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Elevated Carbon Dioxide

Combined Effects of Water Shortage and CO_2 Enrichment on Yield and Water Use Properties of Barley (*Hordeum vulgare* L.) Genotypes

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ABSTRACT

A selection of winter barley genotypes was investigated for their responses to water shortage under different CO_2 concentrations in a greenhouse. The experiment was conducted in a 2*2 factorial design. Plants were grown either with optimum water supply or simulated drought in the BBCH 59 stage at current (~400 ppm CO_2) or elevated CO_2 concentrations (~700 ppm CO_2) with identical climatic conditions. Yield parameters (aboveground biomass and grain yield), water use and water use efficiency (WUE; kg m^{-3}) were calculated. Drought stress induced a decrease in yield production, and the rate of yield reduction was higher at 700 ppm CO_2 concentration. On average for the genotypes studied, water withdrawal resulted in a 20.01% and 22.0% reduction in grain yield at 400 ppm and 700 ppm CO_2 levels, respectively. Under optimum irrigation, BCC570, BCC607, Ketos, BCC887, HOR12808 and Kaskade showed significant positive CO_2 responses. Under a limited water supply, positive CO_2 reactions were only observed for BCC570, Ketos and BCC1585 genotypes; however, a significant positive CO_2 reaction regarding the yield production was observed for Panda. The carbon dioxide enrichment significantly increased water use efficiency under optimum watering conditions by 24.57% and drought-stressed conditions by 21.72%. However, the water shortage significantly increased water use efficiency under both 400 ppm (10.48%) and 700 ppm (12.36%) CO_2.

1 | Introduction

One of the greatest challenges that will face mankind over the coming decades is how to satisfy the food requirements of a growing population (van Dijk et al. 2021). Based on recent trends, even more agricultural land is needed to produce fodder crops (Waha et al. 2018). Barley (*Hordeum vulgare* L.) is primarily used for livestock feed, malt production and only to a lesser extent for human consumption (Newman and Newman 2008). In 2022, 155 million tonnes of barley were produced worldwide on 49 million hectares of land with an average productivity of approximately 3.0 t per hectare (FAO 2023). Barley is more resilient to abiotic stresses than wheat, and some varieties can be efficiently cultivated in arid regions. However, a shortage of water, especially during the vegetative phase, could result in a significant decrease in harvested yield (Kumar et al. 2020). Drought is already a serious problem in many regions of the world and has particularly damaging consequences for agriculture, especially field crop production, which becomes even more serious because it limits plant growth and productivity (Gbadegesin et al. 2022). The predicted climate change scenarios involving rising CO_2 concentration and the associated global warming are likely to further increase the frequency of droughts. Atmospheric CO_2 is predicted to rise continuously from currently 410 ppm up to between 550 and 1000 ppm by 2100, depending on the scenario (Intergovernmental Panel on Climate Change (IPCC) 2019). Recent studies confirm the impact of rising atmospheric CO_2 on plant water use (Brienen et al. 2017; Varga et al. 2017). Plants absorb CO_2 through stomata and transpire water into the atmosphere. A higher CO_2 level stimulates photosynthesis and increases plant growth, while

Key Points

1. The interactions of environmental factors such as water availability and atmospheric carbon dioxide concentration significantly influence the adaptability of winter barley genotypes.
2. The level of water supply is a significant factor in the realisation of the carbon dioxide responses of barley genotypes.
3. Plants grown under elevated carbon dioxide offset much of the negative effects of drought stress and contribute to soil water retention.

decreasing stomatal conductance. This improves water use efficiency (WUE) and alleviates the potentially negative impacts of drought stress (Ainsworth and Long 2020; Farkas et al. 2021). However, breeding of small grain cereals is a relatively slow and expensive process, taking around 6–12 years to produce a new cultivar from the initial cross (Bourgault et al. 2013). An effective approach to selecting genotypes with optimised CO_2 responses would be to test existing varieties or breeding lines. Previous studies have shown that an 8% higher grain yield can be achieved with one barley genotype in a free-air CO_2 enrichment experiment under optimum water supply (Manderscheid et al. 2009), while a 14% higher biomass and a 12.5% higher grain yield have been documented for barley in the same experimental system under 550 ppm CO_2 compared to ambient levels (Weigel and Manderscheid 2012). CO_2 enrichment impacts not only aboveground biomass, but also root length and root biomass. Varga et al. (2022) confirmed that the elevated CO_2 induces root growth in winter wheat varieties, while (Paeßens et al. 2019) demonstrated the positive impact of the free-air CO_2 enrichment on the root growth of maize and sorghum genotypes.

The amount of water taken up by the plants during the vegetation period is a crucial parameter because a shortage of available above- and belowground water resources that can be utilised for agricultural production has been detected in many parts of the world (Sabbaghi et al. 2020; Vargas-Amelin and Pindado 2014). Not only is water use efficiency (WUE) a key indicator of drought tolerance, it also links sustainable agriculture, which focuses on the efficient usage of above- and belowground water resources, with profitable agricultural production (Varga et al. 2015). Crop breeding has already increased WUE and is expected to play a significant role in future (Passioura 2012). In order to assess the abiotic plasticity of individual varieties, their phenotypic and physiological properties must be evaluated under suboptimal climatic conditions (Dahala et al. 2014). Some recent studies have highlighted that stress conditions modify the WUE; however, the direction and size of these changes depend on the variety and stress intensity (Cai et al. 2020; Farkas et al. 2020). Elevated atmospheric CO_2 concentrations typically result in an increased water use efficiency due to intensive photosynthesis and grain production, which could counterbalance the negative impacts of abiotic stresses (Varga et al. 2017; Wang et al. 2018).

The effects of drought stress and increased CO_2 can induce opposite responses in plants. Drought is a stressor, whereas carbon dioxide stimulates plant growth. Therefore, the interaction between these two factors could result in wide variability in the responses of different plants due to inconsistencies in drought tolerance and reactions to carbon dioxide.

A previous experiment (Mitterbauer et al. 2017) examined the CO_2 reactions of 98 barley genotypes in an open-top chamber study under well-watered conditions and found high intraspecific variability in growth responses to elevated CO_2. However, these varieties were only examined under optimum water supply. Therefore, 10 genotypes showing different CO_2 responses were selected for a more detailed examination in climate-controlled greenhouses. The aim was to determine (i) how simulated drought stress influences the CO_2 responses, and in particular (ii) whether there is an interaction between drought and elevated CO_2 on grain yield and water use, (iii) how CO_2 enrichment influences plant water use and water use efficiency and (iv) the extent to which increased CO_2 concentrations can alleviate drought stress in individual genotypes.

2 | Materials and Methods

2.1 | Plant Materials and Experimental Layout

Ten winter barley (*Hordeum vulgare* L.) genotypes originating from GeneBank accessions at the Leibniz Institute of Plant Genetics and Crop Plant Research in Germany were examined in climate-controlled greenhouse chambers at the HUN-REN Centre for Agricultural Research in Martonvásár, Hungary. These genotypes were chosen based on the outcomes of a CO_2 enrichment experiment conducted in open-top chambers (Mitterbauer et al. 2017), which tested the CO_2 reactions of 98 barley genotypes under well-watered conditions. Of the genotypes previously tested, BBC1585 and BBC887 were selected for this study due to their significant positive CO_2 reactions. BBC570, HOR12808 and Gerbel exhibited significant negative CO_2 reactions in the previous experiment. For the remaining genotypes (BCC 607, Ketos, Leonie, Kaskade and Panda), no significant CO_2 reactions were determined. The origin and ear types of the examined genotypes are summarised in Table 1.

Before planting, the plantlets were vernalised at 4°C for 6 weeks. Five uniform vigorous plants were potted in each plastic pot (height 30 cm, diameter: 20 cm) filled with 10 kg of a mixture of soil, sand and humus 3:1:1 (v/v). The actual water content and the maximum water-holding capacity of the soil were determined gravimetrically. First, the metal containers (volume: 0.2 L) filled with soil were weighed and then their total weight was measured. The containers were then over-dried at 70°C until they reached a constant weight. Next, the containers were soaked in water for 48 h to saturate them, after which they were allowed to dry. Once the dripping had stopped, the containers were weighed again. Based on these calculations, the water content of the pots was set at 60% of the soil's maximum water holding capacity, corresponding to a volumetric water content of 22%–25% (v/v). To prevent evaporation, the surface of the pots was covered with black plastic foil. Soil water content was measured using 5TE soil water meters, and the data were recorded by an EM50 data logger (Decagon Devices Ltd., USA). The plants were watered twice a week until they reached the stem elongation stage, and then three times a week. Volldünger Classic complex fertiliser solution

(Gartenhilfe Ltd., Austria) was added once a week in accordance with the manufacturer's recommendations. Water consumption between consecutive irrigations was determined by weighing the pots on a digital scale (Mettler-Toledo Ltd., USA). Drought stress was simulated by withholding water completely once at the end of the heading stage (BBCH 59) (Meyer 2018). The drought treatment (S) was initiated when 50% of the plants had reached the BBCH 59 developmental stage. The fresh aboveground biomass of four calibration pots was weighed every 2 weeks, and the weight corresponding to the 60% soil water holding capacity (control) was recalculated each time. The amount of irrigation water was determined by the differences between the actual weight (measured) and the weight corresponding to the 60% soil water holding capacity. The water content of the control pots (C) was kept constant at 60% of the soil water capacity throughout the growing season. The treated plants received no water during the stress period and were re-watered to control levels (60% soil water capacity) when the soil's volumetric water content dropped to 5–7 v/v%, regardless of the severity of the symptoms. The duration and timing of the stress period were summarised in Table 2.

TABLE 1 | The ear type and the origin of the winter barley genotypes.

No.	Genotype	Ear type	Origin	CO_2 reactions[a]
1	BCC1585	2-row	Russia	+
2	BCC887	6-row	USA	+
3	BCC570	6-row	India	–
4	GERBEL	6-row	France	–
5	HOR12808	2-row	China	–
6	BCC607	6-row	Japan	0
7	LEONIE	2-row	Germany	0
8	KASKADE	2-row	Germany	0
9	KETOS	2-row	Germany	0
10	PANDA	2-row	France	0

[a]CO_2 responses were estimated based on the results of Mitterbauer et al. (2017). + indicates significant positive, − significantly negative and 0 no significant CO_2 responses.

The temperature and supplemental lighting conditions of the greenhouse chambers were automatically controlled. The climatic program applied was developed by (Tischner et al. 1997) based on the analysis of a 50-year time series for Hungary, and is also used routinely in phytotron studies. The air temperature was increased from an initial 10°C–12°C to 24°C–26°C over 16 weeks, while the humidity was maintained at 60% and 80% through greenhouse chamber ventilation. When necessary, natural light intensity was increased to 500 μmol $m^{-2} s^{-1}$ at the beginning of the growing season using artificial lighting, and this was gradually increased to 700 μmol $m^{-2} s^{-1}$. The CO_2 concentration in the control chamber was maintained at 400 ppm (ambient) through ventilation with outdoor air. In the elevated chamber, the CO_2 concentration was maintained at 700 ppm. Carbon dioxide was introduced into the chambers from 37.5-kg cylinders via an SH-VT250 (SH-VT250; CO_2, Temperature, and Humidity Transmitter, Soha Tech Co. Ltd., Seoul, Korea) automatic valve controller through a network of perforated pipes placed 0.5 M above the plant canopy, and an even distribution of the gas was achieved through ventilation. As a double check, in both chambers, the actual CO_2 levels were measured and continuously recorded using a Wöhler CDL 210 (Wöhler CDL Serie 210 CO_2 Messgerät, Wöhler Technik GmbH, Bad Wünnenberg, Germany) CO_2 monitor.

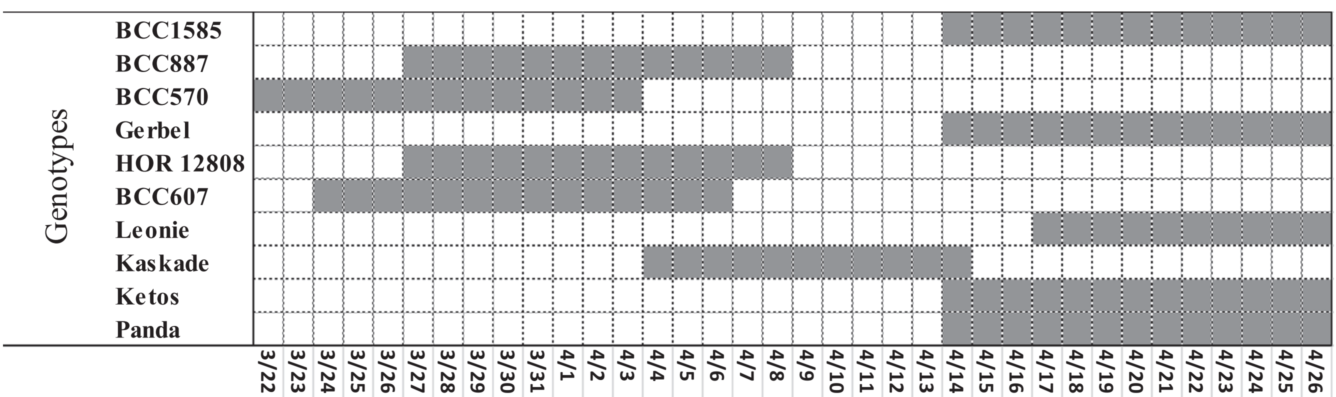
Inside the chambers, the pots (60 pots plus 4 calibration pots) were arranged in four rows (8 pots per row) on two movable plant-growing tables. To minimise the chamber effect, we rotated the pots with each watering, swapping them between the two tables and moving those from the inner two rows to the outer.

Thiovit Jet (Syngenta) (active ingredient: sulphur) was applied twice to control powdery mildew and was combined with Karate Zeon (Syngenta) (active ingredient: lambda-cyhalothrin) for aphid control.

2.2 | Analysis of Plant Material

The effects of the treatment on the phenological parameters were studied when plants had reached the final ripeness stage (BBCH 89). Each plant grown in an individual pot was handled

TABLE 2 | Duration and timing of the drought stress treatments for each genotype.



Note: Gray represents the duration of water withdrawal.

TABLE 3 | The effects of the CO_2 enrichment and water shortage on the aboveground biomass (g/pot) of winter barley genotypes.

Factors	Degree of freedom	Sum of squares	Mean squares	*F* value	Prob	LSD5%
CO_2 (A)	1	414	414	3.66	0.0593	n.s.
Watering (B)	1	8059	8059	71.266	0.000***	9.689
Genotype (C)	9	71,387	7932	70.139	0.000***	11.29
AB	1	15	15	0.13	0.7195	n.s.
AC	9	1391	155	1.367	0.2172	n.s.
BC	9	1517	169	1.491	0.1657	n.s.
ABC	9	980	109	0.963	0.4766	n.s.
Residuals	80	9047	113			

Note: Significance codes: '***' 0.001.
Abbreviation: n.s., non-significant.

and processed together. The grain yield (GY; g) of the plants was measured using a Mettler-Toledo Mx2002 digital balance (Mettler-Toledo GmbH, Budapest, Hungary) with a readability of 0.01 g, after the spikes had been manually harvested and threshed. The plants were cut at the soil level and chopped into pieces. The aboveground biomass, consisting of leaves and stems, was placed in paper bags and dried in an oven at 70°C for 2 days. The weight of the dry aboveground biomass (B; g) was measured using a Mettler-Toledo MX2002 digital balance (Mettler-Toledo GmbH, Budapest, Hungary). Water uptake (WU; kg/pot) of the plants was calculated by multiplying the weight losses measured using the Mettler-Toledo MX16001L balance (readability: 0.1 g) at each irrigation time throughout the vegetation period. Water use efficiency (WUE; kg m^{-3}) was calculated by dividing the grain yield (g/pot) by the water uptake of the plants during the vegetation period (WU; kg/pot). The CO_2 responses of the genotypes were quantified as the quotient of the individual values measured at CO_2 concentrations of 700 ppm and 400 ppm.

2.3 | Statistical Analysis

The experimental design comprised ten genotypes, two watering levels and two CO_2 concentrations. The experimental layout was a 2 × 2 factorial design. Treatments were replicated three times (pots), with 15 biological replications. Multivariate analysis (ANOVA) was used to test for significant main effects of genotype, CO_2, and water supply as well as their interactions. Means were compared using the LSD (least significant difference) test, which was performed with the help of the Agricolae package (Mendiburu 2017) in R software version 3.4.1 (R Core Team 2017). Student's *t*-test ($p < 0.1$) was applied for pairwise comparison, after checking for similarity in variances and normality of the distributions using the Kolmogorov–Smirnov and Bartlett tests, respectively. The effect sizes of the tested factors were determined using the Eta Squared Test with the help of the LSR Package (Navarro and Foxcroft 2019; Richardson 2011). Plots were generated using the ggplot2 (Wichham 2009) and ggpubr (Kassambara 2020) packages in R as well as Microsoft Excel.

3 | Results

3.1 | Biomass

The ANOVA confirmed the significant effects of the watering levels and genotypes on aboveground biomass ($p < 0.001$). However, the impact of the CO_2 concentrations was not significant. No interactions between the tested factors could be detected (Table 3). The Eta Squared test revealed that the factor genotype had a large effect on the biomass, while watering and CO_2 treatments had medium and small effects, respectively.

Differences in watering levels and between the genotypes were the main factors in explaining the observed variability. Water shortage reduced the biomass of BCC570, Leonie, Kaskade and Panda genotypes (asterisks in Figure 1). Under optimum irrigation, the Panda and Gerbel varieties produced the highest biomass at 400 ppm CO_2 concentration, while the aboveground biomass weight of BCC607 was significantly lower than that of the other genotypes. Under limited watering, the biomass of BCC1585 Gerbel, BCC887, Leonie and Panda did not differ significantly. The aboveground biomass of BCC570 and BCC607 was significantly lower than that of the other varieties (Figure 1).

At a CO_2 concentration of 700 ppm, the water shortage resulted in a significant decrease in the aboveground biomass of BCC570, Leonie, Kaskade, and Panda genotypes, while the other genotypes did not respond significantly to drought stress (asterisks in Figure 2). This pattern was similar to the changes observed at 400 ppm CO_2, indicating that CO_2 enrichment did not modify the susceptibility of the studied genotypes to drought. Under well-watered conditions, at 700 ppm CO_2, the highest aboveground biomass was observed for Gerbel, though this did not differ significantly from that of BCC1585 and BCC887. No significant differences were found between Ketos, BCC1585, BCC887, and Leonie. As with the trends observed at 400 ppm, at the 700 ppm CO_2 level, the BCC570 and BCC607 genotypes exhibited the lowest biomass production (Figure 2).

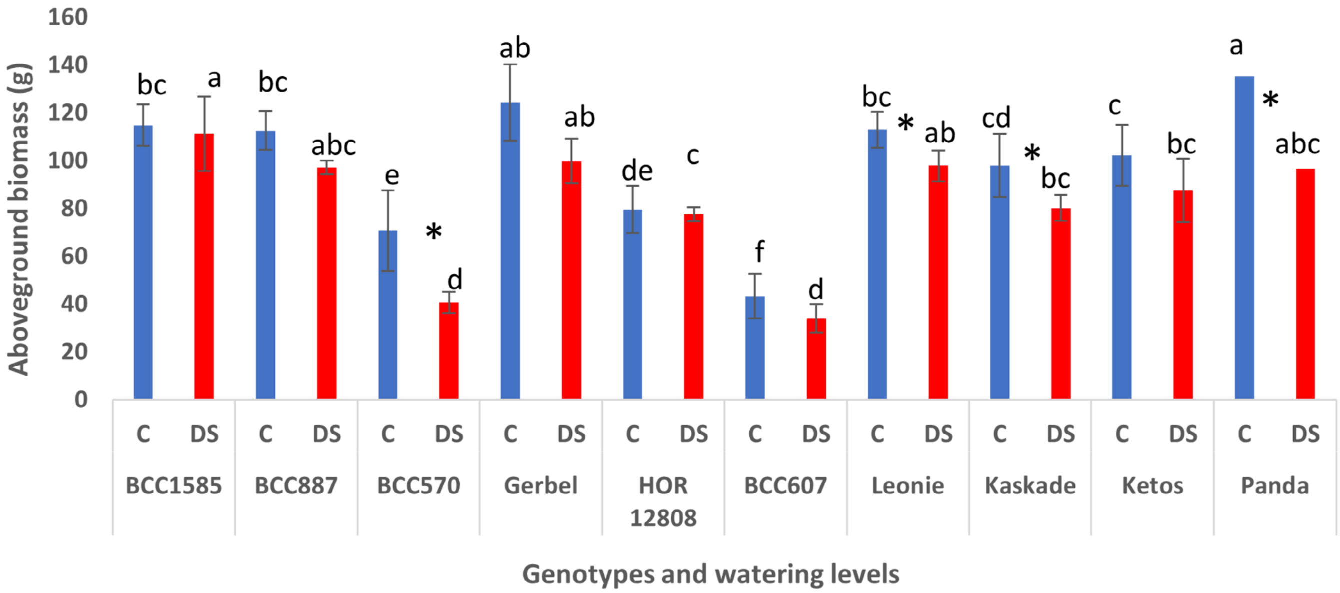


FIGURE 1 | Aboveground biomass (g/pot) production of winter barley genotypes under well-watered (C) and drought-stressed (DS) conditions at ambient (400 ppm) CO_2 concentrations. Error bars represent standard deviations. Different lowercase letters indicate significant differences between the genotypes within the watering levels (LSD test) ($p < 0.05$), and asterisks indicate significant differences between the biomass of the individual genotypes under control and drought-stressed conditions (LSD test) ($p < 0.05$).

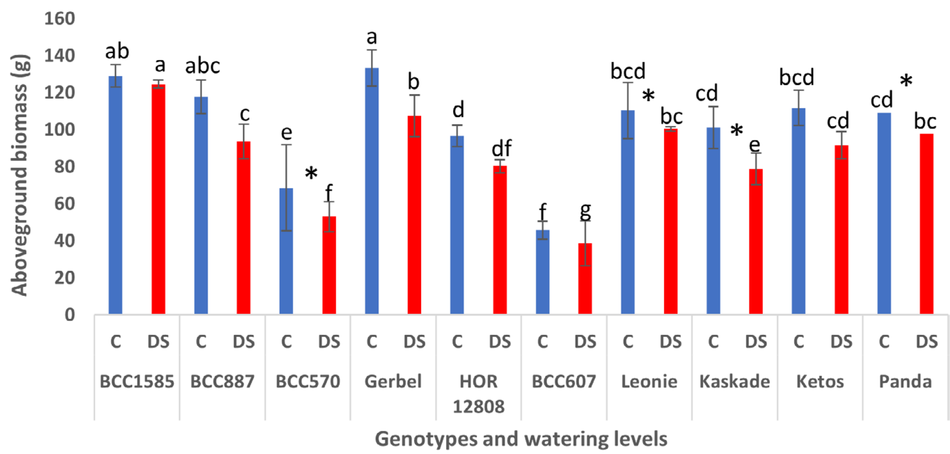


FIGURE 2 | Aboveground biomass (g/pot) production of winter barley genotypes under well-watered (C) and drought-stressed (DS) conditions at elevated (700 ppm) CO_2 concentrations. Error bars represent standard deviations. Different lowercase letters indicate significant differences between the genotypes within the watering levels (LSD test) ($p < 0.05$), and asterisks indicate significant differences between the biomass of the individual genotypes under control and drought-stressed conditions (LSD test) ($p < 0.05$).

ANOVA did not confirm any significant impact of CO_2 on biomass production when considering the full set of experiments. However, when the impacts of the CO_2 enrichment on the biomass weight of individual genotypes were evaluated by Student's *t*-test, significant differences were observed. There was a significant increase in biomass for two varieties (BCC887 and BCC570) at 700 ppm CO_2 concentration under both watering levels, compared with the biomass at 400 ppm (filled bars in Figure 3). The increase in the biomass of BCC607 induced by the CO_2 enrichment was only significant under optimum watering, while positive CO_2 reactions were also detected for Leonie and Ketos, but the impacts of CO_2 enrichment were only significant under drought-stressed conditions (Figure 3).

3.2 | Grain Yield

The grain weight was significantly influenced by all the tested factors (CO_2 levels, watering levels and genotypes) at $p < 0.001$. The interaction between CO_2 and genotypes was significant at the $p < 0.001$ level, and the interaction between watering and genotypes was significant at the $p < 0.05$ level. According to the Eta Squared test, both genotype and watering had large effects on the grain yield in the experiment; however, CO_2 only had a small effect (Table 4).

At 400 ppm CO_2 concentration, the highest grain yields were observed for BCC1585 and Gerbel under optimum watering conditions, 45.1 g/pot and 42.09 g/pot, respectively. The lowest yield was

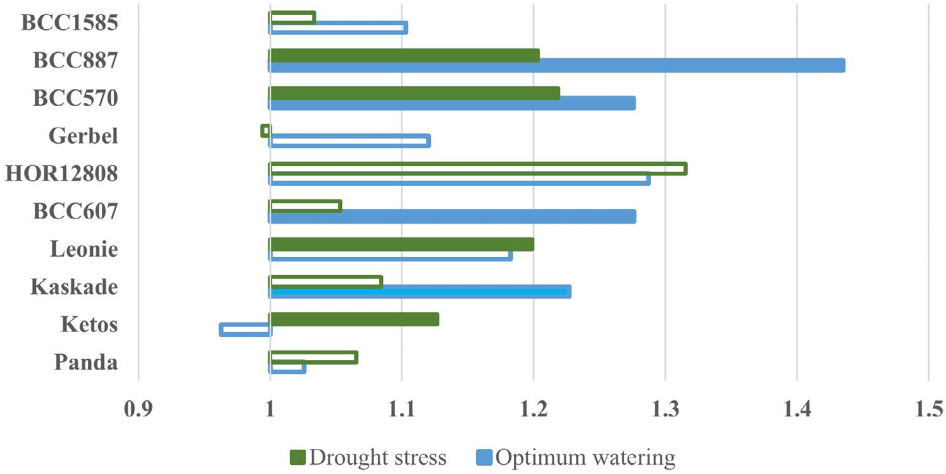


FIGURE 3 | Response of elevated CO_2 on aboveground biomass (g/pot) of winter barley genotypes under optimum watering and drought-stress conditions relative difference between elevated and ambient CO_2 concentrations. Solid bars represent significant CO_2 responses according to Student's *t*-test ($p < 0.1$).

achieved by the BCC607 genotype (13.62 g/pot). At 700 ppm CO_2, the genotypes with the highest yields were Ketos, Gerbel, BCC1585 and BCC887. There were no significant differences between them under optimum irrigation. The same trends were observed under drought-stressed conditions; only the above-listed genotypes and HOR12808 were complete. Under both irrigation levels, Leonie produced significantly lower yields than the other genotypes. On average of the genotypes, the water withdrawal resulted in a 20.01% and 22.0% reduction in grain yield at CO_2 levels of 400 ppm and 700 ppm, respectively. On average of the genotypes, under optimal irrigation conditions, the CO_2 enrichment resulted in an 11.1% increase in yield; however, significant differences were observed in the CO_2 responses of the genotypes. Under optimum irrigation conditions, the CO_2 responses were significantly positive for BCC570, BCC607, Ketos, BCC887, HOR12808 and Kaskade, with yield increases ranging from 13.6% to 40.96%.

Under drought stress conditions, CO_2 enrichment induced a significant increase in grain yield on average across genotypes studied, but the rate of change (8.34%) was lower than under optimal irrigation. Under a limited water supply, the CO_2 fertilisation effects remained stable only for the BCC570, Ketos and BCC1585 genotypes. However, a significant positive CO_2 reaction in terms of yield production was observed for Panda. Leonie showed significant negative CO_2 responses under optimum and limited water supplies, with yield reductions of 13.1% and 17.9%, respectively (Table 4).

3.3 | Water Uptake

The effects of genotype, watering and CO_2 enrichment were statistically significant in relation to water uptake at the $p < 0.001$ level. However, no interactions were observed when the effects of the factors were tested in combination (Table 5). The Eta Squared test revealed that each factor, including genotype, watering and CO_2 enrichment, had a significant impact on the water uptake of plants. Genotype explains the highest proportion of unpredictability, while water supply and CO_2 explain smaller proportions.

The water uptake of the BCC607 variety was significantly lower than that of the other genotypes in all treatments. At a carbon dioxide concentration of 400 ppm, no significant difference in water uptake was detected between the water-demanding genotypes under optimum water supply: Gerbel, Panda, Leonie BCC1585, and Kaskade. Under drought stress conditions, the water uptake of Leonie's water uptake was significantly higher than that of the other genotypes, while no significant differences could be observed between Gerbel, Panda, BCC1585 and Kaskade. At an elevated CO_2 concentration (700 ppm), Leonie and Gerbel took up the highest amount of water under well-watered conditions. In the drought stress treatment, however, no significant differences could be observed in the water use of Panda, Leonie, Gerbel, Ketos and BCC1585. Simulated water withdrawal reduced water uptake by an average of 11.9% and 12.6% across varieties at CO_2 concentrations of 400 ppm and 700 ppm, respectively. CO_2 enrichment reduced the water uptake of the plants by 8.89% and 9.07% under well-watered and drought-stressed conditions, respectively. An increase in the atmospheric CO_2 concentration led to a significant reduction in the water use of the BCC1585, HOR12808, Leonie, Kaskade and Panda genotypes when water supply was at the optimum level. However, under the water-limited conditions, a significant decrease in water uptake was observed in the Gerbel, BCC887, Leonie and Kaskade genotypes (Table 5).

3.4 | Water Use Efficiency

All CO_2 treatments, water supply, and genotype have significant effects on water use efficiency at $p < 0.001$. The interactions between CO_2 treatments and genotypes, and between watering and genotypes, were statistically significant at $p < 0.05$ and $p < 0.001$ levels, respectively. The Eta Squared test confirmed that genotype and CO_2 enrichment have a large effect on water use efficiency, while watering has a medium effect. The ANOVA showed that, in addition to differences in genotype-specific water usage, the impacts of carbon dioxide enrichment dominate the variability in water use efficiency. This also highlights alteration in genotypic CO_2 reactions (Table 6).

TABLE 4 | The effects of the CO_2 enrichment and water shortage on the grain yield (g/pot) of winter barley genotypes.

		Watering levels (B)								
		Control			Drought stress					
		CO_2 levels (A)			CO_2 levels (A)			Mean (B)		
		400 ppm	**700 ppm**	**Mean (A)**	**400 ppm**	**700 ppm**	**Mean (A)**	**400 ppm**	**700 ppm**	**Mean (A × B)**
Cultivars (C)	BCC1585	45.1	43.87	**44.485**	31.47	36.45	**33.96**	**38.29**	**40.16**	39.22
	BCC887	37.95	43.17	**40.56**	31.42	33.54	**32.48**	**34.69**	**38.36**	36.52
	BCC570	22.64	29.68	**26.16**	12.48	17.39	**14.935**	**17.56**	**23.54**	20.55
	Gerbel	42.09	44.51	**43.3**	34.96	37.48	**36.22**	**38.53**	**41.00**	39.76
	HOR 12808	30.95	35.16	**33.055**	28.05	29.62	**28.835**	**29.50**	**32.39**	30.95
	BCC607	13.62	19.2	**16.41**	13.81	11.88	**12.845**	**13.72**	**15.54**	14.63
	Leonie	30.77	24.02	**27.395**	26.74	19.71	**23.225**	**28.76**	**21.87**	25.31
	Kaskade	25.87	33.13	**29.5**	23.52	24.13	**23.825**	**24.70**	**28.63**	26.66
	Ketos	32	44.91	**38.455**	26.04	33.99	**30.015**	**29.02**	**39.45**	34.24
	Panda	40.19	39.02	**39.605**	28.45	34.2	**31.325**	**34.32**	**36.61**	35.47
	Mean	C	C	A × C	C	C	A × C	B × C	B × C	A × B × C
		32.12	**35.67**	***33.89***	**25.69**	**27.84**	***26.77***	***28.91***	***31.75***	30.33

Analysis of variance

Factors	**Degrees of freedom**	**Sum of squares**	**Mean squares**	***F* value**	**Prob**	**LSD 5%**
CO_2 (A)	1.0	277	276.8	29.3	**0.000*****	**3.43**
Watering (B)	1.0	1523	1523.3	161.2	**0.000*****	**4.14**
Genotype (C)	9.0	7547	838.6	88.8	**0.000*****	**4.49**
AB	1.0	8	7.7	0.8	0.370	n.s.
AC	9.0	498	55.3	5.9	**0.000*****	**4.18**
BC	9.0	193	21.5	2.3	**0.025***	**3.22**
ABC	9.0	135	15.0	1.6	0.134	n.s.
Residuals	80.0	756	9.4			

Note: Significance codes: '***' $p < 0.001$ '*' $p < 0.05$. Bold represent significant effect of a single factor and italic represent significat interactions of two factors.
Abbreviation: n.s., non-significant.

TABLE 5 | The effects of the CO_2 enrichment and water shortage on the water use (ml/pot) of winter barley genotypes.

		Watering levels (B)								
		Control			Drought stress					
		CO_2 levels (A)			CO_2 levels (A)			Mean (B)		
		400 ppm	700 ppm	Mean (A)	400 ppm	700 ppm	Mean (A)	400 ppm	700 ppm	Mean (A × B)
Cultivars (C)	BCC1585	25,144	22,878	**24,011**	21,565	19,798	**20,682**	**23,355**	**21,338**	22,346
	BCC887	22,516	21,695	**22,106**	21,094	18,095	**19,595**	**21,805**	**19,895**	20,850
	BCC570	12,552	12,159	**12,356**	12,711	13,017	**12,864**	**12,632**	**12,588**	12,610
	Gerbel	26,929	25,839	**26,384**	23,425	20,659	**22,042**	**25,177**	**23,249**	24,213
	HOR 12808	20,780	17,804	**19,292**	17,619	15,916	**16,768**	**19,200**	**16,860**	18,030
	BCC607	8989	9607	**9298**	7054	7128	**7091**	**8022**	**8368**	8195
	Leonie	26,098	22,969	**24,534**	26,504	21,419	**23,962**	**26,301**	**22,194**	24,248
	Kaskade	25,012	20,269	**22,641**	21,485	17,335	**19,410**	**23,249**	**18,802**	21,025
	Ketos	24,421	22,634	**23,528**	19,416	20,394	**19,905**	**21,919**	**21,514**	21,716
	Panda	26,864	24,669	**25,767**	22,395	21,651	**22,023**	**24,630**	**23,160**	23,895
	Mean	C	C	A × C	C	C	A × C	B × C	B × C	A × B × C
		21,931	**20,052**	20,475	**19,327**	**17,541**	18,434	20,629	18,797	19,713

Analysis of variance table

Factors	Degrees of freedom	Sum of squares	Mean squares	*F* value	Prob	LSD 5%
CO_2 (A)	1.0	110,000,000	110,000,000	23.25	**0.000*****	**2037.8**
Watering (B)	1.0	183,600,000	183,600,000	38.79	**0.000*****	**2017.7**
Genotype (C)	9.0	3,030,000,000	336600000.0	71.12	**0.000*****	**2220.9**
AB	1.0	41,033	41033.1	0.01	0.926	n.s.
AC	9.0	60,210,000	6690135.4	0.01	0.926	n.s.
BC	9.0	77,940,000	8660295.9	1.83	0.075	n.s.
ABC	9.0	18,390,000	2043725.8	0.43	0.914	n.s.
Residuals	80.0	379,000,000	4733562.0			

Note: Significance code: '***' $p < 0.001$. Bold represent significant effect of a single factor and italic represent significncat interactions of two factors.
Abbreviation: n.s., non-significant.

Under atmospheric CO_2 concentrations (400 ppm), the genotype Kaskade (1.034 kg m^{-3}) and Leonie (1.18 kg m^{-3}) exhibited the highest water use efficiencies under optimum water supply. The most favourable water use efficiencies were observed in the BCC570 (1.863 kg m^{-3}), BCC1585 (1.791 kg m^{-3}) and BCC887 (1.687 kg m^{-3}) genotypes. Under elevated CO_2 concentrations (700 ppm), Leonie (1.047 kg m^{-3}) exhibited the lowest water use efficiency, while BCC 570 (2.446 kg m^{-3}) exhibited the highest. Overall, the carbon dioxide enrichment significantly increased water use efficiency under optimum watering conditions by 24.57% and drought-stressed conditions by 21.72%. However, the water shortage significantly increased water use efficiency under both 400 ppm (10.48%) and 700 ppm (12.36%) CO_2 concentrations (Table 6).

Under optimum water supply, carbon dioxide enrichment induced significant changes in all genotypes except BCC1585 and Panda. While most genotypes showed positive CO_2 reactions (from 14.4% to 57.9%); a significant decrease in water use efficiency (−11.3%) was observed in the Leonie variety. Under drought-stressed conditions, the water use efficiency improved significantly as a result of the CO_2 enrichment (from 13.5% to 52.1%) for all genotypes except Leonie, whose water use efficiency decreased by 10.58% under a CO_2 concentration of 700 ppm compared to 400 ppm. Of the 10 genotypes studied, positive effects of CO_2 enrichment on water use efficiency were confirmed in seven varieties. Leonie was the only genotype to consistently show negative CO_2 responses, regardless of irrigation levels (Table 6).

4 | Discussion

Agriculture is a water-intensive industry, and field crop production is particularly vulnerable to the effects of climate change, socioeconomic development, and population growth (Gerten et al. 2020). Hexaploid wheat (*Triticum aestivum* L.) and diploid barley (*Hordeum vulgare* L.) are among the most important cereal crops in terms of feeding the growing human population and serving as animal feed. Consequently, an examination of the stress responses and water use properties of the cultivated varieties is essential to propose appropriate varieties that can support the effectiveness of the water-conservation-oriented agronomic practices (Giraldo et al. 2019). Barley varieties have been found to be more adaptable to adverse environmental conditions than wheat genotypes (Varga et al. 2013). Therefore, it can be presumed that cultivating barley can be more effective in regions where water availability is the main limiting factor in achieving the genetically determined yield potential (Arenas-Corraliza et al. 2019).

(Bardehji et al. 2023) examined the physiology and production of four Iranian local barley varieties in water-limited environments. They found that the two-rowed varieties responded positively to CO_2 enrichment under both normal and limited water supply. However, no positive CO_2 reactions were observed in the six-rowed varieties under drought stress. In our study, the BCC570 and Ketos genotypes responded positively to CO_2 enrichment when grain yield was taken into account; however, they were six-rowed and two-rowed genotypes, respectively. Furthermore, Leonie yielded significantly decreased at elevated CO_2 concentrations compared to the ambient levels under both well-watered and drought-stressed conditions. Bourgault et al. (2013) examined the CO_2 reactions of twelve wheat genotypes, and calculated water use efficiency based on exchange intensity. However, no evidence of genotypic variability in the response to elevated CO_2 was observed. Our study showed significant variety-specific differences in CO_2 reactions and confirmed the positive effects of higher carbon dioxide concentrations on yield production and reduced water uptake. While only two barley genotypes showed an increase in yield, the water use efficiency of all varieties except Leonie improved significantly at 700 ppm CO_2 compared to the ambient concentrations. Fitzgerald et al. (2016) published that elevated CO_2 can increase wheat yield and counterbalance the negative impacts of heat stress. However, our results highlight that, in the case of barley, the CO_2 enrichment can only partly counterbalance the impacts of the water shortage. The observed reduction in the water demand could support the sustainability of the field crop production. Ingvordsen et al. (2015) tested 138 barley genotypes in a combination with elevated temperature and atmospheric carbon dioxide concentrations. They discovered that carbon dioxide enrichment increased grain yield by 16%. However, the combined treatment resulted in a 29% decrease in productivity. Droughts are often accompanied by severe water shortage and high air temperature, but Plants are able to survive prolonged periods of extreme heat as long as there is water available in the soil. Our study focuses on the role of a water-limited environment, and we confirmed that water withdrawal alone resulted in a 20.01% and 22% decrease in grain under ambient and elevated CO_2 concentrations, respectively. In the long term, rising atmospheric CO_2 concentrations will also become a significant factor in field production. Our study confirmed that, for some genotypes, CO_2 enrichment can offset the negative effects of simulated drought on grain yield. However, large variability between genotypes must be emphasised: for example, the yield of the Ketos genotype was 6.2% higher under drought conditions at 700 ppm CO_2 concentration than well-watered plants at 400 ppm. Conversely, the negative impacts of the elevated CO_2 concentrations on the grain yield of Leonie genotype were more severe under drought conditions (−26.3%) than under optimum watering (21.9%). Mitterbauer et al. (2017) determined that the aboveground biomass increased by 15% as a result of the CO_2 enrichment (700 ppm) across 98 barley varieties in well-irrigated open-top chambers. In our study, CO_2 enrichment did not generally modify aboveground biomass production, however positive CO_2 reactions were observed in three genotypes under well-watered conditions.

Increasing harvest index could significantly contribute to the improvement of crop productivity, solving the problem of food supplies for the rapidly increasing population (Asefa 2019). Grain yield is the primary product of barley cultivation, so our results have significant practical implications as we confirmed that CO_2 enrichment generally has more intensive impacts on grain yield than on the by-products. Mitterbauer et al. (2017) ranked the examined genotypes based solely on biomass production, but grain yield and water use efficiency are more relevant properties in field crop production. A comparison with the previously published results shows that BCC570, Gerbel and HOR12808 were identified as varieties insensitive to elevated CO_2; but in our experiment, the biomass of BCC570 and Ketos

TABLE 6 | The effects of the CO_2 enrichment and water shortage on the water use efficiency (kg m^{-3}) of winter barley genotypes.

		Watering levels (B)								
		Control			Drought stress					
		CO_2 levels (A)			CO_2 levels (A)			Mean (B)		
		400 ppm	**700 ppm**	**Mean (A)**	**400 ppm**	**700 ppm**	**Mean (A)**	**400 ppm**	**700 ppm**	**Mean (A×B)**
Cultivars (C)	BCC1585	1.791	1.916	**1.854**	1.461	1.843	**1.652**	**1.626**	**1.880**	1.753
	BCC887	1.687	2.070	**1.879**	1.486	1.852	**1.669**	**1.587**	**1.961**	1.774
	BCC570	1.863	2.446	**2.155**	0.886	1.347	**1.117**	**1.375**	**1.897**	1.636
	Gerbel	1.562	1.787	**1.675**	1.496	1.814	**1.655**	**1.529**	**1.801**	1.665
	HOR 12808	1.493	2.004	**1.749**	1.593	1.870	**1.732**	**1.543**	**1.937**	1.740
	BCC607	1.532	2.012	**1.772**	1.688	1.916	**1.802**	**1.610**	**1.964**	1.787
	Leonie	1.180	1.047	**1.114**	1.030	0.921	**0.976**	**1.105**	**0.984**	1.045
	Kaskade	1.034	1.633	**1.334**	1.108	1.395	**1.252**	**1.071**	**1.514**	1.293
	Ketos	1.312	1.997	**1.655**	1.363	1.672	**1.518**	**1.338**	**1.835**	1.586
	Panda	1.494	1.582	**1.538**	1.271	1.579	**1.425**	**1.383**	**1.581**	1.482
	Mean	C	C	A×C	C	C	A×C	B×C	B×C	A×B×C
		1.495	**1.849**	*1.672*	**1.338**	**1.621**	*1.480*	*1.417*	*1.735*	1.576

Analysis of variance

Factors	Degrees of freedom	Sum of squares	Mean squares	*F* value	Prob	LSD 5%
CO_2 (A)	1.0	3.0460	3.0463	67.1530	**0.000*****	**0.1285**
Watering (B)	1.0	1.1160	1.1165	24.6110	**0.000*****	**0.1365**
Genotype (C)	9.0	6.3060	0.7007	15.4450	**0.000*****	**0.2632**
AB	1.0	0.0390	0.0393	0.8670	0.3546	n.s.
AC	9.0	0.9340	0.1038	2.2880	**0.024***	**0.224**
BC	9.0	2.5480	0.2831	6.2410	**0.000*****	**0.256**
ABC	9.0	0.3360	0.0373	0.8220	0.5972	n.s.
Residuals	80.0	3.6290	0.0454			

Note: Significance codes: '***' 0.001; '*' 0.05. Bold represent significant effect of a single factor and italic represent signifincat interactions of two factors.
Abbreviation: n.s., non-significant.

increased significantly under both well-watered and drought-stressed conditions. Our results support those of (Mitterbauer et al. 2017) because we also detected no significant CO_2 reactions for Gerbel and HOR12808 in terms of biomass and grain yield under either optimum or limited water supply.

Water use efficiency is a useful indicator of the interaction between environmental factors, water use and the yield production (Hoover et al. 2023). Previous studies have confirmed that there is significant variability in the WUE of cereal varieties, which is affected by various abiotic factors (Farkas et al. 2021; Hussain et al. 2022). In our experiment, genotypic differences dominated the determination of the WUE; however, the impacts of the watering and the CO_2 enrichment were also significant. The WUE of BCC570, BCC607, Gerbel, Ketos, BCC887, HOR12808 and Kaskade improved significantly as a result of the CO_2 enrichment under both watering regimes. A significant positive CO_2 response was also detected for the BCC1585 and Panda genotypes under drought stress conditions.

The mechanism of action of elevated carbon dioxide concentrations manifests through numerous plant physiological processes during plant development (Seneweera and Norton 2011). To determine exactly how simulated CO_2 enrichment occurs and through which regulatory processes, very complex experimental systems, including plant physiological measurements, are required (Leakey and Lau 2012). Our experiment was suitable for the selection of genotypes that were suitable for further studies because of their reactions to elevated CO_2 concentration under limited water availability in comparison with optimal watering. Our study also highlighted how genotypic responses altered under regulated environmental conditions and under constant CO_2 supply in a comparison with the previous study (Mitterbauer et al. 2017).

The most direct effect of elevated CO_2 is the stimulation of photosynthesis, especially in C3 plants such as wheat and barley, in which Rubisco plays a key role by catalysing the fixation of CO_2 in the Calvin cycle (Ekele et al. 2025). However, Rubisco also has oxygenase activity, which reacts with O_2 to initiate photorespiration. Rubisco can react with O_2 under low CO_2 conditions that favour photorespiration, so by inhibiting photorespiration, this enzyme can protect previously fixed carbon and increase net photosynthetic efficiency (Busch 2020). Based on our results, the differences between genotypes can be inferred from the differences in Rubisco activity that can shift the balance between photosynthesis and photorespiration.

In our study, we tried to measure the photosynthetic activity of the genotypes during the drought stress period using the CIRAS-2 Portable Photosynthesis System (PP Systems Inc., Hertfordshire, U.K.), but we could not obtain comparable results because the stomata were closed quickly, especially when the plants were exposed to drought stress. But special investigations could answer the open questions of whether the elevated CO_2 induced a faster stomatal closure and how the regulation of stomata was induced by the water shortage. Elevated atmospheric CO_2 affects stomata primarily by altering guard cell physiology and plant water relations (Xu et al. 2010). However, in a CO_2-rich environment, plants can maintain sufficient CO_2 uptake while losing less water through transpiration, thereby improving energy and water balance. de Haan et al. (2021) confirmed that there is no significant correlation in the major field crops between water use efficiency (WUE), which was calculated by dividing the plant's water uptake and harvested yield, and the intrinsic water use efficiency (WUE_i), determined based on gas exchange intensity; therefore, even though some physiological processes influence plant physiology at a given time, it does not necessarily impact the ratio of productivity and photorespiration at a growing cycle level.

Elevated CO_2 can activate several signalling pathways that may play a role in the regulation of stomatal closure (Xu et al. 2016). HT1 (High Leaf Temperature 1) normally suppresses CO_2-induced closure under ambient CO_2, but its elevated level inhibits HT1 activity, allowing downstream closure signalling (Hõrak et al. 2016; Tian et al. 2015). The OST1 (Open Stomata 1) pathway is a central component of abscisic acid (ABA) signalling, demonstrating an overlap between the ABA and CO_2 pathways (Negi et al. 2014). However, CO_2 signalling is not entirely ABA-dependent; stomata can respond directly to elevated CO_2 without large increases in ABA (Hsu et al. 2018). The complexity of the physiological processes behind the stomata regulations shows that, besides reactive oxygen species (ROS) and calcium signalling, numerous other regulatory mechanisms modulate stomata and influence gas exchange (Liu et al. 2022). Our experiment was not suitable for measuring the physiological background behind these processes, even if, because many gene functions have already been confirmed only in Arabidopsis. Our experiment can support the selection of genotypes showing specific CO_2 reactions for more specific studies.

However, it was also confirmed that long-term exposure to elevated CO_2 can also lead to photosynthetic acclimation, which may result in a decrease in the initial stimulation level over time (Xu et al. 2015; Krämer et al. 2022). In our study, the observed genotypic variability could be induced by such an acclimation process, as plants were grown under elevated CO_2 concentrations since planting, because this experimental setup simulates well future environmental conditions. In the previous experiment conducted by (Mitterbauer et al. 2017), the CO_2 was supplied only during daylight hours, which can also result in differences in the genotypic responses between the two experiments. However, the effects of potential acclimation should be evaluated in future experiments.

Elevated atmospheric CO_2 can strongly influence plant osmotic adjustment by altering carbon metabolism and solute accumulation (López-Jurado et al. 2025). Under elevated CO_2, plants can accumulate more carbohydrates and starch degradation products (glucose and maltose), which act as osmolytes and can help reduce cellular osmotic potential (Hendrix et al. 1994; Darko et al. 2019). The most intense osmolyte accumulation may contribute to increased drought tolerance in some genotypes. Elevated CO_2, particularly under abiotic stress conditions, may enhance the accumulation of compatible osmolytes such as proline, glycine betaine, organic acids, and soluble amino acids. These compounds contribute to osmotic adjustment while stabilising proteins and membranes and maintaining enzyme activity without interfering with normal cellular metabolism (Ashraf and Foolad 2007; Ghosh et al. 2021).

5 | Conclusions

Our results highlight that the interactions between different factors can significantly modify the adaptability of winter barley genotypes. Therefore, multifactorial experiments conducted under controlled conditions are necessary to properly characterise individual varieties' responses to water-limited environments and atmospheric CO_2 concentrations. Our results show that water withdrawal generally decreases yield production and that the rate of yield reduction is significantly higher at 700 ppm CO_2 concentration than at 400 ppm. However, it has been confirmed that counterbalancing the negative impact on yield, an elevated CO_2 concentration reduces water uptake and increases water use efficiency, resulting in a water-saving effect. Plants grown under elevated CO_2 conditions can compensate for many of the negative effects of drought stress and contribute to water sequestration in the soil. This could be a key factor in implementing sustainable field crop production.

In the open-top chamber experiment that served as the basis for variety selection, the two varieties (BCC1585 and BCC887) that exhibited positive CO_2 responses also reacted positively to increased atmospheric carbon dioxide under controlled climatic conditions, even under optimal water supply and simulated drought stress, although the magnitude of the change was not significant for BCC1585. The biomass production of the BCC570 genotype, which showed negative CO_2 responses in the preliminary experiment, was significantly positive in both treatments in our trial, while there was no difference in trends between the two experiments for the Gerbel and HOR12808 varieties. Regarding grain yield, we confirmed this trait in varieties that showed positive CO_2 responses in the preliminary experiment; however, for BCC1585, this was only the case under simulated water deficit, while for the BCC887 genotype, it was observed under optimal water supply. Similar to biomass production, the BCC570 variety exhibited opposite responses in the grain yield analysis across the two experiments; under both water supply conditions, we observed a significant increase in yield at 700 ppm CO_2 compared to plants grown at 400 ppm CO_2.

The Leoni variety stood out, as its biomass production showed significant positive CO_2 responses; in contrast, during the grain yield assessment, we observed a significant decrease in yield for this variety at both water supply levels. As a result of these characteristics, this was the only variety whose water-use efficiency decreased at both water supply levels. Regardless of the previously determined CO_2 responses, each tested variety, except for Leonie, exhibited a significantly positive CO_2 effect on water-use efficiency.

Based on our results, we recommend BCC570 and Leonie varieties for future investigations, as these genotypes exhibited different responses to elevated carbon dioxide levels compared to the other genotypes, or their responses differed significantly from those observed in previous experiments.

Author Contributions

Balázs Varga: conceptualisation, writing – original draft, visualisation, methodology. **Zsuzsanna Farkas:** investigation, writing – review and editing, validation. **Emese Varga-László:** writing – review and editing, software, data curation. **Ottó Veisz:** funding acquisition, project administration, resources, supervision. **Máron György:** visualisation, formal analysis, writing – review and editing.

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Ethics Statement

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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