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Annual ryegrass (*Lolium rigidum* Gaud) competition altered wheat grain quality: A study under elevated atmospheric CO_2 levels and drought conditions

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Title page

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Abstract

Annual ryegrass is one of the most serious, costly weeds of winter cropping systems in Australia. To determine whether its competition-mediated plant defence mechanisms effect on wheat grain quality, wheat (cv. Yitpi) and annual ryegrass were grown under two levels of CO_2 (400 ppm; (a[CO_2]) *vs* 700 ppm; (e[CO_2]), two levels of water (well-watered *vs* drought) and two types of competition (wheat only; (W), and wheat × annual ryegrass; (W × R) with four replicates. The competition × [CO_2] interaction had a significant effect on wheat grain protein content, where it was increased in W×R under both e[CO_2] (+ 17%) and a[CO_2] (+ 21%). Grain yield, total grain reducing power and phenolic content were significantly affected by [CO_2] × drought × competition. In a summary, annual ryegrass competition significantly altered the wheat grain quality under both [CO_2] levels (depending on the soil water level), while also decreasing the grain yield.

Key Words: Wheat grain quality, Wheat, Annual ryegrass, weed competition, grain protein concentration, grain total reducing power, grain total phenolics

1. Introduction

Wheat (*Triticum aestivum* L.) is a staple food crop for almost half the world's population, and is one of the main sources of protein and minerals in most developing regions (Cakmak, 2004). In 2012, worldwide wheat production was 672 million tonnes (FAOSTAT, 2014), and it is thus imperative that any threat to the efficiency of production and quality of grain is addressed as soon as it is practicable. In this respect, there are two immediate issues which need attention. These issues are (i) weeds, which are found to compete with wheat for water and nutrients and thus reduce yield and quality, and (ii) climate change, which alters the parameters of growth for competing species in different ways, which makes crop management problematic. This investigation looks at the issue of annual ryegrass (*Lolium rigidum* Gaud) as a significant competitor for wheat crops, and does so in the context of rising CO_2 levels.

Annual ryegrass is one of the most serious weeds found in annual winter cropping systems in Southern Australia and Victoria, and it has significant economic consequences (Gill, 1996). Of particular concern is the observation that infestations of annual ryegrass in wheat cropping systems have been shown to reduce the number of grain-bearing ears of wheat plants, and it is accepted that competition for nitrogen with weeds is the most important single factor underlying this reduction (Levick, 1969).

In addition, increasing environmental stress associated with climate change will affect both the yield and quality of wheat production. Since 1959, rapid fossil fuel consumption and deforestation have steadily raised atmospheric CO_2 concentration (hereafter abbreviated as a[CO_2]) from 315 µmol mol^{-1} to approximately 400 µmol mol^{-1} in 2015. Moreover, currently a[CO_2] is likely to increase up to 550 µmol mol^{-1} by 2050 according to the Intergovernmental Panel on Climate Change (IPCC) in accord with most of the accepted emission scenarios. This increase is likely to affect the global and regional climates and weather patterns. For example, global temperature is predicted to increase by an average of 1.5-4.5 ºC with more frequent occurrences of extreme climatic events, such as heat waves and/or droughts (Carter, Jones & Lu, 2007). As noted earlier, maintaining grain quality under changing climate conditions is critical for providing an essential source of human nutrition, with high quality end-use functional properties and sustained commodity value. In this respect, many studies have now shown that elevated atmospheric CO_2 levels (hereafter abbreviated as e[CO_2]) decrease the grain protein content while altering the grain mineral composition (Fernando et al., 2015; Fernando, Panozzo, Tausz, Norton, Fitzgerald & Seneweera, 2012; Högy & Fangmeier, 2008). Grain protein level is one of the major factors that determine the grain quality; hence this will intimately affect the price of the harvested grains. Additionally, in recent years, grains and pulses have attracted a great deal of attention because some of their constituents have been shown to be bioactive (Yanping, Chang, Yan & Qian, 2011). These bioactive compounds can be categorized into phenolic acids, flavanols, flavonoids, soyasaponins, phytic acid and condensed tannins (Campos-Vega, Loarca-Pina & Oomah, 2010). Of relevance here is that recent epidemiological studies have found that these bioactive compounds in grains and pulses confer protection against chronic diseases through a multitude of biological activities (Ha et al., 2014) including antioxidant and anticancer activities, angiotensin I-converting enzyme inhibition, reduction of blood lipid, and reduction of the risk of cardiovascular diseases (Bazzano et al., 2001; Duane, 1997; Ha et al., 2014; Shepherd et al., 1995). Thus, as a consequence of the nutritional and bioactive properties of wheat grain, any input to the understanding of its economic production will contribute to a globally sustainable future.

However, as a complicating factor in this investigation, there is evidence that suggests the nature of the competition between weeds and crops alters under e[CO_2] (Patterson & Flint, 1990; Ziska & Dukes, 2011), bringing a level of uncertainty to traditional management techniques. This means that, in the light of the predicted change in future climates as well as atmospheric conditions, significant agronomic and genetic adjustments will be required.

In designing this investigation, it was recognised that weed competition with crop species is generally evaluated by growth and yield characteristics of the target plant, such as plant height, leaf area, tillering, vegetative biomass production and crop yield (Ziska & Dukes, 2011). It has also been shown that weeds negatively affect crops in many ways other than economic yield levels (Gallandt & Weiner, 2015).Work presented here specifically focuses on whether weed competition has an impact on wheat grain quality, a question which has been paid only minor attention in most previous studies. In this respect, there are three components of community assembly that are potentially relevant to crop-weed interactions: species richness/abundance, functional traits diversity and polygenetic diversity (Gibson, Young & Wood, 2017). These authors assert that the effects of these three interrelated components are mediated via physiological responses affecting crop quality. Of these three components, the link between species richness and relative abundance of different weed species in a crop system is relatively well studied (Zimdahl, 2004). However, what is required for future production is a greater understanding of the link between abundance of weed species in relation to crop quality (Gibson et al., 2017).

Therefore, whilst it is important to understand how competition level of annual ryegrass with wheat changes under e[CO_2], an understanding of how annual ryegrass competition impacts on wheat grain quality under the predicted climate change scenario is also important. This is a complex system, and as such a need to consider both the indirect climate effects on global food production, as well as the direct effects of increasing [CO_2] and other extreme climate events on crop-weed competition, is crucial.

The objectives of the current study were to (i) determine the individual and interactive effects of annual ryegrass competition, e[CO_2], and periodic drought events on wheat grain yield, and (ii) to determine the individual and interactive effect of e[CO_2], periodic drought and annual ryegrass competition on wheat grain quality parameters represented by grain protein, grain total reducing power (TRP) and grain total phenolic content (TP).

2. Materials and methods

2.1. Growth chambers and growing conditions

This study was conducted during the period January-August 2015, in two identical environmental control growth chambers (*Steriudium e2400*; 3.1 m long × 2.4 m wide × 2.6 m high) located at Federation University Australia, Mount Helen (37.6298° S, 143.8835° E), Victoria, Australia. The [CO_2] in one chamber was held at 400 μmol mol^{-1} (ambient; a[CO_2]), whereas the other was maintained at 700 μmol mol^{-1} (elevated; e[CO_2]), throughout the experiment. The average day/night temperature of the chambers was maintained to approximately replicate the field conditions of wheat grown in south-east Australia. From the time of seed sowing to the 3-5 leaf-seedling stage, the average day/night temperatures were set at 12ºC/8ºC, and then increased to 18ºC/10ºC until the tiller production stage of the wheat. From the tiller production stage to the flowering stage, the average day/night temperatures of the chambers were maintained at 22ºC/15ºC, and during grain filling, the chamber's average day/night temperature was increased to 24ºC/18ºC. The humidity inside the chambers was maintained at 40-50%, and a light 12h: dark 12h photoperiod was maintained, with a light intensity of 1000 μmol m^{-2} s^{-1} photosynthetic photon flux density provided during the light time.

2.2. Plants and growing conditions in the growth chambers

Wheat cultivar (*Triticum aestivum* L. cv. Yitpi) and annual ryegrass (*Lolium rigidum* Gaud.) were grown in 5 l opaque polyethylene cylindrical pots (40 cm height × 18 cm diameter). The experimental design was factorial with two [CO_2] treatments, (a[CO_2] *vs* e[CO_2]), and two treatments of soil water (80% soil field capacity *vs* periodic drought). Two levels of competition were invoked: (i) wheat only (W), with two wheat plants per pot without competition, and (ii) wheat × annual ryegrass (W × R), with two wheat and two ryegrass plants per pot to provide the competition. Eight replicates were used.

Soil was collected on the 7th September 2014 from the top plough layer (top 0.20 m) from four fields within places of a wheat cropping area in Lismore, Ballarat, Victoria. The soil was air dried, crushed into < 10 mm fractions and then filtered through a 2 mm mesh. Plant residues, pebbles and bigger soil particles were removed before mixing the soil thoroughly. Six soil samples were taken and soil field capacity, soil water level and soil nitrogen (N) content were estimated. Each of the 5 l opaque polyethylene cylindrical pots was filled with 5.5 kg of air dried soil before re-wetting the soil with reverse osmosis (RO) water to 80% of field capacity prior to sowing. To ensure the soil was wet throughout the column, the 5.5 kg soil was added in two portions; a 2 kg initial layer, then a top portion of 1.5 kg. After the introduction of each soil portion, addition of an appropriate amount of RO water was made.

Four seeds of wheat (cv. Yitpi) were sown 2 cm below the soil surface in each of the pots on the 2nd of February 2015. Ryegrass seedlings were raised in an environmentally controlled glasshouse at Federation University Australia. A week prior to the sowing of the wheat seeds, ryegrass seeds were sown into 7 cm deep seed trays filled with the same soil mixture a week prior to the sowing of the wheat seeds. Two weeks after wheat seeds were sown, annual ryegrass seedlings were transplanted into the (W × R) wheat pots. Based on wheat plant density under field grown conditions, seedlings were thinned to two plants per pot in the treatment of wheat grown without weed competition (W). In the pots of wheat grown with ryegrass competition, wheat seedlings were thinned to two plants per pot and two ryegrass seedlings were transplanted per pot. For fertilisation a total nitrogen (N; 60 kg/ha) as urea, phosphorus (P; 20 kg/ha) as triple super phosphate and potassium (K; 20 kg/ha) as muriate of potash was prepared and portioned based on the surface area of the pots. This was top-dressed at three stages of plant growth: (i) the seedling stage, (ii) the tillering stage after two nodes had expanded, and (iii) at the booting stage. The proportion of each nutrient added was based on surface area of the pot, and this rate and method of fertiliser application reflected local agronomic practices. To reduce any chamber effect on plant growth, pots were randomised and rearranged at weekly intervals within the chamber. In addition, plants and [CO_2] treatments were alternated between plant growth chambers at monthly intervals.

2.3. Water treatment

All the pots were watered with RO water to a constant weight (80% field capacity (FC)) by weighing each pot every second day until the drought treatment commenced. During the drought treatment, pot weight with plants was measured daily and the water level of the well-watered treatment was adjusted to 80% of FC. During the flowering stage of wheat, plants grown under drought were not watered for eight consecutive days, by which time the plants wilted. Drought treated plants were subsequently well-watered to 80% FC for a week and all plants were then watered every second day until the upper most leaves of wheat started turning yellow. Subsequently, the watering frequency was decreased to once in every five days until grain maturity based on the reduced requirement of water during grain maturity of wheat.

2.4. Plant harvesting and grain sample preparation

Ears were harvested at wheat grain maturity and wheat grains were separated and aspirated (Vacuum separator, Kimseed, Australia) to remove the remaining husk and dust, and stored at 20 ºC in plastic containers to avoid moisture absorption until further analyses.

2.5. Grain protein analysis

Total protein content in the whole grain was determined by near Infrared Reflectance Spectroscopy (NIR, Foss, Sweden) (AACC method 39-25). Whole-grain total protein content was expressed on grain dry weight basis.

2.6. Grain total reducing power measurements

Wheat grain samples were homogenised separately using a conical burr grinder to produce a fine powder. Fifteen millilitres of a 95% methanol, 5% acetic acid extraction solvent was added to a centrifuge tube along with 1g of the powdered material, which were then agitated for 20 minutes at 220 rpm using an orbital shaker. This was followed by three centrifugation steps, each involving 10 minutes of centrifugation at 16,800×g followed by the collection of the supernatant and the re-addition of an additional 15 ml of extraction solvent. The combined supernatant was then filtered using a Whatman no. 4 filter paper under vacuum, and made up to 50 ml with extraction solvent and stored in the dark at -20 ºC until required for further analysis. The total reducing power was determined using an adaptation of the CUPRAC method as described by Apak, Gorinstein, Böhm, Schaich, Özyürek & Güçlü, 2013. In this method 1 ml of a 10 mM copper (II) chloride aqueous solution was mixed with 1 ml of 1M ammonium acetate aqueous solution in a test tube followed by 1 ml of a 7.5 mM neocuproine ethanol solution, 100 µµl of adequately diluted sample extract and 1 ml of RO water. Test tubes were capped, vortexed briefly and incubated in a 50 º °C water bath, in the dark, for 30 minutes. Absorbance was subsequently read on a UV-visible spectrophotometer (Shimadzu UV-1800) at 450 nm and standardised with Trolox®. Concentration was derived as a function of Trolox® for equivalent absorbance in the range 50 mg l^{-1} to 650 mg l^{-1} (R^2= 0.998). Total reducing power was expressed as mg Trolox/100 g dry weight (DW).

2.7. Total phenolics (TP)

Determination of total phenolics was achieved using a modification of the Folin-Coicalteu method developed by Singleton and Rossi (1965). Two millilitres of Folin-Coicalteu reagent, diluted 1:10 with reverse osmosis water, was added to 400 µl of sample extract that had been diluted as necessary with the extraction solvent. This was left at room temperature for 10 minutes before the addition of 2 ml of 7.5% (w/v) sodium carbonate aqueous solution. The test tubes were capped and vortexed briefly before incubation in darkness in a 40 °C water bath for 30 minutes. Absorbance was read at 760 nm and standardised using gallic acid. Concentration was again derived as a function for the equivalent absorbance of gallic acid in the range 5 mg l^{-1} to 80 mg l^{-1} GAE (R^2=0.9941). Total phenol was expressed as mg GAE/100 g dry weight (DW).

2.8. Statistical analysis

Data were analysed with MINITAB 17 statistical package using a General Linear Model analysis of variance. Homogeneity of variances was checked with the Levene's test and $\log_e$-transformed where necessary to equalize variances between treatments. The least significant difference (LSD) at p = 0.05 was used to compare the means between treatments unless otherwise stated.

3. Results

3.1. Grain yield

Wheat grain yield in the W×R competition was significantly decreased (- 35%) compared to the wheat in the W only (Fig 1d, Table 1). The effect of drought decreased wheat grain yield by 22% compared to the wheat grown under well-watered conditions. In contrast, wheat grown under e[CO_2] increased grain yield by 22% compared to the wheat grown under a[CO_2] (Fig 1d, Table 1).

3.2. Grain protein content

Competition × [CO_2] interaction had a significant effect on wheat grain protein content. Grain protein content was significantly increased in W×R under both e[CO_2] (+ 17%) and a[CO_2] (+ 21%) (Fig 1a, Table 1). The highest amount of grain protein content (16.3%) was observed in wheat grown under a[CO_2] with annual ryegrass competition (Fig 1a, Table 1). It was a 12% higher grain protein content compared to the wheat grown under e[CO_2] with annual ryegrass competition. Overall, grain protein content was significantly increased in the grains grown under drought conditions (+ 13%) than under the well-watered conditions (Fig 1a, Table 1).

3.3. Total grain reducing power (TRP)

Three way interaction of [CO_2] × drought × competition had a significant effect on TRP of grains. At a[CO_2], TRP of wheat grains grown under drought condition was higher in W×R by 145% compared to the wheat grown in W only (Fig 1c, Table 1). However, at a[CO_2], TRP of wheat grains grown under well-watered conditions was not different in grains grown in W×R and W only (Fig 1c, Table 1).

At e[CO_2], TRP of wheat grains grown under drought conditions in W×R was 10% higher than the wheat grown in W only. Similar to the wheat grown at a[CO_2], TRP of wheat grains grown under well-watered conditions was not different in grains grown in W only and W×R (Fig 1c, Table 1). Total reducing power of grains grown in W only at e[CO_2] was increased by 161% under well-watered conditions while increased by 101% under drought condition than the grains grown at a[CO_2]. Total reducing power of grains grown in W×R, at e[CO_2]

was increased only under well-watered conditions (+131%) compared to the wheat grown at a[CO_2] (Fig 1c, Table 1).

3.4. Total Phenolics

The three way interaction of [CO_2] × drought × competition had a significant effect on total phenolics of wheat grains. At a[CO_2], total phenolics of wheat grains significantly increased by annual ryegrass competition under well-watered conditions (+ 21%) (Fig 1b, Table 1). In contrast, total phenolics of wheat grains were significantly decreased by annual ryegrass competition under drought conditions (- 18%). However, under e[CO_2], total grain phenolics content was not affected by annual ryegrass competition (Fig 1b, Table 1).

Total grain phenolics content of wheat grown under e[CO_2] with annual ryegrass competition was significantly increased by 28% under drought conditions and decreased by 13% under well-watered conditions than wheat grown under a[CO_2] (Fig 1b, Table 1).

4. Discussion

Annual ryegrass competition significantly changed the wheat grain quality under both current and future predicted levels of atmospheric [CO_2] depending on the soil water level. Grain quality is defined by a range of physical and compositional properties where threshold levels are set according to end-user requirements. For staple grains such as wheat, whole-grain physical properties such as size, shape, colour and firmness relate to consumer appeal, influence on milling yield and screening losses, which determines the processing efficiency and value of the grain. Grain compositional properties relate to consumer health and benefits including grain protein concentration, composition, and taste. In addition, potential toxicity from pollutants, heavy metals, nitrates and pesticide residues can influence end-use properties of dough mixing and rheological characteristics, bread making process and product quality.

In our experiment, wheat competition with annual ryegrass increased the level of the grain protein concentration under both a[CO_2] and e[CO_2]. However, this is in contrast to some previous studies conducted under field conditions. For example Peltzer and Bowran (1996) reported that the introduction of a competitive weed such as annual ryegrass into the wheat cropping system decreased the grain protein level at grain maturity. Wheat grain protein level with presence of weeds depend on the N level of the soil, level of weed competition at early growth stage of crop, N uptake level of crop, and competition ability of the crop. Under N limited soil conditions, weeds compete with the crop for the available N (Ponce, 1987) which can significantly reduce the wheat grain protein level. In our experiment, the wheat cultivar that we selected had a high early vigour hence it competed well with annual ryegrass at early growth stage. However, at the later growth stage of wheat, it has been observed that annual ryegrass outcompetes some cultivars of wheat (unpublished data), and in this respect it has been shown that the weed competitive ability of wheat dependent on the genotype with the competitive ability being strongly correlated with mature crop height and early crop vigour (Zerner, Rebetzke & Gill, 2016). In our experiment, wheat being more competitive with annual ryegrass at early growth stage, may have taken up higher amounts of N during the early growth stage and remobilized it into the grains at the grain-filling stage. Moreover, lower grain yield produced by wheat grown with annual ryegrass competition further impacts on higher grain protein content.

The presence of annual ryegrass significantly decreased wheat grain yield under both [CO_2] levels. Weed competition decreases the number of fertile tillers and spikelets of wheat (Reeves, 1976) which decreases the grain yield at maturity. Also, it has been noted in a previous study that grain yield reduction was dependant on the density of annual ryegrass (Smith & Levick, 1974). In our experiment, 0% or 50% density of annual ryegrass was studied to distinguish between intra- and inter-specific competitions, since this best straddles the agricultural growing conditions. Historically, the economic value of preferred grain compositional traits has been a secondary consideration in agronomic crops compared with yield. However, recent commercialisation of crop genetics with traits providing improved seed quality has received significant adoption with an increased value to farmers (Waltz, 2010). The increasing trend in the market towards an emphasis on grain quality may progressively expand into broader opportunities for farmers to extract economic value for their grain (Gibson et al., 2017). Consequently, a grain market that favours quality over the quantity could result in fundamental changes in crop management, with greater emphasis on weed management.

Annual ryegrass competition increased TRP of wheat grains grown under drought conditions, under both levels of [CO_2]. A recent review reported that e[CO_2] increases the level of antioxidants in wheat grains only under stress conditions, and in only about 22% of the studies reviewed (AbdElgawad, Zinta, Beemster, Janssens & Asard, 2016). More specifically, increased C availability under e[CO_2], possibly resulting in increased supply of defence (antioxidant) molecules, is often held primarily responsible for improved protection against oxidative damage under stress at e[CO_2]. Moreover, percentage increase of wheat grains TRP due to annual ryegrass competition, under drought, was much higher at a[CO_2] than at e[CO_2]. Elevated [CO_2] reduced the negative effect of drought stress than observed at a[CO_2] (Ainsworth et al., 2008; Kimball et al., 2001) and led to lower requirements for antioxidative defence systems in wheat grown with competition.

Phenolic acids in cereals exist in free, soluble conjugate, and insoluble bound forms, but are primarily in the latter condition. Ferulic acid is the major phenolic compound in grains, with free, soluble-conjugated, and bound ferulic acids present at a 0.1:1:100 ratio (Acosta-Estrada, Gutiérrez-Uribe & Serna-Saldívar, 2014; Adom & Liu, 2002). In our study, levels of total phenolic compounds were estimated and were found to be significantly affected by annual ryegrass competition under a[CO_2], depending on the soil water level. Lower phenolic content under drought when wheat is grown in competition with annual ryegrass, may be due to higher stress levels that wheat plants without competition experienced, which limits the resource availability to produce phenolic compounds. This is attributed to the amount of substrate available to biosynthesise of phenolic compounds (Bustos, Riegel & Calderini, 2012). Phenolic content was not affected by annual ryegrass competition at both well-watered and drought conditions under e[CO_2], in agreement with previous research on phenolic content of wheat leaves (Li, Shi & Chen, 2008).

We conclude that annual ryegrass competition significantly raised the wheat grain quality under both [CO_2] levels depending on the soil water level, while decreasing the grain yield. Whilst it is recognised that the two important components of sustainable agricultural production are crop quantity (yield) and quality (Triboi & Triboi-Blondel, 2002), a simultaneous increase in these components is difficult because of resource trade-offs experienced by the crop. This is clearly a complex problem because under stress, it appears that crop yield is sacrificed for crop quality, giving crop managers a choice rather than a preferred crop strategy.

One implication of this finding is that because annual ryegrass has developed resistance to a number of the available herbicide groups and therefore has become one of the troublesome weeds to control in winter cropping systems, the positive effects of annual ryegrass on wheat grain quality should be considered in future sustainable and economical weed-crop management strategies.

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	Probability						
	CO_2	Competition (C)	Water treatment (W)	CO_2 × C	CO_2 × W	C × W	CO_2 × C × W
Grain protein content	**0.034**	**0.006**	**0.04**	**0.04**	0.12	0.92	0.66
Grain TRP	**0.000**	**0.002**	**0.000**	**0.012**	**0.005**	**0.002**	**0.029**
Total phenolic	0.819	0.627	0.239	0.451	0.334	0.301	**0.04**
Grain yield	**0.004**	**0.000**	**0.001**	0.971	0.212	0.543	0.736

Table 1: Probability of ANOVA results for CO_2, competition (C), water treatment (W) and their interactions are shown for tested grain quality parameters: grain protein content, grain total reducing power (TRP), total grain phenolic content and grain yield. Significant probabilities at 5% level are highlighted.

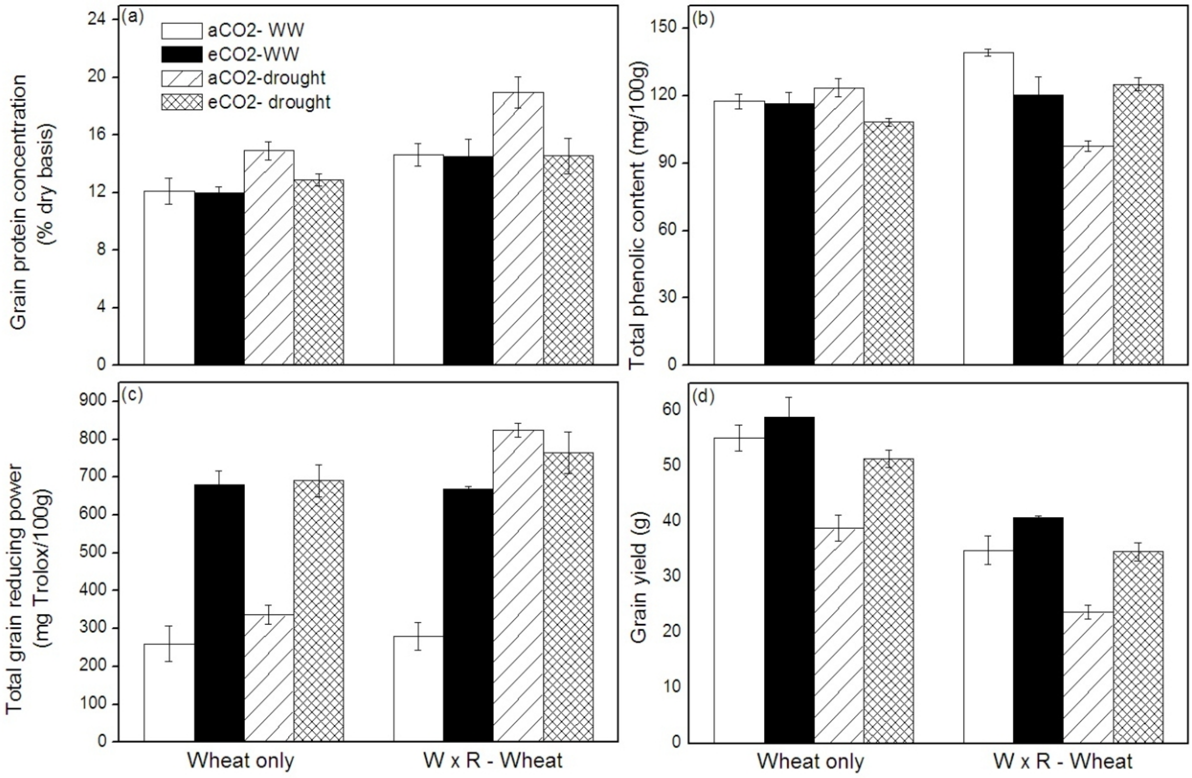
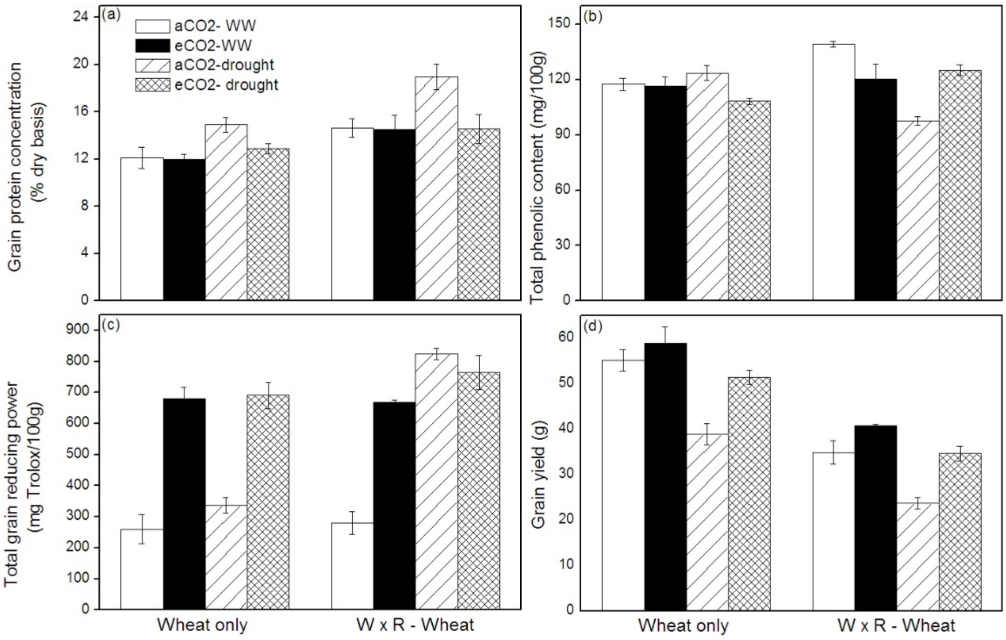


Figure 1: Wheat grain quality parameters of (a) grain protein concentration (b) total phenolic content (c) total reducing power (d) grain yield of wheat grains grown individually (wheat only) and in a competition with annual ryegrass (W × R –wheat) under ambient [CO_2] and elevated [CO_2] at well-watered and drought conditions. Data presented are the mean ±standard errors of n=4 replicates.



Highlights

- Wheat and annual ryegrass were grown with two [CO_2], and two water levels.
- Grain yield, protein content, total grain reducing power and phenolics were measured.
- Annual ryegrass competition increased wheat grain protein content under both [CO_2].
- Grain yield decreased (by 35%) with annual ryegrass competition.
- Total grain reducing power and phenolic content were significantly altered by both treatments.