{wet soil weight} - \text{dried soil weight}) \times 100/\ \text{dried soil weight} \quad (1)$$

$$\text{Soil water storage (mm)} = \Sigma\, SWC_i \cdot D_i \cdot H_i \quad (2)$$

$$ET = W_1 - W_2 + P \quad (3)$$

Note: in Loess Plateau, the soil has deep loess soil layer that has good soil water storage ability and the soil water moved smoothly. The region where we applied our experiment was flat, therefore, the soil water runoff and deep drainage were ignored in this study.

$$WUE = Y/ET \quad (4)$$

where SWC (%) is the soil water content; D_i ($g\,cm^{-3}$) is the soil bulk density; H_i (cm) is the soil depth; ET (mm) is the water consumption during the crop-growing season; W_1 and W_2 (mm) are the water storage at a soil depth of 0–200 cm in the sowing and harvest seasons, respectively; P (mm) is the precipitation during the overall crop growth period; WUE ($kg\,ha^{-1}\,mm^{-1}$) is the water use efficiency in the field; and Y ($kg\,ha^{-1}$) is the grain yield.

2.4. Statistical analysis

Split-plot analysis with three-way ANOVA (fertilization and tillage were set as fixed factors, year (precipitation) was set as random factor) was used to assess the significance of main and interaction effects of the treatments. Three replications were calculated for each measurement, and ANOVA was used to compare the effects of different treatments on the measured variables. F-tests were conducted and multiple comparisons were performed using the least significant difference test (LSD) ($P \le 0.05$). The experimental data were analyzed with SPSS statistical package v.17.0 (SPSS Inst., Cary, NC), and the figures were generated using Origin 2015 (Systat Software Inc.).

3. Results

3.1. Crop yield

3.1.1. Winter wheat

Yield of winter wheat was significantly affected by the interaction effects of fertilization with tillage practices ($P < 0.05$) and year with fertilization ($P < 0.05$) (Table 4). For fertilization treatments, CF and BF had significant yield improvements compared with LF and the increments were 1027 and 929 $kg\,ha^{-1}$, respectively. For tillage practices, NS and CN rotation had significant yield improvements compared with CT, increasing by 7.1% and 6.9%, respectively. In contrast, the difference between SC rotation and CT was not significant.

In the first experimental year of 2008, the difference among tillage practices was not significant and the difference was primarily due to fertilization (Fig. 3). In subsequent years, differences occurred, and

Table 3
Planting details of wheat- maize rotation systems.

Year	Crop rotation	Planting date	Harvest date	Variety	Precipitition (mm)
2007–2008	Winter wheat	Sep 20, 2007	Jun 14, 2008	Jin mai 47	235.3
2008–2009	Fallow	–	–		400.2
2009	Spring maize	Apr 19, 2009	Sep 21, 2009	Yu yu 22	402.6
2009–2010	Winter wheat	Sep 25, 2009	Jun 22, 2010	Jin mai 47	187.3
2010–2011	Fallow	–	–		484.4
2011	Spring maize	Apr 23, 2011	Sep 21, 2011	Yu yu 22	471.6
2011–2012	Winter wheat	Sep 26, 2011	Jun 15, 2012	Jin mai 47	255.4
2012–2013	Fallow	–	–		455.7
2013	Spring maize	Apr 24, 2013	Sep 18, 2013	Yu yu 22	293.0
2013–2014	Winter wheat	Sep 25, 2013	Jun 17, 2014	Jin mai 47	230.1
2014–2015	Fallow	–	–		488.1
2015	Spring maize	Apr 26, 2015	Sep 20, 2015	Zheng dan 958	273.6
2015–2016	Winter wheat	Sep 26, 2015	Jun 18, 2016	Chang 6359	240.0

CCN (5440 kg ha $^{-1}$) and LSC (3799 kg ha $^{-1}$) produced the highest and lowest average wheat yield in ten years, respectively. CNS, BNS, BSC and BCN had nearly the same level of wheat yield as CCN.

3.1.2. Spring maize

Unlike winter wheat, the yield of spring maize was significantly affected by fertilization ($P < 0.01$), tillage practices ($P < 0.05$) and year ($P < 0.001$) during the growth season (Table 4). For fertilization treatments, both BF and CF had significant yield improvements compared with LF, and the increments were 1072 kg ha $^{-1}$ and 675 kg ha $^{-1}$, respectively. For tillage practices, NS rotation had a significant yield improvement compared with CT, and it increased by 6.3%. In contrast, the difference between SC, CN rotation and CT was not significant.

In four maize growth seasons, the difference among fertilization and tillage systems was significant, BNS (8415 kg ha $^{-1}$) and LCT (6849 kg ha $^{-1}$) produced the highest and lowest average maize yield in four years. BSC, BCN, BCT, CNS and CCN had nearly the same level of maize yield as BNS (Fig. 4). In the rainy growth seasons of 2009 and 2011, the maize yield was quite different among treatments. In the dry growth seasons of 2013 and 2015, the differences among different treatments declined.

3.2. ET

3.2.1. Winter wheat

In ten years, ET during winter wheat growth season mainly affected by the interaction effects of year with fertilization and tillage practices ($P < 0.001$) and year with fertilization ($P < 0.001$) or tillage practices($P < 0.01$) (Table 5). For fertilization treatments, there was no significant difference among BF, LF and CF, CF (394 mm) had a

Table 4
Effect of fertilization and tillage practices on average grain yield in winter wheat-spring maize rotation during.2007–2016.

Treatment	Grain yield (kg ha $^{-1}$)	
	Winter wheat	Spring maize
Fertilization		
BF	5115a	8260a
LF	4186b	7188b
CF	5213a	7863a
Tillage		
NS	5014a	8045a
SC	4649b	7654ab
CN	5007a	7810ab
CT	4683b	7572b
ANOVA		
Variation	*P*-value	
Y(df = 4)	0.003**	0.001***
F(df = 2)	0.000***	0.004**
T(df = 3)	0.002**	0.013*
F*T(df = 6)	0.046*	0.265(ns)
Y*F(df = 8)	0.035*	0.058(ns)
Y*T(df = 12)	0.571(ns)	0.671(ns)
Y*F*T(df = 24)	0.956(ns)	0.897(ns)

BF, balanced fertilization, LF, low fertilization, CF, conventional fertilization, NS, no tillage rotated with subsoiling, SC, subsoiling rotated with conventional tillage, CN, conventional tillage rotated with no tillage, CT, conventional tillage annually.
Different letters within a column represent significant differences at the 5% level (LSD).
Y is year (precipitation), T is tillage, F is fertilization, ns represents no significant difference. Asterisks show the significance level of correlation (* for P < 0.05, ** for P < 0.01 and ***for P < 0.001).

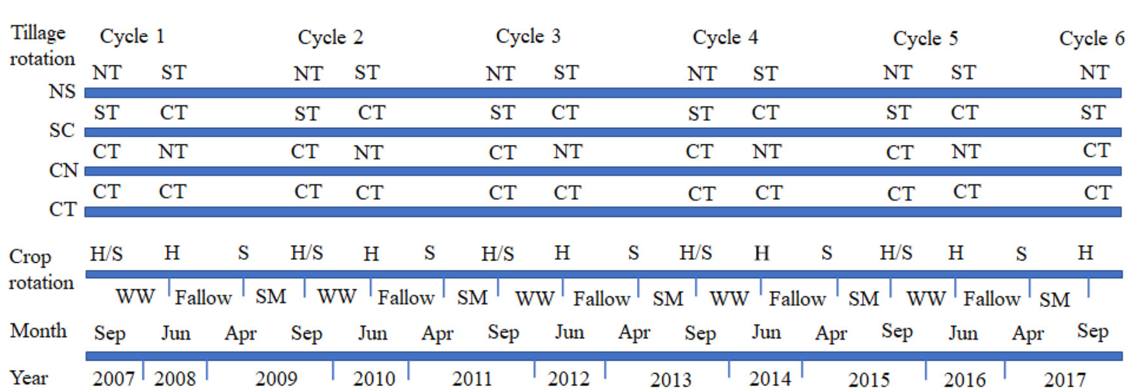

Fig. 2. The details of crop and tillage rotation. BF, balanced fertilization. LF, low fertilization. CF, conventional fertilization. NS, no tillage rotated with subsoiling. SC, subsoiling rotated with conventional tillage. CN, conventional tillage rotated with no tillage. CT, conventional tillage annually. H, crop harvest. S, crop sowing. H/S, spring maize harvest and winter wheat sowing. WW, winter wheat. SM, spring maize.

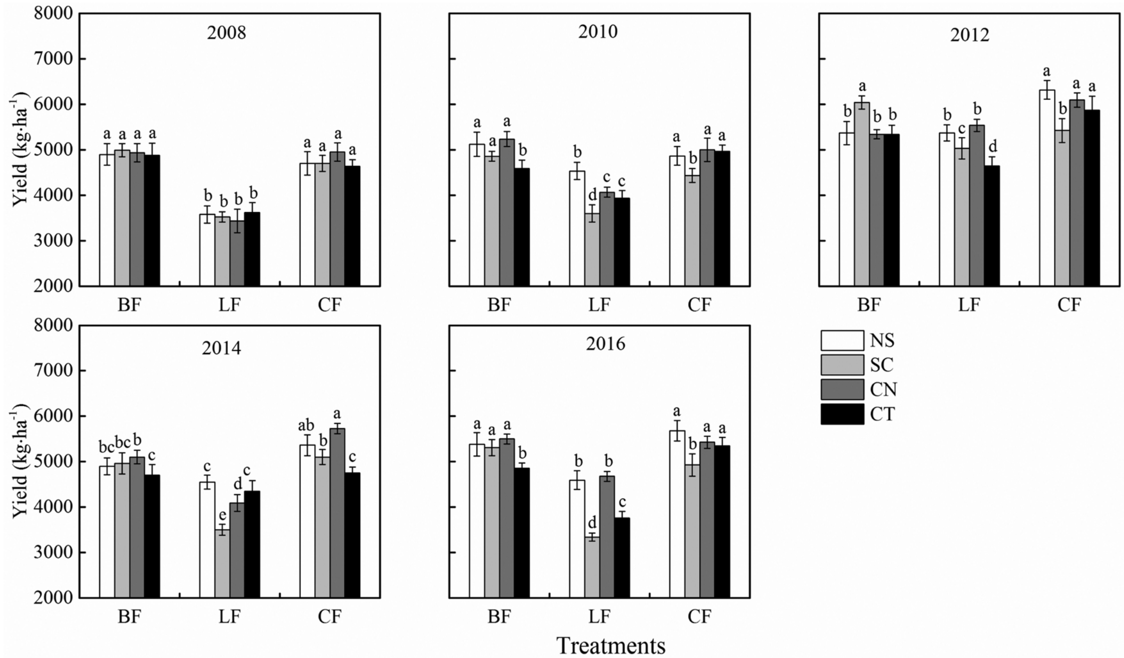

Fig. 3. Yield of winter wheat under different fertilization and tillage systems. BF, balanced fertilization. LF, low fertilization. CF, conventional fertilization. NS, no tillage rotated with subsoiling. SC, subsoiling rotated with conventional tillage. CN, conventional tillage rotated with no tillage. CT, conventional tillage annually. Bars with different letters in the same year are significantly different at $P < 0.05$.

relatively high ET than BF (388 mm) and LF (382 mm). For tillage practices, the difference among tillage systems was not significant, SC (392 mm) had a relatively high ET than other treatments.

Ten-year results showed that CCT (397 mm) had a relatively high ET and LNS (373 mm) showed a relatively low ET (Fig. 5). For the ET of winter wheat, there was significant difference among different fertilization with tillage treatments in year 2008, 2010 and 2016 ($P < 0.05$), but the difference of that in year 2012 and 2016 was not significant.

3.2.2. Spring maize

Different from winter wheat, ET during spring maize growth season mainly affected by the interaction effects of year with fertilization and tillage practices ($P < 0.001$) and year with fertilization ($P < 0.001$) (Table 5). For fertilization treatments, there was no significant

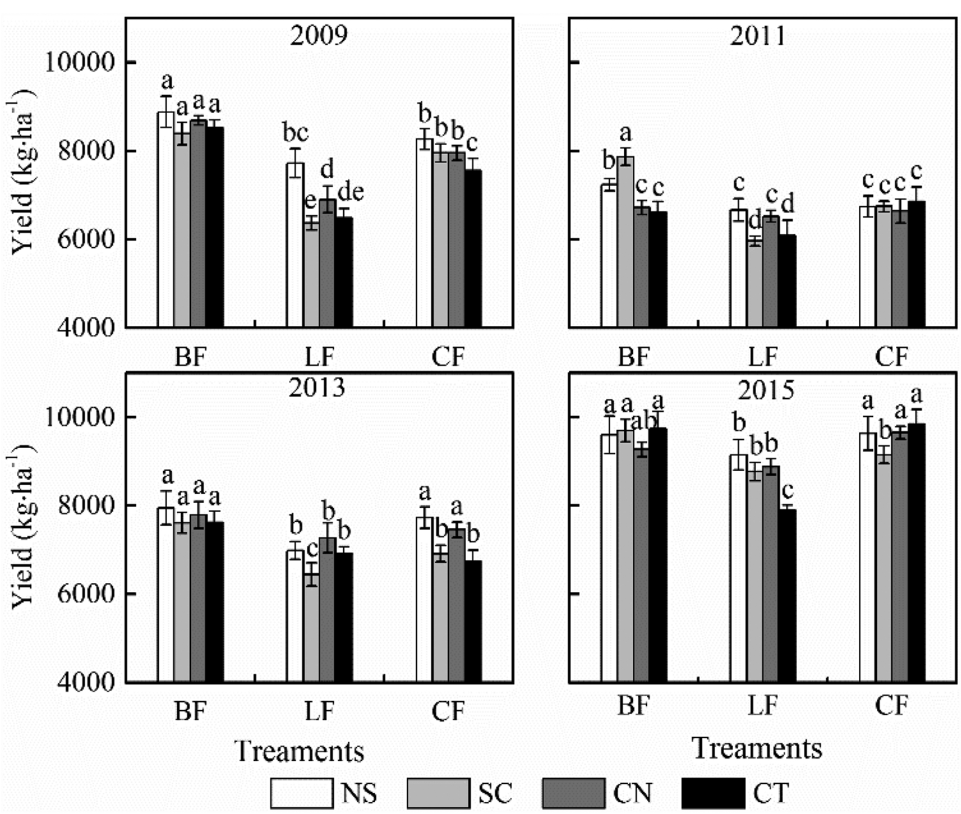

Fig. 4. Yield of spring maize under different fertilization and tillage systems. BF, balanced fertilization. LF, low fertilization. CF, conventional fertilization. NS, no tillage rotated with subsoiling. SC, subsoiling rotated with conventional tillage. CN, conventional tillage rotated with no tillage. CT, conventional tillage annually. Bars with different letters in the same year are significantly different at $P < 0.05$.

Table 5
Effect of fertilization and tillage practices on soil water and WUE in winter wheat-spring maize rotation during.2007–2016.

Treatment	Soil water in fallow period (mm)	ET (mm)		WUE (kg ha^{-1} mm^{-1})	
		winter wheat	Spring maize	Winter wheat	Spring maize
Fertilization					
BF	378b	388a	346a	14.2a	23.9a
LF	405a	382a	349a	11.5b	20.6b
CF	383b	394a	350a	14.6a	22.6a
Tillage					
NS	398a	384a	348a	14.1a	23.2a
SC	380a	390a	345a	12.7a	22.2a
CN	392a	392a	344a	13.8a	22.7a
CT	386a	386a	354a	13.0a	21.4a
ANOVA					
Variation	P-value				
Y(df = 4)	0.002**	0.000***	0.029*	0.000***	0.044*
F(df = 2)	0.020*	0.717(ns)	0.958(ns)	0.018*	0.072(ns)
T(df = 3)	0.036*	0.898(ns)	0.776(ns)	0.056(ns)	0.085(ns)
F*T(df = 6)	0.011*	0.806(ns)	0.668(ns)	0.310(ns)	0.407(ns)
Y*F(df = 8)	0.019*	0.000***	0.001***	0.001***	0.001***
Y*T(df = 12)	0.158(ns)	0.010**	0.052(ns)	0.310(ns)	0.243(ns)
Y*F*T(df = 24)	0.999(ns)	0.000***	0.000***	0.741(ns)	0.846(ns)

BF, balanced fertilization, LF, low fertilization, CF, conventional fertilization, NS, no tillage rotated with subsoiling, SC, subsoiling rotated with conventional tillage, CN, conventional tillage rotated with no tillage, CT, conventional tillage annually.
Different letters within a column represent significant differences at the 5% level (LSD).
Y is year (precipitation), T is tillage, F is fertilization, ns represents no significant difference. Asterisks show the significance level of correlation (* for $P < 0.05$, ** for $P < 0.01$ and ***for $P < 0.001$).

difference among BF, LF and CF, CF (350 mm) had a relatively high ET than BF (346 mm) and LF (349 mm). For tillage practices, the difference among tillage systems was not significant, CT (354 mm) had a relatively high ET than other treatments.

Ten-year results showed that BCT (359 mm) had a relatively high ET and BNS (338 mm) showed a relatively low ET (Fig. 5). For the ET of spring maize, there was significant difference among different fertilization with tillage treatments in year 2015, ($P < 0.05$), but the difference of that in year 2009, 2011 and 2013 was not significant.

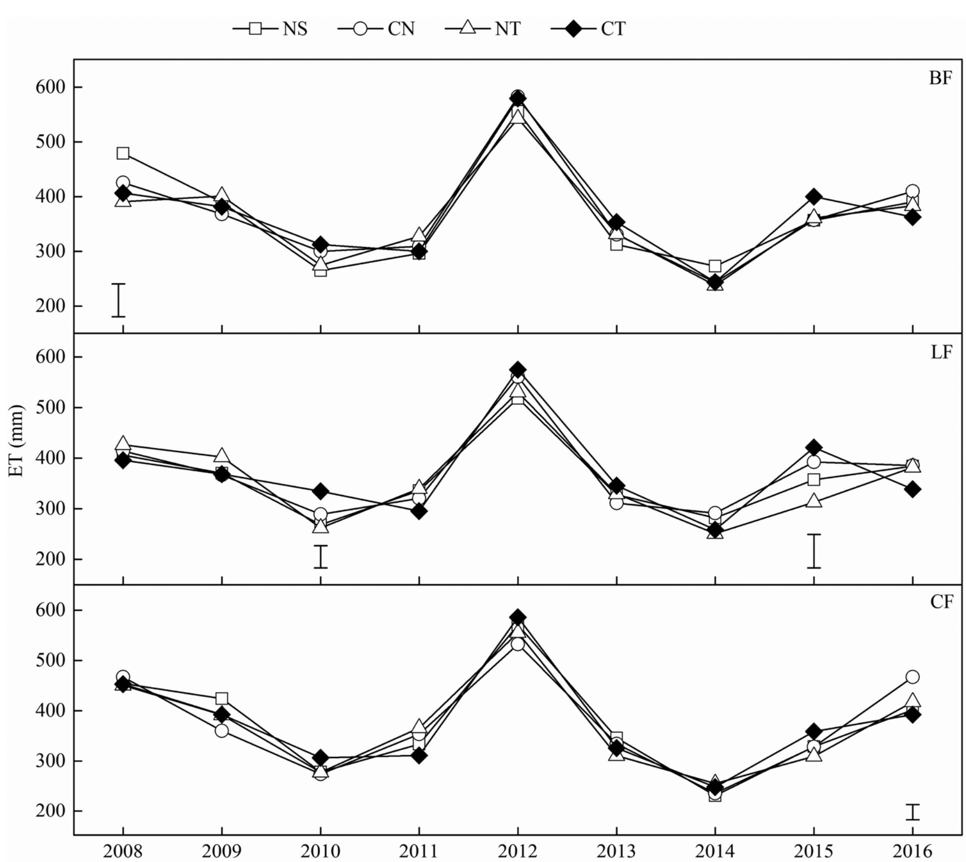

Fig. 5. ET during winter wheat and spring maize growth season under different fertilization and tillage systems. BF, balanced fertilization. LF, low fertilization. CF, conventional fertilization. NS, no tillage rotated with subsoiling. SC, subsoiling rotated with conventional tillage. CN, conventional tillage rotated with no tillage. CT, conventional tillage annually. Vertical bars represent the LSD ($P \leq 0.05$) for different treatments.

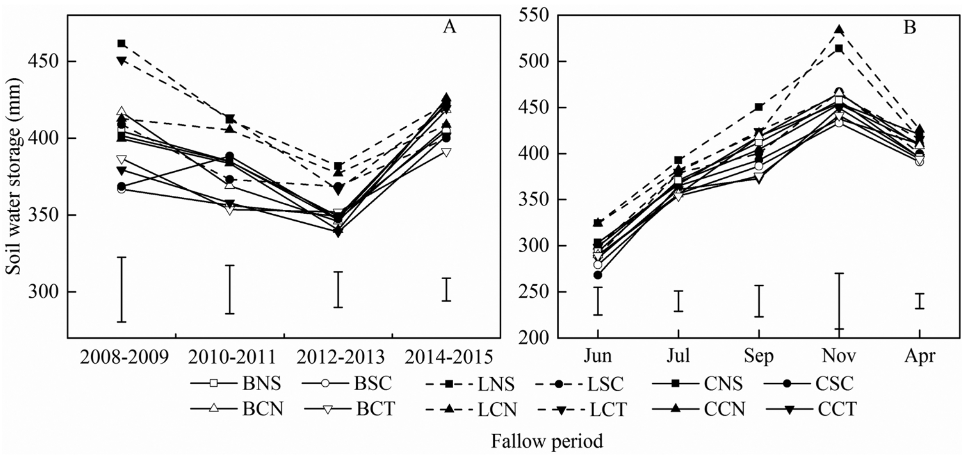

Fig. 6. Soil water storage (A) at end of fallow period and dynamic changes (B) in the fallow period under different tillage systems. BNS, BF + NS. BSC, BF + SC. BCN, BF + CN. BCT, BF + CT. LNS, LF + NS. LSC, LF + SC. LCN, LF + CN. LCT, LF + CT. CNS, CF + NS. CSC, CF + SC. CCN, CF + CN. CCT, CF + CT. Vertical bars represent the LSD ($P \leq 0.05$) for different treatments.

3.3. Soil water in fallow periods

The results showed that soil water storage in fallow periods was significantly affected by the interaction effects of fertilization with tillage practices ($P < 0.05$) and year with fertilization ($P < 0.05$) (Table 5). For fertilization treatments, LF had a significantly better soil water condition than BF and CF. For tillage practices, the difference among tillage systems was not significant.

In four fallow periods, LNS (419 mm) and LCN (412 mm) always showed better soil water conditions at the end of the fallow period ($P < 0.05$) (Fig. 6A). After a spring maize –winter wheat rotation, the soil water storage was relatively low at the beginning of the fallow period. With the arrival of the rainy season (July, August, and September), the water storage increased significantly, and the difference between fertilization and tillage treatments was significant ($P < 0.05$) (Fig. 6B). Following a dry winter season and evaporation, the soil water storage decreased, and this difference declined.

3.4. Soil water variation during crop growth

To further explore the soil water use and redistribution of spring maize-winter wheat rotation, the total soil water storage in 0–2 m at important crop growth stages was studied (Fig. 7).

3.4.1. Winter wheat

During growth season of winter wheat, the soil water storage declined persistently with the wheat growth (Fig. 7). The sowing time (mean value: 435 mm) performed the highest soil water storage and consumed nearly 33.6% of that when it harvested (mean value: 289 mm). From jointing stage to filling time, the soil water storage declined sharply that due to the fast growth of winter wheat.

Differences among different fertilization with tillage systems were significant ($P < 0.05$), and LNS (mean value: 381 mm) showed better soil water storage through the whole wheat growth season, while BCT (mean value: 348 mm) showed less soil water storage.

3.4.2. Spring maize

Unlike the soil water dynamics of winter wheat, the soil water storage of spring maize at sowing stage (mean value: 404 mm) and harvest time (mean value: 415 mm) keep on the same level (Fig. 7). At seedling stage, soil water storage increased that due to the more precipitation at summer season (June, July, August). From seedling to filling stage, soil water storage declined sharply which due to the high evaporation in summer season and emergency requirement for crop growth. But the crop consumed little soil water when it finished filling, with the rainy season at August, the soil water increased from filling stage to harvest time.

During maize growth season, differences among different fertilization with tillage systems were significant ($P < 0.05$), and LNS (mean value: 410 mm) showed better soil water storage through the whole wheat growth season, while BCT (mean value: 391 mm) showed less soil water storage.

3.5. WUE

3.5.1. Winter wheat

The WUE of winter wheat was significantly affected by the interaction effect of year with fertilization ($P < 0.001$) (Table 5). For fertilization treatments, CF and BF had significantly higher WUEs than LF and they increased by 23.5% and 26.9%, respectively. For tillage

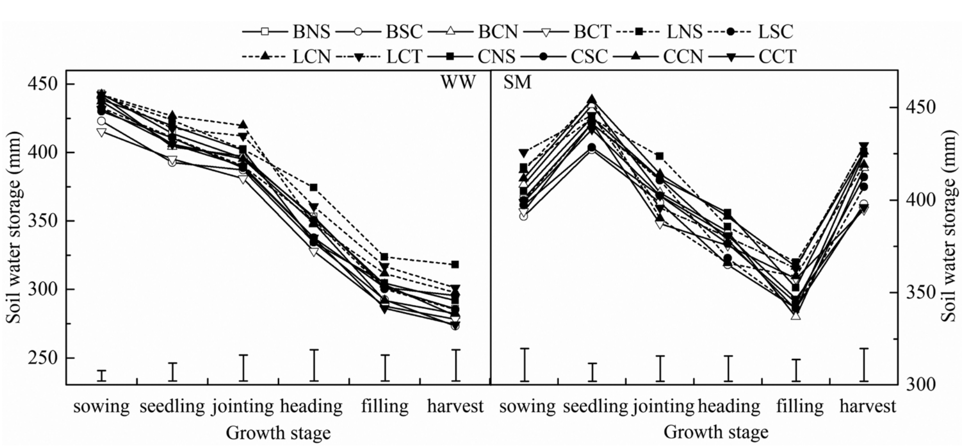

Fig. 7. Soil water variation of winter wheat (WW) and spring maize (SM) under different fertilization and tillage systems. BNS, BF + NS. BSC, BF + SC. BCN, BF + CN. BCT, BF + CT. LNS, LF + NS. LSC, LF + SC. LCN, LF + CN. LCT, LF + CT. CNS, CF + NS. CSC, CF + SC. CCN, CF + CN. CCT, CF + CT. Vertical bars represent the LSD ($P \leq 0.05$) for different treatments.

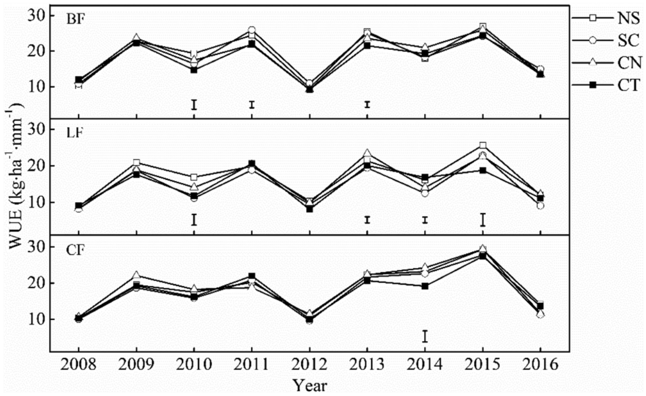

Fig. 8. Water use efficiency (WUE) under different fertilization and tillage systems. BF, balanced fertilization. LF, low fertilization. CF, conventional fertilization. NS, no tillage rotated with subsoiling. SC, subsoiling rotated with conventional tillage. CN, conventional tillage rotated with no tillage. CT, conventional tillage annually. Vertical bars in the same row represent the LSD ($P \leq 0.05$) for different treatments.

practices, the difference was not significant, and NS and CN rotations had higher WUEs than SC rotation and CT.

3.5.2. Spring maize

The WUE of spring maize was also significantly affected by the interaction effect of year with fertilization ($P < 0.001$) (Table 5). The difference of WUE was not significant for fertilization treatments or tillage systems; BF and NS had higher WUEs in this experiment.

In ten years, the average WUE of the spring maize-winter wheat rotation ranked in the following order: BNS > BSC, CCN > BCN, CNS > BCT > CCS and CCT > LNS > LCN > LCT > LSC (Fig. 8). BNS showed a higher WUE of 19.0 kg ha $^{-1}$ mm^{-1} and LSC had a low WUE of 14.5 kg ha $^{-1}$ mm^{-1}. In most years, the difference of WUE among tillage systems was not significant in BF, LF and CF.

4. Discussion

4.1. Soil water conservation

In rainfed areas, crop yield depended on soil water storage at sowing and effective precipitation during the crop growth season (Meng et al., 2012; Sang et al., 2016). This demonstrated that soil water at sowing time plays an important role in crop production in dryland agriculture. In this study, there was a long-term fallow period (from late June after the winter wheat harvest to late April before maize sowing the following year) followed by a consecutive spring maize and winter wheat rotation growth season. Thus, better soil water conservation in the fallow period would provide a better soil condition for the subsequent production of maize and wheat. Due to the better rainfall capture and soil water conservation effect of straw mulching in no tillage (Giller et al., 2009), NS and CN rotations provided higher soil water storage in the fallow period. In addition, tillage rotations that enhanced the soil physical properties also promoted soil water movement that led to better soil water conditions in the deep soil (Hou et al., 2012; Sang et al., 2016). As a result of the combination of bare soil surface and intensive evaporation of CT, SC and CT resulted in low soil water storage. Moreover, the better soil texture and aeration of tillage rotation may cause more evaporation and lead to lower soil water in SC than CT.

Although ET of different tillage rotations and fertilizations had no significant effect in ten years, CF and CT showed a higher average ET than other treatments. This would result in lower soil water storage in long-term crop production. In agreement with a previous study on the Loess Plateau (Zhang et al., 2015b), high fertilization would consume more soil water and lead to less soil water than low fertilization. Moreover, crop rotation and the significant interaction of fertilization and tillage rotation also would affect the soil water storage in this study. This needs to be further explored in the future.

4.2. Soil water use

A good soil water condition is the basis for obtaining a high and sustainable crop yield (Yan et al., 2002), especially in the critical growth period. The sharp decline of soil water from the heading stage to the filling stage illustrated the importance of soil water at a critical stage.

Despite the special case in the filling stage of spring maize, the soil water conditions of NS and CN showed better soil water conditions in the entire winter wheat-spring maize growth season, especially in deep soil layers. This may be attributed to the better soil water condition in the fallow period and soil water movement of tillage rotations. The higher soil water storage in the fallow period provided a better soil water condition for maize sowing in NS and CN rotations. In the following maize growth season, straw mulching reduced evaporation on the soil surface and produced more effective soil water for crop growth (Zhao et al., 2014). In addition, tillage rotations improved the soil structure, promoted the growth of roots and fully utilized the soil water in the surface and deep soil layers (Nie et al., 2015; Zhu et al., 2015). This led to better soil water conditions and high WUEs during the maize growth season in NS and CN rotations. The different soil water distribution at the maize filling stage and the lower difference among tillage systems from sowing time to filling stage was also due to the water use of tillage rotations and the special crop rotation in this study.

Unlike our prediction initially, intensive soil water consumption did not affect subsequent wheat growth. The high rainfall at maize harvest time restored the soil water rapidly and ensured good soil water conditions for wheat, especially in NS and CN rotations. The better soil structure and water movement provided better soil water in the deep soil layer (Hou et al., 2012; Sun et al., 2010). In contrast to spring maize, the soil water in the wheat growth season was greatly diminished. However, the long-term fallow period followed by the wheat growth season can replenish the soil water and provide good soil water conditions for the following crop rotation cycle. This is an advantage of wheat-maize rotation. This typical rotation can restore soil water seasonably and provide sufficient soil water for crop growth. Furthermore, the long-term fallow period in this rotation provided sufficient time for the decomposition of crop residue and enhanced soil properties for crop growth.

Surprisingly, with straw mulching, tillage rotations did not significantly decrease ET during this crop rotation; however, water was utilized effectively and increased the WUE. This does not agree with previous studies on the Loess Plateau (Wang et al., 2015; Zhang et al., 2015a,b). Previous studies showed that tillage rotations have a significant effect on ET. The different results may be due to the typical benefits brought by crop and tillage rotations in this study. No tillage with straw mulching reduced evaporation on the soil surface (Wang et al., 2012a,b), but subsoiling promoted soil water depletion on roots in deep soil layers (Cai et al., 2014). Moreover, the typical crop rotation also affected crop growth and water use. This should be further studied. Regarding fertilization, ET was not significant, but the higher ET during crop growth season and low soil water storage in fallow period in CF that would exaggerate the difference of soil water storage among different fertilization treatments (Zhang et al., 2015b).

4.3. Crop yield increase

Due to the coordination and interaction of tillage rotations and fertilization, the highest average crop yield was observed in BNS and was followed by BCN in this study. The reasonable colocalization of soil water and soil nutrients provided a better crop growth condition for obtaining high crop yields in BF (Meng et al., 2012). Excessive fertilization causes no significant increase in crop yield, but the soil water would decrease (Liu et al., 2013; Zhang et al., 2015b). Additional fertilizer input would cause low fertilizer efficiency, unused fertilizer and environmental pollution (Yousaf et al., 2017). Application of a balanced fertilizer with N, P, and K would increase wheat yield at a rate of 5.8 kg kg^{-1}. N, P, and K fertilizer has the benefit of enhancing each component (Liu et al., 2013). Although the soil in the Loess Plateau is rich in K, it does not facilitate long-term high crop production. A balanced N, P and K treatment would improve the crop yield and avoid further deterioration of soil fertility. The absence of least one major nutrient, i.e., either N, P or K, would cause a specific nutrient deficiency stress and retard crop growth with a concomitant reduction in yield (Yousaf et al., 2017). Thus, the lack of K in CF limited the positive effect of N and P on crop yield (Yousaf et al., 2017).

The factors by which tillage practices influence crop growth are complex (Chen et al., 2011). The changes in soil quality, such as soil moisture, soil temperature and soil nutrient availability, have all been considered as the dominant reasons for crop yield differences among tillage practices (Soane et al., 2012). NS and CN provide favorable water during the crop growth season and ST loosens soil structure (Zhang et al., 2017). This promotes soil water movement and crop root growth in the deep soil layer (Liu et al., 2015). The interaction of water and balanced soil nutrients eventually increases crop yield. Optimized or balanced fertilization with proper tillage practices can effectively increase soil water and crop yield. These practices are an effective way to increase crop yield and income in dryland agriculture.

5. Conclusion

In ten years, LF, NS, and CN showed a better soil water condition in the fallow period. During the spring maize-winter wheat growth season, NS and CN rotations provided higher soil water, especially in the deep soil layers for the entire crop rotation. Despite the negligible economic profit of CNS, BNS and BCN produced relatively high crop yield. Combining the water and fertilizer use, BNS and BCN are recommended for improving fertilization and tillage systems in long-term rainfed dryland agriculture.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.agwat.2019.04.018.

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Research article

Carbon allocation, osmotic adjustment, antioxidant capacity and growth in cotton under long-term soil drought during flowering and boll-forming period

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Responses of plant to drought largely depend on the intensity, duration and developmental stage at which water stress occurs. The purpose of this study was to analyze the dynamic of cotton physiology response to different levels sustained soil water deficit during reproductive growth stage at leaf basis. Three levels of steady-state water regimes [soil relative water content (SRWC) maintained at (75 ± 5)%, (60 ± 5)% and (45 ± 5)%] were imposed when the white flowers had opened on the first fruiting position of the 6–7th fruiting branches ($FB_{6\text{-}7}$), which was the first day post anthesis (i.e. 1 DPA) and lasted to 50 DPA. Results showed decreasing SRWC slowed cotton growth on the base of biomass and leaf area. However, carbon metabolites levels were globally increased under drought despite of notably inhibited photosynthesis throughout the treatment period. Clear diurnal pattern of sucrose and starch concentrations was obtained and sucrose levels were evaluated while starch concentration was reduced with decreasing soil water content during a 24-h cycle. Osmotic adjustment (OA) was observed at most of the sampling dates throughout the drought period. K^+ was the main contributor to osmotic adjustment (OA) at 10 and 24 DPA then turned out to be amino acid at 38 and 50 DPA. The stressed cotton gradually failed to scavenge reactive oxygen species (ROS) with increasing days post anthesis, primarily due to the permanent decrease in SOD activity. Elevated carbohydrates levels suggest cotton growth was more inhibited by other factors than carbon assimilation. OA and antioxidant could be important protective mechanisms against soil water deficit in this species, and transition of these mechanisms was observed with drought intensity and duration increased.

1. Introduction

Soil desiccation is one of the major environmental stresses that limit crop production worldwide. Limiting available soil water content severely altered plant morphological and physiological characters across a range of temporal and spatial scales, and lead to negative effects on plant growth (Tardieu et al., 2000; Chaves et al., 2002; Muller et al., 2011; Carmo-Silva et al., 2012; Deeba et al., 2012). Cotton is grown in a wide region around the world and is negatively affected by water stress (Gerik et al., 1996; Pettigrew, 2004; Lokhande and Reddy, 2014). What's worse, changes in climate might lead to expanding of drought-affected areas and enhancement of drought intensity according to projected increase in global air temperature (Giorgi and Lionello, 2008). Therefore, understanding cotton physiological mechanisms in response to water stress is critical for cotton production improvement via stress-tolerant genotypes identification and management practices.

The balance between carbon assimilation, storage and use is important to plant growth (Smith and Stitt, 2007). Numerous studies have addressed the impact of water deficit on carbon (C) metabolism in various plant species. Most studies (Timpa et al., 1986; Pelleschi et al., 1997; Clifford et al., 1998; Praxedes et al., 2006; Hessini et al., 2009; Muller et al., 2011) demonstrated that drought most often induced carbohydrates accumulation in varying plant organs, even increased carbon availability in root (Hummel et al., 2010; Durand et al., 2016), indicating that carbon demand is decreased more than carbon supply under water stress. These

Abbreviations: DPA, days post anthesis; LWP, leaf water potential; OA, osmotic adjustment; ROS, reactive oxygen species; SRWC, soil relative water content.

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results were contrary to the hypothesis that plants under drought suffer from carbon shortage due to the down-regulation of photosynthesis (Chaves et al., 2002; McDowell et al., 2008). But supported results were obtained under some extreme scenarios like severe and prolonged water deficit or interaction with high temperature (McDowell and Sevanto, 2010).

The increase in carbohydrate concentration was usually considered to be in pace with an increased need for OA under drought (Mccree et al., 1985; Chaves, 1991; Clifford et al., 1998). Some previous studies (Koppenaal et al., 1991; Wang and Stutte, 1992; Bajji et al., 2001; Hessini et al., 2009) supported this hypothesis: OA in *Picea glauca* shoots and *Pinus banksian* roots largely resulted from an increase in fructose and glucose (Koppenaal et al., 1991). Bajji et al. (2001) showed sugars were the main solutes that contributed to OA in wheat plants exposed to water deficit; OA was mainly due to the accumulation of sugars and proline in mild water deficit, while plants failed to develop active osmotic adjustment under severe water stress in. *S. alterniflora* (Hessini et al., 2009). On the other hand, organic acids were the major constituents of the soluble carbon fraction involved in OA in *Fraxinus excelsior* L. and Hummel et al. (2010) reported that not sugars but K^+ was the main contributors to osmotic adjustment in drought stressed *Arabidopsis* plants.

Water stress promoted the production of reactive oxygen species (ROS) as the absorption of excess photons that could not be used by photosynthesis in leaves (Mittler, 2002). In order to keep the balance between ROS production and scavenging, plants developed scavenging systems against ROS, involving both enzymatic and non-enzymatic systems. Under mild short-term water deficit, activities of antioxidant enzymes like SOD, POD and CAT were increased to eliminate excess ROS, but MDA still showed a slight increasing trend, suggesting antioxidant capacity was not sufficient to contribute to resistance to water stress (Reddy et al., 2004; Ge et al., 2006). Zhang et al. (2000) reported that SOD activity was decreased, and CAT activity was enhanced first then declined as the duration of water stress increased, while POD activity was affected differently in various cultivars.

Thus, it was hard to assess the physiological response of plants under drought condition from fore-mentioned reports. This is not surprising, since the consequences of water deficit depend on species, mode of water stress imposed and interaction with other environmental factors (Bray, 2004; Liu et al., 2008; Loka and Oosterhuis, 2012; Tozzi et al., 2013). It was assumed that the adaptive mechanisms of plants change with the intensity or duration of water deficit. Future studies should impose reproducible levels of water deficit and pay attention to interaction with the possible accompanying stresses to explore the consequence of drought (Hummel et al., 2010; Lokhande and Reddy, 2014).

Cotton is sensitive to water stress, particularly during reproductive growth stage which is the most sensitive period to water shortage (Loka and Oosterhuis, 2012). Therefore, we imposed steady-state and reproducible levels of water deficit during the periods of flowering and boll-formation in cotton, aiming to evaluate the dynamic of carbohydrate profiles, OA, antioxidant capacity and their relationship with cotton growth.

2. Materials and methods

2.1. Plant materials and growth conditions

Pot experiment (32 cm high and 37 cm diameter, filled with 25 kg of soil) was conducted at the experimental station (32°02′N and 118°50′E) of Nanjing Agricultural University in Nanjing, Jiangsu Province, China in 2012 and 2013. Cotton seeds (cv. Siza 3) were sown on 25 April 2012 and 8 April 2013, respectively. Two rows of pots were placed together, and then a 40-cm-wide aisle was left. The soil type was clay, mixed, thermic, Typic alfisols (udalfs; FAO luvisol) which was collected from topsoil layer to 30 cm depth from the experimental station. In 2012 and 2013, respectively, the soil averaged 16.4 and 17.7 g kg^{-1} organic matter, 1.12 and 1.14 g kg^{-1} total N, 65.9 and 70.0 mg kg^{-1} mineral N (NH_4^+-N and NO_3^- -N), 18.1 and 20.4 mg kg^{-1} Olsen P, and 122 and 135 mg kg^{-1} exchangeable K (NH_4OAc-K). The pots were covered with a transparent waterproof material above the crop canopy when it rained. Other practices were conducted following the standard commonly performed in the area.

2.2. Experimental designs

Experiments were conducted in a randomized complete block design with three replications. Each plot consisted of 40 pots of cotton plants. Cotton was growth under three levels of soil water regimes: SRWC maintained at (75 ± 5)%, (60 ± 5)% and (45 ± 5)%, denoted as SRWC (75 ± 5)%, SRWC (60 ± 5)% and SRWC (45 ± 5)%, respectively. Soil water treatments were established when approximately 50% of white flowers had opened on the first fruiting position of the 6–7th fruiting branches (FB_{6-7}), which was the first day post anthesis (i.e. 1 DPA) and lasted to 50 DPA. Cotton plants were well-watered before the water deficit event.

2.3. Soil water content and leaf water potential

Soil water content was measured according to the method of Liu et al. (2008). Soil samples at 0–25 cm depth were collected about every 2 day at 18:00–19:00 local time with a punch (2 cm-diameter) from each plot. Fresh weight of the soil samples were determined and then these samples were oven-dried at 105 °C for 8 h. Soil water content was expressed as g water g^{-1} dried soil. Cotton plants would be watered to the upper soil water limit in the early morning. Pre-dawn leaf water potential (LWP) was measured every three days during flowering and boll-forming period, on topmost fully expanded leaves with two samples per plot at 5:00–6:00 local time. Leaves were removed by cutting the petiole, then used the pressure chamber (3005 Pressure Extractor, Soilmoisture Equipment Corp., Goleta, CA, USA) to measure water potential.

2.4. Growth and morphological indices

Cotton plant biomass was measured after determining of leaf area with an area meter (Li-3000A, Li-COR Inc., NE, USA). Shoot and root of cotton plants were killed at 105 °C for half an hour and then maintained at 80 °C until constant weight. Measurements were done every 10 d with three plants per plot from 0 to 40 DPA. Number of bolls and boll sets were counted in ten targeted plants at the same time. Boll shedding rate was also calculated for these ten plants.

2.5. Gas exchange measurements

Gas exchange parameters were measured on the topmost fully expanded leaf on main stem on 10, 24, 38 and 50 DPA. These parameters were measured with a photosynthesis system (Li-6400, Li-COR, Lincoln, NE, USA) under 1500 μmol m^{-2} s^{-2} light intensity at 9:00–11:00 a.m.

The diurnal course of net photosynthesis of the topmost fully expanded leaf was determined at about 17 DPA using the same photosynthesis system but under ambient light intensity. Measurements were conducted on two leaves per plot at intervals of 2 h from 6:00 to 20:00. Light intensity, leaf and air temperatures were documented simultaneously.

2.6. Harvest of samples

Fully extended main-stem leaves that used for solutes concentration and osmotic potential measurement were sampled at about 10:00 a.m. on 10, 24, 38 and 50 DPA. Leaf sample that used for dynamic of metabolite content during 24 h cycle measurement was sampled at intervals 3–4 h on about 17 DPA. Two leaves were sampled in each plot. These leaves were divided into two halves each side of the main vein. One half was washed with distilled water and then placed in liquid N_2 and stored at −80 °C until enzymatic measurement. The other half was weighted and then dipped into distilled water and placed in a dark incubator at 4 °C for 8 h. After the saturated fresh weight was measured, these leaves were oven-dried and then used for metabolites analysis.

2.7. Carbohydrate content

Sucrose, hexose (glucose plus fructose), starch contents were determined spectrophotometric analysis of soluble and residual fractions of ethanol-water extracts according to Liu et al. (2013) and Hu et al. (2015). The soluble sugar content was determined spectrophotometrically at 620 nm using an anthrone reagent. Amino acid and proline contents were measured at 570 nm and 520 nm, respectively, using a ninhydrin reagent according to Bates et al. (1973).

2.8. Nitrate content and inorganic ion assay

Nitrate (NO_3^-) contents were measured at 410 nm using salicylic acid and sulphuric acid according to Hu et al. (2016a). Potassium and sodium content was determined by acid digestion and analyzed using an atomic absorption spectrophotometer (TAS-986, Persee-COR, BJ, CHN).

2.9. Osmotic potential and contribution of solutes to osmotic adjustment

Osmotic potential was measured using a vapor pressure osmometer (Wescor Vapor 5520, ELITechGroup Inc., Logan, UT, USA) according to Hummel et al. (2010). Then osmotic adjustment (OA) was calculated as the difference in Ψs^{100} between the control (Ψs_c^{100}) and the stressed plants (Ψs_s^{100}):

$$OA = \Psi s_c^{100} - \Psi s_s^{100} \quad (1)$$

Soluble metabolites contents were expressed on a saturated fresh weight basis from contents on a fresh weight basis using (RWC) and water content (WC) values according to the method of Hummel et al. (2010). Then contribution of each metabolite to OA (OA_{met}) was calculated according to Hessini et al. (2009):

$$OA_{met} = (((C_{stressed} - C_{contral}) \times 0.1 \times 100)/40)/OA \quad (2)$$

2.10. MDA concentration and antioxidant enzymes activities

Activities of antioxidant enzymes (Superoxide dismutase (SOD), catalase (CAT) and peroxidases (POD)) and contents of reactive oxygen spices (O_2^-, H_2O_2) were determined according to Zhang et al. (2013). MDA content were measured as described by Zheng et al. (2009). Soluble protein content in leaf was determined as described by Bradford (1976).

2.11. Statistical analysis

Data presented are means (±SD) of at least three independent experiments. Data were subjected to an analysis of variance with SPSS statistic package Version 17.0. and difference between mean values greater than the LSD ($P = 0.05$) was determined as significant and are indicated by different letters above bars or numbers.

3. Results

Cotton plants received more solar radiation during the flowering and boll-forming period in 2013 than 2012 (Fig. 1). Temperature and vapor pressure deficit (VPD) were also higher in 2013 than 2012. Soil water content reached the target levels at about 10 DPA and then maintained at the constrained levels (Fig. 2). The course of LWP roughly paralleled with soil water content. LWP of all three water regimes were slightly lower in 2013 than 2012.

Both SRWC (60 ± 5)% and SRWC (45 ± 5)% induced a marked reduction in vegetative growth compared with SRWC (75 ± 5)% (Fig. 3a). The increase of leaf area was notably inhibited by soil water deficit. Leaf area of SRWC (45 ± 5)% even progressively decreased after 20 DPA (Fig. 3b). Boll number declined progressively with increasing soil water deficit (Fig. 3c). At 50 DPA, compared with SRWC (75 ± 5)%, cotton bolls were 29.7%–31.7% and 42.9%–56.3% lower in SRWC (60 ± 5)% and SRWC (45 ± 5)%, respectively. Less plant biomass was allocated to reproductive organs and Root/Plant ratio was increased under soil water stress (Fig. 4). The adverse effects of soil water deficit on cotton growth in 2013 were more pronounced than in 2012.

Compared with SRWC (75 ± 5)%, net photosynthesis (*A*) of cotton leaves was significantly decreased under SRWC (45 ± 5)%, whereas a decrease was only notably from 24 to 50 DPA under SRWC (60 ± 5)% (Fig. 5a). Leaf *A* progressively declined with the increasing days post anthesis under soil water deficit. On the contrary, transpiration rate (*E*) was more inhibited than *A*, WUEi (*A*/*E*)

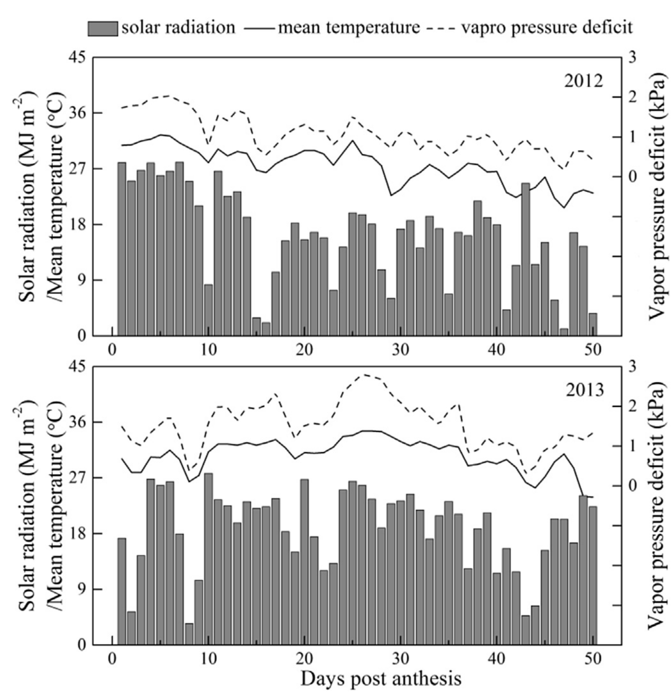

Fig. 1. Daily weather summary at the experimental station in Nanjing during soil water treatment in 2012 and 2013. All data were collected from the Nanjing Weather Station located about 6 km from the experimental site.

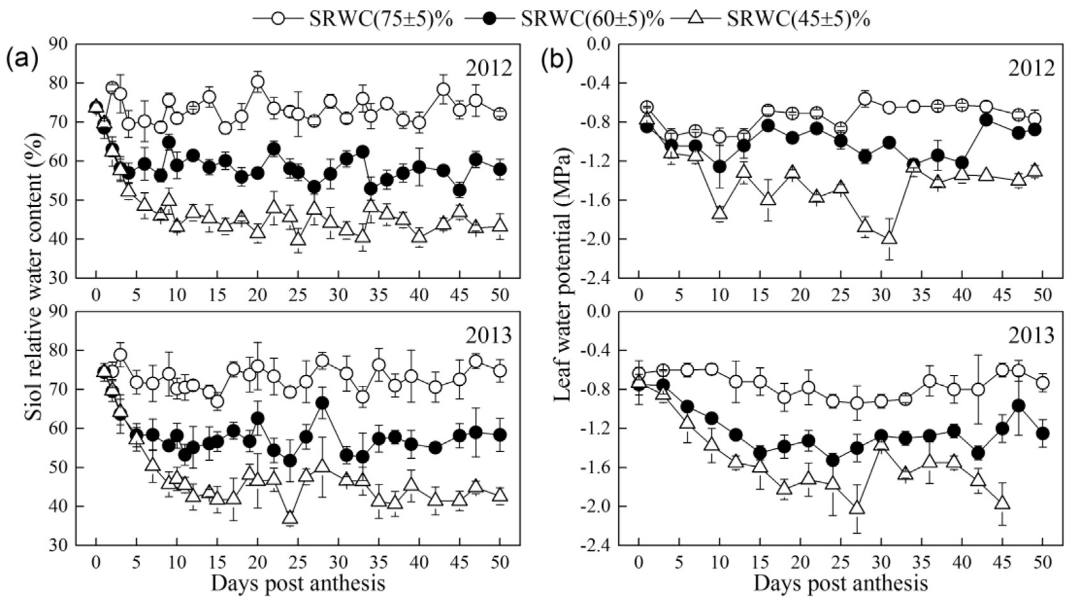

Fig. 2. Temporal trend in (a) soil relative water content and (b) cotton leaf water potential under different soil relative water content in 2012 and 2013. Soil sample at 0–25 cm depth was obtained about every 2 day at 18:00–19:00 local time. Leaf water potential was measured on the topmost fully expanded leaf at 5:00–6:00 local time. Data are the mean (±SD) of three replications.

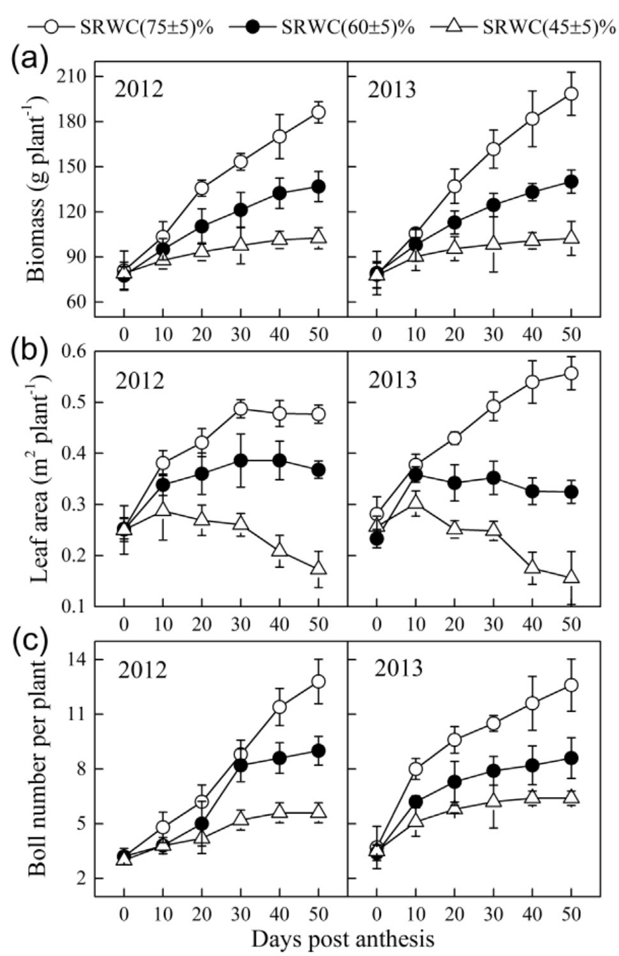

Fig. 3. Changes of (a) cotton biomass, (b) leaf area and, (c) boll number under different soil relative water content in 2012 and 2013. Data are the mean (±SD) of three replications.

increased under soil water deficit conditions (Fig. 5b).

A unimodal pattern of *A* in cotton leaf was observed (Fig. 6a), the highest *A* was recorded at 10:00 a.m. under SRWC (75 ± 5)% and SRWC (60 ± 5)%, while at 8:00 a.m. under SRWC (45 ± 5)%. *A* was reduced gradually with decreasing soil relative water content during a diurnal cycle. The diurnal integral of *A* under SRWC (75 ± 5)% were about 1.5-fold and 2.5-fold greater than SRWC (60 ± 5)% and SRWC (45 ± 5)%, respectively. The most significant differences of *A* in SRWC (75 ± 5)%, SRWC (60 ± 5)% and SRWC (45 ± 5)% were observed at 10:00 a.m. Leaf temperature correlated with air temperature (Fig. 6b). Leaf temperature under SRWC (75 ± 5)% was lower than air temperature during the diurnal cycle, while at SRWC (45 ± 5)% was significantly higher than air temperature from 10:00 to 18:00. Leaf water potential was notably decreased under soil water conditions throughout day and night (Fig. 6c). Daily time-courses of leaf water potential were similar among three soil water regimes: leaf water potential decreased to the minimum at 14:00, and then gradually increased until the next morning.

Accumulation of starch and four soluble C metabolites in leaf during a 24-h cycle were performed simultaneously with diurnal measurement of *A* (Fig. 7). Sucrose, starch and soluble sugar accumulated during the daytime and were remobilized at night. The diurnal course of hexose content paralleled leaf *A* in the daytime and peaked at 10:00 a.m., while the content of sucrose, starch, soluble sugar and amino acid peaked later than hexose content. Soil water deficit reduced the starch accumulation rate during the daytime and the greatest difference among water treatments was evident at the end of day. In contrast, the content of other metabolites increased under water stress.

The concentration of five major Carbon(C) metabolites (sucrose, starch, hexose, proline and amino acid), potassium, sodium and nitrate were measured in cotton leaf sampled at 10:00 a.m. on 10, 24, 38 and 50 DPA (Fig. 8). Soil water deficit induced a global increase in concentration of metabolites expressed on saturated fresh weight basis. Under soil water deficit, cotton leaf potassium concentration showed a decreasing trend while the concentration of proline and sodium showed an increasing trend with later days

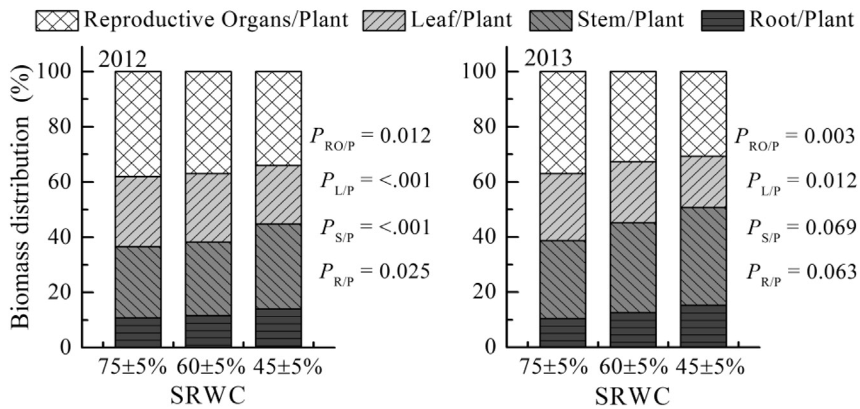

Fig. 4. Biomass proportion of cotton reproductive organs, leaf, main stem and root under different soil relative water content in 2012 and 2013. RO/P, L/P, S/P and R/P stand for reproductive organs/plant, leaf/plant, stem/plant and root/plant, respectively. Data are collected at 40 DPA with three replications.

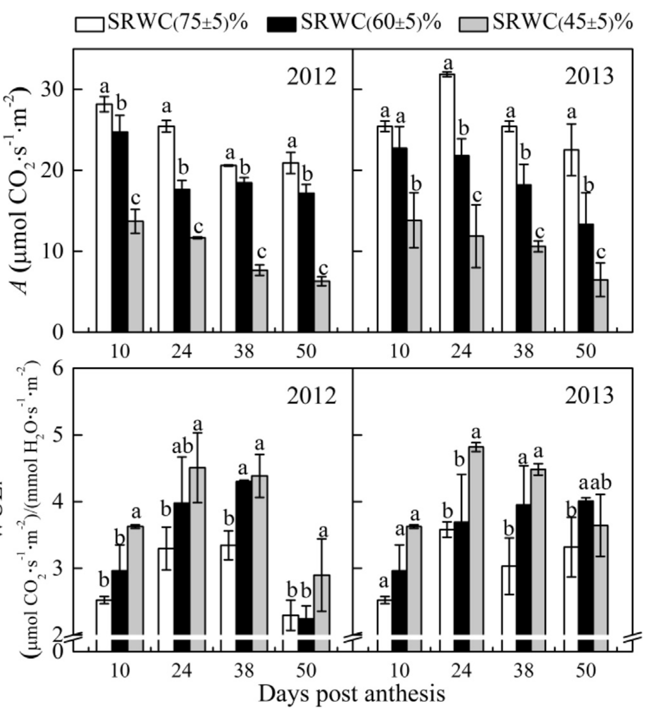

Fig. 5. Changes of (a) net photosynthesis and (b) instantaneous water use efficiency in cotton leaves under different soil relative water content in 2012 and 2013. Data are the mean (±SD) of three replications.

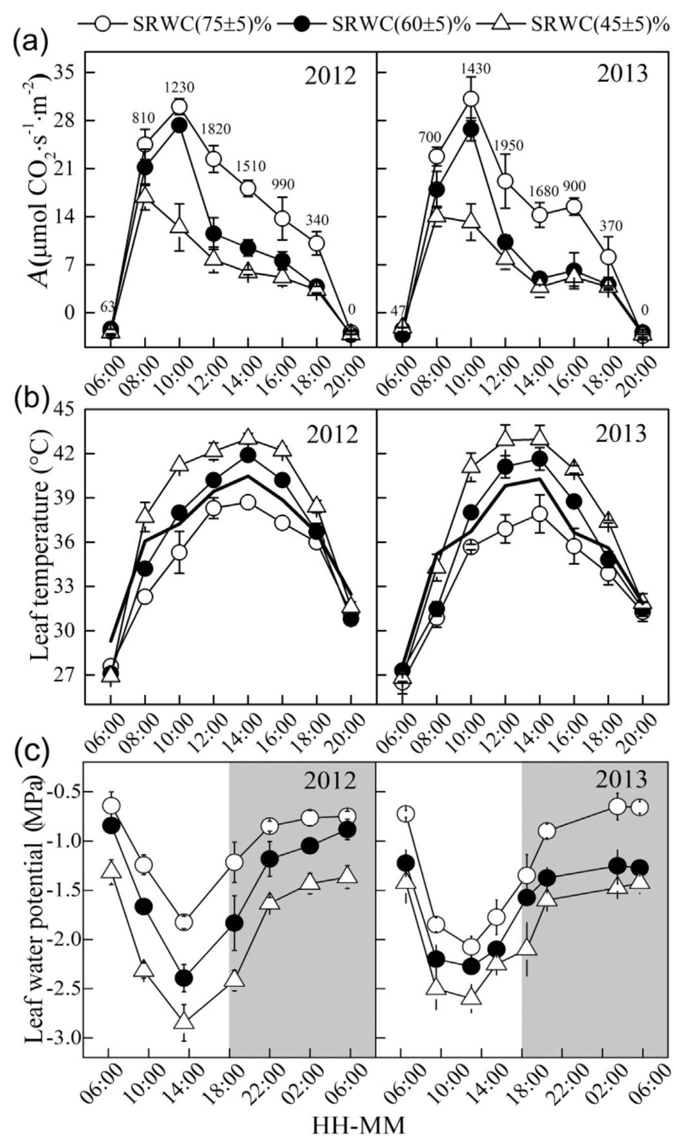

Fig. 6. Diurnal dynamics of (a) photosynthesis, (b) leaf temperature and (c) leaf water potential in cotton leaves under different soil relative water content in 2012 and 2013. Number in (a) stand for the quantities of photosynthesis activity radiation (μmol m^{-2} s^{-1}). The thick line in (b) stand for the diurnal dynamic of air temperature. Data are the mean (±SD) of three replications.

post anthesis.

After 10 DPA, soil relative water content had reached the target levels and the pre-dawn leaf water potential was around −1.2 MPa and −1.5 MPa under SRWC (60 ± 5)% and SRWC (45 ± 5)%, respectively, as compared to −0.8 MPa under SRWC (75 ± 5)%. The decrease in relative water content contributed most to increase leaf water potential as the value of osmotic adjustment (OA) was much lower (Table 1). Potassium contributed 22.8%–31.9% of osmotic adjustment at 10 and 24 DPA. While after 24 DPA, the potassium contribution decreased sharply, especially under SRWC (45 ± 5)%. The contribution of amino acid to OA increased gradually with increasing days post anthesis and became the main contributor to OA at 50 DPA.

Fig. 9 shows the dynamics of O_2^- production rate, H_2O_2 and malondialdehyde (MDA) content in cotton leaves under different

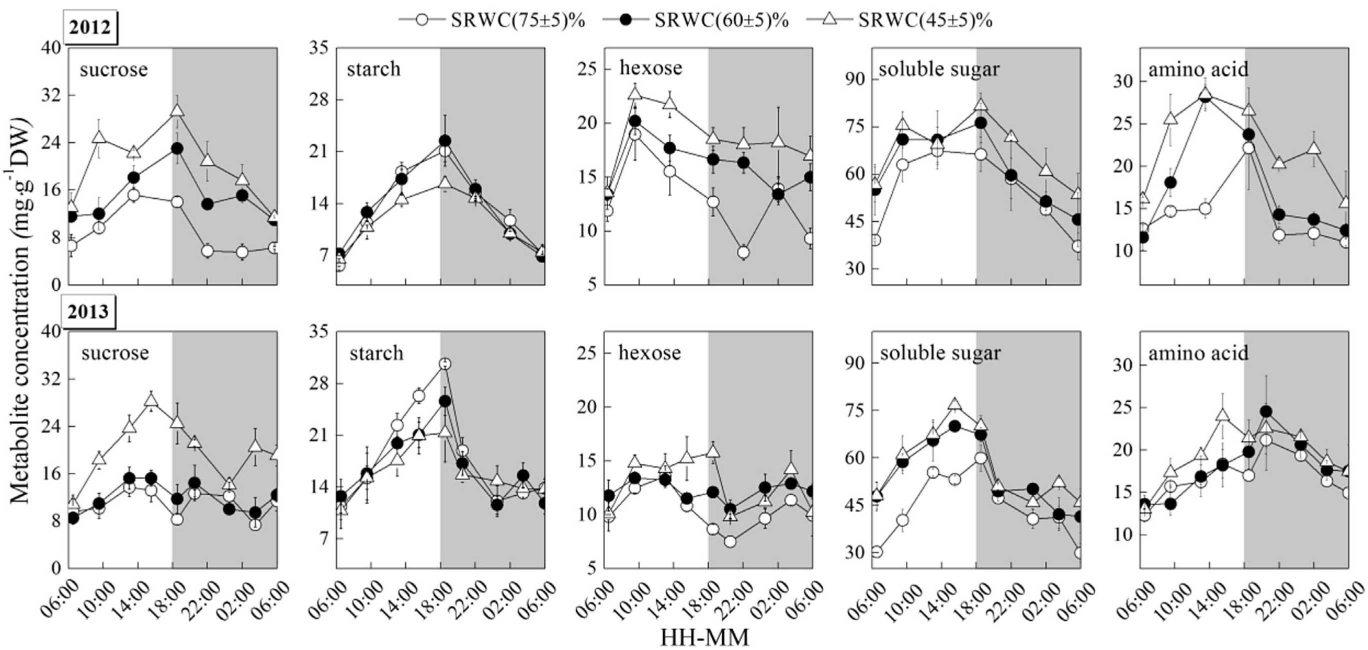

Fig. 7. Diurnal dynamics of carbohydrate concentration in cotton leaves under different soil relative water content in 2012 and 2013. Data are the mean (±SD) of three replications.

soil relative water content. O_2^- production rate was rapidly increased at 10 DPA under SRWC (45 ± 5)% and was significantly improved only after 24 DPA under SRWC (60 ± 5)%. Little difference of H_2O_2 content between SRWC (60 ± 5)% and SRWC (75 ± 5)% was observed. However, SRWC (45 ± 5)% resulted in greater H_2O_2 content at 10 and 24 DPA compared with SRWC (75 ± 5)%. Accumulation of reactive oxygen led to greater MDA concentration under SRWC (45 ± 5)% and SRWC (60 ± 5)% than SRWC (75 ± 5)%.

Soil water deficit dramatically decreased SOD activity in cotton leaf, and POD activity was increased by soil water deficit at 38 and 50 DPA (Fig. 10). CAT activity was inhibited initially, and then increased during later growing periods. The activities of SOD and POD showed an increasing trend on later days post anthesis under all three soil water regimes in 2012.

4. Discussion

Our data revealed changes in cotton growth, dynamics of *A* and carbohydrate profiles in cotton leaves across different temporal scales, and the modification of OA and ROS-scavenging mechanism of cotton in response to the long-term soil water deficit.

The coordination between carbon supply and use is vital for plant growth (Roitsch, 1999; Smith and Stitt, 2007; Muller et al., 2011). Many previous studies (Pelleschi et al., 1997; Praxedes et al., 2006; Hessini et al., 2009; Hummel et al., 2010) documented carbon assimilation decreased while carbohydrate accumulated in plants exposed to drought. Similarly, this research showed that despite of the notable decline in photosynthesis rate, carbohydrate concentration was increased in leaves of cotton under water deficit, being more pronounced with the enhancement of soil water stress level and its prolonged duration (Fig. 8). Meanwhile, cotton growth was significantly inhibited (Fig. 3). This result implied that cotton growth was more inhibited than carbon assimilation. McDowell et al. (2008) reported carbon starvation and hydraulic failure occurred when drought duration and intensity were long and sufficient enough, resulting in plant mortality. But carbon starvation was not observed in the present study, possibly because the water deficit imposed was not severe or prolonged enough as we were more concerned with growth and yield of cotton plants than survival.

Soil water deficit influenced carbohydrate concentrations in cotton but did not alter the diurnal pattern of the main carbon metabolites (Fig. 7). Carbohydrate, especially starch, metabolism acts in accordance with the circadian clock: they accumulate during daytime and are utilized at night (Smith et al., 2004; Zeeman et al., 2007; Hummel et al., 2010; Peng et al., 2014). Similarly, sucrose and starch were accumulated during daytime and remobilized at night in the present study. The maximum leaf sucrose concentration occurred a few hours after the peak in *A*, indicating sucrose production was greater than demand during the day. Sucrose accumulation was promoted by soil water deficit while *A* was strongly reduced, suggesting fewer carbohydrates were exported from water stressed leaves during this interval. Starch accumulation was inhibited under SRWC (45 ± 5)% in two experimental years, and starch concentration was decreased by 21.1–30.4% compared to SRWC (75 ± 5)% at the end of the day. Nevertheless, the decrease in starch content in cotton leaves was much smaller than that in diurnal interval of *A* (Fig. 6). These results suggest that sucrose transport during the day was strongly reduced by osmotic stress, and cotton growth rate in daytime might be more sensitive to water deficit than at night.

Osmotic adjustment (OA) plays an important role in maintaining cell turgor and growth capacity at lower leaf water potential. But OA capacity is different in various species and the main contributors to OA are not consistently observed from previous studies (Bajji et al., 2001; Hessini et al., 2009; Hummel et al., 2010). In the present study, OA, defined as the difference in osmotic potential at saturation between well-watered [SRWC (75 ± 5)%] plants and stressed [SRWC (45 ± 5)% and SRWC (60 ± 5)%] plants, was observed on most sampling dates to be more pronounced in SRWC (45 ± 5)% (Table 1). Soil water deficit was imposed progressively and it took about 10 days for soil relative water content to reach the lower target level (Fig. 2). OA in cotton leaves increased initially and then declined after 38 DPA (Table 1). This suggested that OA degree depends on soil relative water content and its duration and intensity. The decrease of OA during the later growing period also

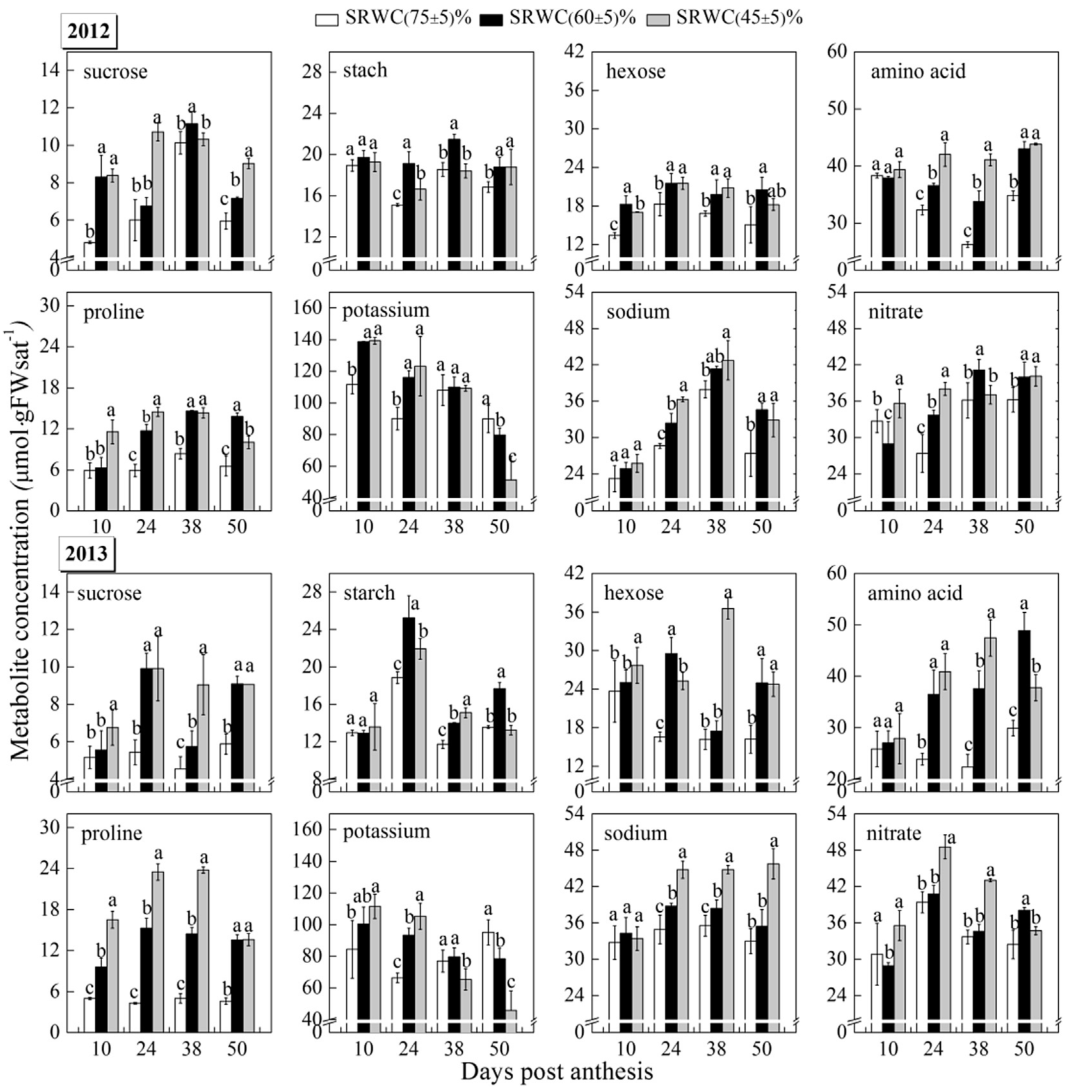

Fig. 8. Dynamics of carbohydrate concentration in cotton leaves under different soil relative water content in 2012 and 2013. Data are the mean (±SD) of three replications.

indicated soil water stress injury of cotton leaves occurred as the duration of soil water stress increased. Soluble metabolites (such as organic acid, sugars) and ions (K^+ and nitrates) have been confirmed to be the main contributor to OA (Hessini et al., 2009; Hummel et al., 2010). In the present study, at 10 and 24 DPA, K^+ in cotton leaf was the main contributor to OA both under SRWC (45 ± 5)% and SRWC (60 ± 5)%. However, the contribution of K^+ sharply declined from 38 DPA, especially under SRWC (45 ± 5)%, and organic acids (amino acids or proline) became the main contributor. Furthermore, the accumulation of sugars contributed little to OA at most sampling dates (Table 1). Since only certain species of solute were measured in the present study, it may be that other solutes, such as fumarate, malate and glycine betaine, might also play an important role in osmotic adjustment in cotton (Chaves et al., 2009).

Leaf senescence was promoted under soil water deficit, as indicated by the decreased leaf area and increased leaf MDA concentration in the present study. Enhanced ROS levels generally causes damage to cellular components and basic metabolism (Zhang et al., 2013; Li et al., 2014) and, thus, is closely related to leaf senescence. O_2^- production rate was increased under soil water deficit while increased H_2O_2 concentration was only be observed at the earlier sampling date under SRWC (45 ± 5)%, presumably due to the permanent decrease in SOD activity and enhanced the activities of CAT and POD at the later growing period (Figs. 9 and 10). In addition, the gradually decreased K^+ concentration and accumulated soluble sugar in cotton leaf also indicated the senescence of leaves (Wright, 1999; Quirino et al., 2000; Koch, 2004; Pourtau et al., 2006; Hu et al., 2016b). Therefore, together with lower leaf area and *A*, earlier leaf senescence likely strongly inhibited cotton growth, being more pronounced under SRWC (45 ± 5)% where plant biomass and boll number were almost constant after 30 DPA (Fig. 3).

Plants were usually exposed to multiple hazards under ambient environmental conditions (Carmo-Silva et al., 2012; Tozzi et al., 2013). Drought is most often accompanied by high temperature and light stress and its adverse influence were usually amplified by concurrent stresses (Chaves et al., 2003; Flexas et al., 2004). In this research, soil water stress level were more severe in 2013 than 2012 on account of leaf water potential (Fig. 2), primarily due to the higher temperature and solar radiation (Fig. 1). Under soil water deficit, cotton biomass, leaf area and boll number were severely decreased (Fig. 3) and less biomass was translocated to reproductive organs in 2013 (Fig. 4).

Table 1
Dynamics of osmotic potential, osmotic adjustment, and relative contribution of metabolites in cotton leaves under different soil relative water content in 2012 and 2013.

Years	Parameters	Days post anthesis											
		10			24			38			50		
		75%[a]	60%	45%	75%	60%	45%	75%	60%	45%	75%	60%	45%
2012	Ψs^{100} (MPa)	1.02b[b]	1.31a	1.33a	1.03b	1.41a	1.43a	1.11b	1.26a	1.37a	0.92a	1.07a	1.02a
	OA (MPa)[c]		0.288	0.305		0.384	0.402		0.158	0.262		0.150	0.103
	Contribution to osmotic adjustment (%)[d]												
	Potassium		31.9	27.8		22.8	28.4		7.4	2.6		−13.4	−109.7
	Sodium		2.2	2.5		5.8	16.7		8.3	6.4		17.9	19.9
	Proline		0.5	5.8		4.9	9.0		13.2	7.5		16.1	11.4
	Amino acid		0.1	0.8		4.0	8.5		16.4	18.7		20.7	31.4
	Nitrate		−3.7	2.8		5.6	9.1		11.4	5.4		11.2	15.6
	Hexose		5.6	3.6		2.9	3.0		6.6	8.0		13.1	11.2
	Sucrose		4.0	3.7		0.7	3.9		2.5	0.4		3.1	10.1
2013	Ψs^{100} (MPa)	1.19a	1.36b	1.49a	1.26c	1.67b	1.78a	1.24b	1.49a	1.62a	1.11b	1.33a	0.98c
	OA (MPa)		0.166	0.295		0.405	0.518		0.255	0.381		0.223	–
	Contribution to osmotic adjustment (%)												–
	Potassium		31.6	31.1		28.3	30.8		5.0	−4.5		−19.0	–
	Sodium		2.6	0.7		4.0	11.2		3.6	8.7		3.7	–
	Proline		9.2	13.2		13.8	18.6		12.2	17.8		13.3	–
	Amino acid		2.0	2.3		12.7	13.1		20.0	25.1		33.3	–
	Nitrate		−4.2	5.5		9.5	8.6		1.9	11.4		12.0	–
	Hexose		2.5	4.7		12.5	6.9		2.0	5.1		15.5	–
	Sucrose		0.7	1.8		4.3	3.4		1.6	4.5		5.7	–

[a] 75%, 60% and 45% stand for SRWC (75 ± 5)%, SRWC (60 ± 5)% and SRWC (45 ± 5)%, respectively.
[b] Mean followed by the same letter within a year and a row are not significantly different according to LSD0.05.
[c] Osmotic potential difference at saturation between well-watered [SRWC (75 ± 5)%] and stressed [SRWC (45 ± 5)% and SRWC (60 ± 5)%] conditions provides an estimate of osmotic adjustment (OA).
[d] The contribution of solute to osmotic adjustment was calculated from concentration differences between stressed and nonstressed conditions.

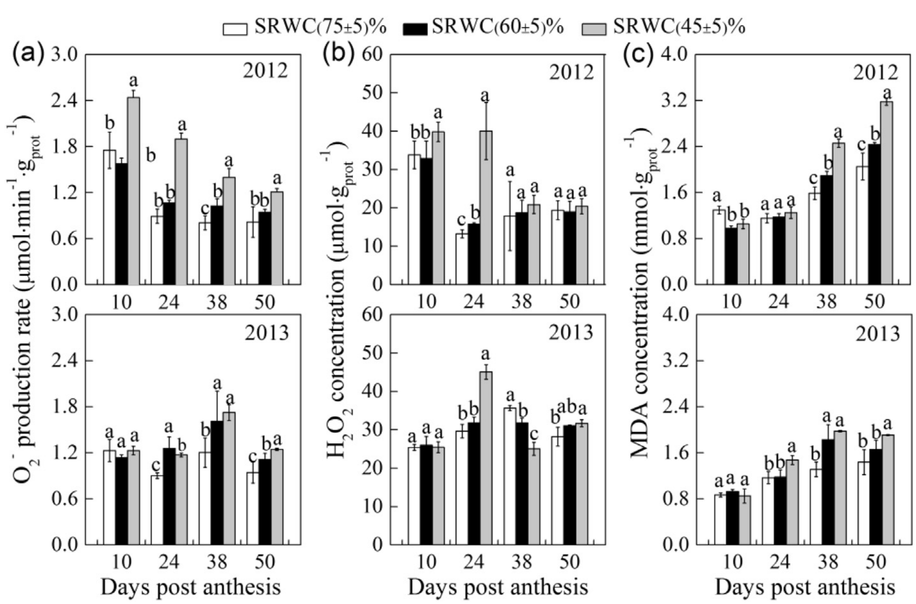

Fig. 9. Dynamics of (a) O_2^-, (b) H_2O_2 and (c) MDA concentrations in cotton leaves under different soil relative water content in 2012 and 2013. Data are the mean (±SD) of three replications.

In conclusion, our study indicates the following in *Gossipum hirsutum* L.

(1) Source capacity of cotton leaf was severely limited by soil water stress as indicated by dramatically decreased *A* at various times and a transitory decline in starch concentration at the end of the photoperiod. Decreased leaf source capacity and leaf area acted in concert to inhibit dry matter accumulation in stressed plants.
(2) Water deficit induced increased soluble carbon metabolite concentrations in cotton leaves, possibly due to cotton growth being affected more strongly than photosynthesis and carbon metabolism.
(3) The major contributor to OA depended on the duration of soil water deficit. K^+ and amino acid in cotton leaf contributed most to OA at the early and later growing periods, respectively, during the water deficit period. Accumulated sugars in cotton leaf contributed less to OA compared with other

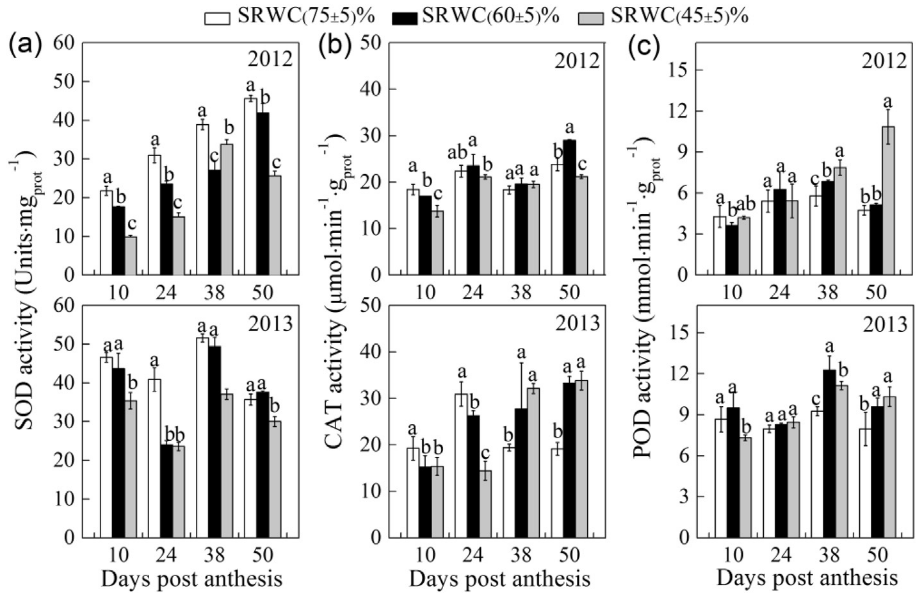

Fig. 10. Dynamics of the activities of (a) SOD, (b) CAT and (c) POD in cotton leaves under different soil relative water content in 2012 and 2013. Data are the mean (±SD) of three replications.

measured solutes but showed a slightly increased trend as the duration of soil water stress increased.

(4) O_2^- production rate in cotton leaves was increased by soil water deficit, possibly because of the decreased SOD activity. The difference in H_2O_2 concentration between well-watered and drought plants was little at most sampling dates, presumably due to the constant CAT activity and increased POD activity. The increased MDA concentration in cotton leaves indicated that the antioxidant level was not sufficient to prevent long-term damage due to soil water deficit.

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关于国家自然科学基金资助项目批准及有关事项的通知

王瑞　　先生/女士：

根据《国家自然科学基金条例》的规定和专家评审意见，国家自然科学基金委员会（以下简称自然科学基金委）决定批准资助您的申请项目。项目批准号：31801300，项目名称：氮素对拔节期受旱玉米根系发育与产量形成的调控机制研究，直接费用：24.00万元，项目起止年月：2019年01月至 2021年 12月，有关项目的评审意见及修改意见附后。

请尽早登录科学基金网络信息系统（https://isisn.nsfc.gov.cn），获取《国家自然科学基金资助项目计划书》（以下简称计划书）并按要求填写。对于有修改意见的项目，请按修改意见及时调整计划书相关内容；如对修改意见有异议，须在计划书电子版报送截止日期前提出。

计划书电子版通过科学基金网络信息系统（https://isisn.nsfc.gov.cn）上传，由依托单位审核后提交至自然科学基金委进行审核。审核未通过者，返回修改后再行提交；审核通过者，打印为计划书纸质版（一式两份，双面打印），由依托单位审核并加盖单位公章后报送至自然科学基金委项目材料接收工作组。计划书电子版和纸质版内容应当保证一致。向自然科学基金委提交和报送计划书截止时间节点如下：

1、提交计划书电子版截止时间为**2018年9月11日16点**（视为计划书正式提交时间）；

2、提交计划书电子修改版截止时间为**2018年9月18日16点**；

3、报送计划书纸质版截止时间为**2018年9月26日16点**。

请按照以上规定及时提交计划书电子版，并报送计划书纸质版，未说明理由且逾期不报计划书者，视为自动放弃接受资助。

附件：项目评审意见及修改意见表

国家自然科学基金委员会
生命科学部
2018年8月16日

ICS 65.020.20
B 05

DB61

陕西省地方标准

DB 61/T 1168—2018

渭北旱地玉米保护性轮耕技术规程

Technical Specifications of Rotational Conservation tillage for Maize Field on Weibei Dryland

2018-08-27 发布　　2018-09-27 实施

陕西省质量技术监督局　发布

前　　言

本标准按照GB/T 1.1—2009给出的规则起草。

本标准由西北农林科技大学提出。

本标准由陕西省农业厅归口。

本标准起草单位：西北农林科技大学、杨凌现代农业产业标准化研究推广服务中心。

本标准主要起草人：李军、王旭东、朱瑞祥、张睿、王长发、王小利、王瑞、陈小莉、周永明。

本标准由西北农林科技大学负责解释。

本标准首次发布。

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ICS 65.020.20
B 05

DB61

陕 西 省 地 方 标 准

DB 61/T 1278—2019

渭北旱地小麦保护性轮耕技术规程

Technical Specifications of Rotational Conservation tillage for Wheat Field on Weibei Dryland

2019-10-29 发布　　2019-11-29 实施

陕西省市场监督管理局　发布

前　　言

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