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Impact of Water Deficit on Primary Metabolism at the Whole Plant Level in Bread Wheat Grown under Elevated CO_2 and High Temperature at Different Developmental Stages †

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Abstract: Predicted increases in the atmospheric CO_2 concentration and the earth's mean surface temperature will be accompanied by a higher incidence of drought events. These environmental changes are likely to adversely affect crop productivity and quality, including wheat, an essential food in the human diet. We investigated the primary C-N metabolism response to drought stress at the whole-plant level and its dependence on plant development in bread wheat grown under combined elevated CO_2 and temperature. With this aim, the content of carbohydrates, nitrate, proteins and amino acids, together with the biomass were assessed in flag leaves and roots of wheat grown in controlled environment chambers at both ear emergence and anthesis stages. Multifactorial analysis revealed that the organ was the main factor explaining data variation. The physiological and biochemical traits in the flag leaves were more affected by drought than growth stage, leading to an accumulation of soluble carbohydrates, nitrate and amino acids. By contrast, roots were affected by the developmental stages but not by the treatment. The root content of fructose, glucose, starch and amino acids was higher at ear emergence than anthesis, whereas the accumulation of sucrose, fructans, proteins and nitrate increased at the latest growth stage. This study provides new insights into the reprograming of primary metabolism at whole plant level throughout the development in response to the future climate scenario, which could help to select traits ensuring sustainable food production systems that strengthen capacity for adaptation to climate change following the Sustainable Development Goals of 2030 Agenda.

Keywords: wheat; climate change; drought; carbohydrates; N compounds; leaves; roots

1. Introduction

Bread wheat (*Triticum aestivum* L.) is the third largest crop in the world, feeding more than 4.5 billion people worldwide [1]. The necessity for increasing food supply by about 70% by 2050 [2], together with the detrimental effects of climate change on global crop production and quality, reveals that more efforts to improve wheat performance under sustainable conditions are required to ensure food security. The atmospheric CO_2 concentrations are expected to rise to 1000 ppm by the end of this century and the Earth's mean surface temperature is also expected to rise by 2.6 and 4.8 °C. These environmental changes may lead to an increase in the frequency of other climate events such as irregular rainfalls and drought, which may spell serious threats to crop yield, including wheat [3–7].

Drought stress has an important impact on wheat performance throughout its different developmental stages, being the reproductive growth stage the most sensitive [8–10]. Morpho–physiological and biochemical changes in leaves and roots provide protection against drought stress, decreasing water losses [11–13]. One of the physiological responses include the stomata closure to avoid water loss through transpiration, which seems to be related to either hydraulic or chemical signals mediated by the abscisic acid, but may also lead to a limitation in carbon fixation and nutrient absorption by the roots [14–17]. At biochemical level, the osmotic adjustment counters the effect of water deficit by accumulating different osmolytes such as carbohydrates or amino acids [18,19]. Nevertheless, elevated CO_2 may mitigate the inhibition of photosynthesis under drought stress improving water use efficiency because a synergistic effect of both factors on stomatal closure [20]. These responses reveal that the regulation of primary metabolism is crucial for drought stress tolerance in higher plants [21]. Thus, the aim of this work was to assess the physiological and biochemical changes in the flag leaves and roots of wheat plants subjected to drought stress under simultaneous increases in atmospheric CO_2 and temperature in interaction with its developmental stages, including ear emergence and anthesis. The results of our study will help to select traits ensuring sustainable food production systems that strengthen the capacity for the adaptation to future climate change.

2. Methods

2.1. Plant Material and Growing Conditions

Seeds of bread wheat (*Triticum aestivum* L. var. Gazul), a high-yielding variety in Castilla y León, were sown in 2-L pots containing a mixture of perlite:vermiculite (3:1, *v*/*v*) and grown in controlled environmental growth chambers (3.6 m length × 4.8 m width × 2.4 m height) under combined elevated CO_2 (900 ppm) and temperature (4 °C above current temperatures of Salamanca region, 26 °C). The daily cycle was 16/8 h light/dark, with a light intensity of 400 μmol m^{-2} s^{-1} and relative humidity of 40%/60% day/night. At the beginning of the experiment, the maximum soil volumetric water content of each pot was evaluated as the difference between pot weight after watering with the excess water drained (field capacity) and the pot dry weight. Pots with two seeds per pot were irrigated from the seed germination until the tillering stage. After that, the water deficit was imposed and one half of the plants was maintained under well-watered conditions until the end of the experiment, whereas the other half was subjected to water deficit conditions (65% of field capacity). Plants were watered with a nutrient solution twice per week and with water once per week to avoid excessive salt accumulation. The nutrient solution contained NO_3^- and it was supplied to a concentration of 10 mM provided by 6 mM KNO_3 and 2 mM $Ca(NO_3)_2$, while the other macro- and micronutrients were supplied as described by Vicente et al. [22].

2.2. Sampling Procedure

Plant material was harvested at ear emergence (Zadoks stage 59) and anthesis (Zadoks stage 65), respectively [23]. Flag leaves of the main shoots and roots were harvested 3–4 h after the onset of the photoperiod. Each leaf was cut and immediately plunged into liquid N_2 under illumination. The roots were separated from the aerial part of the plant and they were collected after washing with water to remove substrate residues and nutrient solution, blotted dry on tissue paper, and quickly immersed in liquid N_2. The samples were stored at −80 °C until use for analyses. The experiments included a total of four replicates for each treatment and developmental growth stage.

2.3. C-N Metabolites Quantification

Frozen leaf and root plant material was used for ethanol/water extraction for carbohydrate determination according to Morcuende et al. [24]. Glucose, fructose, sucrose and fructans were measured in the ethanol/water extracts with a spectrophotometric assay coupled to $NADP^+$ reduction as described by Morcuende et al. [24], while the starch content was measured in the insoluble residue. Total amino acids content was also determined in the ethanol/water extracts by the ninhydrin colorimetric method, as well as NO_3^- and protein contents following the methods described by Vicente et al. [25].

2.4. Determination of Biomass Parameters

At the ear emergence stage, plants were harvested to determine the fresh (FW) and dry matter (DW) accumulation. The aerial part of the plants was separated from the roots. The dry weight of each of the parts separated from the plants was recorded after drying in an oven at 60 °C for 48 h. Humidity (H) and leaf dry matter content (LDMC) were estimated as H = (FW-DW)/FW and LDMC = DW/FW, respectively.

2.5. Statistical Analyses

Descriptive analyses and graphs of variables and individuals from multifactorial analyses were carried out as described by Marcos-Barbero et al. [26].

3. Results and Discussion

Bread wheat (*Triticum aestivum* L.) is one of the most widely grown staple crop in the world threatened by the future climate change conditions. The impact of these conditions on wheat varies across the world, so specific adaptation strategies need to be studied in order to increase crop adaptation to the global climate change [27]. Furthermore, wheat growth and productivity under drought conditions needs an in-depth study of the physiological and biochemical mechanisms involved in the response to the conditions anticipated by global climate change at the end of this century and its dependence on development at the whole plant level. In this work, wheat plants were subjected to drought stress under combined elevated atmospheric CO_2 and temperature and flag leaves and roots were harvested at ear emergence and anthesis. Multifactorial analysis revealed that the organ was the main factor explaining data variation, whereas the developmental stage and drought treatment induced lower effects on the studied parameters (Figure 1A). In addition, the correlation circle showed that the C and N metabolites affected more the flag leaves, while the roots were more affected by fresh and dry weights and humidity (Figure 1B,C). In accordance with these results, Kumar et al. [21] reported that sugars and amino acids increased significantly in the aerial organs of different crops under drought stress. In addition, greater growth reduction was observed in shoots than in roots of barley plants subjected to drought [28].

When the flag leaves and root organs were studied separately, the results reflected that the physiological and biochemical traits in the flag leaves were more affected by drought than by the developmental growth stage, leading to an accumulation of soluble carbohydrates, nitrate and amino acids (Figure 2A–C). These findings are in accordance with those reported by Guo et al. [29], suggesting that sugars, organic acids and amino acids played a pivotal role to enhance drought tolerance of the aerial part of the plant. Furthermore, enhanced accumulation of water soluble carbohydrates (glucose, fructose, sucrose and fructans) was found in drought tolerant wheat genotypes [30,31], whereas a reduction in sucrose, fructose, mannose and tagatose seems to be associated with sensitive genotypes under water deficit stress [32]. This fact suggests that sugars may function as compatible solutes contributing to cell osmoregulation and the maintenance of foliar water content, providing a solvation shell around proteins, stabilizing subcellular structures and protecting membrane integrity [32–34]. In addition, amino acids content was also increased in wheat plants grown under water deficit conditions, in which proline, tryptophan, leucine, isoleucine and valine were notably accumulated in leaf tissues [32], protecting cellular structures during dehydration and contributing to redox balance [35]. The increase in NO_3^- in leaves could be explained by the inability of plants to reduce nitrate, being used as an osmolyte getting a better response to drought stress [36]. By contrast, roots were mainly affected by the developmental stages, but not by the treatment (Figure 2D). The root content of fructose, glucose, starch and amino acids was higher at the ear emergence than at the anthesis, whereas the accumulation of sucrose, fructans, proteins and nitrate increased at the latest growth stage (Figure 2E,F). The increase in glucose and amino acids enhanced the capacity to reduce and transport nitrate to other organs [37]. At anthesis, the increase in nitrate levels could be related to a limitation of nitrate reduction with the evolution of plant development [38]. Moreover, the increase in sucrose levels has been previously reported by Shao et al. [39].

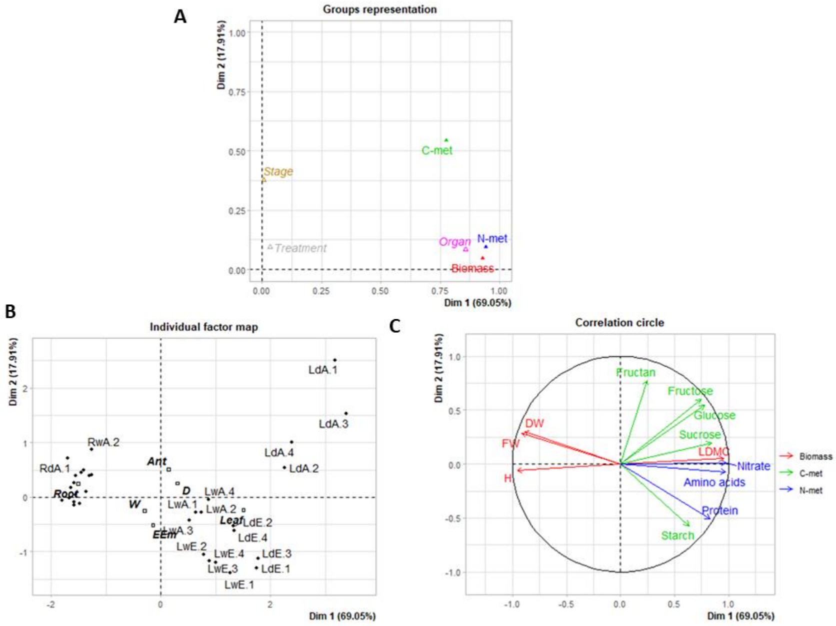

Figure 1. Multifactorial analyses for the C and N metabolites (C-met and N-met) and biomass parameters evaluated in the flag leaves and roots (L; R) of wheat plants subjected to different water regimes (d: drought; w: water) under elevated CO_2 and high temperature at different developmental stages (E: ear emergence; A: anthesis). The considered factors were organ, treatment and stage. The statistical analyses included groups representation (**A**), individual factor map (**B**) and correlation circle (**C**).

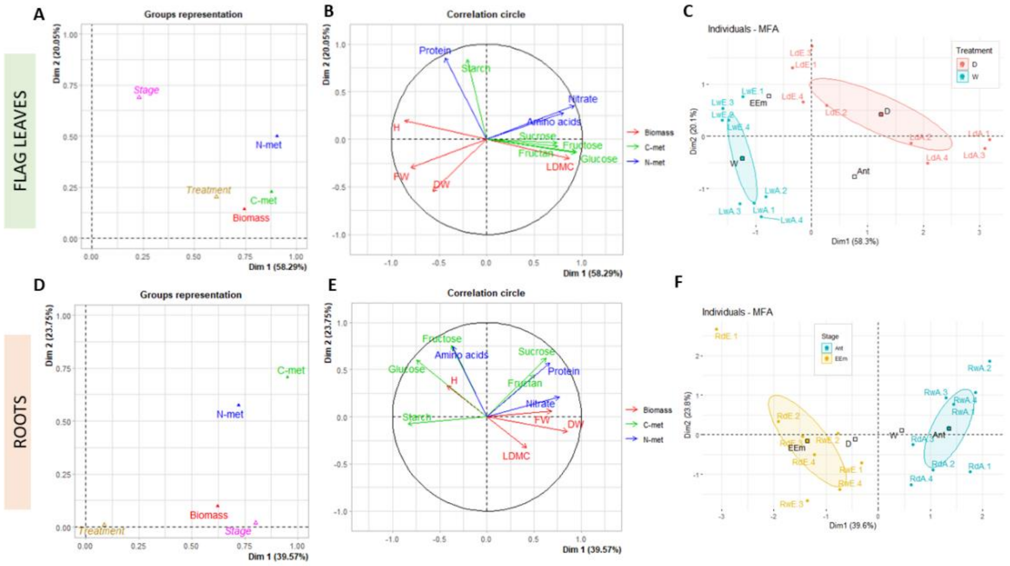

Figure 2. Multifactorial analyses for the primary C and N metabolites (C-met: glucose, fructose, sucrose and starch; N-met: nitrate, amino acids and proteins) and biomass parameters (DW, FW, LDMC and H) evaluated in the flag leaves and roots of wheat plants subjected to different water regimes (D: drought; W: water) under elevated CO_2 and high temperature at different developmental stages (EEm: ear emergence; Ant: anthesis). The statistical analyses for each wheat organ included groups representation (**A**,**D**), correlation circle (**B**,**E**) and MFA (**C**,**F**).

4. Conclusions

The comparative analyses of the physiological and biochemical traits in the flag leaves and roots of wheat plants under concurrent drought stress, elevated CO_2 and high temperature showed that leaves were more affected by the drought treatment than by the growth stage, leading to an accumulation of soluble carbohydrates, nitrate and amino acids, whereas roots were more affected by the developmental stages accumulating more fructose, glucose, starch and amino acids at the ear emergence than at the anthesis, whereas the accumulation of sucrose, fructans, proteins and nitrate increased at the latest growth stage. Thus, this study provides new evidence of the shift of primary C-N metabolism at whole plant level at ear emergence and anthesis developmental stages in response to the predicted elevated CO_2 and temperatures, and more lasting drought events. This could help us to select specific traits that are useful for wheat breeding programs to ensure food production systems with more resilient wheat varieties to climate change conditions following the Sustainable Development Goals of 2030 Agenda.

Author Contributions: J.B.A. and R.M. conceived and designed the experiment. N.B.-R., A.I.G.-H., E.L.M.-B., J.M.-A., J.B.A. and R.M. carried out the experiments and performed the measurements of the different assays. N.B.-R., A.I.G.-H. and E.L.M.-B. carried out the statistical data analyses. O.B and I.G.-F. contributed to the experimental work. N.B.-R. and A.I.G.-H. wrote the proceeding. J.B.A. and R.M. revised the manuscript. All authors have read and agreed to the published version of the manuscript.

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