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Original Research Article

Dynamics and contribution of stem water-soluble carbohydrates to grain yield in two wheat lines contrasted under Drought and elevated CO_2 conditions

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Stem water-soluble carbohydrates (stem WSC) are a source of carbon for grain filling in wheat that become more important in maintaining grain yield during post-anthesis period when current photosynthesis declines due water stress. Stem WSC are therefore expected to be one of the key traits in adapting wheat to water-limited environments. The aim of this study was to determine dynamics and role of stem WSC in maintaining grain yield traits of wheat under post-anthesis water deficit and elevated CO_2 (eCO_2) conditions. A research was conducted at the Australian Grains and Free-Air CO_2 Enrichment Facility (AGFACE) in Horsham, Victoria. Two Seri/Babax (SB) wheat lines, SB03 and SB62, were grown under varying combinations of water and CO_2. Results showed that WSC contributed 20-32 % of grain yield/spike (highest contribution was for SB62 under eCO_2). Elevated CO_2 (550 ppm) increased the accumulation of stem WSC and grain yield m^{-2}, the two lines were not significantly different on the latter variable. Higher WSC increased grain yield/spike under drought conditions regardless of amount of CO_2 available. The stem WSC contributed to grain yield under all conditions and not just under eCO_2 x drought conditions. Replication under different agro-ecological environments is required to confirm these findings.

Key words: Wheat, crop adaptation, grain yield, water-soluble carbohydrates, elevated CO_2, drought.

INTRODUCTION

The demand for food in the world is increasing on at least two main fronts: the increasing world population expected to reach 9 billion by 2050 (Godfray et al., 2010) and on the other hand the rising affluent population in emerging economies that means improved purchasing power and soaring of demand and consumption of improved and expensive diets. These socio-economic dynamics exert challenges on global food production and supply (Godfray et al., 2010; Parry and Hawkesford, 2010). Agro-ecological threats such as climate change and land degradation mean that more food has to be produced per hectare of the available farmland in order to satisfy the rising demand for food (Aranjuelo et al., 2013; Parry and Hawkesford, 2010; Ziska et al., 2012). Climate change, due to greenhouse gas

(GHG) emissions and rising temperature, threatens food security due to its alteration of crop growing conditions through heat stress, drought, salinity and waterlogging (Chapman et al., 2012). Carbon dioxide (CO_2) is the most important GHG causing climate change (Hogy et al., 2009). The rising atmospheric CO_2 concentration is projected to reach 550 parts per million (ppm) by 2050 from the current 389 ppm (in 2012) (IPCC, 2007; Longinelli et al., 2013) Drought is a serious threat to sustainable crop production and is expected to become more severe (Boyer and Westgate, 2004).

Wheat is the most important cereal by global trade (FAOSTAT, 2013, Parry and Hawkesford, 2010). In Australia, wheat is the most important by production and export earnings according to the Australian Bureau of Agricultural and Resource Economics and Sciences (ABARES, 2012). Although its ranking on the world wheat market fluctuates, Australia remains among the top five exporters (Qureshi et al., 2013). Although drought has affected the Australian wheat industry for the past 200 years, developments in the past decade have been unprecedented (Head et al., 2011). Increasing temperature has worsened the impact of droughts by enhancing the evapotranspiration rate (Nicholls, 2004). Australia receives very little rainfall; for example between 2000 and 2009, the mean annual rainfall was 486 mm (ABS, 2012).

Since Australia is an important wheat exporter, any factors that limit wheat productivity in the cropping regions would have significant consequences on world food security (Qureshi et al., 2013). The impact would have an immediate and most severe effect on the top importing countries of its wheat, consequently leading to increased prices on the world market. These challenges beckon agronomists and plant breeders to provide appropriate wheat adaptation technologies.

Adapting cereals to drought and elevated carbon dioxide (eCO_2) conditions is critical to global food security (Tester and Langridge, 2010). Plant breeding is one strategy of crop adaptation but requires prior identification of relevant traits driving economic yield. Understanding relevant bases of grain yield is key to successful breeding (Bennett et al., 2012). One area of crop physiology that has potential to make significant contribution to wheat adaptation to climate change is stem water-soluble carbohydrates (WSC). The ability to accumulate high stem WSC has been suggested as an important trait in breeding cultivars for water-limited environments (Foulkes et al., 200; Shearman et al., 2005).

WSC are non-structural carbohydrates that include fructose, glucose, sucrose and fructans, with fructans as the major component (Ruuska et al., 2006). WSC are accumulated during vegetative growth up to just after anthesis and are remobilised to the growing grains during grain filling (Ehdaie et al., 2008; Ruuska et al., 2006). Remobilisation of WSC to grains increases with post-anthesis water stress, when photosynthesis declines (Ruuska et al., 2006; Yang et al., 2000). Leaf stomatal conductance and net CO_2 assimilation decrease under water-stressed conditions and therefore account for the diminished photosynthetic role (Blum et al., 1988; van Herwaarden et al., 1998). Water-stressed conditions post-anthesis induce early senescence, which in turn increases grain filling rates but shortens grain-filling periods (Yang and Zhang, 2005). Under these conditions, remobilisation of WSC from stems and leaf sheaths to the grains begins earlier and hence the increased role of WSC in grain yield (Gupta et al., 2011). Therefore the contribution of WSC to grain yield is higher under water-stressed conditions than under well-watered conditions (Ehdaie et al., 2008; Foulkes et al., 2002; Yang et al., 2003). There are other factors that also influence yield benefits derived from WSC.

The percentage contribution of WSC to grain weight varies with type of cultivars, timing and severity of water stress in relation to crop phenology (Blum 1998). Because of this, values reported also vary, (e.g. 10 to 20% (Dreccer et al., 2009) and 50% (Rebetzke et al., 2008). Since the frequency of drought and rising atmospheric CO_2 concentration are expected to worsen, there is need for further research to understand the role of WSC in maintaining grain yield in cereals. Elevated CO_2 stimulates photosynthetic carbon fixation (Leakey et al., 2009) and this can offset crop productivity losses due to drought (Ainsworth et al., 2008, Bourgault et al., 2013). Availability of carbon depends on photosynthetic rate and remobilisation of reserves (Ruuska et al., 2006). Therefore, the yield stimulation reported under eCO_2 is attributable to an increase in current photo-assimilates and WSC concentration as indicated by an increase in fructans and other WSC in wheat grains (Hogy et al., 2009). Nie et al. (1995) found that eCO_2 increased accumulation of WSC. It is expected that increased WSC accumulation will be one of the key adaptive traits for dry-land farming under eCO_2. The role of stem WSC in maintaining yield of cereals under dry conditions has been documented (Dreccer et al.,_2009; Ruuska et al., 2006). However, there is limited information about their role under the combined influence of drought and free air CO_2 enrichment conditions. This study was carried out to determine the dynamics and role of stem WSC under these factors, as simulated under field conditions at the Australian grains free-air carbon dioxide enrichment (AGFACE) site in Horsham. The free-air carbon-dioxide enrichment (FACE) technology, practised under intact ecosystems simulates more closely the expected future climatic conditions (Nowak et al., 2004). FACE therefore provides a more reliable tool on which to base important decisions than studies done in enclosed environments (Long et al., 2006). The research is expected to indicate whether the accumulation of large stem WSC is an important adaptive trait for wheat grown under drought and eCO_2 conditions in the field as opposed to enclosed environments. The results obtained in this study are expected to be useful to wheat breeders when breeding cultivars to dry-land Australia. Since the Seri-Babax (SB) 62 line has higher stem WSC, it was expected to have higher grain yield than the SB03 line that has lower stem WSC under drought and elevated CO_2 conditions.

MATERIALS AND METHODS

Experiment Site

The research was conducted using the AGFACE facility in Horsham (36°45′07″S lat., 142°06′52″E long., 127 m elevation). The facility sits on a Murtoa Clay, which has approximately 35% clay at the surface increasing, to 60% at a 1.4 m depth; and the soil is a vertisol according to the Australian Soil Classification (developed by Isbell (1996), as cited in Mollah et al., 2009,). The experimental site has a Mediterranean type of climate (Hutchinson et al., 2005). According to the Australian Bureau of Meteorology (BoM)'s historical records (http://www.bom.gov.au), the mean annual rainfall for 20 years (between 1981 and 2010 was 435 mm. This was almost the same as the long term (137 years) average, which was 436 mm. In 2012, both the growing season rainfall and the total annual rainfall recorded at the site were less than their corresponding long term averages, being 214 mm and 287 mm respectively. As for temperature during the growing season, the minimum on average was 5˚C while the maximum was 20˚C. An atmosphere with eCO_2 just above crops was obtained by directly discharging pure CO_2 into the air on the upwind side through eight unconnected stainless steel tubes of an octagonal ring. CO_2 was released during daytime when there was ample light for photosynthesis.

Experiment layout and treatments

The experimental layout was a split-split-plot design. With 4 blocks, 2 varieties, 2 levels of CO_2 treatment as main plots and 2 levels of water treatment, there were 4×2×2×2 (32) experimental units. Two recombinant wheat lines from the Seri-Babax population, SB62 and SB03 (described by Olivares-Villegas et al., 2007), were grown at various CO_2 and water combinations in the 2012 growing season. The lines were selected on the basis of known differences in stem WSC. SB03 has lower WSC than SB62 (Dreccer et al., 2009). CO_2 was at two levels namely ambient (aCO_2) and elevated (eCO_2). The aCO_2 was ~389 ppm which according to Longinelli et al., (2013) was the concentration in the period 2011-2012 in this region of the globe stretching from South Africa to New Zealand; eCO_2 was targeted at 550 ppm. The experiment involved four blocks. In each block there were two rings - one of which had eCO_2 treatment and the other with aCO_2 treatment. Elevated CO_2 was turned on at crop emergence (13th June), which was 14 days after sowing, and turned off 10 days after physiological maturity (decimal code, DC, 90) (Zadoks et al., 1974). The plants reached physiological maturity on 30th November. Since each block had two rings, a total of eight rings (of 16 m diameter each) were used for the four blocks in the experiment. Rings constituted whole plots of either aCO_2 or eCO_2.

Each ring was divided into halves for two levels of water treatment. One half was under rainfed conditions and the other was under supplementary irrigation. The half rings, also known as bays, were separated with a polythene barrier to avoid seepage of water from the supplemental half to the rainfed half. Half rings constituted subplots. In each half ring, there was one sub-subplot of SB62 and another of SB03. All treatments were under rain-fed (RF) conditions until supplementary irrigation (SI) was effected on the same date (34 days before anthesis for SB62 and 37 days before anthesis for SB03). The 2012 growing season was a dry year with only 287 mm of annual rainfall received. Irrigation water was applied at a rate of 30 mm each time for a maximum of four times. This means that with growing season rainfall of 214 mm, plants under SI had an additional 120 mm of water (or can be regarded as having received 334 mm of growing season water). There were 14-day intervals between irrigation water applications. The last application of water was done 22 days before each line attained its physiological maturity.

Stem water soluble carbohydrate analysis

Individual plants were randomly harvested from each subplots at anthesis, 18 days after anthesis (18 DAA) and at physiological maturity (40DAA). For each plant harvested, spikes, peduncle, penultimate and lower internodes from main stems were collected in different envelopes and oven-dried at 70˚C and internode and grain dry weights obtained. Later, peduncle, penultimate and lower internodes harvested at anthesis and at 18 DAA were again oven-dried and ground with a Qiagen Retsch Tissue Lyser at a vibration frequency of 24 Hz and analysed for WSC. Sugar extraction was done using water as a solvent. Oven-dry weights ranging between 0.009 and 0.0155 g were determined and collected in 2 ml Eppendorf tubes to which 1.5 ml deionised water was added per tube and vortexed . The tubes were heated in a water bath at 65˚C for a total of 60 minutes, with vortexing in the process. After centrifugation at 12,000 revolutions per minute (rpm) for 5 minutes, 100 µl of supernatant was collected from each tube and diluted in 300 µl of deionised water. Calibration was done using fructose sequentially diluted to obtain fructose solutions of 1.00 mg/ml, 0.50 mg/ml, 0.25 mg/ml, 0.125 mg/ml, 0.063, 0.031 mg/ml and 0.00 mg/ml (deionised water only). These solutions were vortexed and anthrone – sulphuric acid reagent added just like was done with the extracts; and then used for the preparation of a standard curve.

The anthrone method as described by Yemm and Willis (1954) was used to determine WSC concentrations but with water extraction only, as the extraction with ethanol was not found to extract additional sugars. The anthrone - sulphuric acid reagent was prepared by adding 100 ml of 70% sulphuric to 0.2 g of anthrone. From each supernatant diluted at the end of the extraction process and the standard fructose solutions, an aliquot of 100 µl was obtained and added to 1.5 ml of anthrone - sulphuric acid reagent collected in Eppendorf tubes. The Eppendorf tubes were then vortexed and transferred to the water bath for heating at 85°C for 10 minutes. Finally, 300 µl was collected from each Eppendorf tube and transferred to a microplate

Table 1. Analysis of Variance Output for Stem Sugar Content at Anthesis

Variate: Stem Sugar Content					
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Rep stratum	3	39099.	13033.	3.00	
Rep. CO_2 stratum					
CO_2	1	79598.	79598.	18.35	0.023
Residual	3	13016.	4339.	0.23	
Rep. CO_2.Water stratum					
Water	1	408.	408.	0.02	0.887
CO_2.Water	1	957.	957.	0.05	0.828
Residual	6	112086.	18681.	1.74	
Rep. CO_2.Water.Cultivar stratum					
Cultivar	1	644933.	644933.	60.02	<.001
CO_2.Cultivar	1	21881.	21881.	2.04	0.179
Water.Cultivar	1	293.	293.	0.03	0.872
CO_2.Water.Cultivar	1	3745.	3745.	0.35	0.566
Residual	12	128941.	10745.		
Total	31	1044956			

well for absorbance reading at 620 nm using a Tecan – Sunrise™ absorbance reader.

Parameters analysed included sugar concentrations, sugar contents in the stems, number of grains/spike (i.e. grains/spike), grain weight, grain yield/spike, number of spikes per m^2 and grain yield per m^2.

Statistical Analysis

Data were analysed using GENSTAT (15th edition) statistical package and means compared using least significant differences (LSD at $p = 0.05$).

RESULTS

Sugar concentrations used in calculating the stem sugar contents were determined at the two growth stages. The analysis of variance for sugar contents at anthesis indicated that there were significant cultivar and CO_2 effects (Table 1). SB62 had significantly ($p< 0.001$) higher WSC than SB03 with more sugars having been stored under eCO_2 ($p < 0.05$) than under aCO_2 treatment. Water treatment was non-significant as well as all the interactions. Even at 18 DAA, SB62 had significantly ($p < 0.001$) higher stem sugar contents than SB03 (Figure 1). The other main effect that was significant was water ($p < 0.05$). However, the Water x Cultivar interaction was not significant but the CO_2 x Water x Cultivar interaction was ($p < 0.05$).

Water soluble carbohydrates dynamics

Table 2 indicates WSC dynamics between anthesis and 18 DAA with remobilisation occurring only in the stems of plants under RF conditions and net accumulation in those under SI conditions. This was irrespective of the other independent factors (both cultivar and CO_2 treatments). However, there were large differences in percentages of WSC remobilised; with SB03 remobilising more than eight times (-25%) of its WSC under aCO_2 than SB62 (-3%). On the other hand, SB62 remobilised a higher percentage (more than two times under RF) of its WSC under eCO_2 conditions than SB03.

Grain Yield Components**:** The grain yield components that were analysed included grains per spike (grains/spike), grain yield/spike, individual grain weight, spikes/m^2 and yield/m^2.

Grains/Spike**:** Water treatment significantly ($p< 0.001$ at 18 DAA and $p < 0.01$ at physiological maturity) affected the number of grains/spike, with more grains/spike occurring under SI conditions than under RF conditions. And from 18 DAA to physiological maturity, the significance of type of cultivar increased (from $p = 0.022$ to $p = 0.001$) with SB62 developing more grains/spike than SB03 (Figure 2). The levels of CO_2 applied did not influence the number of grains/spike under all treatment combinations. In addition, there were no significant interactions between factors at both growth stages.

Grain Yield/Spike**:** At 18 DAA, grain yield/spike was influenced by genotype. SB62 had a significantly ($p<0.001$) higher grain yield/spike than SB03 (Figure 3). Water and CO_2 treatments effects were not significant as well as all the interactions. However, at physiological maturity, water treatment as well as genotype significantly affected grain yield/spike ($p < 0.05$ and $p < 0.001$, respectively).

Grain Weight: The average grain weight at both 18 DAA and physiological maturity was significantly influenced by cultivar type ($p < 0.01$ and $p < 0.001$, respectively) and not by water and CO_2. (Figure 4).

Spike density: As opposed to all other grain yield components studied, number of spikes per m^2 was the only one where SB03 had a significantly ($p < 0.05$)) higher number of spikes than SB62 (Figure 5). All other studied factors and their interactions did not significantly affect

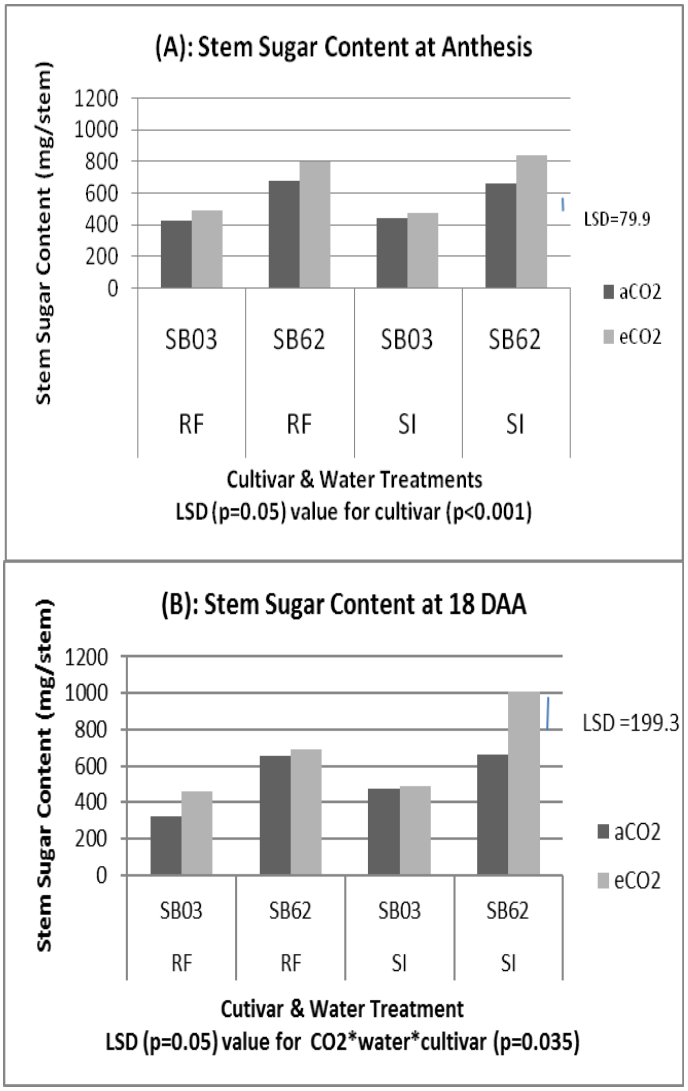

Figure 1: Stem Sugar Content at Anthesis (A) and at 18DAA (B)

the number of spikes per m^2.

Grain yield per M^2: The main factors that significantly influenced grain yield per m^2 were water and CO_2. Under SI conditions, both cultivars accumulated significantly ($p < 0.05$) higher WSC than under RF conditions (Figure 5). In addition, more WSC were accumulated under eCO_2 ($p = 0.041$) than under aCO_2 conditions. Genotype effect was not significant. Table 3 shows the responses of grain yield to eCO_2 and SI.

Potential contribution of stem water soluble carbohydrates to grain yield

The potential contribution of stem WSC to grain yield was obtained by expressing stem sugar content at 18 DAA as a percentage of grain weight at physiological maturity (Table 4). However, this could also have been determined at the anthesis or a later stage than 18 DAA. Although potential contributions were higher for SB62, the values were comparable with those of SB03.

DISCUSSION

WSC Dynamics: The stem sugar contents increased from anthesis to 18 DAA under SI conditions but decreased under RF conditions during the same period in both cultivars. With this trend being observed in both cultivars, it was likely that the maximum stem WSC concentration under RF conditions was attained at anthesis or somewhere close, which would make it consistent with other reports. For example, van Herwaarden et al. (1998) observed that under terminal drought, WSC reached their maximum at anthesis. Ehdaie et al. (2008) found that for plants under water stress, the maximum stem WSC content was reached at 10 DAA while under well-watered conditions it was 20 DAA. Therefore, under well-watered conditions, both cultivars accumulated more sugars in the stems than under water-limited conditions. This should be expected as well-watered environments are favourable for photosynthesis. The photosynthetic rate must have been high enough to satisfy the demand for carbon for grain filling and allow for storage of the rest of it in the stems.

Under RF conditions (water stress) the sharpest percentage decrease in stem WSC (-25% under a CO_2 for SB03) was an indication of senescence which was noticeable in the field at 18 DAA. With a senescing photosynthetic apparatus, stomatal conductance and net CO_2 assimilation are negatively affected (van Herwaarden et al., 1998; Schnyder, 1993). This is the likely explanation for the net remobilisation under RF conditions and suggests that demand for grain filling was not satisfied by assimilation from current photosynthesis alone. Furthermore, the results clearly showed that for both accumulation and remobilisation of stem WSC, SB03 had higher efficiencies than SB62 under aCO_2 conditions, while SB62 ranked higher under eCO_2 conditions. Therefore, judging the two cultivars on the basis of stem WSC dynamics alone between anthesis and 18 DAA, the aCO_2 treatment was more favourable to SB03 than the eCO_2 treatment while the opposite was true for SB62. In fact, SB62 was the more interesting cultivar because it exhibited more WSC remobilisation under RF and eCO2 conditions.

As remobilisation of stem WSC was already underway between anthesis and 18DAA under RF conditions but not under SI conditions, it was an indication that the contribution of stem WSC to grain growth was higher under RF than SI conditions as also revealed in a number of studies in the past (Ehdaie et al., 2008; Ruuska et al., 2006; Yang et al., 2000). Indeed, Yang et al. (2002) found that in situations where senescence was delayed (e.g. due to excessive N application), much of the WSC reserves accumulated remained unused in stems and sheaths and therefore did not contribute to grain filling. However, under SI conditions, it is a desirable characteristic to have delayed senescence as assimilation of more WSC through current photosynthesis contributes to grain filling (Plaut et al., 2004). The net assimilation in stems of plants under SI at 18 DAA showed that the supply of carbon from current photosynthesis was higher than the demand for grain filling

Table 2. Percentage Changes in Average Stem Sugar Contents (mg) between Anthesis and 18 DAA

Factors		SB03			SB62		
CO_2 Treatment	**Water Treatment**	**Anthesis**	**18DAA**	**% Change**	**Anthesis**	**18 DAA**	**% Change**
aCO_2	RF	428.11	322.68	-25	675.33	655.23	-3
	SI	439.90	473.90	+8	655.95	660.00	< +1
eCO_2	RF	486.26	459.04	-6	794.80	691.00	-13
	SI	476.65	485.68	+2	840.56	1005.42	+19

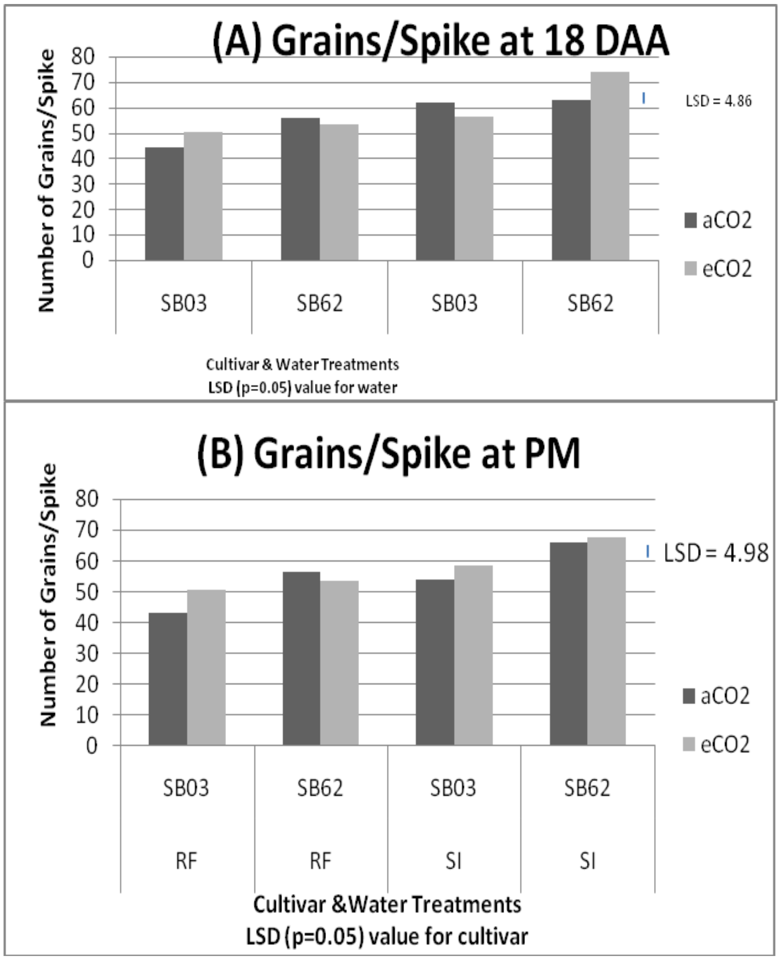

Figure 2: Number of Grains per Spike at 18DAA (A) and Physiological Maturity (B)

and therefore there was no need for remobilisation of stem WSC.

Potential Contribution of WSC to Grain Yield: The potential contribution of stem WSC to grain yield at physiological maturity was in the range of 20 - 32 % (the highest contribution being for SB62 under eCO_2). These values are on a much higher side than for non-stressed conditions (10-20%) cited by Dreccer et al. (2009) and (8-27%) reported by Gebbing and Schnyder (1999), which included both sheaths and stems as opposed to stems only in this study. Therefore, it can be said that on the basis of stem WSC alone in eCO_2 and water-limited environments, SB62 would be a preferable cultivar to SB03. van Herwaarden et al. (1998) found that the capacity to accumulate high WSC minimized negative effects of post-anthesis water stress through increased remobilization.

Yield Components: For number of grains/spike, between 18 DAA and physiological maturity, the number of grains/spike declined. The range of number of grains/spike, which was from 44.4 (SB03) to 74.1 (SB62) at 18 DAA, reduced to 43.1 (SB03) and 67.8 (SB62) at physiological maturity. These were much higher Figures than those recorded by Dreccer et al. (2009) for four SB lines that included SB03 and SB62, grown under fully irrigated and moderate water stress conditions. Nevertheless, the number of grains/spike was more

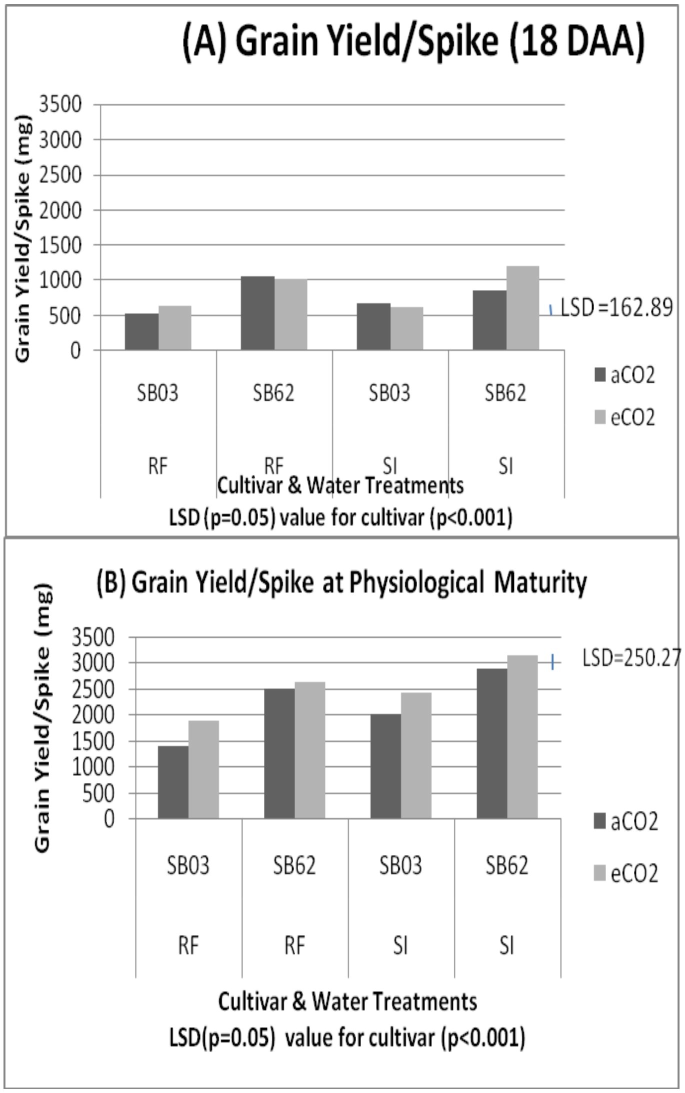

Figure 3: Grain Yield Per Spike at 18DAA (A) and Physiological Maturity (B)

adversely affected by water stress at 18 DAA but by the genotype at physiological maturity. The reason SB62 had more grains/spike than SB03 can be attributed to higher WSC accumulation by the former – there were more sugar reserves available for grain filling for SB62. For both cultivars, the heaviest grains were developed under SI conditions. Well-watered conditions prolong grain filling and thus stimulate large grain development (Li et al., 2000). Thus smaller grains under RF conditions underscore the negative effects water stress has on grain filling and consequently on grain weight. Furthermore, the maximum values of stem WSC accumulated at 18 DAA were 485.68 mg/stem for SB03 and 1005.42 mg/stem for SB62. And relating these amounts to their corresponding numbers of grains/spike at physiological maturity, there was 8.33 mg of stem WSC potentially available per grain for SB03 against 14.83 mg for SB62. Therefore, despite SB62 having had more grains/spike than SB03, there were still more stem reserves (1.78 times higher) potentially available for grain

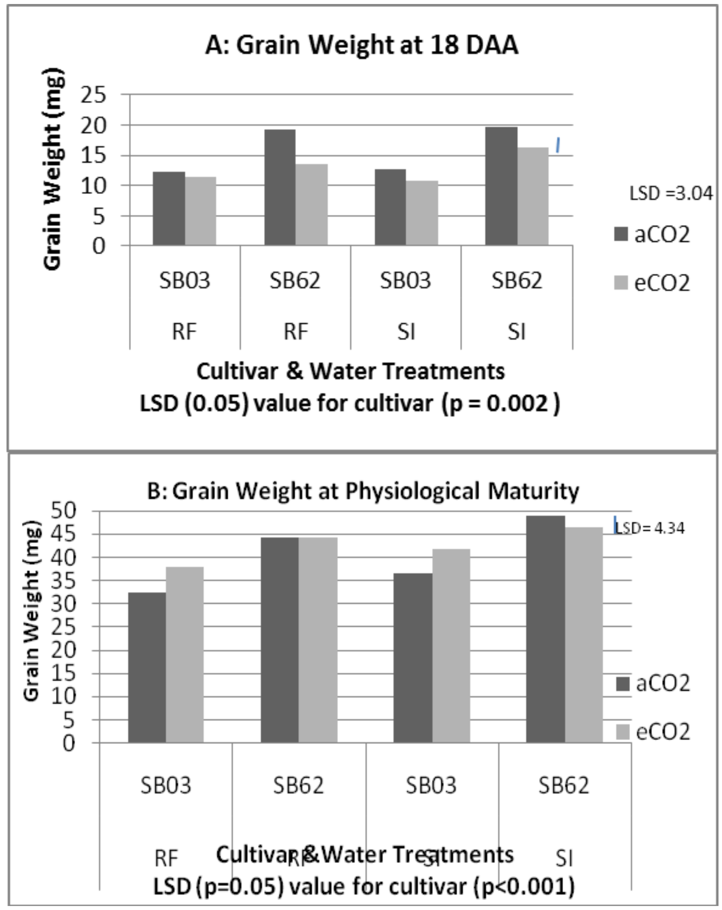

Figure 4: Grain Weight at 18 DAA (A) and Physiological Maturity (B)

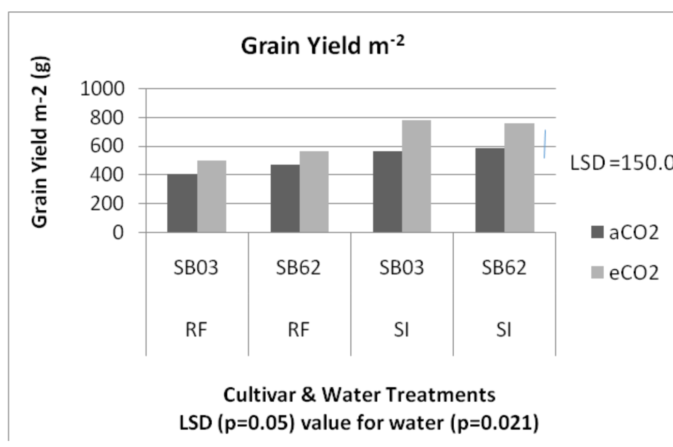

Figure 5: Grain yield per m^2 at Physiological Maturity

filling per grain for SB62 than for SB03. The relationship between stem WSC and grains/spike in this study contrasts with Dreccer et al. (2009) who found that lines with high stem WSC also had lower grain numbers than those with lower stem WSC. This contrast might be due to differences in agro-ecological factors under which the studies were carried out.

Grain Yield/Spike: At 18 DAA, the grain yield/spike for

Table 3. Average grain yield per m^2 (g) stimulation due to eCO_2 and SI

Factors	SB03			SB62		
	aCO_2	**eCO_2**	**Percentage eCO_2 Stimulation (Above aCO_2)**	**aCO_2**	**eCO_2**	**Percentage eCO_2 Stimulation (Above aCO_2)**
RF	408.2	497.1	**+22**	469.02	568.13	**+21**
SI	566.68	781.64	**+38**	590.28	761.05	**+29**
Percentage SI Stimulation (Above RF)	**+39**	**+57**	-	**26**	**34**	**-**

plants grown under the SI treatment was not significantly different from that of plants grown under RF conditions. Most likely, the soil under RF conditions still had adequate plant available water and therefore water stress had not yet become serious. The differences observed in grain yield/spike can therefore be wholly attributed to genotypic effects. The picture did not remain the same by the time of physiological maturity. Moving further away from the beginning of SI application, it is clear that water stress become severe under RF conditions. This explains why water treatment, apart from genotypic effects ($p < 0.001$), became significant ($p = 0.044$) at physiological maturity. This is not surprising since from anthesis to 18 DAA there was net accumulation of stem WSC under SI conditions as opposed to net remobilisation under RF conditions. Crops grown in environments with adequate soil moisture have higher attainable grain yields than those in water-limited environments (Saeidi et al., 2012).

As SB62 had more grains/spike than SB03, it had a larger sink size and therefore a greater demand for carbon. Grain yield sink size is an important factor in determining remobilization of WSC (Blum 1998), therefore there must have been a higher contribution of stem WSC to grain filling for SB62 than for SB03. The larger amount of stem WSC and the larger grain yield sink explain the significantly higher grain yield/spike for SB62. Considering yield performance under drought conditions, higher WSC increased grain yield/spike regardless of the cultivar and of the amount of CO_2 available. This result was also observed in other studies (Ruuska et al., 2006; Yang et al., 2002). Finally, with more grains/spike and heavier grains, it explains why SB62 had higher grain yield/spike than SB03. Grain yield/spike is affected by both grain weight and grain number, both of which are its subcomponents (Saeidi et al., 2012)

The fact that eCO_2 increased the WSC between anthesis and 18 DAA under SI conditions but that eCO_2 did not significantly affect the number of grains/spike as well as grain weight and grain yield may be an indication of its negative effect on photosynthesis. This may relate to the finding by Azcon-Bieto (1983) that increased WSC accumulation in leaves had a negative feedback on net CO_2 assimilation. If Azcon-Bieto (1983) was right, it means that when stem WSC accumulation by plants under eCO_2 reached their maximum, current photosynthesis got inhibited but that is was still more active under aCO_2 conditions. Thus WSC under aCO_2 increased to the same level as under eCO_2 conditions. Consequently, the effect of eCO_2 on these three yield components, as tested on main stem spikes alone, was not statistically different from that of aCO_2.

Soil moisture limits crop response to eCO_2(Sun et al., 2009). In this study, grain yield m^{-2} responded to both eCO_2 and SI. For SB03, irrigation increased grain yield m^{-2} by 39% under aCO_2 against 57% under eCO_2 (Table 3). The figures were lower for SB62 being 26% and 34% respectively. Holding the level of water applied constant, eCO_2 stimulated grain yield m^{-2} by 22% under RF and 38% under SI for SB03, and 21% and 29% respectively for SB62. These comparisons show that the percentage response to eCO_2 was higher under well-watered conditions than under water stress. Reports by Kimball et al. (2002,) and Sun et al. (2009) were contrary, namely that stimulation by eCO_2 under water stress is higher than under well-watered conditions. This finding was consistent with the stem WSC dynamics between anthesis and 18 DAA that indicated net accumulation under SI conditions as opposed to net remobilisation under RF conditions (Table 2). This shows that current photosynthesis was still more active under SI but negatively affected under RF.

Besides the stem WSC at both anthesis and 18 DAA, SB62 also ranked higher than SB03 on all the yield components considered above except for spikes m^{-2}. That tendency was also observed for grain yield m^{-2} but the difference was not significant. Apart from stem WSC and grain yield m^{-2}, other dependent variables were not affected by the level of CO_2 applied. Results in this study were all based on main stem spikes, with the exception of grain yield m^{-2} and spikes m^{-2}.

With grain yield m^{-2} responding to eCO_2, it implies that tiller spikes had a significantly higher response to eCO_2 than main stem spikes. There were definitely more tiller spikes than main stem spikes m^{-2}. This inconsistency in grain yield response to eCO_2 between main stem and tiller spikes in wheat was also observed by Li et al. (2000). But whether this was due to increased WSC accumulation in tillers or not warrants further investigation.

Although grain yield m^{-2} responded to eCO_2, the cultivars did not. SB62 had significantly higher grain yield/spike, but this ranking, based on the capacity to accumulate more WSC, was diminished by the propensity for SB03 to develop a higher spike density (due to its higher tillering ability than SB62). Consequently, the grain yield m^{-2} was not significantly different between the two cultivars. It can be said that if the two cultivars had the same number of spikes m^{-2}, SB62 was going to have a significantly higher grain yield m^{-2} than SB03.

Agronomic implications of WSC and spike density relationship: As noted, SB62 had less spikes m^{-2}, which according to Dreccer et al. (2009) is characteristic of lines with higher stem WSC. This negative relationship between higher stem WSC and spikes m^{-2} was also reported by Rebetzke et al. (2008). SB62 appears promising for water-limited regions such as Australia, because it has a lower number of spikes m^{-2} and a higher stem WSC. In such regions, having a combination of a higher number of spikes m^{-2} and lower stem WSC would result in poorer grain filling in times of post- anthesis water-stress. Where grain filling is poor, grain yield is expected to have a large percentage of screenings. But in well-watered environments, lines like SB62 would need to be sown at higher sowing rates to obtain similar spike densities to existing free-tillering cultivars like SB03.

In conclusion, the role of stem WSC in maintaining grain yield in two wheat lines (SB03 and SB62) under drought by eCO_2 interactions was studied. The study showed that stem WSC contributed to grain yield under all conditions and not specifically under drought by eCO_2 interactions. Stem WSC potentially contributed 20 to 32 % of grain yield/spike (the highest contribution was for SB62 under eCO_2). Accumulation of stem WSC was responsive to eCO_2 and SI with SB62 accumulating higher WSC than SB03. Grain yield m^{-2} was the only yield component that also responded to both eCO_2 and SI but the findings showed that it was not due to high WSC. This was apparently attributed to the fact that SB03 had a significantly higher spike density that compensated for lower stem WSC and lower grain yield/spike. Therefore WSC will still play a role in wheat adaptation for drought environments. However, for the grain yield per unit area of high WSC cultivars like SB62 to respond to eCO_2, the agronomic implication is that sowing rates would need to be increased in order to obtain higher spike densities similar to the free-tillering lines like SB03.

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Competing interests

The authors declare that they have no competing interests

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