Journal of Plant Nutrition

ISSN: 0190-4167 (Print) 1532-4087 (Online) Journal homepage: http://www.tandfonline.com/loi/lpla20

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To cite this article: Wei Chen, Xi-Ping Deng, Sang-Soo Kwak & Anthony Egrinya Eneji (2015) The Relationship Between Yield and Fructan Exo-Hydrolases Activity in Two Drought Resistant Wheat Cultivars Grown Under Different Fertilizer and Tillage Treatments, Journal of Plant Nutrition, 38:1, 13-27, DOI: 10.1080/01904167.2014.892989

To link to this article: http://dx.doi.org/10.1080/01904167.2014.892989

Published online: 29 Sep 2014.

Journal of Plant Nutrition, 38:13–27, 2015

ISSN: 0190-4167 print / 1532-4087 online
DOI: 10.1080/01904167.2014.892989

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THE RELATIONSHIP BETWEEN YIELD AND FRUCTAN EXO-HYDROLASES ACTIVITY IN TWO DROUGHT RESISTANT WHEAT CULTIVARS GROWN UNDER DIFFERENT FERTILIZER AND TILLAGE TREATMENTS

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□ *Using two drought resistant wheat (*Triticum aestivum *L.) cultivars, 'Changwu134' and 'Changhan58,' a field experiment was conducted in ChangWu Agro-ecological Experiment Station on China's Loess Plateau during 2008 and 2009 to compare the effects of different fertilizer and tillage treatments on the fructan contents as well as fructan exo-hydrolase (FEH) activity in relation to wheat yield. We found that 'Changhan58' had greater yield and fructan content in the penultimate internode and higher FEH activity than did 'Changwu134.' For 'Changhan58.' applying 195 kg·ha^{-1}(120 + 75) N plus 45000 kg·ha^{-1} of pig manure and 120 kg·ha^{-1} phosphate under conservation tillage produced the highest yield (6769 ka/ha), fructan content in penultimate internode, water use efficiency (WUE), as well as FEH activity among the fertilizer and tillage treatments. Therefore, routine soil management for wheat should focus on combined use of manures and inorganic fertilizer to enhance the amount and transportation efficiency of WSC and ultimately ensure greater yield.*

Keywords: conservation tillage, fructan metabolism, soil nutrient management, wheat yield

Received 1 February 2012; accepted 13 April 2012.
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INTRODUCTION

Grain filling is the final stage of growth in cereals, and is an important stage determining economic yield. At this stage, the carbohydrates fixed by photosynthetic organs are mobilized and/or turned into grains. Carbohydrates are the main products of plant photosynthesis and can be divided into structural carbohydrates and non-structural carbohydrates (NSC). The former, which include cellulose and lignin are mainly used for plant metabolism; the latter, which include glucose, fructan, sucrose, fructan and starch, also play an important role in the metabolism (Pan et al., 2002). Grain growth and development in wheat depend on carbohydrates produced: (i) after anthesis and translocated directly to the grains, (ii) after anthesis but stored temporarily in the stem before being remobilized to the grains, and (iii) before anthesis and stored mainly in the stem and remobilized to grains during grain filling (Edhaie et al., 2006).

In wheat, carbohydrates available for mobilization at the stage of maximum concentration of water-soluble carbohydrate consist of 85% fructans and 10% sucrose (Cruz-Aguado et al., 2000). Some varieties of wheat exhibited non-structural carbohydrate levels of up to 40% to 45% (Bancal and TriboI, 1993) when 70% or more of grain dry matter was exposed to drought stress (Wardlaw and Willenbrink 1994). Under adequate moisture conditions, pre-anthesis assimilate reserves in the stems and sheaths of wheat and rice (*Oryza sativa*) contributed 10% to 40% of the final grain weight (Schnyder, 1993; Gebbing and Schnyder, 1999; Gebbing et al., 1999; Loggini et al., 1999; Yang and Zhang, 2006; Kirigwi et al., 2007). During drought when photosynthetic activity was inhibited by stress conditions after anthesis and the photosynthetic organs became increasingly senescence, grain filling was more dependent on mobilized stem reserves, which then represented 40% to 60% of the dry matter that accumulated in grains (Wagner and Wiemken, 1987; Blum, 1996, 1998; Vijn and Smeekens, 1999). Under normal conditions, the accumulation of water soluble carbohydrate (WSC) can reach more than 40% of the stem dry weight (Jeong and Housley, 1990). Wheat cultivars capable of synthesizing and storing a high concentration of soluble carbohydrate in the stems prior to anthesis are more likely to exhibit improved grain yield under conditions of water stress (Michiels et al., 2004), as stem carbohydrates can be mobilized to the grain in the absence of photosynthetic carbon assimilation. The stems of rain-fed wheat have significantly higher average total carbohydrate (1.8-fold) and fructan (2.5-fold) accumulations than irrigated wheat (Kerepesi and Galiba, 2000; Michiels et al., 2004). It has been estimated that pre-anthesis reserves contribute up to 74% and 57% of the grain yield of barley and wheat, respectively (Wagner and Wiemken, 1987; Loggini et al., 1999; Vijn and Smeekens, 1999; Kirigwi et al., 2007), when crops suffered from post-anthesis water deficit.

The main storage forms of non-structural carbohydrates (NSC) in the stem (culm + sheath) of wheat are sucroses and fructans (Wagner and Wiemken, 1987; Vijn and Smeekens, 1999). Sucrose is the diurnal carbohydrate reserve form and is stored mainly in vacuoles of photosynthetic cells of leaf blades, glumes and the photosynthesizing parts of the stem and leaf sheaths. On the other hand, longer-term carbohydrate reserves are mainly in the form of fructans; the fructan is the main form of water soluble sugar and is stored in the lower portions of the internodes, which are enclosed by leaf sheaths (Jeong and Housley, 1990;). At the stage of maximum WSC content, fructans represent 85% of the WSC in wheat stem internodes (Turner et al., 2008).

Fructans are synthesized by fructosyltransferases (FTs) and depolymerized by fructan exohydrolsaes (FEHs) (del Viso et al., 2009). The synthesis of fructan in the form of triaccharide and beyond, is catalyzed by sucrose:sucrose fructosyltransferase (SST; EC 2.4.1.99) and fructan: fructan fructosyltransferase (FFT; EC 2.4.1.100) (Wagner and Wiemken, 1987; Jeong and Housley, 1990). Fructan exohydrolase (FEH; EC 3.2.1.80), on the other hand, catalyzes the hydrolysis of fructans, leading to the release of fructose, which, in turn, has to be converted to the precursors required for the resynthesis of sucrose before phloem loading (Willenbrink et al., 1998). The FEHs include 1-FEHs and 6-FEHs (Van Riet et al., 2006). The 1-FEHw1, w2, and w3 are very important for maintaining the flow of carbon required for grain filling. By contrast, 6-FEH is not inhibited by sucrose, suggesting that it might not be involved in reserve mobilization. The expressions of FEHs forms in plant are very different (Gebbing, 2003). The accumulation of the 1-FEHw3 mRNA was 300 fold greater than that of 1-FEHw1 and 1-FEHw2. The mRNA accumulation continued after the stem water soluble carbohydrate concentrations reached a peak, consistent with the role of 1-FEHw3 in the breakdown of water soluble carbohydrates (Zhang et al., 2008).

Nitrogen is the major nutrient influencing grain yield and protein concentration (Novoa and Loomis, 1981; Martre et al., 2003). Prior to anthesis, N supply affects crop growth and photosynthetic capacity. In winter wheat, N application had large effects on grain yield (Thomas and Howarth, 2000). Conservation tillage increased crop yield (from 8% to 35%) and WUE (2 to 36%) (He et al., 2007). Many studies (Ge et al., 2010; Liu et al., 2010) showed that the application of manure not only enhanced the fertility of soil but also minimized the potential negative impact on surroundings ecosystems.

We studied the activity of 1-FEH, weight of penultimate internode, and the contents of total sugar in penultimate internode to determine the relationship between the activity of 1-FEH and yield of wheat under different fertilizer and tillage treatments.

TABLE 1 Details of experimental treatments

Treatment code	Cultivars	Nitrogen (kg/ha)	Phosphate (kg/ha)	Pig manure (kg/ha)	Tillage methods
T1	Changwu134	150 (as urea)	120(as ssp)		Conventional
T2	Changhan58	120 + 75*	120		Conservation
T3	Changhan58	120 + 75*	120	45000	Conservation
T4	Changhan58	150	120		Conventional

*Representative plus nitrogen at 4 April 2009.

MATERIAL AND METHODS

Growth Conditions

Field experiments were conducted during 2008 to 2009 growing season at the ChangWu Experimental Station of Chinese Academy of Sciences (107°40′ to 107°42 E, 35°12′ to 35°16′N, 1200 m asl). The station is located in ChangWu County, Shannxi Province, a typical semi-arid area of the Loess Plateau. The Mean annual air temperature is 9.1°C, the cumulative temperature above 10°C covers 302d, and the annual frost free period is 171d. The average annual precipitation (rain and snow) in the area is 584 mm, with 68% of the precipitation occurring between June and September (Ma et al., 2006; Li et al., 2007). The soil is dark loessial soil (Calcic Kastanozems, FAO) locally classified as Heilu soil.

The wheat cultivation methods used by local farmers in Changwu County involves conventional tillage—farmers' labor—intensive tillage practice: once at the end of the wheat maturity and again in the following July. The field is either tilled manually or with a tractor, depending on the size of land involved. The depth of rotary tillage is about 20 to 30 cm. The HP of the tractor ranges from 40 to 70 with the diesel engine. In this study, we compared the farmers' tillage practice with conservation (minimum) tillage in which plots were rotor-tilled only once. Conservation tillage has many benefits, such as increasing yield and water use efficiency (WUE) (Aase and Pikul Jr 1995), enhancing the contents of soil carbon, nutrient retention (He et al. 2007; Tian et al., 2010) and reducing soil erosion and degradation.

Experimental Design

Seeds of two winter wheat cultivars ('Changhan58' and 'Changwu134') were sown on 3 October 2008 on the plots. The inorganic fertilizer and pig manure treatments were imposed prior to sowing. Plots were 24 m long and 6 m wide with 20 rows spaced 0.20 m apart. The four treatments studied are shown in Table 1. Each treatment was replicated three times in a randomized complete block design.

Sampling

Prior to crop harvest, the number of spikes (per/m^2) was recorded and 40 main spikes from each plot were sampled to determine kernel number per spike. Crop harvest was completed by using a plot combine. After harvest, grains were air-dried, and plot yield and 1000 seed weight were determined.

The number of days to heading was determined when 50% of the spikes partially emerged from the flag leaf sheaths; number of days to anthesis was determined when 50% of the spikes had extruded anthers. Physiological maturity was determined when 50% of the spikes lost their green color (Ehdaie et al., 2006). Three hundred to 450 main tillers from different treatments were tagged as spikes emerged from the flag leaf sheaths. For yield determination, a $1m^2$ area was harvested at random with 5 replications per plot. Thirty main tillers were sampled at 6-day intervals after anthesis until physiological maturity. After each sampling, the main tiller was divided into spike and stem; then leaf sheaths were removed from the stem. A third of the samples was divided into three segments, namely peduncle (first internode below the spike including the distal node), penultimate internode (the internode below the peduncle including the distal node), and the lower internodes. Samples were immediately preserved in liquid nitrogen for use in measuring the activity of FEH. The rest of the samples were immediately dried in a force-air oven at 80°C for 48 h.

Measurement of Water Use Efficiency

Soils were sampled from the surface to a depth of 200 cm before sowing and after harvesting. Samples were collected at intervals of 10 cm within the first 100 cm depth and at 20 cm intervals from 101 cm to 200 cm. The fresh weight of the samples was determined immediately and the dry weight determined after drying in a forced-air oven at 105°C until a constant weight was achieved. Soil moisture, soil water-storage, water consumption and WUE were determined according to the following equations:

$$SW = (D \cdot R \cdot Wm) \cdot 100 \quad (1)$$

$$WUE = Y/ET = Y/(SWB + Pr - SWL) \quad (2)$$

where for Equation 1: SW, D, R and Wm are soil-water-storage (m^3), soil depth (m), soil bulk density (mg/m^3), and soil moisture at different depths (%), respectively. For Equation 2: WUE, Y, ET, SWB, Pr and SWL are water use efficiency (kg·m^{-3}·ha^{-1}), yield (kg ha^{-1}), amount of consumption water (m^3), the total precipitation in the whole period of wheat growth, soil water storage before sowing (m^3) and soil water storage after harvest (m^3), respectively.

Total Water Soluble Sugar in Penultimate Internodes

Dried main stem samples were ground to pass a 0.5 mm sieve and 0.5 g of the sieved samples was transferred into a 10 mL tube, adding 5 mL of 80% (v/v) alcohol water-heating at 80°C for 30 min, followed by centrifugation at 4,000 g at room temperature for 15 min. The supernatant was put into 25 mL flask. The sediment was repeated twice as the above procedures. Finally the supernatant was settled with sterile distilled water to 25 mL. A standard curve was prepared using appropriate dilutions of pure glucose in distilled water. The WSC was determined according to procedures described previously (Gao 2006).

Determination of Fructans in Penultimate Internodes

The hydrolysates (glucose, fructose, sucrose and fructan) were separated as anions through an UltimateTM NH_2, eluent flow contained in Methyl cyanide and deionized-water (70:30). The eluent flow rate was 1 μL min^{-1}, and the column temperature was 25°C. Chromatographic signals were analyzed by Waters 2420 detector with glucose, fructose and sucrose determined based on reference internal standard and blank samples (Wang, 2009).

Extraction and Measurement of the Activity of FEH in Penultimate Internodes

All chemicals and enzymes used for enzymatic measurements were from Sigma Chemical Company (St. Louis, Mo, USA). The method for FEH extraction was modified from Yang (Yang et al., 2004). Briefly, the frozen stems were ground with a mortar and pestle in 10 mL of ice-cold 50 mM citrate-phosphate (pH 5.5), containing 5 mM magnesium chloride ($MgCl_2$), 5 mM dithiothreitol (DTT), 0.2% (w/v) polyvinylpolypyrrolidone (PVPP) and 0.1% (w/v) bovine serum albumin (BSA). The homogenate was centrifuged at 12,000 g for 10 min (<4°C). The supernatant was immediately desalted by centrifugal filtration on a Sephadex G-25 column equilibrated with 50 mM citrate-phosphate buffer (pH 5.5). As substrate for the assay of FEH, fructan was extracted from young leaves of wheat that had been induced to accumulate fructan. The extraction method was as described previously (Wang, 2009). For FEH assay, a 0.5 mL reaction mixture, containing 50 mM citrate-phosphate buffer (pH 5.5), 5 mM $MgCl_2$, 5 mM DTT, 0.2%(w/v) PVPP, 0.1% (w/v) BSA, 1.5% (w/v) fructan (extracted from young leaves of wheat) and 0.3 mL enzyme solution, was incubated for 60 min at 30°C. Incubation was stopped by transferring the sample tubes to boiling water for 3 min then holding them on ice. Fructose from hydrolysis by FEH was determined enzymatically as described above.

Statistical Analysis

Data were analyzed using the analysis of variance and means were separated using Duncan's multiple range test at the 5% level of significance.

RESULTS

Dry Weight of Penultimate Internodes

Figure 1 illustrates changes in the dry weight of the penultimate internode at different times in the grain filling stage. The dry weight of 'Changhan58' was consistently heavier than that of 'Changwu134.' For 'Changhan58,' the T3 treatment produced the heaviest DW, which ranged from 200 to 450 mg. At the beginning of grain filling, T3 had the greatest dry weight among the treatments, suggesting that plants under T3 accumulated the greatest amount of dry matter. The peak weight of the penultimate internodes, on overage, was reached 20 days after anthesis (DAA), and then decreased progressively until maturity.

Total Soluble Sugar (TSS) Content of the Penultimate Internode

Concentrations of total soluble sugars (TSS) analyzed during the grain filling period to assess developmental trends in carbohydrate metabolism

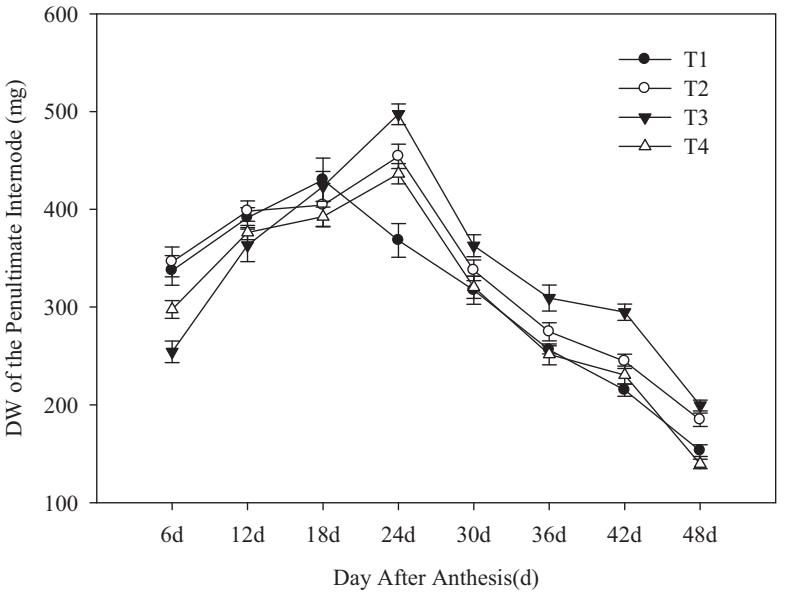

FIGURE 1 Changes in dry weight of the penultimate internode of two wheat cultivars under different fertilizer and tillage treatments after anthesis. T1:Changwu134 N150 + Conventional tillage; T2:Changhan58 N (120+75) + Conservation tillage T3:Changhan58 N (120+75) + Pig manure + Conservation tillage T4:Changhan58 N150 + Conventional tillage.

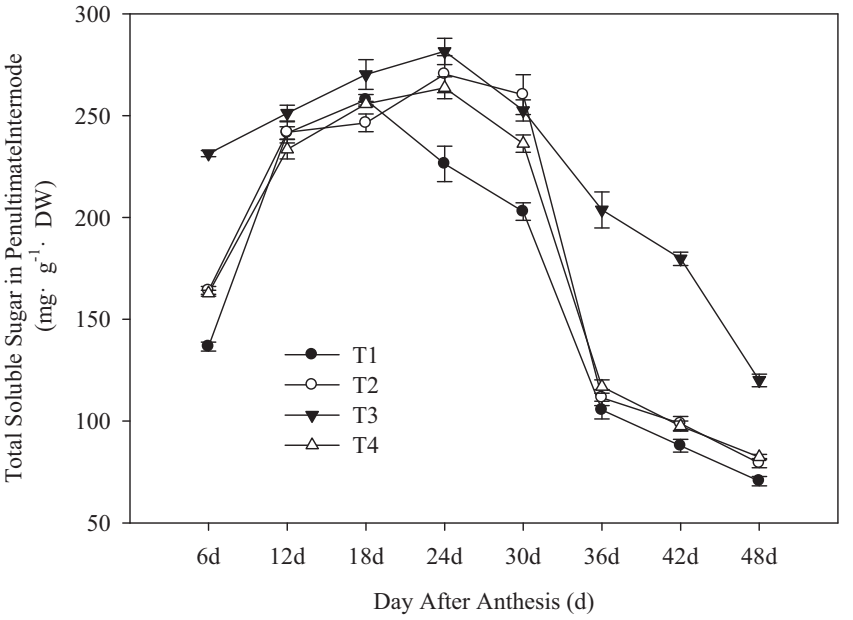

FIGURE 2 Changes in total soluble sugar concentration in the penultimate internode of two wheat cultivars under different fertilizer and tillage treatments after anthesis. Treatments have same definitions as Figure 1.

among the treatments are shown in Figure 2. Variations in TSS followed a similar trend as dry weight. At the beginning of the grain filling period, the TSS was very low. For example, under T3 (which had the highest concentration of TSS among the treatments), the TSS peaked in the middle of grain filling, and then decreased slowly thereafter. The TSS as a form of NSC in the penultimate internodes increased from 1 to 22 DAA, and slowly declined thereafter, irrespective of treatment. 'Changwu134' showed substantially reduced NSC from 12 to 48 DAA. A very similar pattern was observed for fructan concentration in the penultimate internode from 6 to 48 DAA (Figure 3), suggesting that changes in the penultimate internode during this period was mainly due to the change in fructan level. The peak TSS concentration in the penultimate internodes was achieved 18 DAA for 'Changwu134' and 24 DAA for 'Changhan58.' The concentration of TSS increased initially and then decreased. However, the concentrations differed according to treatment with T3 showing the highest content.

Fructan Concentration in the Penultimate Internode

Fructan is the main carbohydrate in the penultimate internode. The fructan content in 'Changhan58' increased several-fold compared to that of 'Changwu134' in the grain filling period (Figure 3). The maximum content in 'Changwu134' occurred at 18 DAA, being about 6d earlier than that

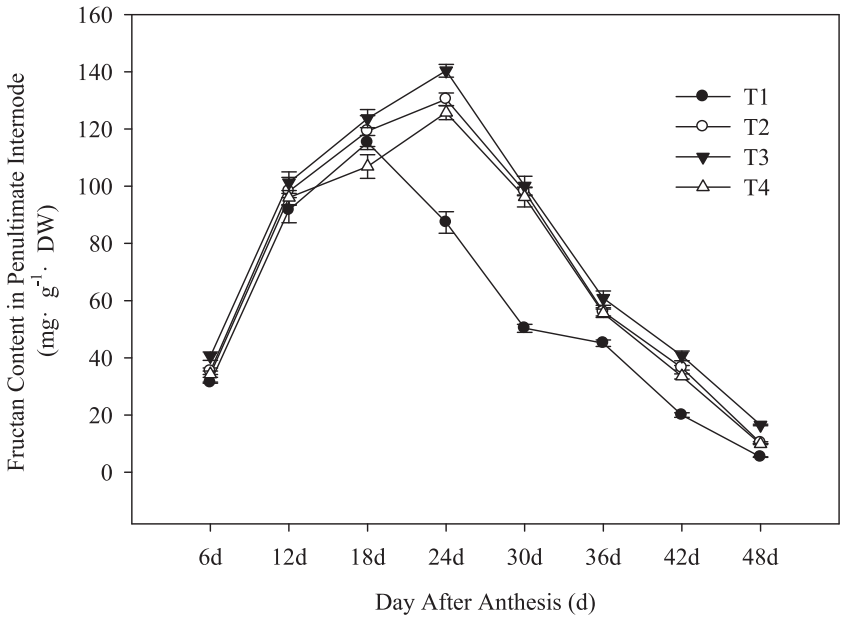

FIGURE 3 Changes in the fructan content in the penultimate internode of two wheat cultivars under different fertilizer and tillage treatments after anthesis. Treatments have same definitions as Figure 1.

of 'Changhan58.' For 'Changhan58,' the fructan content was very low at the onset of grain filling, then increased in the middle of grain filling, particularly, under the T3 treatment and thereafter dropped progressively until maturity. In the T3, the maximum fructan content was 139 $mg{\cdot}g^{-1}{\cdot}DW$.

Changes in FEH Activities in the Penultimate Internodes

In wheat, the FEH is responsible for the degradation of fructan into fructose and sucrose when the supply of carbohydrate exceeds the demand. The grain filling period requires more carbohydrate. The FEH changed similarly across treatments (Figure 4). The activities of FEH in 'Changwu134' were lower than those in 'Changhan58' during the grain filling period. The peak FEH in T3 was 24.52 at 30 DAA, being late by 6d compared with 'Changwu134.' In 'Changhan58,' the different fertilizer and tillage treatments only changed the activities of the FEH during the grain filling period and had no influence on the time of peak FEH activities. For 'Changwu134,' the timing of the drop in activity of FEH occurred 6d earlier than 'Changhan58.'

Grain Yield and Water Use Efficiency

The different fertilizer treatments had different effects on WUE, although there was no significant difference in wheat yield between T2 and

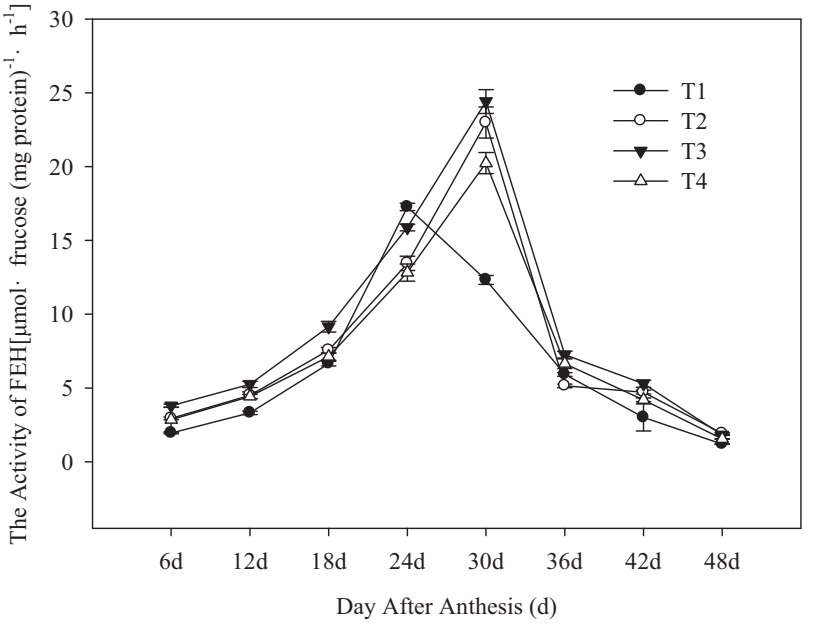

FIGURE 4 FEH Activity in the penultimate internode of two wheat cultivars under different fertilizer and tillage treatments after anthesis. Treatments have same definitions as Figure 1.

T4. Manure and conservation tillage increased the grain weight under T3. In T2, the grain weight was similar to that of T4 and higher than that of T1 (Table 2). For T3, the wheat yield was 6769 kg ha^{-1}, being 657 and 889 kg ha^{-1} (or 10.7 and 15.1%) greater than yields under T2 and T4, respectively. The 1000-grain weight was the main factor influencing the yield and harvest index (HI). The T3 produced the heaviest individual grains, grain yield and the highest HI. The T2 had more yield, heavier grains and higher WUE and HI than T4. This implies that split fertilizer application was superior to one time application. The WUE ranged from 15.50 to 18.36 [kg/ (hm^2·mm)] and was highest under T3 and lowest WUE under T1.

Wheat yield is determined by spike number, grain number and grain weight. None of the treatments had a significant effect on number of spikes at maturity; T3 had the highest grain weight, WUE and HI (Table 2). As a result, it also had the highest grain yield among the treatments.

'Changhan58' had higher yield than 'Changwu134,' and T3 was the best yielder with the heaviest individual grains. However, its grain number was significantly lower than that of T2 and T4.

DISCUSSION

The remobilization of pre-anthesis assimilate stored in penultimate internode is important for grain filling, particularly for wheat under abiotic

TABLE 2 Grain yield, yield components and WUE of two wheat cultivars under different fertilizer and tillage treatments

Cultivating model	Yield (kg/ha)	1000-grain weight(g)	WUE [kg/(hm^2•mm)]	HI	Grain Number per spike	Number of spike (10000/ha^{-1})
T1	5423 ± 12.81*C**	44.52 ± 1.023C	15.50 ± 0.9641C	0.425 ± 0.036C	22.80 ± 2.754A	551 ± 1.2A
T2	6112 ± 14.75*B*	46.93 ± 0.639B	18.24 ± 1.3561B	0.506 ± 0.026B	19.90 ± 2.082B	542 ± 2.2A
T3	6769 ± 17.17*A*	50.46 ± 1.818A	18.36 ± 1.3656A	0.584 ± 0.035A	16.75 ± 3.697C	572 ± 2.3A
T4	5880 ± 14.26*B*	46.87 ± 0.147B	15.79 ± 1.3396C	0.461 ± 0.048C	20.12 ± 3.403B	554 ± 1.2A

*Different letters in each row indicate significant difference at $p < 0.05$ as analyzed by the Duncan's multiple range test.

stress such as drought and heat during the grain filling period. There are two ways of monitoring the amounts of accumulated and mobilized stem reserves. The indirect way is to monitor changes in internode dry weight (Ehdaie et al., 2006); the direct way is to measure internode WSC content during the grain-filling period (Blum, 1998; Vijn and Smeekens, 1999).

Ehdaie et al. (2006) insisted that changes in stem dry weight could be used to estimate stem reserves and related characters instead of measuring stem WSC content. Thus, the weight of penultimate internode is a vital index for dry matter accumulation and mobilization. In this study, we observed the change in the content of DW (indirect approach) and TTS (direct approach) of the penultimate internodes. Both parameters changed similarly during the grain filling stage. The grain filling period can be divided into three phases: the first phase is slow-increase duration—at the onset of grain filling when the weight and concentration of total soluble sugars in the penultimate internode increased slowly. The second phase is the fast increase duration—at the middle of the grain filling period, when both the dry weight and concentration of total soluble sugars in the penultimate internode increased sharply. The third phase of the grain filling period is the slight increase duration, where the dry weight and concentration of the penultimate internode increased slightly.

From Figure 1, we note that the maximum weight occurred at 18 DAA. The ratio of decrease for T3 between the 18 DAA and 36 DAA was 8.29 mg d^{-1}, which was very fast relative to other treatments. The chlorophyll content of the flag leaf (data not show) changed similarly, illustrating that the use of manure and conservation tillage can enhance the fixing of more carbohydrate and storage of water soluble carbohydrates in the penultimate internode, thereby maintaining a stronger "source" for grain filling and prolonging the duration of the fast increase phase of the grain filling period.

Measuring the WSC content in the internode during the grain-filling period is a direct way of monitoring the amounts of accumulated and mobilized stem reserves. Figure 2 illustrated the changes in water soluble carbohydrates in the penultimate internode. The maximum penultimate internode dry weight and WSC content across treatments was reached 24 and 10 DAA, which then declined progressively over time (Figure 2). A large portion of the drop in stem dry weight in the period between maximum and minimum stem dry weight could be accounted for by the mobilization of stem WSC.

Fructan storage can rapidly be induced in photosynthetically active tissues, when the export of carbohydrates is restricted. But in most fructan storing species, under more 'natural' growth conditions, long-term fructan storage occurs mainly in tissues of low or negligible photosynthetic activity (Gebbing, 2003). During the grain filling stage, the activities of FEH increased at DAA 20, and then declined progressively over time (Figure 4). During the same stage, particularly in the middle of that period, sole dependence on the flag leaf could not supply enough carbohydrates for grain

filling, so the fructan stored in the penultimate internode was remobilized and transferred to the grain. The change in fructan content of the penultimate internode was similar to that of the grain weight, except that the maximum grain weight occurred about 6 days later than the maximum fructan content. We consider that the contribution of fructan to yield requires more time.

Crop WUE is an important trait in breeding programs. In almost all crops, the greater WUE for grain was not due to an improvement in biomass accumulation, but, rather surprisingly, almost entirely to an improved HI (Richards et al., 1993). Grain yield ranged from 5423 to 6769 kg ha^{-1}, being lowest in T1 and highest in T3. Our results indicated that T3 (conservation tillage plus combined use of inorganic and organic fertilizer) optimized yield and WUE better than other treatments. The fertilizer treatments in T2 and T4 were different and the results showed differences in HI and WUE, but not in grain yield. We observed at the beginning of the wheat growth period, namely the vegetative stage, that both cultivars had same growth conditions, however, in the late reproductive stage, T2 received a split fertilization, which supplied N at 75 kg ha^{-1}, thus improving the nutrient level of the soil, which translated to a yield advantage of 232 kg ha^{-1} over T4.

Nitrogen is the major nutrient influencing grain yield and protein concentration (Novoa and Loomis, 1981; Martre et al., 2003; McDonald et al., 2008). Prior to anthesis, N supply affects crop growth and photosynthetic capacity. In winter wheat, N application has large effects on leaf area expansion and duration; which have been associated frequently with grain yield (Thomas and Howarth, 2000). Pig manure and conservation tillage might improve the WUE, grain yield and HI of winter wheat, especially for 'Changhan58.' Organic manures are a vital resource not only for supplying plant nutrients but also for replenishing organic matter content of most agricultural soils (Manna et al., 2007). Pig manure can enhance the storage of soil water and improve soil fertility for wheat growth. The different fertilizer treatments had different effects on WUE, although there was no significant difference in wheat yield between T2 and T4.

CONCLUSIONS

During the grain filling stage, the activities of FEH first increased at 20 days after anthesis, then declined progressively. During this time same stage, particularly in the middle of this stage, sole dependence on the flag leaf could not supply enough carbohydrates for grain filling, so the fructan stored in the penultimate internode was remobilized and transferred to the grain. The application of N and P, in combination with organic manure, would be an efficient practice for increasing wheat yield (through positive changes in plant eco-physiology) and soil fertility.

FUNDING

This work was conducted in the State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau and Changwu Experimental Station of Chinese Academy of Sciences and supported by National"973" Project (2009CB118604), the International Science & Technology Cooperation Program of China (2010DFA91930), and the China 111 project (No. B12007).

REFERENCES

Aase, J. K., and J. L. Pikul Jr. 1995. Crop and soil response to long-term tillage practices in the northern Great Plains. *Agronomy Journal* 87: 652–656.

Bancal, P., and E. TriboI. 1993. Temperature effect on fructan oligomer contents and fructan-related enzyme activities in stems of wheat (*Triticum aestivum* L.) during grain filling. *New Phytologist* 123: 247–253.

Blum, A. 1996. Crop responses to drought and the interpretation of adaptation. *Plant Growth Regulation* 20: 135–148.

Blum, A. 1998. Improving wheat grain filling under stress by stem reserve mobilisation. *Euphytica* 100: 77–83.

Cruz-Aguado, J. A., R. Rodes, I. P. Perez, and M. Dorado. 2000. Morphological characteristics and yield components associated with accumulation and loss of dry mass in the internodes of wheat. *Field Crops Research* 66: 129–139.

del Viso, F., A. F. Puebla, H. E. Hopp, and R. A. Heinz. 2009. Cloning and functional characterization of a fructan 1-exohydrolase (1-FEH) in the cold tolerant Patagonian species Bromus pictus. *Planta* 231: 13–25.

Edhaie, B., G. A. Alloush, M. A. Madore, and J. G. Waines. 2006. Genotypic variations for stem reserves and mobilization in wheat: II. Postanthesis changes in internode water-soluble carbohydrates. *Crop Science* 46: 2093–2103.

Gao, J. F. 2006. *Experimental Guidance for Plant Physiology*. Beijing: Higer Education Press.

Ge, G..F., Z. F. Li, F. L. Fan, G. X. Chu, Z. N. Hou, and Y. C. Liang. 2010. Soil biological activity and their seasonal variations in response to long-term application of organic and inorganic fertilizers. *Plant and Soil* 326: 31–44.

Gebbing, T. 2003. The enclosed and exposed part of the peduncle of wheat (*Triticum aestivum*)–spatial separation of fructan storage. *New Phytologist* 159: 245–252.

Gebbing, T., and H. Schnyder. 1999. Pre-anthesis reserve utilization for protein and carbohydrate synthesis in grains of wheat. *Plant Physiology* 121: 871–878.

Gebbing, T., H. Schnyder, and W. Kühbauch. 1999. The utilization of pre-anthesis reserves in grain filling of wheat. Assessment by steady-state 13CO2/12CO2 labelling. *Plant, Cell and Environment* 22: 851–858.

He, J., H. W. Li, X. Y. Wang, A. D. McHugh, W. Y. Li, H. W. Gao, and N. J. Kuhn. 2007. The adoption of annual subsoiling as conservation tillage in dryland maize and wheat cultivation in northern China. *Soil and Tillage Research* 94: 493–502.

Jeong, B. R., and T. L. Housley. 1990. Fructan metabolism in wheat in alternating warm and cold temperatures. *Plant Physiology* 93: 902–906.

Kerepesi, I., and G. Galiba. 2000. Osmotic and salt stress-induced alteration in soluble carbohydrate content in wheat seedlings. *Crop Science* 40: 482–487.

Kirigwi, F. M., M. Van Ginkel, G. Brown-Guedira, B. S. Gill, G. M. Paulsen, and A. K. Fritz. 2007. Markers associated with a QTL for grain yield in wheat under drought. *Molecular Breeding* 20: 401–413.

Li, Z., W. Z. Liu, Q. K. Yang, S. J. Li, Zh.T. Gan, B. Wang, and R. Wang. 2007. Land use/land cover change and their ecological effect in small watershed on gully region of the Loess Plateau. *Chinese Journal of Applied Ecology* 18: 1299–1304 (in Chinese).

Liu, X., F. M. Li, D. Q. Liu, and G. J. Sun. 2010. Soil organic carbon, carbon fractions and nutrients as affected by land use in semi-arid region of Loess Plateau of China. *Pedosphere* 20: 146–152.

Loggini, B., A. Scartazza, E. Brugnoli, and F. Navari-Izzo. 1999. Antioxidative defense system, pigment composition, and photosynthetic efficiency in two wheat cultivars subjected to drought. *Plant Physiology* 119: 1091–1099.

Ma, S. C., B. C. Xu, Zh. B. Huang, L. Liu, X. H. Zhang, W. Z. Liu, and F. M. Li. 2006. Effects of partial root excision at the Rr-greening stage of winter wheat on root/shoot ration, yield and water use efficiency in Loess Plateau Region, China. *Journal of Plant Ecology* 30: 976–982 (in Chinese).

Manna, M. C., A. Swarup, R. H. Wanjari, B. Mishra, and D. K. Shahi. 2007. Long-term fertilization, manure and liming effects on soil organic matter and crop yields. *Soil and Tillage Research* 94: 397–409.

Martre, P., J. R. Porter, P. D. Jamieson, and E. Triboi. 2003. Modeling grain nitrogen accumulation and protein composition to understand the sink/source regulations of nitrogen remobilization for wheat. *Plant Physiology* 133: 1959–1967.

McDonald, G. K., Y. Genc, and R. D. Graham. 2008. A simple method to evaluate genetic variation in grain zinc concentration by correcting for differences in grain yield. *Plant and Soil* 306: 49–55.

Michiels, A., A. Van Laere, W. Van den Ende, and M. Tucker. 2004. Expression analysis of a chicory fructan 1-exohydrolase gene reveals complex regulation by cold. *Journal of Experimental Botany* 55: 1325–1333.

Novoa, R., and R. S. Loomis. 1981. Nitrogen and plant production. *Plant and Soil* 58: 177–204.

Pan, Q. M., X. G. Han, Y. F. Bai, and J. C. Yang. 2002. Advances in physiology and ecology studies on stored non- structure carbohydrates in plants. *Chinese Bulletin of Botany* 19: 30–38 (in Chinese).

Richards, R. A., C. Lopez-Castaneda, H. Gomez-Macpherson, and A. G. Condon. 1993: Improving the efficiency of water use by plant breeding and molecular biology. *Irrigation Science* 14: 93–104.

Schnyder, H. 1993. The role of carbohydrate storage and redistribution in the source-sink relations of wheat and barley during grain filling—a review. *New Phytologist* 123: 233–245.

Thomas, H., and C. J. Howarth. 2000. Five ways to stay green. *Journal of Experimental Botany* 51: 329–337.

Tian, S. Z., T. Y. Ning, Y. Wang, H. J. Li, W. L. Zhong, and Z. J. Li. 2010. Effects of different tillage methods and straw-returning on soil organic carbon content in a winter wheat field. *Chinese Journal of Applied Ecology* 21: 373–378 (in Chinese).

Turner, L. B., A. J. Cairns, I. P. Armstead, H. Thomas, M. W. Humphreys, and M. O. Humphreys. 2008. Does fructan have a functional role in physiological traits? Investigation by quantitative trait locus mapping. *New Phytologist* 179: 765–775.

Van Riet, L., V. Nagaraj, W. Van den Ende, S. Clerens, A. Wiemken, and A. Van Laere. 2006. Purification, cloning and functional characterization of a fructan 6-exohydrolase from wheat (*Triticum aestivum* L.). *Journal of Experimental Botany* 57: 213–223.

Vijn, I., and S. Smeekens. 1999. Fructan: more than a reserve carbohydrate? *Plant Physiology* 120: 351–359.

Wang, Z. H. 2009. Regulation of Drought Stress on Assimilate Translocation during Grain Filling in Wheat. Dissertation. Northwest A&F University, Yangling, Shaanxi, China (in Chinese).

Wagner, W., and A. Wiemken. 1987. Enzymology of fructan synthesis in grasses: properties of sucrose-sucrose-fructosyltransferase in barley leaves (*Hordeum vulgare* L. cv Gerbel). *Plant Physiology* 85: 706–710.

Wardlaw, I. F., and J. Willenbrink. 1994. Carbohydrate storage and mobilisation by the culm of wheat between heading and grain maturity: the relation to sucrose synthase and sucrose-phosphate synthase. *Functional Plant Biology* 21: 255–271.

Willenbrink, J., G. D. Bonnett, S. Willenbrink, and I. F. Wardlaw. 1998. Changes of enzyme activities associated with the mobilization of carbohydrate reserves (fructans) from the stem of wheat during kernel filling. *New Phytologist* 139: 471–478.

Yang, J. C., and J. H. Zhang, 2006. Grain filling of cereals under soil drying. *New Phytologist* 169: 223–236.

Yang, J. C., J. H. Zhang, Z. Q. Wang, G. W. Xu, and Q. S. Zhu. 2004. Activities of key enzymes in sucrose-to-starch conversion in wheat grains subjected to water deficit during grain filling. *Plant Physiology* 135: 1621–1629.

Zhang, J. J., B. Dell, E. Conocono, I. Waters, T. Setter and R. Appels, 2008. Water deficits in wheat: Fructan exohydrolase (1-FEH) mRNA expression and relationship to soluble carbohydrate concentrations in two varieties. *New Phytologist* 181: 843–850.