Contents lists available at ScienceDirect

Journal of Cereal Science

journal homepage: www.elsevier.com/locate/jcs

Effect of elevated carbon dioxide on plant biomass and grain protein concentration differs across bread, durum and synthetic hexaploid wheat genotypes

Michael Thompson^a, Dananjali Gamage^{a,b}, Disna Ratnasekera^b, Anton Perera^b, Anke Martin^a, Saman Seneweera^{a,c,*}

^a Centre for Crop Health, University of Southern Queensland, Toowoomba, QLD, Australia

^b Faculty of Agriculture, University of Ruhuna, Mapalana, Sri Lanka

^c National Institute of Fundamental Studies, Kandy, Sri Lanka

ARTICLE INFO

Keywords: Future climate Total grain protein Climate stress Shoot biomass

ABSTRACT

Atmospheric carbon dioxide conditions predicted for future climates cause increases in wheat biomass, but also decreases wheat grain protein concentration. We investigated the response of grain protein concentration of wheat to elevated carbon dioxide in nineteen wheat genotypes, including five tetraploid, eleven hexaploid and three synthetic hexaploid genotypes to test whether decreased grain protein is genotype dependent and whether it is caused by biomass dilution. These were grown in ambient and elevated carbon dioxide conditions simultaneously. Shoot biomass and grain samples were taken at maturity. The grain protein concentration, grain biomass, shoot biomass and harvest index were analysed for each genotype. Despite most genotypes and total grain protein (g), the majority of genotypes decreased in grain protein concentration (%) under elevated carbon dioxide. Elevated carbon dioxide caused an increase in grain biomass for all genotypes and total shoot biomass for most genotypes, with harvest index increasing for all genotypes were not statistically significant, suggesting that the individual genotype of wheat plants determines the response to elevated carbon dioxide rather than the wheat type.

1. Introduction

One of the main components of global climate change is the increasing concentration of carbon dioxide (CO₂) in the atmosphere. Under future climates, the increased atmospheric CO₂ concentration ([CO₂]) will directly affect the yield, growth and development of crop plants (Ainsworth and Long, 2005; Leakey et al., 2009). For wheat (*Triticum aestivum*), although elevated [CO₂] (e[CO₂]) usually improves plant biomass and grain yield (Thilakarathne et al., 2013), the nutritional aspects of the grain suffer the opposite effect, where the concentration of protein and many macro and micronutrients declines (Fernando et al., 2012). With the global human population expected to increase, there will be a greater demand on food production. As such, the effect of climate change on food crops is of great concern.

Wheat is one of the most important food crops in the world,

accounting for nearly a third of the global cereal production in the 2015/2016 season (FAO, 2017). Wheat species typically belong to three different ploidy levels, consisting of diploids (2n = 2x = 14), tetraploids (2n = 4x = 28) and hexaploids (2n = 6x = 42). The hexaploid wheat genome is comprised of seven pairs of chromosomes each in three genomes, called the A, B and D genomes. Hexaploid wheat was created from the hybridisation of the tetraploid *T. turgidum* (containing the A and B genomes) with the D donor *Aegilops tauschii* (Matsuoka, 2011). Synthetic hexaploid wheat is created by hybridising these two species, followed by amphidiploidisation (Yang et al., 2009). With this method, breeders are able to develop synthetic hexaploid wheat genotypes which incorporate genes from *T. turgidum* and *Ae. tauschii* that were not maintained during hexaploid wheat evolution, including traits such as drought tolerance (Reynolds et al., 2007), increased nutrient uptake (Calderini and Ortiz-Monasterio, 2003) and pathogen resistance

https://doi.org/10.1016/j.jcs.2019.03.007

Received 3 August 2018; Received in revised form 4 March 2019; Accepted 9 March 2019 Available online 14 March 2019 0733-5210/ © 2019 Elsevier Ltd. All rights reserved.







Abbreviations: e[CO2], elevated carbon dioxide concentration; a[CO2], ambient carbon dioxide concentration; HI, harvest index; GPC, grain protein concentration; GPY, grain protein yield

^{*} Corresponding author. Centre for Crop Health, University of Southern Queensland, Toowoomba, QLD, Australia. *E-mail address:* saman.seneweera@usq.edu.au (S. Seneweera).

(Wang et al., 2016). These synthetic hexaploids can then be crossed with bread wheat cultivars to transfer across the elite genes and improve upon the bread wheat cultivar (Li et al., 2014).

Growth under $e[CO_2]$ causes increased yields in wheat (Amthor, 2001; Högy et al., 2009), but many studies have shown that it also causes a decline in nitrogen stored in the grain at maturity (Taub et al., 2008; Högy et al., 2013; Fernando et al., 2015). Protein composition of wheat grain grown under $e[CO_2]$ is also affected, resulting in lower bread making quality in some cultivars (Fernando et al., 2015). Of the proteins in the grain, storage proteins (glutens), rather than structural or metabolic proteins, appear to be the most affected by $e[CO_2]$ (S. Arachchige et al., 2017).

Previous studies have looked at the effect of $e[CO_2]$ across diploid, tetraploid and hexaploid wheat species (Sinha et al., 2009; Uprety et al., 2009). Uprety et al. (2009) observed that the responses of each species to $e[CO_2]$ was different depending on the physiological variable measured. For example, variables such as photosynthesis, leaf area, dry weight, grain yield and harvest index (HI) had a greater response to e $[CO_2]$ in hexaploids and tetraploids than diploids. Sinha et al. (2009) also found differing responses of each ploidy level for their variables studied. Protein concentration in grains decreased for all ploidy levels, though the decrease was lowest in tetraploids and highest in hexaploids. How synthetic wheat responds to $e[CO_2]$ has not previously been determined.

A major goal for wheat breeders has been to develop cultivars with improved HI. As such, identifying wheat with a high HI is important for the continual improvement of commercial wheat cultivars. Elevated [CO₂] increases both the grain yield (Amthor, 2001) and shoot biomass (Kimball, 2016) of wheat, with the ratio of these two components determining the plant's HI. The stimulation of both biomass and yield at the same magnitude can lead to no change in HI, which has been seen in both hexaploid bread wheat and tetraploid durum wheat (Wang et al., 2013: Aranjuelo et al., 2015: Fitzgerald et al., 2016). Furthermore, some studies have shown HI to both increase and decrease in some wheat cultivars (Uddling et al., 2008; Wang et al., 2013). Thilakarathne et al. (2013) found that increases in grain yield are associated with increases to leaf mass area due to e[CO2]. As such, the degree that e [CO₂] increases grain yield, and in turn HI, may rely partly on how leaf mass area is affected. Increased HI, however, may lead to decreased grain protein concentration (GPC) in wheat due to dilution of N with increased carbohydrates (Taub et al., 2008).

In this study, we aimed to identify whether the effect of $e[CO_2]$ on wheat GPC is dependent on wheat type and whether GPC decline is affected by HI and/or biomass dilution. We also investigated how e $[CO_2]$ affects the GPC of synthetic hexaploid wheats. To achieve these aims we grew nineteen wheat cultivars under $e[CO_2]$ and $a[CO_2]$, consisting of five tetraploid, eleven hexaploid and three synthetic hexaploid genotypes, and analysed their biomass and protein content. One-Way ANOVA analysis was used to determine the significance of $[CO_2]$ on the traits measured in the study.

2. Materials and methods

2.1. Plant material and growth conditions

Nineteen wheat genotypes were grown in an environmentally controlled glasshouse at the University of Southern Queensland, Toowoomba, Queensland, Australia, in 2014. The genotypes consisted of five tetraploid durum wheats (Tjilkuri, WID802, Hyperno, Jandaroi and Caparoi), eleven hexaploid bread wheats (Hartog, Sunbri, Longreach Dart, Sunvale, Longreach Crusader, Aus29259, LRC2010-157, Longreach Scout, Longreach Lincoln, Sunguard and Longreach Spitfire) and three synthetic hexaploid wheats (CPI133814, CPI133811 and CPI133898) (Supplementary Table 1). All three synthetic hexaploid genotypes share a similar pedigree, with CPI133814 and CPI133811 being derived from the same parental cultivars. The number of



Fig. 1. Effect of $e[CO_2]$ on A) GPC and B) total grain protein. Data represents the difference between $a[CO_2]$ and $e[CO_2]$ values. Positive values indicate greater values for $e[CO_2]$ than $a[CO_2]$. Genotypes are organised into tetraploids (Caparoi, Jandaroi, WID802, Hyperno and Tjikuri), hexaploids (Sunbri, Spitfire, Lincoln, Hartog, Crusader, Scout, Sunvale, LRC2010-157, Aus29259, Dart and Sungard) and synthetic hexaploids (CPI133814, CPI133898, CPI133811).

genotypes used for each wheat type was limited by the availability of genotypes at the time of the experiment and therefore, is not consistent among wheat types.

The average day/night temperatures of the glasshouse chambers were maintained at 20 ± 2 °C and 17 ± 2 °C, respectively, with 60–70% relative humidity. During the experiment, the light inside the glasshouse ranged from 700 to 1000 µmol m⁻² s⁻¹ during midday. A large compartmented glasshouse was used, where ambient [CO₂] (a [CO₂]) (~389 µmol mol⁻¹) and e[CO₂] (~700 ± 20 µmol mol⁻¹) were maintained in their respective treatments. All nineteen genotypes were grown in both CO₂ conditions (a[CO₂] & e[CO₂]) at the same time. For each CO₂ treatment all genotypes were grown as four replicates within the same glasshouse chamber, where each replicate consisted of one pot containing four plants. Seeds were pre-germinated and planted into pots containing 2.5 kg top soil. Each pot had a diameter of 175 mm

Table 1

Grain protein concentration (%) and total grain protein (g) of tetraploid, hexaploid and synthetic hexaploid genotypes in ambient [CO₂] and elevated [CO₂]. ANOVA results show differences between each wheat type. Data represent values per plant. Abbreviations: P_T , AVOVA results against tetraploids; P_H , ANOVA results against hexaploids; P_S , ANOVA results against synthetic hexaploids; ns, not significant; *, $P \le 0.05$; **, $P \le 0.01$.

Wheat type	Genotype	Grain Pro		Total Grain Protein (g)										
					ANO	VA						ANOVA		
		e[CO ₂]	a[CO ₂]	e[CO ₂] – a[CO ₂]	\mathbf{P}_{T}	\mathbf{P}_{H}	$\mathbf{P}_{\mathbf{S}}$	e[CO ₂]	a[CO ₂]	e[CO ₂] – a[CO ₂]	\mathbf{P}_{T}	\mathbf{P}_{H}	$\mathbf{P}_{\mathbf{S}}$	
Tetraploid	Caparoi	11.08	13.80	-2.72	-	ns	ns	1.67	1.40	0.27	-	ns	ns	
	Jandaroi	17.42	19.66	-2.24				3.22	2.12	1.10				
	WID802	11.86	13.16	-1.30				2.29	1.51	0.78				
	Hyperno	11.83	12.13	-0.30				2.33	1.53	0.80				
	Tjilkuri	18.49	13.89	4.61				3.64	1.44	2.20				
Hexaploid	Sunbri	9.68	14.72	- 5.04	ns	-	ns	2.56	2.07	0.49	ns	-	ns	
	Spitfire	11.81	15.11	-3.30				2.08	1.78	0.29				
	Lincoln	12.11	15.33	-3.22				2.13	2.57	-0.44				
	Hartog	11.13	14.01	-2.89				2.30	1.78	0.52				
	Crusader	13.86	15.47	-1.61				2.98	2.15	0.83				
	Scout	11.90	13.40	-1.51				2.47	2.12	0.34				
	Sunvale	13.65	13.70	-0.05				2.43	1.53	0.89				
	LRC/2010/157	13.29	12.78	0.51				2.69	2.00	0.69				
	Aus 29259	13.66	12.92	0.74				2.80	1.90	0.90				
	Dart	16.17	15.42	0.76				2.83	1.78	1.05				
	Sunguard	14.37	12.54	1.83				1.65	1.26	0.39				
Synthetic Hexaploid	CPI 133814	14.53	14.55	-0.02	ns	ns	-	1.78	1.29	0.49	ns	ns	-	
	CPI 133898	12.09	12.29	-0.20				1.02	0.74	0.28				
	CPI 133811	19.62	15.54	4.08				2.46	1.56	0.90				

and were placed with 15 cm between each pot, resulting in a plant density of 36 plants m^{-2} . All pots were randomized and rearranged weekly to eliminate chamber effects.

2.2. Biomass analysis

Plants were sampled at physiological maturity and separated into leaf blades, stems (including sheaths) and heads, and dried at 60 °C for 48 h. Heads were hand threshed to obtain the grain. The grains were weighed to obtain total seed weight. Plant tissues were weighed to obtain total shoot biomass. Grain number per plant was counted.

2.3. Nitrogen analysis

Grain from each genotype was ground using a Millser IFM-800DG grinder (Iwatani, Japan). A 100 mg sample of the ground grain was analysed for its nitrogen concentration using a CN analyser (LECO CN628 analyser, Michigan, USA). The total GPC was calculated by multiplying the total N concentration of grain by the conversion factor of 5.7. Protein content was analysed by multiplying the GPC by total grain biomass.

2.4. Harvest index

Harvest index was calculated by dividing the total grain biomass by the total plant biomass (grain biomass + shoot biomass):

2.5. Statistical analysis

Statistical analysis to determine significant difference between means of the dependent variables (Grain biomass, total shoot biomass, GPC and HI) was performed using Compare Means in IBM SPSS Statistics ver. 23 (IBM Corp., 2015, Armonk, NY). Statistical significance was determined using a One-Way ANOVA with the wheat type (tetraploid, hexaploid and synthetic hexaploid) as the independent factor and CO₂ response (GPC, grain biomass, total shoot biomass and HI) as the dependent variable. CO₂ response was calculated as the difference between the e[CO₂] data and the a[CO₂] data. Results were regarded as significant at $P \leq 0.05$.

IBM SPSS Statistics ver. 23 was also used to perform a Pearson product-moment correlation test using Correlate to analyse any correlation between grain biomass and total grain protein. Statistical significance was determined with Bivariate Correlations using grain biomass and total grain protein as the variables. Results were regarded as significant at $P \leq 0.01$.

3. Results

3.1. Grain protein concentration and total grain protein

Of the 19 genotypes tested thirteen had a lower GPC under $e[CO_2]$ compared to $a[CO_2]$ (Fig. 1, Table 1). Among the tetraploid wheat genotypes, Caparoi, Jandaroi, WID802 and Hyperno had a lower GPC in plants grown under $e[CO_2]$. Similarly, of the hexaploid genotypes, GPC declined in Sunbri, Spitfire, Lincoln, Hartog, Crusader, Scout and Sunvale, while it also declined in the synthetic hexaploid genotypes CPI133814 and CPI133898 under $e[CO_2]$ (Fig. 1). Despite the majority of genotypes decreasing in GPC under $e[CO_2]$, however, a paired-samples *t*-test found no significant difference between $e[CO_2]$ and a $[CO_2]$ grown wheats for any of the wheat types.

Tetraploids showed the largest variation of GPC between the least and most responsive genotypes in response to $e[CO_2]$ between the three wheat types, with Caparoi incurring the greatest reduction of GPC among tetraploids. The largest decrease in GPC occurred in the hexaploid genotype Sunbri where the GPC at $e[CO_2]$ was 5.04% lower than at $a[CO_2]$. On average, synthetic hexaploids had the least decline in GPC under $e[CO_2]$, while hexaploids incurred the greatest reduction of GPC. On the other hand, some genotypes in each wheat type increased in GPC. For both tetraploids and synthetic hexaploids only one genotype was found to increase in GPC under $e[CO_2]$, while there were four hexaploid genotypes. Statistical analysis by One-Way ANOVA showed that the effect of $e[CO_2]$ on GPC was not significantly different between the tetraploid, hexaploid and synthetic hexaploid wheat genotypes examined in this study.

Despite the majority of genotypes decreasing in GPC in response to e $[CO_2]$, the total grain protein content increased in 18 out of 19 genotypes when grown under $e[CO_2]$ (Fig. 1, Table 1). Only Lincoln (a hexaploid genotype) decreased in total grain protein content. Similarly





Fig. 2. Effect of $e[CO_2]$ on A) grain biomass and B) grain number. Data represents the difference between a[CO2] and $e[CO_2]$ values. Positive values indicate greater values for $e[CO_2]$ than $a[CO_2]$. Genotypes are organised into tetraploids (Caparoi, Jandaroi, WID802, Hyperno and Tjikuri), hexaploids (Sunbri, Spitfire, Lincoln, Hartog, Crusader, Scout, Sunvale, LRC2010-157, Aus29259, Dart and Sungard) and synthetic hexaploids (CPI133814, CPI133898, CPI133811).

to GPC response, Tjilkuri increased the most in total grain protein content when grown in $e[CO_2]$ (Fig. 1). For those genotypes which increased in protein in response to $e[CO_2]$, total grain protein content of tetraploids increased on average by 1.03g and by 0.64g and 0.55g for hexaploids and synthetic hexaploids, respectively. There was no significant difference between wheat types for total grain protein, however, a pairwise *t*-test showed that $[CO_2]$ significantly affected the tetraploids (p = 0.033) and hexaploids (p = 0.001).

3.2. Grain biomass and number

Total grain biomass increased in all genotypes grown under e[CO₂]

compared to a[CO₂] (Fig. 2, Table 2). In addition, the grain number per plant also increased in all genotypes (Table 2). On average, e[CO₂] stimulated the greatest increase in grain biomass for tetraploid genotypes compared to both hexaploid and synthetic hexaploid genotypes, with the increase in tetraploids averaging about 67% compared to 44% for hexaploids and 34% for synthetic hexaploids. Tetraploids also had, on average, a greater response of grain number. Both of the genotypes which displayed the greatest and least increase in grain biomass, respectively, were Sunbri and Lincoln, both of which are hexaploids (Fig. 2). Despite the differences in average grain biomass response to e [CO₂], there was no significant difference between the three groups. On the other hand, the three synthetic hexaploid genotypes were significantly different in grain number compared to both tetraploids and hexaploids. A paired-samples t-test revealed that the effect of e[CO₂] on grain biomass was significant for all three wheat types (tetraploids, p < 0.001; hexaploids, p < 0.001; synthetic hexaploids, p = 0.012). This was also the case for grain number (tetraploids, p = 0.043; hexaploids, p < 0.001; synthetic hexaploids, p = 0.002). Pearson productmoment correlation analysis revealed that there was a moderately positive correlation between grain biomass and total grain protein (r = 0.584, n = 19, p = 0.009). Grain number had a strong positive correlation with both grain biomass (r = 0.820, n = 19, p < 0.001) and HI (r = 0.698, n = 19, p = 0.001), while there was a moderately negative correlation with shoot biomass (r = -0.494, n = 19,p = 0.032).

3.3. Total shoot biomass

Total shoot biomass increased in all genotypes grown under $e[CO_2]$ compared to $a[CO_2]$ except for the tetraploid wheat Jandaroi (Fig. 3, Table 3). Synthetic hexaploid genotypes had the greatest increase in total shoot biomass under $e[CO_2]$, with an average increase of 71.1%, compared to tetraploids and hexaploids, which increased on average by 17.4 (excluding Jandaroi) and 14.9%, respectively. Among the synthetic hexaploids CPI133811 increased the most under $e[CO_2]$. Statistical analysis by One-Way ANOVA found that synthetic hexaploids were significantly different from hexaploids (P = 0.001) and tetraploids (P = 0.002), however, no significant difference was found between tetraploids and hexaploids. A paired-samples *t*-test found that the effect of $e[CO_2]$ on total shoot biomass was only significant for hexaploids (p < 0.001).

3.4. Harvest index

Harvest index increased in all plants when grown under e[CO₂] compared to $a[CO_2]$, except for the two synthetic hexaploid genotypes CPI133814 and CPI133811 (Fig. 3, Table 3). These synthetic hexaploid genotypes share the same pedigree. The genotype with the greatest increase in HI under e[CO2] was the tetraploid genotype WID802, with an increase of 26.28%. Of the two genotypes which declined in response to e[CO₂], CPI133814 declined the most, decreasing in HI by 19.25%. The tetraploids had an average increase in HI of 12.84%, while hexaploids increased on average by 6.23%. On the other hand the synthetic hexaploids had an average decrease of 15.51%, excluding CPI133898, which increased in HI in response to e[CO₂] by 1.20%. Like total shoot biomass, statistical analysis by One-Way ANOVA found that synthetic hexaploids were significantly different from hexaploids (P = 0.007) and tetraploids (P = 0.001), however, no significant difference was found between tetraploids and hexaploids. Paired-samples t tests were carried out for each wheat type, which revealed the effect of $e[CO_2]$ to be significant for both tetraploids (p = 0.022) and hexaploids (p = 0.001), but not for synthetic hexaploids (p = 0.219). In addition, we also analysed the correlation between HI and GPC. Statistical analysis by Pearson product-moment correlation found there was no significant correlation between these two variables.

Table 2

Grain biomass (g) and grain number of tetraploid, hexaploid and synthetic hexaploid genotypes in ambient [CO₂] and elevated [CO₂]. ANOVA results show differences between each wheat type. Data represent values per plant. Abbreviations: P_T , AVOVA results against tetraploids; P_H , ANOVA results against hexaploids; P_S , ANOVA results against synthetic hexaploids; ns, not significant; *, $P \le 0.05$; **, $P \le 0.01$.

Wheat type	Genotype	Grain Biomass (g)					Grain Number							
						VA						ANOVA		
		e[CO ₂]	a[CO ₂]	e[CO ₂] – a[CO ₂]	\mathbf{P}_{T}	\mathbf{P}_{H}	$\mathbf{P}_{\mathbf{S}}$	e[CO ₂]	a[CO ₂]	e[CO ₂] – a[CO ₂]	\mathbf{P}_{T}	\mathbf{P}_{H}	$\mathbf{P}_{\mathbf{S}}$	
Tetraploid	Caparoi	15.07	10.15	4.92	-	ns	ns	262.00	182.75	79.25	-	ns	ns	
	Jandaroi	18.48	10.78	7.70				296.00	221.75	74.25				
	WID802	19.35	11.50	7.85				397.50	317.50	80.00				
	Hyperno	19.70	12.65	7.05				417.25	308.75	108.50				
	Tjilkuri	19.70	10.40	9.30				388.75	239.50	149.25				
Hexaploid	Sunbri	26.43	14.07	12.36	ns	-	ns	639.50	491.67	147.83	ns	-	ns	
	Spitfire	17.58	11.80	5.78				356.00	288.00	68.00				
	Lincoln	17.60	16.75	0.85				396.75	346.00	50.75				
	Hartog	20.68	12.73	7.95				391.75	292.50	99.25				
	Crusader	21.50	13.90	7.60				456.50	325.50	131.00				
	Scout	20.73	15.83	4.90				360.75	294.75	66.00				
	Sunvale	17.78	11.20	6.58				357.25	301.75	55.50				
	LRC/2010/157	20.23	15.67	4.56				422.00	381.75	40.25				
	Aus 29259	20.50	14.70	5.80				303.50	301.75	1.75				
	Dart	17.50	11.55	5.95				455.75	386.25	69.50				
	Sunguard	11.50	10.08	1.43				238.25	233.50	4.75				
Synthetic Hexaploid	CPI 133814	12.23	8.88	3.35	ns	ns	-	198.75	186.25	12.50	ns	ns	-	
	CPI 133898	12.52	10.03	2.50				308.00	307.00	1.00				
	CPI 133811	8.40	6.00	2.40				172.50	157.75	14.75				

4. Discussion

It is currently unclear to which extent e[CO₂] affects different types of wheat, and thus our research aimed to investigate how the GPC of wheat is affected by e[CO₂] across three different wheat types: tetraploid, hexaploid and synthetic hexaploid. In addition, we aimed to investigate the relationship between GPC, HI and biomass of each wheat type, in order to elucidate the mechanism behind GPC decline under e [CO₂]. Our results suggest that rather than the wheat type determining GPC, it is specific genotypes within and between wheat types that determine GPC. On the other hand, we found that the HI of the studied wheat genotypes was significantly different between tetraploids and synthetic hexaploids, showing that while GPC may not be affected by type specific differences, wheat type may affect HI. Our results did not show any significant link between GPC and HI. As the number of genotypes differed between each wheat type, these results may have varied if further genotypes were available for analysis. In particular, the similar pedigree shared by the synthetic hexaploids limits the applicability of these results for other genotypes, which may show a larger variability in the traits studied.

We examined the response of GPC, grain biomass, total shoot biomass and HI to e[CO₂] for 19 wheat genotypes, consisting of five tetraploids, eleven hexaploids and three synthetic hexaploids. Overall, the majority of genotypes decreased in GPC. This is a typical response of bread wheat to e[CO₂] (Taub et al., 2008) and along with lower pasta quality, GPC has also been found to decrease in tetraploid wheat under e[CO₂] (Fares et al., 2016). However, GPC response to e[CO₂] is scarcely studied in tetraploid wheats, and to our knowledge, this is the first study to observe the effect of e[CO₂] on the GPC of synthetic hexaploids. The synthetic hexaploids in this study, however, share similar pedigrees, which may not represent other synthetic hexaploids of more diverse origins. The effect of e[CO₂] on GPC was not consistent within each wheat type. While most genotypes decreased in GPC, at least one genotype of each type increased in GPC. Tjilkuri was the only tetraploid genotype to increase in GPC and although it had the greatest increase of all genotypes, there were more hexaploid genotypes than tetraploid for which we observed an overall increase in GPC. As such, our study can't confirm the results of Sinha et al. (2009), who found tetraploid wheat had the lowest decline in GPC compared to hexaploid and diploid

wheats, however, these differences can likely be attributed in part to the variation in number of genotypes studied. When looking solely at the genotypes which decreased in GPC, we found that the decrease in GPC for hexaploids on average was greater than for tetraploids, thus supporting Sinha et al. (2009). This study was limited to the availability of genotypes and experimental conditions. For a more comprehensive picture, more genotypes need to be studied in addition to a greater number of replicates. Furthermore, repeating this experiment using Free-air CO₂ Enrichment (FACE) facilities would allow the comparison of glasshouse experiments with field data.

Despite the amount of genotypes with lower GPC under e[CO₂], nearly all genotypes (16 of 19) increased in total grain protein (g) per plant. We found that there was a significant correlation between grain biomass and total grain protein. This suggests that as the grain biomass increases due to the greater carbon availability from e[CO₂], the plant remobilizes or takes up greater amounts of nitrogen to the grain. However, even though most genotypes have greater grain protein and biomass under e[CO₂], there is a wide variation in GPC. Most genotypes have a lower GPC under e[CO₂], which means that while more protein is being stored in grains of plants grown under e[CO₂] than a[CO₂], the stimulation of grain biomass is too great for nitrogen uptake, transport or assimilation to keep up with. A study by Pleijel and Uddling (2011) observed a growth dilution effect on grain protein accumulation due to e[CO₂], where the increase in grain protein yield (GPY; the amount of grain protein accumulated per unit area) was lower than the increase in grain yield. Furthermore, GPC also declined as grain yield increased. An important observation, however, was that the decline in grain protein could not be completely explained by growth dilution, as e[CO₂] negatively affected GPY and GPC in plants with no increase in grain yield. For our study, we acknowledge the variation in number of genotypes per wheat type in this experiment and as such, a larger number of cultivars is needed to further support this conclusion. Repeating this experiment under FACE conditions with larger numbers of plants per genotype would give more conclusive data relevant to plants grown under field conditions.

Synthetic hexaploids differ from other wheats in that they are derived from crosses between *Ae. tauschii* and *T. turgidum*. The resulting GPC of each synthetic hexaploid then, is likely dependent on the responsiveness of both parent genotypes to $e[CO_2]$. For instance, the



Fig. 3. Effect of e[CO₂] on A) total shoot biomass and C) HI. Data represents the difference between a[CO2] and e[CO2] values. Positive values indicate greater values for e[CO2] than a[CO2]. Genotypes are organised into tetraploids (Caparoi, Jandaroi, WID802, Hyperno and Tjikuri), hexaploids (Sunbri, Spitfire, Lincoln, Hartog, Crusader, Scout, Sunvale, LRC2010-157, Aus29259, Dart and Sungard) and synthetic hexaploids (CPI133814, CPI133898, CPI133811).

increased GPC of CPI133811 is likely a trait inherited from either its Ae. tauschii parent or the T. turgidum parent. As it shares the same T. turgidum parent as the other synthetic hexaploids in this study, it is likely to be a trait derived from its Ae. tauschii parent. As such, identifying the GPC response to e[CO₂] for the parents of each synthetic hexaploid examined in this study could explain the differences in CO₂ responsiveness of the synthetic hexaploids. In order to develop more synthetic hexaploid cultivars with improved responsiveness to e[CO₂], it may be crucial to screen genotypes of Ae. tauschii and T. turgidum to identify genotypes with high GPC responses to e[CO₂] that could allow breeders to develop synthetic hexaploid lines with a similar responsiveness. These elite synthetic hexaploid cultivars could then be crossed with bread wheat cultivars to transfer the improved traits, as has been done for other types of traits (Li et al., 2014). Synthetic hexaploids could be a solution for improving the bread-making quality of bread wheat grown under e[CO2]. Ae. tauschii has displayed a greater variety in high

molecular weight and low molecular weight glutenin subunits, encoded by the $Glu-D^t1$ and $Glu-D^t3$ loci respectively, compared to the glutenin subunits of bread wheat (Pflüger et al., 2001). Within this variety of alleles in *Ae. tauschii* we may find alleles capable of overcoming the poorer bread-making quality of wheat grown under e[CO₂].

One of the main hypotheses explaining the decline in grain protein is the dilution hypothesis, whereby e[CO₂] causes greater biomass stimulation in wheat compared with that observed under a[CO₂], and this increase is too great for the uptake and assimilation of N to keep up with (Taub et al., 2008). We investigated whether HI, a measurement of biomass allocation, was linked with GPC across tetraploid, hexaploid and synthetic hexaploid wheats. Harvest index, like total grain protein, increased in the majority of genotypes (17 of 19). Unlike total grain protein, however, where a tetraploid, hexaploid and synthetic hexaploid decreased under e[CO2] (Jandaroi, Lincoln and CPI133811, respectively), the two genotypes which declined in HI were both synthetic hexaploids (CPI133814 and CPI133811). This is likely due to both genotypes sharing the same pedigree. We found that there was a significant difference between tetraploids and synthetic hexaploids, however, hexaploids were not found to be significantly different to either of the other wheat types. This may be influenced by the lower number of genotypes for both the tetraploids and synthetic hexaploids than for the hexaploids. Harvest index is determined by the total shoot biomass and total grain biomass of the plant. These components are each affected by e[CO₂], which means that HI will be determined by the extent that either component is affected. For example, a genotype which is greatly affected by e[CO₂] in both grain biomass and total shoot biomass will have a much different change in HI than a genotype which is mostly affected in grain biomass. Amthor (2001) found the grain yield of wheat increased by 31% on average in response to e[CO₂]. This would lead to greater harvest indices for plants with low total shoot biomass responses to e[CO₂]. However, as reported with the meta-analyses of Wang et al. (2013). HI remained the same because shoot biomass increased proportionally to the increase in grain biomass. In our experiment, total shoot biomass and total grain biomass both increased for all genotypes in response to e[CO₂], except in Jandaroi, which declined in total shoot biomass. Synthetic hexaploids were significantly different from both tetraploids and hexaploids in total shoot biomass response to e[CO₂]. While the differences between means of tetraploids, hexaploids and synthetic hexaploids were not significant for total grain biomass, the synthetic hexaploids showed the lowest response to e[CO₂]. It appears that the main factor contributing to the lower HI of the synthetic hexaploids is the response of total shoot biomass to e[CO2] rather than grain biomass. This indicates that the extra carbon being assimilated under e[CO₂] is being stored largely in the shoot, compared to grain, of synthetic hexaploids.

The increase in biomass, both shoot and grain, can be explained by the effect of $e[CO_2]$ on photosynthesis. While the rate of photosynthesis was not measured for the plants in this experiment, it is accepted that e [CO₂] generally increases the photosynthetic rate of C3 plants (Ainsworth and Long, 2005). An increase in photosynthesis means there is greater carbon fixation, resulting in increased biomass in the plant. Increasing the HI of a plant involves increasing how much carbon is stored in the grain as opposed to the shoot, as can be seen in this study's results when comparing the HI of each genotype with the two components of grain biomass and total shoot biomass. This increase in carbon could partly explain the decline in GPC for plants grown under e[CO₂]. As previously mentioned, one of the main hypotheses for the decline in protein under e[CO₂] is dilution by carbohydrates, where the increase in biomass is greater than the increase in nitrogen (Loladze, 2002; Taub and Wang, 2008). However, as with Taub and Wang (2008), we argue that biomass dilution cannot be the only explanation for the decline in GPC. Our results showed that the change in GPC did not correlate with the change in grain biomass or HI. While some genotypes, such as Sunbri and Hartog had a clear decline in GPC and increase in both grain biomass and HI, others were not as consistent. Some genotypes

Table 3

Total shoot biomass (g) and harvest index of tetraploid, hexaploid and synthetic hexaploid genotypes in ambient [CO₂] and elevated [CO₂]. ANOVA results show differences between each wheat type. Data represent values per plant. Abbreviations: P_T , AVOVA results against tetraploids; P_H , ANOVA results against hexaploids; P_S , ANOVA results against synthetic hexaploids; ns, not significant; *, $P \le 0.05$; **, $P \le 0.01$.

Wheat type	Genotype	Total Sho		Harvest Index											
					ANO	VA							ANOVA		
		e[CO ₂]	a[CO ₂]	e[CO ₂] – a[CO ₂]	\mathbf{P}_{T}	\mathbf{P}_{H}	$\mathbf{P}_{\mathbf{S}}$	e[CO ₂]	a[CO ₂]	e[CO ₂] – a[CO ₂]	\mathbf{P}_{T}	\mathbf{P}_{H}	$\mathbf{P}_{\mathbf{S}}$		
Tetraploid	Caparoi	6.9	5.1	1.80	_	ns	**	0.69	0.67	0.02	-	ns	**		
	Jandaroi	6.0	5.1	0.90				0.75	0.68	0.08					
	WID802	6.1	7.6	-1.50				0.76	0.60	0.16					
	Hyperno	6.3	5.5	0.80				0.76	0.70	0.06					
	Tjilkuri	4.2	4.1	0.10				0.82	0.72	0.11					
Hexaploid	Sunbri	6.6	6.1	0.50	ns	-	**	0.80	0.70	0.10	ns	-	**		
	Spitfire	6.4	5.3	1.10				0.73	0.69	0.04					
	Lincoln	7.1	6.8	0.30				0.71	0.71	0.00					
	Hartog	7.0	6.1	0.90				0.75	0.68	0.07					
	Crusader	6.7	5.8	0.90				0.76	0.71	0.06					
	Scout	7.2	6.7	0.50				0.74	0.70	0.04					
	Sunvale	7.0	6.3	0.70				0.72	0.64	0.08					
	LRC/2010/157	6.1	5.8	0.30				0.77	0.73	0.04					
	Aus 29259	7.3	5.3	2.00				0.74	0.74	0.00					
	Dart	5.5	4.2	1.30				0.76	0.73	0.03					
	Sunguard	5.3	4.9	0.40				0.68	0.67	0.01					
Synthetic Hexaploid	CPI 133814	8.5	4.4	4.10	**	**	-	0.59	0.67	-0.08	**	**	-		
-	CPI 133898	9.6	7.9	1.70				0.57	0.56	0.01					
	CPI 133811	15.7	7.9	7.80				0.35	0.43	-0.08					

increased in GPC despite the stimulation of grain biomass, most notably Tjilkuri, which had the greatest increase in GPC and the second greatest increase in grain biomass. This means that there are factors that are affecting the GPC of each genotype other than carbon dilution itself. In addition, Lincoln declined in GPC despite a small change to grain biomass and HI. As such, while dilution might explain part of the decline in GPC of some genotypes, there are very likely other factors controlling the protein response of wheat to $e[CO_2]$. Other explanations have been proposed, such as altered nitrogen assimilation (Bloom et al., 2014). Our results also found some correlation between grain biomass and total grain protein, suggesting that as e[CO₂] stimulates grain biomass, it also causes the plant to transport more nitrogen to the grain. This was not always the case, however, as Jandaroi in particular put less nitrogen into grain under e[CO₂] despite its increase in grain biomass. This supports the idea that there are other mechanisms being affected by e [CO2] which control the transport of protein. Lincoln decreased in total grain protein despite the low stimulation of grain biomass. This further suggests that lower total grain protein is not controlled by how great e [CO₂] stimulates grain biomass.

In addition to a decline in protein concentration, the composition of protein is altered and ultimately the baking quality of grain harvested from plants grown under $e[CO_2]$ is affected (Fernando et al., 2014; Panozzo et al., 2014). Therefore, it is important to not only identify hexaploid genotypes with greater responses to $e[CO_2]$ with regards to GPC, but also those which will not have decreased baking quality. As previously mentioned it will be important to screen a wider range of wheat genotypes for those which are highly responsive to $e[CO_2]$ for their GPCs, but to ensure the end product quality it will also be necessary to screen the highly responsive genotypes for baking quality.

Our results have identified a number of wheat genotypes that increased in both HI and GPC. Repeated confirmation of these results could provide breeders with genotypes that would benefit breeding programs for developing wheat cultivars capable of maintaining or improving upon current GPCs and HIs for future CO_2 conditions. Future studies would also benefit from increasing the number of genotypes, particularly tetraploids and synthetic hexaploids, as well performing this experiment in FACE conditions to gain data from field trials. The genotypes in the current study could be used in further research to investigate the mechanisms of GPC decline by providing wheat with contrasting CO₂ responsiveness. The tetraploid genotype Tjilkuri, which increased in GPC in response to $e[CO_2]$, may be a potential parent for generating synthetic hexaploid genotypes. However, in addition to the generation of synthetic hexaploids, there exists another possibility for developing wheat genotypes with improved GPC and HI under $e[CO_2]$. While there are many barriers to success, crossing tetraploid genotypes with hexaploids can result in pentaploid wheats (Padmanaban et al., 2017). Pentaploid wheat can be a source of great genetic variability and has shown promise for improving resistance to both biotic and abiotic stress (Padmanaban et al., 2017). Crossing highly [CO₂] responsive tetraploid and hexaploid genotypes together could lead to pentaploid genotypes with improved GPC and HI under $e[CO_2]$. These pentaploid genotypes, thus allowing the transfer of durum genes into bread wheat and vice versa.

Nitrogen is not the only nutrient which is affected in wheat grain. A meta-analysis by Broberg et al. (2017) investigated the effect of $e[CO_2]$ on grain mineral concentration and found significantly reduced concentrations of Ca, Cd, Cu, Fe, Mg, Mn, P, S, and Zn. Deficiencies in two of these minerals in particular, Fe and Zn, is a problem for a large portion of the global population, where wheat is one of the main sources of these minerals in their diets (Myers et al., 2014). As such, it is crucial to study the effect $e[CO_2]$ has on these minerals in addition to nitrogen. Future studies could therefore identify how the effect of e $[CO_2]$ on the concentrations of these minerals differs between the three wheat types.

In summary, our results suggest that wheat type is not a major factor for determining GPC or HI response to $e[CO_2]$, although this could be due to the limitation on number of genotypes. GPC and HI both varied among the cultivars within each wheat type and no significant difference could be found between wheat types, except for the difference between the HI of tetraploids and synthetic hexaploids. The difference in HI for the synthetic hexaploids was due to the high response of total biomass to $e[CO_2]$, which itself was significantly different from both tetraploids and hexaploids. However, more synthetic hexaploids with greater genetic variation need to be studied to confirm this for other genotypes. There also does not appear to be a strong connection between the CO_2 effects on GPC and HI regardless of wheat type. Our results suggest that biomass dilution is not the sole cause of the decline in GPC seen in this study. Ultimately, we think that the individual genotype is more important than wheat type in determining the response of wheat GPC and HI to $e[CO_2]$, however, more genotypes need to be studied to arrive at a definitive conclusion.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

This work was supported by the University of Southern Queensland and an Australian Postgraduate Award. The genotype LRC2010-157 was obtained from Dr Cassy Percy from the Centre for Crop Health, University of Southern Queensland.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcs.2019.03.007.

References

- Ainsworth, E.A., Long, S.P., 2005. What have we learned from 15 years of free-air CO2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO2. New Phytol. 165, 351–371.
- Amthor, J.S., 2001. Effects of atmospheric CO 2 concentration on wheat yield: review of results from experiments using various approaches to control CO2 concentration. Field Crop. Res. 73, 1–34.
- Arachchige, S., M, P., Ang, C.-S., Nicolas, M.E., Panozzo, J., Fitzgerald, G., Hirotsu, N., Seneweera, S., 2017. Wheat (Triticum aestivum L.) grain proteome response to elevated [CO2] varies between genotypes. J. Cereal Sci. 75, 151–157.
- Aranjuelo, I., Erice, G., Sanz-Sáez, A., Abadie, C., Gilard, F., Gil-Quintana, E., Avice, J.-C., Staudinger, C., Wienkoop, S., Araus, J.L., Bourguignon, J., Irigoyen, J.J., Tcherkez, G., 2015. Differential CO2 effect on primary carbon metabolism of flag leaves in durum wheat (Triticum durum Desf.). Plant Cell Environ. 38, 2780–2794.
- Bloom, A.J., Burger, M., Kimball, B.A., Pinter, P.J., 2014. Nitrate assimilation is inhibited by elevated CO2 in field-grown wheat. Nat. Clim. Change 4, 477–480.
- Broberg, M., Högy, P., Pleijel, H., 2017. CO2-induced changes in wheat grain composition: meta-analysis and response functions. Agronomy 7, 32.
- Calderini, D.F., Ortiz-Monasterio, I., 2003. Are synthetic hexaploids a means of increasing grain element concentrations in wheat? Euphytica 134, 169–178.
- FAO, 2017. FAO Cereal Supply and Demand Brief [Online]. Food and Agriculture Organization of the United Nations. Available: http://www.fao.org/ worldfoodsituation/csdb/en/, Accessed date: 21 December 2017.
- Fares, C., Menga, V., Badeck, F., Rizza, F., Miglietta, F., Zaldei, A., Codianni, P., Iannucci, A., Cattivelli, L., 2016. Increasing atmospheric CO2 modifies durum wheat grain quality and pasta cooking quality. J. Cereal Sci. 69, 245–251.
- Fernando, N., Panozzo, J., Tausz, M., Norton, R.M., Fitzgerald, G.J., Myers, S., Walker, C., Stangoulis, J., Seneweera, S., 2012. Wheat grain quality under increasing atmospheric CO2 concentrations in a semi-arid cropping system. J. Cereal Sci. 56, 684–690.
- Fernando, N., Panozzo, J., Tausz, M., Norton, R.M., Neumann, N., Fitzgerald, G.J., Seneweera, S., 2014. Elevated CO2 alters grain quality of two bread wheat cultivars grown under different environmental conditions. Agric. Ecosyst. Environ. 185, 24–33.
- Fernando, N., Panozzo, J., Tausz, M., Norton, R., Fitzgerald, G., Khan, A., Seneweera, S., 2015. Rising CO2 concentration altered wheat grain proteome and flour rheological characteristics. Food Chem. 170, 448–454.
- Fitzgerald, G.J., Tausz, M., O'leary, G., Mollah, M.R., Tausz-Posch, S., Seneweera, S., Mock, I., Löw, M., Partington, D.L., Mcneil, D., Norton, R.M., 2016. Elevated

atmospheric [CO2] can dramatically increase wheat yields in semi-arid environments and buffer against heat waves. Glob. Chang. Biol. 22, 2269–2284.

- Högy, P., Wieser, H., Kohler, P., Schwadorf, K., Breuer, J., Franzaring, J., Muntifering, R., Fangmeier, A., 2009. Effects of elevated CO2 on grain yield and quality of wheat: results from a 3-year free-air CO2 enrichment experiment. Plant Biol. 11 (Suppl. 1), 60–69.
- Högy, P., Brunnbauer, M., Koehler, P., Schwadorf, K., Breuer, J., Franzaring, J., Zhunusbayeva, D., Fangmeier, A., 2013. Grain quality characteristics of spring wheat (Triticum aestivum) as affected by free-air CO2 enrichment. Environ. Exp. Bot. 88, 11–18.
- Kimball, B.A., 2016. Crop responses to elevated CO2 and interactions with H2O, N, and temperature. Curr. Opin. Plant Biol. 31, 36–43.
- Leakey, A.D., Ainsworth, E.A., Bernacchi, C.J., Rogers, A., Long, S.P., Ort, D.R., 2009. Elevated CO2 effects on plant carbon, nitrogen, and water relations: six important lessons from FACE. J. Exp. Bot. 60, 2859–2876.
- Li, J., Wan, H.-S., Yang, W.-Y., 2014. Synthetic hexaploid wheat enhances variation and adaptive evolution of bread wheat in breeding processes. J. Syst. Evol. 52, 735–742. Loladze, I., 2002. Rising atmospheric CO2 and human nutrition: toward globally imbalanced plant stoichiometry? Trends Ecol. Evol. 17, 457–461.
- Matsuoka, Y., 2011. Evolution of polyploid Triticum wheats under cultivation: the role of domestication, natural hybridization and allopolyploid speciation in their diversification. Plant Cell Physiol. 52, 750–764.
- Myers, S.S., Zanobetti, A., Kloog, I., Huybers, P., Leakey, A.D., Bloom, A.J., Carlisle, E., Dietterich, L.H., Fitzgerald, G., Hasegawa, T., Holbrook, N.M., Nelson, R.L., Ottman, M.J., Raboy, V., Sakai, H., Sartor, K.A., Schwartz, J., Seneweera, S., Tausz, M., Usui, Y., 2014. Increasing CO2 threatens human nutrition. Nature 510, 139–142.
- Padmanaban, S., Zhang, P., Hare, R.A., Sutherland, M.W., Martin, A., 2017. Pentaploid wheat hybrids: applications, characterisation, and challenges. Front. Plant Sci. 8, 358.
- Panozzo, J.F., Walker, C.K., Partington, D.L., Neumann, N.C., Tausz, M., Seneweera, S., Fitzgerald, G.J., 2014. Elevated carbon dioxide changes grain protein concentration and composition and compromises baking quality. A FACE study. J. Cereal Sci. 60, 461–470.
- Pflüger, L.A., D'ovidio, R., Margiotta, B., Peña, R., Mujeeb-Kazi, A., Lafiandra, D., 2001. Characterisation of high- and low-molecular weight glutenin subunits associated to the D genome of Aegilops tauschii in a collection of synthetic hexaploid wheats. Theor. Appl. Genet. 103, 1293–1301.
- Pleijel, H., Uddling, J., 2011. Yield vs. Quality trade-offs for wheat in response to carbon dioxide and ozone. Glob. Chang. Biol. 18, 596–605.
- Reynolds, M., Dreccer, F., Trethowan, R., 2007. Drought-adaptive traits derived from wheat wild relatives and landraces. J. Exp. Bot. 58, 177–186.
- Sinha, P.G., Kapoor, R., Uprety, D.C., Bhatnagar, A.K., 2009. Impact of elevated CO2 concentration on ultrastructure of pericarp and composition of grain in three Triticum species of different ploidy levels. Environ. Exp. Bot. 66, 451–456.
- Taub, D.R., Wang, X., 2008. Why are nitrogen concentrations in plant tissues lower under elevated CO2? A critical examination of the hypotheses. J. Integr. Plant Biol. 50, 1365–1374.
- Taub, D.R., Miller, B., Allen, H., 2008. Effects of elevated CO2 on the protein concentration of food crops: a meta-analysis. Glob. Chang. Biol. 14, 565–575.
- Thilakarathne, C.L., Tausz-Posch, S., Cane, K., Norton, R.M., Tausz, M., Seneweera, S., 2013. Intraspecific variation in growth and yield response to elevated CO2 in wheat depends on the differences of leaf mass per unit area. Funct. Plant Biol. 40, 185–194.
- Uddling, J., Gelang-Alfredsson, J., Karlsson, P.E., Selldén, G., Pleijel, H., 2008. Source–sink balance of wheat determines responsiveness of grain production to increased [CO2] and water supply. Agric. Ecosyst. Environ. 127, 215–222.
- Uprety, D.C., Dwivedi, N., Raj, A., Jaiswal, S., Paswan, G., Jain, V., Maini, H.K., 2009. Study on the response of diploid, tetraploid and hexaploid species of wheat to the elevated CO2. Physiol. Mol. Biol. Plants 15, 161–168.
- Wang, L., Feng, Z., Schjoerring, J.K., 2013. Effects of elevated atmospheric CO2 on physiology and yield of wheat (Triticum aestivum L.): a meta-analytic test of current hypotheses. Agric. Ecosyst. Environ. 178, 57–63.
- Wang, Y., Wang, C., Quan, W., Jia, X., Fu, Y., Zhang, H., Liu, X., Chen, C., Ji, W., 2016. Identification and mapping of PmSE5785, a new recessive powdery mildew resistance locus, in synthetic hexaploid wheat. Euphytica 207, 619–626.
- Yang, W., Liu, D., Li, J., Zhang, L., Wei, H., Hu, X., Zheng, Y., He, Z., Zou, Y., 2009. Synthetic hexaploid wheat and its utilization for wheat genetic improvement in China. J. Genet. Genom. 36, 539–546.