CO₂ Responses of Winter Wheat, Barley and Oat Cultivars under Optimum and Limited Irrigation

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Abstract: Field crop production must adapt to the challenges generated by the negative consequences of climate change. Yield loss caused by abiotic stresses could be counterbalanced by increasing atmospheric CO₂ concentration, but C₃ plant species and varieties have significantly different reactions to CO₂. To examine the responses of wheat, barley and oat varieties to CO₂ enrichment in combination with simulated drought, a model experiment was conducted under controlled environmental conditions. The plants were grown in climate-controlled greenhouse chambers under ambient and enriched (700 ppm and 1000 ppm) CO₂ concentrations. Water shortage was induced by discontinuing the irrigation at BBCH stages 21 and 55. Positive CO₂ responses were determined in barley, but the CO₂-sink ability was low in oats. Reactions of winter wheat to enriched CO₂ concentration varied greatly in terms of the yield parameters (spike number and grain yield). The water uptake of all wheat cultivars decreased significantly; however, at the same time, water-use efficiency improved under 1000 ppm CO₂. Mv Ikva was not susceptible to CO₂ fertilization, while no consequent CO₂ reactions were observed for Mv Nádor and Mv Nemere. Positive CO₂ responses were determined in Mv Kolompos.

Keywords: winter cereals; CO₂ enrichment; drought stress; WUE; climate change

1. Introduction

The Industrial Revolution had significant environmental and social impacts. Due to its enormous agricultural, hygienic and medical achievements, the human population is projected to exceed 10 billion by the end of the century [1]. Among others, the greatest challenges of the upcoming decades will be to maintain food security and to ensure drinking water supply. The inventions of the Industrial Revolution accelerated not only the rate of population growth, but also the burning of fossil fuels, increasing the concentration of atmospheric CO₂ from 280 ppm [2] to ~416 ppm [3]. If CO₂ emission remains at the current level, in 30 years its atmospheric concentration will reach 550 ppm [4]. Although CO₂ is part of the atmosphere and is necessary for normal plant functions, it has become one of the most significant greenhouse gases due to its level, which has almost doubled [5] since its first measurement. Increased CO₂ affects photosynthesis, decreases water use, improves the growth and production of the plants [6], has direct implications for plant metabolism and decreases photorespiration [7]. In relation to this, increasing carbon-nitrogen rates can be observed to have changed the chemical processes in leaves, thus also reshaping the eating habits of herbivores [8,9]. In addition, an increase in CO₂ level can reduce stomatal conductance, resulting in better water-use efficiency [10]. In C₃ crops, an elevated CO₂ level can stimulate net photosynthetic CO₂ assimilation, leading to greater biomass production and yield [11]. Although the ‘CO₂ fertilization effect’ on C₃ crops is
well-known phenomenon [12–14], it depends heavily on various environmental growing conditions, such as air temperature or the availability of nutrients and soil water [11,15–17]. Scenarios have forecasted that drought will be more frequent and more severe in the next decades in many crop-growing areas [18]. Drought is one of the major stress factors that limit cereal production worldwide, and may affect about 40–60% of the world’s agricultural lands [19]. It can severely influence the growth and development of plants, causing various physiological and biochemical damage. For example, it can lead to stomatal closure and can reduce photosynthesis, transpiration, growth and antioxidant production, and can also change hormonal composition [20–23]. Increased atmospheric CO$_2$ may contribute to climate change, including changes in precipitation and evapotranspiration, and may increase the risk of drought in many areas, as seen in Central Europe [24–26]. Several studies have indicated that plants in their reproductive phases (i.e. from elongation to anthesis stages) are less tolerant to water stress [27–29]. Especially in the case of wheat, the effects of water shortage depend on onset time, duration and intensity. Aside from the developmental stages and the severity of the stress, the effects of water shortage on cereals depend on soil type, environmental conditions [30,31], the cultivated varieties or species [32,33] and also the cultivation technologies employed [34,35]. Drought can cut wheat yields by up to 92% [27], but sometimes extreme drought at the right time can lead to a total yield loss. Since wheat (Triticum aestivum L.) is one of the most important cereals in human and animal nutrition, and as it is one of the most extensively grown crops [36,37], drought can cause serious damage to food security. In addition to wheat, barley (Hordeum vulgare L.) is an important cereal, contributing nearly 157 million metric tons to cereal production worldwide [38]. The most negative correlation was observed between yield and drought stress at the heading and flowering stages in barley [39]. Although in the last few decades the demand for oats (Avena sativa L.) in human consumption has increased because of their dietary benefits, compared to other cereal crops, their production is more suited to marginal environments, such as cool–wet climates and soils with low fertility [40,41]. Among cereals, oats are the most sensitive regarding drought stress at germination and heading developmental stages [42]. Water-use efficiency (WUE; kg·m$^{-3}$) reflects the relationship between carbon and water cycles, and it is a key indicator of drought tolerance. WUE is an essential parameter for assessing the reactions of plants to climate change. It is a well-known phenomenon that there are considerable differences between the WUE values of the cereal species [43].

Although temperature, water availability and atmospheric CO$_2$ are important regulators of plant growth, function and development, their impacts on different species and varieties show great variability. In this study, we examined the effects of different CO$_2$ concentrations combined with simulated water shortage at different developmental stages on four Hungarian winter wheat varieties, one winter oat and one winter barley variety. The aims of our study were: (1) to determine how water shortage and different CO$_2$ concentrations influence the phenological and yield parameters of some widely cultivated cereal varieties in the Carpathian Basin, (2) to determine the water uptake and water-use efficiency of plants under different environmental conditions and (3) to quantify the specific CO$_2$ responses of the examined varieties.

2. Materials and Methods

2.1. Experimental Design

Four winter wheat (Triticum aestivum L.) varieties (‘Mv Ikva’, ‘Mv Nádor’, ‘Mv Nemere’, ‘Mv Kolompos’), one winter barley (Hordeum vulgare L.) (‘Mv Initium’) and one winter oat (Avena sativa L.) (‘Mv Hópehely’) cultivar were examined in a model experiment at the Agricultural Institute Centre for Agricultural Research, Eötvös Loránd Research Network in Martonvásár, Hungary. All varieties were bred locally. The study was carried out in climate-controlled greenhouse chambers in 2020. The experiment was begun on 3rd February and ended at the end of June, when the plants were harvested manually. ‘Mv Ikva’ and ‘Mv Initium’ are early-ripening varieties; ‘Mv Nádor’ and ‘Mv Nemere’ are...
middle-ripening, while ‘Mv Kolompos’ and ‘Mv Hópehely’ are late-ripening varieties. The experimental design consisted of three water-supply treatments (control, water shortage at tillering, and water-shortage at heading developmental stage). Control (‘C’) plants (54 pots, 216 plants in total,) were watered until reaching 60% of soil water-holding capacity (WHC). Drought stress was simulated in one-third of the plants (54 pots, 216 plants in total) by stopping the irrigation completely at BBCH stage 21 (‘T’) [44], and the other one-third (54 pots, 216 plants in total) were similarly stressed at BBCH stage 55 (‘H’). The WHC was determined each day at 9:00 during the stress treatments in the centre of the pot, using 5TE sensors (Decagon Devices Ltd., Pullman, WA, USA), and pots were re-watered when the soil water content dropped below 5 v/v%. In this way, the plants were continuously exposed to the same level of stress intensity. The experiment was carried out in three similar climate-controlled greenhouse chambers under three different atmospheric CO$_2$ levels. Aside from the ambient level chamber (~400 ppm) (control), CO$_2$ concentrations in the other two chambers were enriched to 700 ppm or 1000 ppm, respectively. Pure CO$_2$ was introduced into the chambers through a perforated pipe network placed 0.5 m above the plants. Uniform gas distribution was achieved by ventilation. Carbon dioxide concentration was controlled by the SH-VT250 device (SH-VT250 CO$_2$, Temperature and Humidity Transmitter, Soha Tech Co., Ltd., Soul, Korea) and the CO$_2$ level was measured and verified by the Wöhler CDL 210 (Wöhler CDL Serie 210 CO$_2$ Messgerät, Wöhler Technik GmbH, Bad Wünnenber, Germany) logger device in the chambers where the plants were grown under elevated CO$_2$ concentration.

Four vernalized plants of each variety were planted in plastic pots (depth: 27 cm; diameter: 24 cm) as described by Varga et al. [28]. The experimental design involved 162 pots in total; 54 pots in each of the three different greenhouse chambers with different levels of CO$_2$. We examined 684 plants in total. 12 plants of each variety were treated the same way at every irrigation level and every CO$_2$ level. At full maturity, the dry weight of the aboveground biomass (shortly biomass, BM), spike numbers and yields per pot were measured. The exact water uptake of the plants/pot was monitored by a digital balance (ICS689g-A15, Mettler Toledo Ltd., Budapest, Hungary) from the planting to the final harvest. Grain yield and biomass were measured using a digital scale (440-45N, KERN & SOHN GmbH, Balingen, Germany). The harvested aboveground biomass (BM) was oven-dried for two days at 70 °C, then the dry weight of the plant material was measured.

Water-use efficiency (WUE) was calculated using Equation (1)

$$WUE = \frac{GY}{WU}$$  \hspace{1cm} (1)

where WUE is water-use efficiency (kg·m$^{-3}$), GY is grain yield (kg), and WU is water use (m$^3$).

The harvest index (HI) was calculated as described in Equation (2).

$$HI = \frac{GY}{BM} \times 100$$  \hspace{1cm} (2)

where HI is harvest index (%), GY is grain yield (kg) and BM is dry aboveground biomass (shortly biomass) (kg).

Relative changes of the different parameters to elevated carbon dioxide level were calculated using Equation (3)

$$\frac{Ex}{A} \text{ or } \frac{Ey}{A}$$  \hspace{1cm} (3)

where $A$ is the different parameters’ values on 400 ppm CO$_2$ level, $Ex$ is the different parameters’ values at 700 ppm CO$_2$ level and $Ey$ is the different parameters’ values at 1000 ppm CO$_2$ level.

2.2. Plant Growth Conditions

Seeds of each variety were germinated on 14 December 2019. The seeds were kept at room temperature (22 °C) in plastic boxes in darkness for two days; after that, the
plants were transferred into a vernalization chamber (temperature: 4 °C) for 48 days. Four seedlings were planted into each pot on 3 February 2020. Each pot contained 10 liters of a 3:1:1 (v/v) homogenous mixture of soil, sand and humus. Climatic conditions were automatically regulated using the Spring–Summer climatic program [45]. Air temperature was increased from a range of 10–12 °C to one of 24–26 °C during the growing period, and relative humidity was kept between 60% and 80%. When necessary, natural light was enhanced by artificial illumination to 500 µmol·m⁻²·s⁻¹ at the beginning of the vegetation period, and gradually increased to 700 µmol·m⁻²·s⁻¹. Nutrient solution was provided once a week. To each pot, 22 mL water-soluble fertilizer (14% N, 7% P₂O₅, 21% K₂O, 1% Mg, 1% B, Cu, Mn, Fe, Zn; Volldünger Classic; Kwizda Agro Ltd., Vienna, Austria) was added before irrigation. The plants were watered with tap water two times a week until the tillering stage, and three times a week afterwards. The soil was covered with non-transparent foil to prevent soil evaporation. Sulphur (Thiovit Jet) and lambda-cyhalothrin (Karate Zeon 5 CS, Syngenta Ltd. Switzerland) were applied two times.

2.3. Statistical Processing

The experimental design involved four winter wheat, one winter barley and one winter oat variety, three watering treatments and three CO₂ levels in three replicates. A multi-way ANOVA was performed to determine the effects of the tested factors (variety, water supply and CO₂) and Tukey’s post hoc test was used to compare means. The SPSS 16.0 program (IBM, Armonk, NY, USA) and Microsoft Excel (Microsoft, Redmond, WA, USA) were used for the statistical analysis and visualization. The significance level was set at $p \leq 0.05$. ANOVA tables are presented in Tables A1–A6.

3. Results

At 400 ppm CO₂ level, the drought stresses (water shortage at BBCH 21 [‘T’] and at BBCH 55 [‘H’]) caused a significant decrease in biomass compared to the control treatment in each variety, except for Mv Kolompos (Table 1). Furthermore, a significant difference was observed between the two stress treatments in Mv Höpehely: compared to the early stress, the late drought reduced biomass to a greater extent. The highest decrease in biomass compared to the control was observed for Mv Ikva (−29% and −33% in ‘T’ and ‘H’ treatments, respectively). At 700 ppm CO₂ concentration, in barley, oat, Mv Ikva and Mv Kolompos, significant differences in biomass were observed between the treatments (‘C’, ‘T’ and ‘H’), and the water shortage at BBCH 55 caused significant reductions in biomass values in all examined varieties. In Mv Kolompos and Mv Nádor, both stress treatments significantly lowered the biomass of the plants compared to the control. The most pronounced decrease was observed for the oat cultivar (−19% and −48% compared with the control in ‘T’ and ‘H’ treatments, respectively) (Table 1). At 1000 ppm CO₂, water withdrawal at BBCH 21 decreased the biomass of Mv Ikva (−10%) and Mv Kolompos (−9%). The simulated drought at BBCH 55 decreased the biomass of Mv Höpehely (−35%), Mv Nemere (−13%) and Mv Kolompos (−11%) compared to the control treatment (Table 1).

Mv Initium responded positively to CO₂ enrichment under stress, but this reaction was not observed under well-watered conditions (Figure 1). The tested oat variety appeared to be susceptible to the level of atmospheric CO₂ concentration. Either in the control treatment or with water withdrawal at BBCH 21 stage, CO₂ enrichment to 700 ppm increased biomass, while 1000 ppm CO₂ concentration inhibited plant growth. When oat plants suffered from drought at BBCH stage 55, both levels of CO₂ enrichment influenced biomass production negatively. Significant and negative CO₂ reactions were observed for Mv Ikva in the control and the ‘T’ treatments, but no significant CO₂ responses could be observed under drought stress conditions induced at BBCH stage 55. The other three wheat varieties (Mv Nádor, Mv Kolompos and Mv Nemere) showed positive CO₂ responses in terms of biomass under 700 ppm CO₂ level in each treatment but this tendency was not detected under 1000 ppm (Figure 1).
### Table 1. Biomass (g) of the tested varieties.

| Variety                  | Treatment | ~400 ppm | 700 ppm | 1000 ppm |
|--------------------------|-----------|-----------|----------|----------|
| **Mv Initium (winter barley)** | C         | 55.46Ba1  | 53.78Cc1  | 53.86Aa1  |
|                          | T         | 47.26Bb3  | 58.59Aa1  | 54.56Aa2  |
|                          | H         | 45.3Ab2   | 54.61Ab1  | 52.32Aa3  |
| **Mv Hopehely (winter oat)** | C         | 61.70Aa2  | 73.67Aa1  | 55.82Aa3  |
|                          | T         | 54.55Ab2  | 59.82Ab1  | 52.52Aa2  |
|                          | H         | 49.05Ac1  | 38.59Cc2  | 36.08Cb2  |
| **Mv Ikva (winter wheat)**     | C         | 50.15Da1  | 43.41Ea2  | 36.97Ca3  |
|                          | T         | 35.73Cb1  | 33.02Ac2  | 33.24Cb2  |
|                          | H         | 33.82Cb12 | 36.92Db1  | 33.36Cb2  |
| **Mv Nador (winter wheat)**     | C         | 40.05Ea2  | 44.82Ec1  | 38.19Ca2  |
|                          | T         | 35.79Cb12 | 38.24Cb1  | 34.88Ca2  |
|                          | H         | 34.62Cb2  | 37.21Db1  | 34.94Da2  |
| **Mv Nemere (winter wheat)**     | C         | 40.93Ea2  | 45.86Ca1  | 36.87Ca2  |
|                          | T         | 37.91Cb12 | 41.32Db1  | 35.19Ab3  |
|                          | H         | 36.74Cb2  | 39.34Cb1  | 31.97Cb3  |
| **Mv Kolompos (winter wheat)**   | C         | 46.49Ca3  | 55.91Ba1  | 48.03Ba2  |
|                          | T         | 45.65Ba2  | 54.83Ba1  | 43.82Bb2  |
|                          | H         | 45.00Ba2  | 49.94Bb1  | 42.96Bb2  |

'C': control treatment; 'T': drought stress at BBCH 21; 'H': drought stress at BBCH 55. Capital letters indicate the statistical significance between the varieties; lowercase letters indicate the statistical significance between the treatments; the numbers show the statistical significance between the different CO₂ levels at $p \leq 0.05$ level ($n = 3$).

**Figure 1.** Relative changes in cultivars in response to elevated CO₂ in terms of biomass; 'C': control treatment; 'T': drought stress at tillering stage; 'H': drought stress at heading stage. 'INI': Mv Initium, (winter barley); 'HOP': Mv Hopehely (winter oat); 'IKVA': Mv Ikva (winter wheat); 'NAD': Mv Nador (winter wheat); 'NEM': Mv Nemere (winter wheat); 'KOL': Mv Kolompos (winter wheat). Full bars represent significant differences compared to the control (400 ppm) $p \leq 0.05$ level ($n = 3$).
Under ambient CO₂ concentration, drought induced at BBCH stage 21 caused a significant increase in spike numbers in Mv Nemere (+30%) and Mv Nádor (+18%), but a decrease was observed for Mv Initium (−13%) (Table 2). Water shortage at BBCH 55 resulted in a decrease in spike numbers in Mv Ikva (−24%) and Mv Initium (−13%). At 700 ppm CO₂ level, simulated drought at the tillering stage (BBCH 21) significantly reduced spike numbers in Mv Ikva (−12%) but increased the number of productive tillers in Mv Nemere (+23%) and Mv Initium (+13%) (Table 2). Even drought stress at BBCH stage 55 influenced spike numbers by improving tillering ability. Spike numbers increased in Mv Nádor (+43%) and decreased in Mv Ikva (−12%) compared with the well-watered control. At 1000 ppm CO₂ level, water shortage at tillering stage increased spike numbers only in Mv Nemere (+30%), and at heading stage only in Mv Ikva (+31%). In Mv Ikva, Mv Nemere and Mv Kolompos, there were significant differences between the two stress treatments. Higher spike numbers were observed for Mv Nemere and Mv Kolompos when water shortage occurred at the early stage of development, while in Mv Ikva, the effect of the late drought was more pronounced (Table 2).

Table 2. Average spike number per pots of the tested varieties.

| Variety            | Treatment | ~400 ppm | 700 ppm | 1000 ppm |
|--------------------|-----------|-----------|----------|----------|
| Mv Initium (winter barley) | C         | 15Ab1     | 15Abb1   | 14Aa1    |
|                    | T         | 13bb2     | 17Aa1    | 16Aa1    |
|                    | H         | 13Ab2     | 14Bb12   | 15Ab1    |
| Mv Hópehely (winter oat)     | C         | 13Bc1     | 14Ab1    | 12Ab1    |
|                    | T         | 13Bb1     | 14Ab1    | 14Aa1    |
|                    | H         | 13Aa1     | 12Ca1    | 12Ab1    |
| Mv Ikva (winter wheat)        | C         | 17Aa1     | 16Aa1    | 13Ab2    |
|                    | T         | 15Aa1     | 14Ab12   | 13Ab2    |
|                    | H         | 13Ab2     | 14Bb2    | 17Aa1    |
| Mv Nádor (winter wheat)       | C         | 11Cb2     | 14Ab1    | 13Ab12   |
|                    | T         | 13Bb2     | 16Ab1    | 13Aa2    |
|                    | H         | 12Ab2     | 10Aa1    | 14Ba2    |
| Mv Nemere (winter wheat)      | C         | 10Cb2     | 13Bb1    | 10Bb2    |
|                    | T         | 13Bb2     | 16Ab1    | 13Aa2    |
|                    | H         | 11Ab12    | 13Bc1    | 10C12    |
| Mv Kolompos (winter wheat)    | C         | 11Ca1     | 12Ba1    | 11Abab1  |
|                    | T         | 11Ca12    | 14Bb1    | 13Aa12   |
|                    | H         | 11Aa1     | 12Bc1    | 10Cb1    |

'C': control treatment; 'T': drought stress at BBCH 21; 'H': drought stress at BBCH 55. Capital letters indicate the statistical significance between the varieties; lowercase letters indicate the statistical significance between the treatments; the numbers show the statistical significance between the different CO₂ levels at p ≤ 0.05 level (n = 3).

Generally, CO₂ enrichment had positive effects on tillering ability and spike numbers in cereals (Figure 2). Only the 1000 ppm CO₂ concentration influenced the spike numbers of Mv Ikva negatively, and this phenomenon could be observed only for the stress-treated plants. The CO₂ fertilization did not influence the spike numbers of Mv Hópehely significantly. Mv Nádor showed the most favorable CO₂ reactions among wheat varieties in terms of spike numbers: under 700 ppm CO₂ concentration, spike numbers in each treatment were significantly greater than under ambient conditions. This beneficial effect in Mv Nádor could not be detected under 1000 ppm concentration, and similar trends were observed for Mv Nemere and Mv Kolompos (Figure 2).
Figure 2. Relative changes in cultivars in response to elevated CO$_2$ in terms of spike number ‘C’: control treatment; ‘T’: drought stress at tillering stage; ‘H’: drought stress at heading stage. ‘INI’: Mv Initium (winter barley); ‘HOP’: Mv Hópehely (winter oat); ‘IKVA’: Mv Ikva (winter wheat); ‘NAD’: Mv Nádor (winter wheat); ‘NEM’: Mv Nemere (winter wheat); ‘KOL’: Mv Kolompos (winter wheat). Full bars represent significant differences compared to the control (400 ppm) $p \leq 0.05$ level ($n = 3$).

At ambient CO$_2$ level (~400 ppm), in the early (BBCH 21) and late (BBCH 55) developmental stages, simulated drought stress decreased the plants’ grain yield in each examined variety except for Mv Kolompos (Table 3). The most considerable changes were observed for Mv Hópehely (~24% in ‘T’ and ~54% in ‘H’ treatment, respectively). Furthermore, in Mv Initium, Mv Ikva and Mv Hópehely, significant differences were observed between the stress treatments; the lowest grain yield was observed for treatment ‘H’. Under elevated CO$_2$ concentration (700 ppm), the drought stress at BBCH stage 21 decreased the grain yield of Mv Hópehely (~47%), Mv Ikva (~20%), Mv Nádor (~12%) and Mv Nemere (~10%). At the heading stage (BBCH 55), the drought stress decreased the grain yield of each cultivar except for Mv Initium. The most exposed variety was Mv Hópehely with 77% yield loss. The drought treatment significantly lowered the grain yield in Mv Nádor (~34%), Mv Kolompos (~26%), Mv Ikva (~25%) and Mv Nemere (~23%). Significant differences were observed between the two stress treatments in Mv Kolompos, Mv Nádor, Mv Nemere and Mv Hópehely: significantly lower grain yield values were observed when the drought occurred at heading (BBCH 55) (Table 3). At 1000 ppm CO$_2$ level, water withdrawal at BBCH stage 21 decreased the grain yield only in Mv Ikva (~15%). The late-stage stress (BBCH 55) reduced grain yield in each cultivar except for Mv Kolompos. The highest rate of yield reduction was detected in Mv Hópehely (~64%). Furthermore, significant differences were observed between the two stress treatments in the barley and oat varieties: in these cases, the consequences of late-stage drought stress were even more severe (Table 3).
Table 3. Average grain yield values per pot (g) of the tested varieties.

| Variety               | Treatment | ~400 ppm | 700 ppm | 1000 ppm |
|-----------------------|-----------|-----------|---------|----------|
| Mv Initium (winter barley) | C         | 21.38 Ba2 | 23.01 Ba1 | 23.42 Aa1 |
|                       | T         | 19.08 ABb2| 22.68 Aa1 | 23.70 Aa1 |
|                       | H         | 18.28 Abc2| 20.86 Aa1 | 21.28 Ab1 |
| Mv Hópehely (winter oat) | C         | 25.89 Aa2 | 31.14 Aa1 | 16.31 Ca3 |
|                       | T         | 19.60 Ab1 | 16.40 Bb2 | 16.07 Cc2 |
|                       | H         | 11.97 Cc1 | 7.10 De2  | 5.94 Db2  |
| Mv Ikva (winter wheat) | C         | 27.72 Aa1 | 24.49 Ab2 | 22.46 Aa2 |
|                       | T         | 20.41 Ab1 | 19.54 Bb1 | 19.12 Ab1 |
|                       | H         | 18.63 Abc1| 18.38 Cb1 | 18.50 Bb1 |
| Mv Nádor (winter wheat) | C         | 21.10 Ba1 | 22.81 Ba1 | 20.61 Aa1 |
|                       | T         | 18.63 Ab2 | 20.16 Ab1 | 18.51 Bab2 |
|                       | H         | 18.06 Abc1| 15.03 Bc2 | 16.02 Ch2 |
| Mv Nemere (winter wheat) | C         | 21.16 Ba2 | 24.98 Aa1 | 19.47 Aa2 |
|                       | T         | 18.39 ARb2| 22.43 Ab1 | 18.68 Bab2 |
|                       | H         | 19.22 Ab1 | 19.35 Cc1 | 17.37 Bc2 |
| Mv Kolompos (winter wheat) | C         | 17.34 Ca3 | 23.44 Ba1 | 20.38 Aa2 |
|                       | T         | 17.70 Bb2 | 22.88 Aa1 | 18.81 Ba2 |
|                       | H         | 16.54 Ba2 | 17.40 Cb1 | 18.32 Aa1 |

‘C’: control treatment; ‘T’: drought stress at BBCH 21; ‘H’: drought stress at BBCH 55. Capital letters indicate the statistical significance between the varieties; lowercase letters indicate the statistical significance between the treatments; the numbers show the statistical significance between the different CO$_2$ levels at $p \leq 0.05$ level (n = 3).

Significantly positive CO$_2$ responses were observed for Mv Initium in terms of grain yield at each watering level, but the stimulating effects of CO$_2$ fertilization were more intense under drought stress conditions (Figure 3). Opposite tendencies were observed for Mv Hópehely: CO$_2$ fertilization (1000 ppm) reduced the yield significantly. Under 700 ppm CO$_2$, a positive response was observed only under well-watered conditions, but CO$_2$ enrichment combined with water withdrawal reduced yield more intensely in a high-CO$_2$ environment. Negative CO$_2$ responses were observed for Mv Ikva under control watering, while the CO$_2$ enrichment did not influence the grain yield significantly. Positive CO$_2$ reactions were found in Mv Nádor, Mv Nemere and Mv Kolompos under 700 ppm concentration when plants were grown under optimum irrigation or stressed at BBCH stage 21. The 1000 ppm concentration induced positive responses in Mv Kolompos, which was statistically confirmed in the control and in the stressed treatments at BBCH 55. At the heading stage, Mv Nádor was especially susceptible to CO$_2$ enrichment, but yield responses were negative under both concentrations (Figure 3).
Figure 3. Relative changes in cultivars in response to elevated CO\textsubscript{2} in terms of grain yield ‘C’: control treatment; ‘T’: drought stress at tillering stage; ‘H’: drought stress at heading stage. ‘INI’: Mv Initium (winter barley); ‘HOP’: Mv Hópehely (winter oat); ‘IKVA’: Mv Ikva (winter wheat); ‘NAD’: Mv Nádor (winter wheat); ‘NEM’: Mv Nemere (winter wheat); ‘KOL’: Mv Kolompos (winter wheat). Full bars represent significant differences compared to the control (400 ppm) \(p \leq 0.05\) level (\(n = 3\)).

At atmospheric CO\textsubscript{2} level, drought stress induced at BBCH stage 21 decreased harvest index in Mv Hópehely (−15%) and increased it in Mv Kolompos (+8%), while water shortage at BBCH stage 55 decreased the harvest index of Mv Hópehely (−45%) and increased it in Mv Initium by 7% compared to the well-watered control treatment. Furthermore, in oats, the drought stress at BBCH 55 resulted in a significant decrease in harvest index values compared to the other two treatments (Table 4). At 700 ppm CO\textsubscript{2} level, the drought stress at tillering (BBCH 21) increased the harvest index of Mv Ikva (+9%), Mv Nemere (+4%) and Mv Nádor (+4%) and decreased it in Mv Hópehely (−31%) and Mv Initium (−8%) significantly. At the heading stage (BBCH 55), water shortage reduced the harvest index of each variety; the most considerable reduction was observed for Mv Hópehely (−62%). Water withdrawal at BBCH stage 55 reduced the harvest index significantly in Mv Nádor, Mv Initium, Mv Kolompos, Mv Ikva and Mv Nemere by −19%, −18%, −16%, −15% and −8%, respectively, compared with the control. Furthermore, there were significant differences between the two stress treatments (drought stress at BBCH 21 and BBCH 55) in each variety: late-stage drought stress reduced the grain yield more intensively than the aboveground biomass; therefore, the reduction in harvest index was more intensive in this treatment (Table 4). Under 1000 ppm CO\textsubscript{2} concentration, drought stress at tillering induced no significant changes in the harvest index, but stress conditions at the heading stage significantly decreased the harvest index in Mv Hópehely (−43%), Mv Nádor (−15%) and Mv Ikva (−9%) (Table 4).
Table 4. Average harvest index values (%) of the tested varieties.

| Variety          | Treatment | 400 ppm | 700 ppm | 1000 ppm |
|------------------|-----------|---------|---------|----------|
| Mv Initium       | C         | 38.5Db2 | 43.7Da1 | 43.5Ca1  |
| (winter barley)  | T         | 40.4Ca1 | 40.3Db2 | 43.4Ca1  |
|                  | H         | 41.4Ba1 | 36.0Da2 | 40.7Ca1  |
| Mv Hópehely      | C         | 42.0Ca1 | 42.3Da1 | 29.2Da2  |
| (winter oat)     | T         | 35.5Ob1 | 29.3Db2 | 30.6Da2  |
|                  | H         | 22.9Ccl | 15.9Ec2 | 16.5Ob2  |
| Mv Ikva          | C         | 55.3Aa2 | 56.9Ab2 | 60.8Aa1  |
| (winter wheat)   | T         | 57.1Aa2 | 62.2Aa1 | 57.5Aab2 |
|                  | H         | 55.2Aa1 | 48.4Bc2 | 55.5Ab1  |
| Mv Nádor         | C         | 52.7Ba1 | 50.9Ab1 | 54.0Aa1  |
| (winter wheat)   | T         | 52.0Ba1 | 52.7Ca1 | 53.1Ba1  |
|                  | H         | 52.2Aa1 | 41.1Cc3 | 45.8Bb2  |
| Mv Nemere        | C         | 51.7Ba1 | 53.1Ab1 | 53.0Bab1 |
| (winter wheat)   | T         | 49.9Bb2 | 55.4Ba1 | 52.2Ba12 |
|                  | H         | 52.3Aa1 | 49.2Ac2 | 54.3Ca1  |
| Mv Kolompos      | C         | 35.8Bb2 | 41.9Ba1 | 42.4Ca1  |
| (winter wheat)   | T         | 38.8Ca2 | 42.5Da12| 42.9Ca1  |
|                  | H         | 36.7Bab2| 35.4Db2 | 42.6BCa1 |

'C': control treatment; 'T': drought stress by tillering; 'H': drought stress by heading. Capital letters indicate the statistical significance between the varieties at $p \leq 0.05$ level; lowercase letters indicate the statistical significance between the treatments at $p \leq 0.05$ level. The numbers in the indexes indicate the statistical significance between the different CO$_2$ levels at $p \leq 0.05$ level ($n = 3$).

In terms of the harvest index, consistent CO$_2$ responses were observed only for Mv Hópehely (Figure 4). Under 1000 ppm CO$_2$, harvest index was significantly lower in each water treatment group, and even the 700 ppm level resulted in a decrease under drought stress conditions. Both enriched CO$_2$ concentrations had a positive influence on the harvest index of Mv Initium, but this trend could be detected only in the well-watered plants. No subsequent CO$_2$ responses could be observed in Mv Ikva and Mv Nemere, but CO$_2$ fertilization significantly decreased the harvest index of Mv Nádor when water availability was reduced at BBCH stage 55. Mv Kolompos showed positive CO$_2$ reactions, which were statistically significant either at 700 or 1000 ppm CO$_2$ under well-watered conditions, but this tendency was significant in drought stress treatments only under 1000 ppm CO$_2$ (Figure 4).
At ambient CO\textsubscript{2} level, drought stress at the early developmental stage (BBCH 21) caused no significant changes in the plants’ water use, but water shortage at BBCH stage 55 significantly decreased the water uptake of Mv Ikva (−24%) and Mv Nemere (−12%) compared with the control treatment. Significant differences between the two drought stress treatments were also observed for Mv Nemere (the lowest water use was observed for treatment ‘H’) (Figure 5). Under 700 ppm CO\textsubscript{2} concentration, drought stress at the early stage decreased the water use of Mv Ikva (−18%), and water withdrawal at BBCH stage 55 decreased water uptake values in each wheat variety; the highest decrease (−20%) was observed for Mv Ikva. Furthermore, significant differences were observed between the two stress treatments in Mv Kolompos (drought stress at BBCH 55 reduced water uptake by 6% compared to the water shortage simulated at BBCH 21) (Table 5). At 1000 ppm CO\textsubscript{2} level, both stress treatments decreased water use in Mv Ikva significantly (−15% and −12% in ‘T’ and ‘H’ treatments, respectively) (Figure 5).
Figure 5. Average water use values (m³) of the tested varieties. ‘C’: control treatment; ‘T’: drought stress at BBCH 21; ‘H’: drought stress at BBCH 55. Capital letters indicate the statistical significance between the varieties; lowercase letters indicate the statistical significance between the treatments; the numbers show the statistical significance between the different CO₂ levels at $p \leq 0.05$ level (n = 3) (Mv Initium is winter barley; Mv Hőpehely is winter oat; Mv Ikva, Mv Nádor, Mv Nemere and Mv Kolompos are winter wheat).
Table 5. The average water-use efficiency values (kg·m$^{-3}$) of the tested varieties.

| Variety               | Treatment | ~400 ppm | 700 ppm  | 1000 ppm |
|-----------------------|-----------|-----------|-----------|-----------|
| **Mv Initium**        |           |           |           |           |
| (winter barley)       | C         | 1.115$^{Ba2}$ | 1.227$^{Ca2}$ | 1.555$^{Ca1}$ |
|                       | T         | 1.130$^{Ba2}$ | 1.169$^{Da2}$ | 1.515$^{Da1}$ |
|                       | H         | 1.040$^{Ca2}$ | 1.174$^{Ba2}$ | 1.538$^{Ca1}$ |
| **Mv Hópehely**      |           |           |           |           |
| (winter oat)          | C         | 0.985$^{Ba12}$ | 1.217$^{Ca1}$ | 0.746$^{Da2}$ |
|                       | T         | 0.656$^{Ca1}$ | 0.628$^{Eb1}$ | 0.724$^{Ba1}$ |
|                       | H         | 0.461$^{Db1}$ | 0.291$^{Cc2}$ | 0.300$^{Db2}$ |
| **Mv Ikva**           |           |           |           |           |
| (winter wheat)        | C         | 1.762$^{Aa2}$ | 1.867$^{Aa2}$ | 2.271$^{Aa1}$ |
|                       | T         | 1.789$^{Aa2}$ | 1.905$^{Aa2}$ | 2.262$^{Aa1}$ |
|                       | H         | 1.686$^{Aa2}$ | 1.745$^{Aa2}$ | 2.126$^{Aa1}$ |
| **Mv Nádor**          |           |           |           |           |
| (winter wheat)        | C         | 1.480$^{Aa2}$ | 1.516$^{Ba2}$ | 1.889$^{Ba1}$ |
|                       | T         | 1.389$^{Aa2}$ | 1.422$^{Cb2}$ | 1.723$^{Bcab1}$ |
|                       | H         | 1.416$^{Ba2}$ | 1.126$^{bc3}$ | 1.576$^{cb1}$ |
| **Mv Nemere**         |           |           |           |           |
| (winter wheat)        | C         | 1.516$^{Aa3}$ | 1.720$^{Aa2}$ | 1.970$^{Ba1}$ |
|                       | T         | 1.289$^{Ba2}$ | 1.664$^{Ba1}$ | 1.933$^{Ba1}$ |
|                       | H         | 1.567$^{Aa2}$ | 1.555$^{Ab2}$ | 1.833$^{Ba1}$ |
| **Mv Kolompos**       |           |           |           |           |
| (winter wheat)        | C         | 0.957$^{Ba2}$ | 1.269$^{Ca1}$ | 1.556$^{Ca1}$ |
|                       | T         | 1.051$^{Ba2}$ | 1.290$^{Da1}$ | 1.453$^{Da1}$ |
|                       | H         | 0.927$^{Ca2}$ | 1.077$^{Bb2}$ | 1.445$^{Ca1}$ |

'C': control treatment; 'T': drought stress at BBCH 21; 'H': drought stress at BBCH 55. Capital letters indicate the statistical significance between the varieties; lowercase letters indicate the statistical significance between the treatments; the numbers show the statistical significance between the different CO$_2$ levels at p ≤ 0.05 level (n = 3).

There were differences between the plants’ reactions in terms of water uptake at the two levels of CO$_2$ enrichment (Figure 6). No significant changes in water uptake could be observed under 700 ppm, but the 1000 ppm CO$_2$ level reduced the water demand of plants during vegetation in each variety and each water treatment group, except for Mv Hópehely under optimum watering. Under optimum watering, the water demand of the cultivars decreased by 24% on average, and when water shortage was simulated at BBCH stages 21 and 55, the reduction in water use was 23% less in both stress treatments than under control conditions (400 ppm) (Figure 6).
Figure 6. Relative changes in cultivars to elevated CO$_2$ in terms of water use ‘C’: control treatment; ‘T’: drought stress at tillering stage; ‘H’: drought stress at heading stage. ‘INL’: Mv Initium (winter barley); ‘HOP’: Mv Hópehely (winter oat); ‘IKVA’: Mv Ikva (winter wheat); ‘NAD’: Mv Nádor (winter wheat); ‘NEM’: Mv Nemere (winter wheat); ‘KOL’: Mv Kolompos (winter wheat). Full bars represent significant differences compared to the control (400 ppm) $p \leq 0.05$ level ($n = 3$).

Under 400 ppm CO$_2$ concentration, the water shortage induced at BBCH stage 21 caused no significant changes in the plants’ water-use efficiency (WUE), but limited water availability at BBCH stage 55 decreased the WUE of Mv Hópehely from the initial 1.115 kg/m$^3$ (control) to 1.040 kg/m$^3$ (Table 5). At the 700 ppm CO$_2$ level, simulated drought at tillering decreased water-use efficiency in Mv Hópehely by 48% and in Mv Nádor by 6%, but no significant changes were observed for the other varieties. In Mv Hópehely, water shortage at heading (BBCH 55) had very serious consequences: its water-use efficiency (0.291 kg/m$^3$) decreased by 76% compared to the well-watered treatment (1.217 kg/m$^3$). Even the WUE of Mv Nádor, Mv Kolompos and Mv Nemere decreased by 25%, 15% and 10%, respectively, compared to the well-watered control. However, the best adaptability was observed in Mv Nemere, as its water-use efficiency was significantly higher under drought conditions at the heading stage than that of Mv Nádor and Mv Kolompos, and it did not differ from the early-ripening cultivar (Mv Ikva). Furthermore, in Mv Hópehely, Mv Nádor, Mv Nemere and Mv Kolompos, significant differences were observed between the two drought stress treatments: water shortage at the heading stage induced a more intensive decrease in water-use efficiency than the shortage simulated at tillering (Table 5). At 1000 ppm CO$_2$ level, water withdrawal at the tillering stage did not induce changes in the WUE, indicating that CO$_2$ fertilization could counterbalance the negative impacts of a limited-water environment. Water shortage at the heading stage reduced water-use efficiency significantly only in Mv Hópehely and Mv Nádor by 60% and 17%, respectively, which indicated the sensitivity of these varieties to drought stress (Table 5).

As a consequence of the positive responses in grain yield and the moderated water uptake of Mv Initium, 1000 ppm CO$_2$ resulted in an improved water-use efficiency
in each treatment (39%, 34% and 48% in control, ‘T’ and ‘H’ treatments, respectively) (Figure 7). The negative trends determined by the grain yield of Mv Hópehely under elevated CO₂ were counterbalanced by the reduced water uptake in the control and by the water shortage at BBCH 21; therefore, CO₂ enrichment did not influence water-use efficiency significantly. The negative impacts of the late drought stress at BBCH 55 was extremely serious in the oat variety; therefore, a declined water-use efficiency was found under both elevated CO₂ concentrations as well. The 1000 ppm CO₂ level improved the water-use efficiency of each wheat variety, but the best responses were determined in Mv Kolompos with 163%, 138% and 156% in the control, ‘T’ and ‘H’ treatments, respectively. In terms of CO₂ reactions, no significant differences were observed between Mv Nádor, Mv Nemere and Mv Ikva. An increased WUE was detected in Mv Kolompos under optimum irrigation, and in the drought-stressed plants at BBCH stage 21. A similar trend was observed for Mv Nemere but only in the control treatment (Figure 7).

![Figure 7. Relative changes in cultivars in response to elevated CO₂ in terms of water-use efficiency ‘C’: control treatment; ‘T’: drought stress at tillering stage; ‘H’: drought stress at heading stage. ‘INI’: Mv Initium (winter barley); ‘HOP’: Mv Hópehely (winter oat); ‘IKVA’: Mv Ikva (winter wheat); ‘NAD’: Mv Nádor (winter wheat); ‘NEM’: Mv Nemere (winter wheat); ‘KOL’: Mv Kolompos (winter wheat). Full bars represent significant differences compared to the control (400 ppm) p ≤ 0.05 level (n = 3).]

4. Discussion

In our experiment, the drought stress induced in the vegetative (BBCH 21) and the generative (BBCH 55) phases of development decreased the biomass of five out of six winter cereal varieties at atmospheric CO₂ level. When the CO₂ level was enriched to 700 ppm, a similar tendency was observed in Mv Hópehely, Mv Ikva, Mv Nemere and Mv Nádor. Furthermore, the biomass of Mv Ikva and Mv Nádor decreased as an effect of drought stress at the 1000 ppm CO₂ level, regardless of the developmental stage at which the stress occurred. In the case of the examined barley cultivar, elevation of the CO₂ level (700 ppm or 1000 ppm) resulted in increased biomass values when the plants were
When the CO₂ content was increased in ambient CO₂ conditions, the CO₂ content at 700 ppm was increased to 1000 ppm. We found that the highest biomass values were in well-watered plants under elevated CO₂ levels. However, at the tillering stage, drought stress increased biomass at 700 ppm CO₂ level. It was found that under elevated CO₂ (700 ppm) water limitation at the terminal growing stage reduced biomass in durum wheat, according to Garmendia et al. [51], and in barley, according to Bista et al. [52]. In the case of the examined barley cultivar, we found increased biomass values compared to the control. In disagreement with our findings, Varga et al. [28] found no significant differences in the plants’ biomass between the well-watered and drought-stressed winter wheat at different developmental stages at elevated CO₂ levels (1000 ppm), which fact confirms the variety-specific CO₂ responses. In this study, we observed increased biomass values at both elevated CO₂ levels (700 ppm, 1000 ppm) in barley when the plants were stressed. At different developmental stages (tillering or heading), induced drought stress increased the biomass values in Mv Nádor and Mv Kolompos at elevated CO₂ levels (700 ppm) compared to the ambient concentration. Increased biomass production was observed in Mv Hópehely, Mv Nemere and Mv Kolompos in well-watered plants under 700 ppm CO₂ levels, and also in Mv Kolompos under 1000 ppm (compared to ~400 ppm). Ulfat et al. [53] stressed winter wheat with drought by anthesis at ambient and elevated CO₂ levels (800 ppm). They found the highest biomass values in well-watered plants at ambient CO₂ levels, and the second highest in well-watered plants under elevated CO₂. However, we observed higher biomass at elevated CO₂ levels (700 ppm) compared to the ambient level in well-watered or drought-stressed Mv Nádor, Mv Nemere and Mv Kolompos.

According to our findings, water shortage at the tillering stage under ~400 ppm increased spike numbers in Mv Nemere and decreased them in the barley variety. The drought at heading decreased spike numbers in the barley and Mv Ikva. Our findings are in agreement with other results [47,54–56]. Samarah et al. [54] also found that late-terminal drought stress decreased the spike number of barley. According to Khakwani et al. [55], water deficit at the reproductive stage reduces the number of panicles per plant in winter wheat. Ding et al. [47] claimed that the drought stress either at elongation or at the heading stage reduced the spikes per plant in winter wheat. Rollins et al. [56] also found a significantly lowered number of spikes in barley in drought-stress treatment at the generative stage compared to the control. When the CO₂ level was increased to 700 ppm, drought at tillering increased spike numbers in the examined barley and Mv Nemere, and decreased them for Mv Ikva. The water shortage at BBCH stage 55 increased the numbers of panicles per plants in Mv Nádor and decreased them in Mv Ikva. According to the findings of Garmendia et al. [51], the number of spikes in durum wheat slightly increased as an effect of terminal drought stress and elevated CO₂ level (700 ppm). We had a similar result for one winter wheat variety (Mv Nádor). In our experiment, at 1000 ppm CO₂ level, water shortage at the tillering or heading stages increased spike numbers only in Mv Ikva. Sionit et al. [57] found the opposite effect: late-stage drought stress decreased spike numbers in spring wheat. We found that neither drought treatments nor changes in the levels of CO₂ produced significant differences in the number of panicles in the examined oat cultivar. In Mv Nádor, higher spike numbers were observed at the 700 ppm CO₂ level in every watering regime compared to the ambient CO₂ level. This was in line with the results of Thilakaratne et al. [58], who found a higher spike number in spring wheat at an elevated CO₂ level (700 ppm) compared to the ambient CO₂ level under optimum watering.

In our experiment, water shortage at BBCH 21 and BBCH 55 decreased the grain yield of Mv Ikva compared to the well-watered plants under all tested CO₂ concentrations (~400 ppm, 700 ppm, 1000 ppm). The two drought treatments also decreased the yield of Mv Hópehely, Mv Nádor and Nemere at ambient and elevated CO₂ levels (700 ppm) and for the barley at ~400 ppm. At 1000 ppm CO₂, drought stress at the heading stage decreased the grain yield of all examined varieties except for Mv Kolompos. Zhao et al. [48] also found decreased
grain yield values in oats as an effect of drought-induced stress at different developmental stages. Quaseem et al. [39] found similar results in wheat as an effect of simulated water shortage at pre-anthesis. Our results are in agreement with Varga et al. [28], Manderscheid and Weigel [49] and Ulfat et al. [53]; these studies also observed a decrease in the grain yield values of wheat as an effect of early-stage or late-stage water shortage at elevated CO₂ levels (700 ppm, 800 ppm or 1000 ppm). Positive CO₂ fertilization effects in barley and negative CO₂ fertilization effects in the oat cultivar were observed for grain yield at both elevated CO₂ levels (700 ppm and 1000 ppm) in every treatment (control, drought at BBCH stage 21 and drought at BBCH stage 55). Additionally, higher grain yield values were observed for the well-watered and early-stage drought-stressed plants in Mv Nemere and Mv Kolombo varieties when the CO₂ concentration was elevated to 700 ppm (compared to the ambient CO₂ level). Thilakarathne et al. [58] also found elevated grain yield values in spring wheat at elevated CO₂ level (700 ppm) under optimal watering treatment.

According to our results, water withdrawal at tillering increased the harvest index in Mv Kolombo at ambient concentrations and in Mv Ikva, Mv Nádor and Mv Nemere at 700 ppm CO₂ levels, and decreased it in Mv Hópehely at ambient and elevated (700 ppm) levels and in Mv Initium at 700 ppm CO₂ level. Water shortage at heading decreased the harvest index of the oat cultivar at each CO₂ level. Late-stage water shortage decreased the harvest index of every examined variety at 700 ppm CO₂ level, and of Mv Hópehely and Mv Nádor at 1000 CO₂ level. Increments in the CO₂ (700 ppm and 1000 ppm) affected the harvest index negatively in Mv Hópehely compared to the ambient CO₂. In contrast to our findings, Zhao et al. [48] observed that the harvest index of the examined oat was improved as an effect of the applied drought stress at ambient levels of CO₂. Samarah et al. [54] stated that the harvest index of barley decreased as an effect of late-terminal drought stress, however, we noted an opposite tendency. Ding et al. [47] found that drought stress at the elongation stage under ~400 ppm CO₂ improved the harvest index of winter wheat; our results were in line with this but only with one wheat cultivar (Mv Kolombo). Wu et al. [60] found slightly higher harvest index values under drought conditions in spring wheat compared with the control (80% of field water capacity) under elevated CO₂ (~700 ppm). Compared to the control treatment, Ulfat et al. [53] found decreased harvest index values for winter wheat as a result of drought stress at anthesis under 800 ppm CO₂ concentration. We also found decreased harvest index values at 700 ppm CO₂ level as a result of drought at heading. Varga et al. [28] also found lower harvest index values due to the effect of water withdrawal compared to the control under 700 ppm and 1000 ppm.

At ambient CO₂ level, drought stress at BBCH stage 21 caused no significant changes in the plants’ water use, and water shortage at BBCH stage 55 significantly decreased water use of Mv Ikva and Mv Nemere compared with the control treatment. Under elevated CO₂ concentrations (700 and 1000 ppm), both drought stresses decreased the water use of Mv Ikva, and water withdrawal at BBCH stage 55 decreased water use values in each wheat cultivar at 700 ppm. Although CO₂ enrichment to 700 ppm had no significant effect on the water uptake of the examined varieties, the elevation of CO₂ to 1000 ppm caused a positive CO₂ effect. Namely, the water use of each examined variety decreased significantly in all treatments (control, drought stress at tillering and drought stress at heading stage). According to Varga et al. [28], an elevated CO₂ level (1000 ppm) can decrease the water uptake of plants in both optimum watering and drought stress at heading.

In our experiment, the drought stress simulated at BBCH 21 decreased the water-use efficiency of Mv Hópehely and Mv Nádor, but only at an elevated CO₂ (700 ppm) level. Water withdrawal at BBCH 55 decreased the WUE of the examined oat in every CO₂ treatment, of Mv Nádor under the elevated CO₂ concentrations (700 ppm and 1000 ppm) and of Mv Nemere and Mv Kolombo under 700 ppm CO₂. A positive CO₂ fertilization effect was observed also in terms of water-use efficiency in barley and all wheat varieties under all water treatments when the CO₂ level was elevated to 1000 ppm. However, this positive response was only observed for Mv Kolombo and Mv Nemere under 700 ppm CO₂, when the plants were well-watered or the drought stress was initiated at BBCH stage...
21. Liu et al. [61] found increased water-use efficiency values in oats when the CO$_2$ level was elevated (700 ppm) and the plants were grown under optimum conditions, but in our experiment, there was no significant difference between the well-watered plants’ WUE at the different CO$_2$ levels. Li et al. [50] found that the water-use efficiency of winter wheat was slightly increased (compared to the control) by drought treatment in the elongation phase at elevated CO$_2$ levels (800 ppm), and WUE was significantly affected by CO$_2$ treatment in that experiment. In disagreement with the results of Li et al. [50], we found decreased water-use efficiency values for the examined wheat cultivars under 700 ppm when the plants were stressed at tillering or heading stage. Robredo et al. [62] observed that the highest WUE was observed when the plants (winter and spring wheat or barley) were treated with drought at an elevated CO$_2$ level (700 ppm). Medeiros and Ward [63] found the highest water-use efficiency under moderate and severe drought stress at an elevated (700 ppm) CO$_2$ level. We also found increased WUE values when the CO$_2$ level was elevated, but only at 1000 ppm (compared to ambient CO$_2$ levels and different treatments).

5. Conclusions

Genotypic differences related to elevated atmospheric CO$_2$ concentration were confirmed in this study, however, the role of environmental factors was significant in this regard. Generally, positive CO$_2$ fertilization effects were found for barley (Mv Initium); CO$_2$ enrichment induced higher biomass and grain yield, decreased water uptake and better water-use efficiency. Low CO$_2$ sink ability was determined for the oat (Mv Hópehely) because an elevated CO$_2$ concentration resulted in a reduction in grain yield and harvest index, as well as in water-use efficiency, regardless of the gas concentration. High variability was observed in the CO$_2$ responses of the four winter wheat varieties. The biomass and grain yield of Mv Kolompos were increased by CO$_2$ fertilization. The water uptake of all wheat varieties decreased significantly, and at the same time, their water-use efficiency improved, but only under 1000 ppm CO$_2$. Mv Ikva was not susceptible to CO$_2$ fertilization. The reason behind this phenomenon might be that this cultivar was an early-ripening variety among the tested plants. No consistent CO$_2$ reactions were observed for Mv Nádor and Mv Nemere. Positive CO$_2$ fertilization effects were found for Mv Kolompos; CO$_2$ enrichment induced higher biomass, grain yield and harvest index, decreased water uptake and improved water-use efficiency. The present study suggests that the effects of different CO$_2$ concentrations should be tested by taking into consideration the determined genotypic differences of cereal species and varieties.

The CO$_2$ responses of genotypes can differ significantly, as confirmed by our study. However, the number of genotypes was not high enough to be able to confirm correlation between the ripening times of the varieties and their CO$_2$ reactions. Our study may be a good base for a more detailed examination in which the physiological background of the CO$_2$ responses can be determined.

Author Contributions: Conceptualization, B.V.; methodology, Z.F and B.V.; software, Z.F. and B.V.; validation, Z.F. and B.V.; formal analysis, Z.F. and B.V.; investigation, Z.F. and B.V.; resources, G.V. and O.V.; data curation, Z.F. and B.V.; writing—original draft preparation, Z.F.; writing—review and editing, B.V.; visualization, Z.F.; supervision, A.A., G.V. and O.V.; project administration, Z.F. and B.V.; funding acquisition, G.V. and O.V. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the GINOP-2.3.2.-15-2016-00029 (Multifunkcionálisan hasznos ítható növények, mint alternatívák a fenntartható mezőgazdaság szolgálatában) project.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: Not applicable.
**Conflicts of Interest:** The authors declare no conflict of interest. The funders had no role in the design of the study, in the collection, analyses, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

**Appendix A**

**Table A1.** Effects of the examined factors on biomass.

| Df          | Sum Sq | Mean Sq | F Value | Pr(>F)   | Signif. |
|-------------|--------|---------|---------|----------|---------|
| CO\textsubscript{2} level | 2 | 976 | 488.1 | 351.26 | <2\texttimes10\textsuperscript{-16} | *** |
| Watering    | 2 | 1801 | 900.6 | 648.13 | <2\texttimes10\textsuperscript{-16} | *** |
| Variety     | 6 | 8191 | 1365.2 | 982.49 | <2\texttimes10\textsuperscript{-16} | *** |
| CO\textsubscript{2} level:Watering | 4 | 95 | 23.8 | 17.14 | 7.59\texttimes10\textsuperscript{-11} | *** |
| CO\textsubscript{2} level:Variety | 10 | 561 | 56.1 | 40.38 | <2\texttimes10\textsuperscript{-16} | *** |
| Watering:Variety | 12 | 1436 | 119.7 | 86.14 | <2\texttimes10\textsuperscript{-16} | *** |
| CO\textsubscript{2} level:Watering:Variety | 20 | 758 | 37.9 | 27.29 | <2\texttimes10\textsuperscript{-16} | *** |
| Residuals   | 105 | 146 | 1.4 | | | 1 |

Signif. codes: *** 0.001 ** 0.01 * 0.05 . 0.1 ‘ 1

**Table A2.** Effects of the examined factors on spike number per pot.

| Df          | Sum Sq | Mean Sq | F Value | Pr(>F)   | Signif. |
|-------------|--------|---------|---------|----------|---------|
| CO\textsubscript{2} level | 2 | 101.26 | 50.63 | 49.782 | 6.22\texttimes10\textsuperscript{-16} | *** |
| Watering    | 2 | 38.65 | 19.33 | 19.001 | 9.05\texttimes10\textsuperscript{-08} | *** |
| Variety     | 6 | 214.76 | 35.79 | 35.193 | <2\texttimes10\textsuperscript{-16} | *** |
| CO\textsubscript{2} level:Watering | 4 | 10.18 | 2.55 | 2.503 | 0.0467 | * |
| CO\textsubscript{2} level:Variety | 10 | 80.67 | 8.07 | 7.932 | 2.14\texttimes10\textsuperscript{-09} | *** |
| Watering:Variety | 12 | 76.39 | 6.37 | 6.259 | 3.15\texttimes10\textsuperscript{-08} | *** |
| CO\textsubscript{2} level:Watering:Variety | 20 | 107.53 | 5.38 | 5.286 | 5.92\texttimes10\textsuperscript{-09} | *** |
| Residuals   | 105 | 106.79 | 1.02 | | | 1 |

Signif. codes: *** 0.001 ** 0.01 * 0.05 . 0.1 ‘ 1

**Table A3.** Effects of the examined factors on grain yield.

| Df          | Sum Sq | Mean Sq | F Values | Pr(>F)   | Signif. |
|-------------|--------|---------|----------|----------|---------|
| CO\textsubscript{2} level | 2 | 117 | 58.5 | 73.69 | <2\texttimes10\textsuperscript{-16} | *** |
| Watering    | 2 | 987.3 | 493.6 | 21.76 | <2\texttimes10\textsuperscript{-16} | *** |
| Variety     | 6 | 402.2 | 67 | 84.44 | <2\texttimes10\textsuperscript{-16} | *** |
| CO\textsubscript{2} level:Watering | 4 | 108.3 | 27.1 | 34.11 | <2\texttimes10\textsuperscript{-16} | *** |
| CO\textsubscript{2} level:Variety | 10 | 319.5 | 32 | 40.24 | <2\texttimes10\textsuperscript{-16} | *** |
| Watering:Variety | 12 | 633.4 | 52.8 | 66.49 | <2\texttimes10\textsuperscript{-16} | *** |
| CO\textsubscript{2} level:Watering:Variety | 20 | 198 | 9.9 | 12.47 | <2\texttimes10\textsuperscript{-16} | *** |
| Residuals   | 105 | 83.4 | 0.8 | | | 1 |

Signif. codes: *** 0.001 ** 0.01 * 0.05 . 0.1 ‘ 1
Table A4. Effects of the examined factors on harvest index.

| Df   | Sum Sq | Mean Sq | F Value | Pr(>F)    | Signif. |
|------|--------|---------|---------|-----------|---------|
| CO2_level | 2     | 37      | 18.7    | 10.075    | 9.94 × 10^{-5} *** |
| Watering | 2     | 1116    | 557.9   | 300.277   | <2 × 10^{-16} *** |
| Variety  | 6     | 13650   | 2274.9  | 1224.323  | <2 × 10^{-16} *** |
| CO2_level:Watering | 4     | 373     | 93.3    | 50.224    | <2 × 10^{-16} *** |
| CO2_level: Variety | 10    | 558     | 55.8    | 30.031    | <2 × 10^{-16} *** |
| Watering: Variety | 12    | 1147    | 95.6    | 51.457    | <2 × 10^{-16} *** |
| CO2_level:Watering:Variety | 20    | 331     | 16.5    | 8.897     | 8.02 × 10^{-15} *** |
| Residuals | 105   | 195     | 1.9     |           |         |

Signif. codes: ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Table A5. Effects of the examined factors on water use.

| Df   | Sum Sq | Mean Sq | F Value | Pr(>F)    | Signif. |
|------|--------|---------|---------|-----------|---------|
| CO2_level | 2     | 501     | 250.6   | 329.001   | <2 × 10^{-16} *** |
| Watering | 2     | 61      | 30.6    | 40.108    | 1.15 × 10^{-13} *** |
| Variety  | 6     | 3229    | 538.2   | 706.466   | <2 × 10^{-16} *** |
| CO2_level:Watering | 4     | 5       | 1.3     | 1.681     | 0.159995 |
| CO2_level: Variety | 10    | 27      | 2.7     | 3.491     | 0.000533 *** |
| Watering: Variety | 12    | 47      | 3.9     | 5.176     | 9.3210 × 10^{-7} *** |
| CO2_level:Watering:Variety | 20    | 22      | 1.1     | 1.435     | 0.122577 |
| Residuals | 105   | 80      | 0.8     |           |         |

Signif. codes: ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Table A6. Effects of the examined factors on water-use efficiency.

| Df   | Sum Sq | Mean Sq | F Value | Pr(>F)    | Signif. |
|------|--------|---------|---------|-----------|---------|
| CO2_level | 2     | 3.459   | 1.73    | 195.49    | <2 × 10^{-16} *** |
| Watering | 2     | 1.157   | 0.579   | 65.397    | <2 × 10^{-16} *** |
| Variety  | 6     | 25.725  | 4.287   | 484.559   | <2 × 10^{-16} *** |
| CO2_level:Watering | 4     | 0.189   | 0.047   | 5.336     | 0.000596 *** |
| CO2_level: Variety | 10    | 1.57    | 0.157   | 17.749    | <2 × 10^{-16} *** |
| Watering: Variety | 12    | 1.19    | 0.099   | 11.205    | 0.001285 ** |
| CO2_level:Watering:Variety | 20    | 0.446   | 0.022   | 2.519     | 0.001285 ** |
| Residuals | 105   | 0.929   | 0.009   |           |         |

Signif. codes: ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

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