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Proceeding Paper

Potential Implications of Elevated CO_2 on Physiochemical Parameters in Peanut (*Arachis hypogaea* L.) Genotypes †

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Abstract: Three peanut genotypes were cultivated under ambient and elevated CO_2 conditions. Elevated CO_2 treatment was associated with higher total phenolic content (TPC) values in the shoots and roots of all genotypes. Seeds showed lower levels of TPC, compared to the other plant tissue, with showing a decline in all three genotypes, with no difference between CO_2 treatments. Crude Protein (CP) content in seed varied significantly among the genotypes, and elevated CO_2 treatment was associated with mild increases (not significant) in CP, consisting of 4 and 6% in Holt, and Alloway cultivars, respectively. It is noteworthy to mention that Kairi contained the highest CP content under elevated CO_2 (29%).

Keywords: nitrogen content; protein content; total phenolic content; antioxidant capacity

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1. Introduction

Elevated atmospheric carbon dioxide concentration ([CO_2]) has been linked to declines in the nutritional quality of grains [1,2]. The decrease in grain nutritional quality is especially troubling for populations where grains are the primary protein source [2]. Despite these detrimental effects, much of the physiological processes by which reduced grain and forage protein is induced by elevated [CO_2] remains unexamined, and the role of both nitrogen derived from the atmosphere and nitrogen taken up from the soil in grain quality has yet to be fully elucidated [3].

Previous studies have documented increases in phenolic compounds concentrations and antioxidative capacity as a response to high [CO_2] exposure with different magnitudes reported in various crops [4] and this increment has often been associated with increased foliar nitrogen (N) concentration in crops such as, i.e., *Brassica rapa* [5] and arboreal species [6]. However, other studies have reported that elevated [CO_2] has a neutral effect on plants' N content or, worst, a detrimental impact, suggesting that the response is subjected to species-specific variability.

Thus, it is increasingly important to better understand the metabolic response of crops and plants to this stimulation. This understanding can aid in the selection of genotypes that have the potential for increased productivity under elevated CO_2 conditions.

The present work aims to evaluate the performance of three peanut genotypes, focusing on changes in their physiochemical composition when grown under both ambient (400 ± 50 µmol mol^{-1}) and enriched CO_2 (650 ± 50 µmol mol^{-1}) conditions in view of identifying the most promising genotype for dual-purpose (grain and graze) cropping under elevated CO_2.

2. Materials and Methods

2.1. Site Description

The research was conducted at the Central Queensland Innovation Research Precinct in Rockhampton, Queensland, Australia. The climate in the region is classified as humid subtropical, according to the Köppen climate classification system, and is represented by the code Cfa/Cwa. The project design consisted of eight plots equipped with octagonal-shaped Open-Top Chambers (OTCs), four of which were assigned to ambient carbon dioxide concentration (AC) at ~400 μmol mol^{-1}, while the other four were allocated for elevated carbon dioxide concentration (EC) at 650 μmol mol^{-1} ± 50 μmol mol^{-1}.

2.2. Planting Pattern and Crop Management

Three types of high-oleic runner peanut varieties (Kairi, Holt and Alloway) were grown in clay loamy soil from seed material provided by Peanut Company of Australia. The sowing density was ~190,000 seeds h^{-1}. The seeds of the three varieties were pre-treated with fungicide and inoculated with rhizobia group P and planted simultaneously in the same plots distributed in three linear meters. Before planting, the clay loamy soil was fertilized with 20 g m^{-2} of phosphorus (P as water soluble and citrate soluble) and 20 g m^{-2} of potassium (K as sulphate of potassium). Additionally, microelements, including boron (B), zinc (Zn), magnesium (Mg), manganese (Mn), and copper (Cu), were added to the soil at a rate of 0.4 g m^{2}. Water was applied through dripper irrigation at variable intervals during the season according to the crop growing stage and rainfall events, maintaining soil moisture >70% field capacity. The temperature in the OTC chambers was recorded with temperature data loggers. The OTCs' glasshouse plastic had a light transmissibility of 91%. The gas concentration inside the OTC was maintained at 650 ± 50 μmol mol^{-1} for the EC treatment and ~400 μmol mol^{-1} for the AC treatment. The CO_2 enrichment started 12 days after sowing (DAS), when plants had completely emerged, and it was continuously operated during daytime (0530–1800) until harvest conducted at 132 DAS.

2.3. Total Phenolic Content and Antioxidant Capacity

Kernels, above- and below-ground biomass samples were oven-dried for a minimum of 72 h at 65 ° C and finally ground to powder. Following Johnson et al. [7], plant matrices were extracted using approximately 0.5 g of fine powder of plant material in 90% methanol. The methanolic extracts were stored in the dark at 4 °C until required for further analyses. In order to investigate the impact of elevated CO_2 as a potential stress mitigator, the total phenolic content (TPC) and antioxidant capacity of the vegetative matter and peanut seed material were analyzed using the Folin-Ciocalteu [8] assay for TPC, and the Ferric Reducing Antioxidant Potential (FRAP) and Cupric Ion Reducing Antioxidant Capacity (CUPRAC) assays for antioxidant capacity.

2.4. Nitrogen and Protein Percentage

Nitrogen concentrations of dried ground kernels were analyzed through combustion method using a LECO TruMac elemental analyzer (LECO Corporation, St. Joseph, MI, USA). The protein percentage of kernels and the above- and below-ground biomass samples was calculated by multiplying the nitrogen concentration by a nitrogen-to-protein conversion factor of 6.25 [9] while the kernel concentrations were calculated using a peanuts-specific conversion factor (5.46) [10].

3. Results

The EC treatment impacted the vegetative growth parameters (not shown in this publication) by enhancing the total above-ground biomass, which recorded increases of 5, 23 and 26% for Alloway, Kairi and Holt, respectively. The exposure to elevated CO_2 concentration positively impacted also yield traits, namely, the number of pods and the pods' mass plant^{-1} in all tree genotypes. The plants grown under EC conditions showed an increment in pods plant^{-1} consisting of 43, 24 and 16% in Kairi, Holt and Alloway respectively.

3.1. Total Phenolic Content

The total phenolics content (TPC) varied among genotypes, plant tissue type, and [CO_2] (Table 1). However, the ANOVA tests failed to provide significance between genotypes, [CO_2], and the interactions [CO_2] × genotypes ($p > 0.05$). The TPC showed a mild (albeit non-significant) decrease in EC compared to the control plants in all genotypes' seeds, consisting of −10% in Kairi, −5% in Alloway, and −3% in Holt. A similar pattern was observed in Kairi's roots, whereas in Holt and Alloway, the CO_2 treatment gave a positive response. Between the three plant tissues investigated in this study, the shoots displayed the highest overall phenolic content out of all plant parts. EC moderately enhanced the total phenolic content in shoots of Kairi (4%) and Alloway (2.5%) genotypes, while Holt showed a mild negative response (−1.6%).

Table 1. Mean of the total phenolic content (mg GAE 100 g^{-1} DW ± SD), ferric reducing antioxidant potential and cupric reducing antioxidant capacity reported as mg 100 g^{-1} DW ± under ambient (AC) and elevated CO_2 conditions (EC).

Genotype	[CO_2]	TPC (mg GAE 100 g^{-1} DW)			FRAP (mg TE 100 g^{-1} DW)			CUPRAC (mg TE 100 g^{-1} DW)		
		Seeds	Roots	Shoots	Seeds	Roots	Shoots	Seeds	Roots	Shoots
Kairi	AC	156 ± 33	456 ± 159	691 ± 114	84 ± 17	492 ± 191	798 ± 145	234 ± 108	1750 ± 661	2593 ± 833
	EC	140 ± 3	398 ± 108	717 ± 65	99 ± 22	473 ± 177	786 ± 88	240 ± 94	1424 ± 550	2986 ± 262
Holt	AC	127 ± 12	347 ± 120	685 ± 70	83 ± 9	434 ± 191	954 ± 244	224 ± 86	1205 ± 444	3077 ± 791
	EC	123 ± 10	363 ± 120	674 ± 68	82 ± 5	455 ± 213	942 ± 53	163 ± 16	1434 ± 601	3317 ± 411
Alloway	AC	132 ± 5	334 ± 96	732 ± 73	84 ± 3	394 ± 143	823 ± 37	220 ± 78	1374 ± 297	2795 ± 344
	EC	125 ± 4	366 ± 99	750 ± 140	88 ± 4	454 ± 193	754 ± 155	196 ± 17	1422 ± 429	2796 ± 322

Abbreviations: TPC, total phenolic content; FRAP, ferric reducing antioxidant potential; CUPRAC, Cupric reducing antioxidant capacity; DW, dry weight; GAE, gallic acid equivalents; TE, Trolox equivalents; SD (standard deviation).

3.2. Ferric Reducing Antioxidant Potential

In the root material, the ferric antioxidant potential (FRAP) increased in Holt and Alloway with EC and decreased in Kairi; however, there was no significant correlation between [CO_2], genotypes, and the interactions [CO_2] × genotypes ($p > 0.05$). Similarly, the FRAP measured in seed samples highlighted no significant differences between genotypes and CO_2 treatments. The shoots' response significantly differed among the three genotypes ($p < 0.01$), with Holt showing the highest values, which declined from 954 in AC conditions to 942 mg TE 100 g^{-1} DW in EC conditions.

3.3. Cupric Reducing Antioxidant Capacity

Statistical analysis performed on the cupric reducing antioxidant capacity (CUPRAC) of all sample types in interaction with the [CO_2] and genotypes showed no significant correlation in roots and seeds. However, a positive correlation ($p < 0.05$) between CUPRAC and genotypes was observed in the shoot samples, with Holt having the highest value (3317 mg TE 100 g^{-1} DW, under EC). As similarly seen in the FRAP assay, Holt recorded the highest values (954 mg TE 100 g^{-1} DW, under AC conditions) between the three genotypes declined as a response to enhanced CO_2 in shoot samples. However, in contrast to FRAP for shoot material, Holt CUPRAC results showed an increase of 8% with elevated CO_2.

Table 2. Summary of total phenolic content (mg GAE 100 g^{-1} DW ± SD), ferric reducing antioxidant potential (mg TE 100 g^{-1} DW ± SD) and cupric reducing antioxidant capacity (mg TE 100 g^{-1} DW ± SD) under ambient (AC) and elevated CO_2 conditions (EC).

Plant Material	Parameter	AC	EC	*p* Value	Alloway	Holt	Kairi	*p* Value	[CO_2] × Genotype Interaction
Root	CUPRAC	1465 ± 503	1427 ± 481	NS	1615 ± 767	1371 ± 422	1548 ± 525	NS	NS
	FRAP	441 ± 162	461 ± 176	NS	480 ± 217	463 ± 169	456 ± 147	NS	NS
	TPC	382 ± 129	376 ± 100	NS	395 ± 145	374 ± 101	399 ± 113	NS	NS
Shoot	CUPRAC	2945 ± 537	3033 ± 379	*	2717 ± 486	2895 ± 720	2865 ± 594	NS	NS
	FRAP	859 ± 166	828 ± 129	**	480 ± 151	463 ± 242	456 ± 135	NS	NS
	TPC	703 ± 83	714 ± 94	NS	713 ± 96	652 ± 94	691 ± 104	NS	NS
Seed	CUPRAC	226 ± 83	200 ± 60	NS	236 ± 72	247 ± 130	233 ± 103	NS	NS
	FRAP	84 ± 10	89 ± 14	NS	87 ± 4	90 ± 22	90 ± 17	NS	NS
	TPC	139 ± 24	134 ± 19	NS	138 ± 17	136 ± 20	155 ± 38	NS	NS

Note. NS = not significant ($p > 0.05$), * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Abbreviations: TPC, total phenolic content; FRAP, ferric reducing antioxidant potential; CUPRAC, Cupric reducing antioxidant capacity; DW, dry weight; GAE, gallic acid equivalents; TE, Trolox equivalents; SD, standard deviation.

3.4. Nitrogen and Protein Content

The mean values of N and protein content in kernels, above and below-ground biomass of the three peanut genotypes considered in this study are reported in Table 3. The statistical analysis showed that protein content in all plant material was not significantly impacted by elevated CO_2 ($p > 0.05$). However, there was a significant ($p < 0.05$) variability between genotypes in the root material for all parameters. Kairi showed the highest protein content (29%) in the seed material under EC conditions, followed by Holt (28%) and Alloway (27%). Holt had the highest protein levels in the roots under EC conditions, whereas the protein content in the shoot material was relatively consistent between the genotypes.

Table 3. Mean of nitrogen and protein percentage (on a dry-weight basis) ± SD (standard deviation) in cultivars grown under ambient (AC) and elevated CO_2 conditions (EC).

Variety	Treatment	Nitrogen %			Protein %		
		Seeds	Roots	Shoots	Seeds	Roots	Shoots
Kairi	AC	5.36 ± 0.20	1.11 ± 0.29	2.51 ± 0.25	29.3 ± 1.07	6.95 ± 1.79	15.7 ± 1.56
	EC	5.32 ± 0.15	0.99 ± 0.14	2.48 ± 0.10	29.0 ± 0.83	6.21 ± 0.89	15.5 ± 0.63
Holt	AC	5.08 ± 0.24	1.32 ± 0.21	2.50 ± 0.17	27.70 ± 1.32	8.24 ± 1.34	15.6 ± 1.08
	EC	5.27 ± 0.17	1.23 ± 0.33	2.47 ± 0.06	28.80 ± 0.94	7.70 ± 2.09	15.4 ± 0.37
Alloway	AC	4.63 ± 0.13	0.89 ± 0.17	2.35 ± 0.11	25.30 ± 0.71	5.55 ± 1.08	14.7 ± 0.69
	EC	4.93 ± 0.18	1.06 ± 0.19	2.31 ± 0.10	26.90 ± 0.97	6.65 ± 1.19	14.4 ± 0.64

Table 4. Impact of elevated CO_2 on root nitrogen and protein content in three peanut genotypes.

Parameter	AC	EC	*p* Value	Alloway	Holt	Kairi	*p* Value	Treatment × Variety Interaction
N	1.08 ± 0.28	1.09 ± 0.25	NS	0.97 ± 0.24	1.25 ± 0.22	1.03 ± 0.21	*	NS
Protein	6.69 ± 1.68	6.85 ± 1.48	NS	6.06 ± 1.43	7.68 ± 1.35	6.55 ± 1.18	*	NS

NS = not significant ($p > 0.05$), * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

4. Discussion

This study found no significant impact of CO_2 treatment, genotype, or CO_2 × genotype interaction for TPC content on roots, shoots, and seeds. The shoots had the highest TPC compared to roots and seeds. Similar results have been reported in other crops [7]. A similar pattern was observed by Gillespie et al. [11] in soybean (*Glycine max*; another legume), where elevated CO_2 had no impact on the total phenolic content. Again, Fernando et al. [12] saw no significant effect of elevated CO_2 on the TPC of wheat (*Triticum aestivum*).

Despite this lack of variation seen in TPC, there was a significant impact of CO_2 elevation on the antioxidant capacity of the shoot material, as measured by FRAP ($p < 0.01$) and CUPRAC ($p < 0.05$). Somewhat surprisingly, the trend was not consistent, increasing under EC for CUPRAC, but decreasing for FRAP. Although these assays do have different reaction potentials and measure slightly different aspects of antioxidant activity, further investigation into this trend is required. It is possible that very specific compounds are being up- or down-regulated by the plant's physiological responses to EC, leading to an increase seen in CUPRAC (+8% average increase) but not in FRAP (−3.6% average decline). Both Fernando et al. [12] and Gillespie et al. [11] reported an increase in antioxidant capacity under elevated CO_2. There were no significant differences between genotypes on the antioxidant capacity for any plant part. However, there was a significant genotypic difference in the root protein content—where Holt showed the highest protein content (7.7%) and Alloway the lowest (6.1%).

5. Conclusions

Atmospheric CO_2 represents the primary substrate for photosynthesis, and as such, in high concentrations, it is known to boost plants' growth and biomass build-up by increasing plants' carbohydrate content. However, this process, known as the fertilization effect, could lead to a mismatch with N content in plants tissues, with consequent reduction of N-based compounds such as proteins. Importantly, in this study, there was no significant effects of the CO_2 level on protein, TPC, CUPRAC or FRAP of the peanut seed material, suggesting that peanuts grown under EC should have a similar level of nutritional and health benefits (in terms of protein, phenolics and antioxidants) to those grown under current ambient CO_2 levels.

Additionally, the three peanut genotypes explored here did not vary significantly from one another in their TPC or antioxidant capacity (for any plant part) when grown under either AC or EC levels. This suggests that aside from their root composition, they are quite comparable in terms of their phytochemical composition.

In our future work, we will explore the impact of EC on the morphology and physiology of these three peanut genotypes, allowing a definitive selection of the best genotype(s) for commercial production under future EC scenarios. This will enable peanut producers to be better prepared for potentially altered environmental conditions.

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