Improved responses to elevated CO_2 in durum wheat at a low nitrate supply associated with the upregulation of photosynthetic genes and the activation of nitrate assimilation

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ARTICLE INFO

ABSTRACT

Elevated CO_2 often leads to photosynthetic acclimation, and N availability may alter this response. We investigated whether the coordination of shoot-root N assimilation by elevated CO_2 may help to optimize the whole-plant N allocation and maximize photosynthesis in hydroponically-grown durum wheat at two NO_3^- supplies in interaction with plant development. Transcriptional and biochemical analyses were performed on flag leaves and roots. At anthesis, the improved photosynthetic acclimation response to elevated CO_2 at low N was associated with increased Rubisco, chlorophyll and amino acid contents, and upregulation of genes related to their biosynthesis, light reactions and Calvin-Benson cycle, while a decrease was recorded at high N. Despite the decrease in carbohydrates with elevated CO_2 at low N and the increase at high N, a stronger upward trend in leaf NR activity was found at low rather than high N. The induction of N recycling-related genes was accompanied by an amino acids decline at high N. At the grain-filling stage, the photosynthetic acclimation to elevated CO_2 at high N was associated with the downregulation of both N assimilation, mainly in roots, and photosynthetic genes. At low N, enhanced root N assimilation partly compensated for slower shoot N assimilation and maximized photosynthetic capacity.

1. Introduction

Current climate change predictions involve increases in atmospheric [CO_2] due to anthropogenic activities, and the global mean [CO_2] is expected to rise over the course of this century, exceeding the level of 700 μmol mol^{-1} by 2100 [1]. Rising atmospheric [CO_2] is altering global temperature and precipitation patterns, which may adversely affect plant growth, yield and crop quality [2]. Therefore, climate change could seriously threaten food security in many parts of the world, particularly in more vulnerable regions, such as the Mediterranean [1]. Durum wheat accounts for more than 50% (reaching 90% in some countries) of total wheat production area in the Mediterranean, where it is used to make pasta and other food products [3]. Hence, understanding how this important crop will respond to the rapid change in [CO_2] is essential for tackling the challenge of ensuring food security in the face of a growing population and changing climate.

An increase in atmospheric [CO_2] is expected to stimulate photo-synthesis in C_3 plants such as wheat, and consequently increase crop growth and yield. However, most C_3 plants fail to sustain the maximum stimulation of net photosynthesis rates over prolonged exposure periods [2]. This shortcoming is often quantified by monitoring changes in the maximum carboxylation rate ($V_{c,max}$) of Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase) and the rate of photosynthetic electron transport (J) contributing to regeneration of ribulose-1,5-bisphosphate (RuBP) through leaf gas-exchange measurements [4], being considered an acclimation response [2]. This phenomenon, known as photosynthetic acclimation to elevated CO_2, has frequently been observed in wheat [5–7], and is accompanied by a reduction in Rubisco activity and content [8,9], and by the accumulation of carbohydrates [5,8]. Several factors have been proposed to explain this phenomenon: (i) the repression of photosynthetic genes by carbohydrates [2,10], (ii) limitation by nitrogen (N) availability [11], (iii) insufficient sink capacity [2,6] and accelerated leaf senescence [12]. In recent years, significant emphasis has been placed on the lower protein and total N

content often found in the leaves of plants grown under elevated CO_2 [8,13–16], suggesting that the acclimation of photosynthesis is connected with the N status of plants [16]. It has thus been shown that the degree of acclimation of photosynthetic capacity to elevated CO_2 is greater in N-limited plants than in well-fertilized ones [2,7,11], although no acclimation to elevated CO_2 has been found in wheat grown hydroponically with a low N supply added in direct proportion to its growth rate [17]. The mechanisms by which elevated CO_2 decreases tissue N concentrations and protein have yet to be clarified. CO_2 enrichment can affect multiple processes, from N uptake to metabolism and resource allocation, and some explanations for this decline include N dilution by increased concentrations of non-structural carbohydrates [18], restricted N uptake due to decreased transpiration [9,14], and the inhibition of N assimilation into proteins [7,13,15,16]. The latter derives in part from the inhibitory effect of CO_2 on photorespiration and the dependence of shoot N assimilation on photorespiration [19].

N is the mineral nutrient required in greatest amounts by plants, being a major constituent of many cell components, and it is often the growth-limiting nutrient [20]. Furthermore, N assimilation is closely linked to C metabolism because photosynthesis provides C skeletons and reducing power for assimilating inorganic N into amino acids. Plants usually acquire N from the soil in the form of ammonium (NH_4^+) and nitrate (NO_3^-), although in temperate climates and cultivated soils NO_3^- is the most important source of N available for crops [21]. Depending on the species and NO_3^- abundance, NO_3^- can be either assimilated in the root or translocated through the xylem for assimilation in the aerial part of plants. Typically, the shoot dominates NO_3^- assimilation in a high soil NO_3^- environment [22]. The first and rate limiting step in the NO_3^- assimilation pathway is the reduction of NO_3^- catalysed by nitrate reductase (NR). At the transcriptional level, NR is highly regulated by NO_3^-, sugars and downstream metabolites of N assimilation [23], and a hierarchy of post-transcriptional and post-translational regulation adjusts the activity of the enzyme to the plant's N status [24]. NO_3^- acts as a signalling molecule, and modulates the expression not only of the genes involved in NO_3^- reduction and assimilation, but also of those related to photosynthesis, glycolysis, the TCA cycle, and other metabolic pathways [24,25]. Decreases in NR activity have been reported in tobacco and wheat plants grown at elevated CO_2 [7,16,26–28], together with increases in enzyme activity [29,30]. Contradictory results have also been obtained for N uptake at elevated CO_2 [28,31,32], revealing that N uptake and assimilation do not keep pace with photosynthesis and growth under enhanced CO_2 [11], as shown by the changes in the expression pattern of the genes involved in C and N metabolism [7,33]. The coordination of shoot photosynthetic C fixation with root inorganic N uptake optimizes plant performance in a fluctuating environment [22]. Hence, plant responses to elevated CO_2 will not be understood unless molecular and biochemical aspects are integrated and studied beyond photosynthesis, biomass and C-N allocation at whole-plant level.

Most of the studies evaluating the combined effects of N supply and CO_2 have focused on the aerial part of the plant, with much less attention being paid to root responses and their involvement in whole-plant performance, probably because the bulk of the NO_3^- taken up by plants is reduced in the leaves. A few recent studies provide an integrated snapshot of the whole-plant's response to elevated CO_2 at different N supplies and sources for the model plant Arabidopsis [34,35]. However, in crop species, most studies have addressed the response of a limited number of metabolic and molecular parameters [26–30,36], and only a limited number have included gene expression analyses for the important steps of C and N metabolism in the whole-plant [37] in interaction with its developmental stages [31].

For wheat grown hydroponically using NO_3^- as the sole N source, we have previously reported that, at anthesis, elevated CO_2 increased N allocation to aboveground plant organs and leaf N concentration per unit area at low N supply (0.5 mM), but not at high N (5 mM) [31]. This

shift alleviated photosynthetic acclimation to elevated CO_2 at low N supply. However, when comparing CO_2 effects at lower N concentrations (0.25 and 2.5 mM, respectively, as low and high N supply) applied less frequently, CO_2 acclimation of photosynthesis was recorded at low N [37]. We complement the first of these studies to test the hypothesis that enhanced NO_3^- assimilation by elevated CO_2 in the flag leaves of low N plants (0.5 mM) was a key adaptive mechanism to optimize N allocation within the leaves and maximize photosynthesis when N is limited at late developmental stages. To do so, we assessed the acclimatory responses to elevated CO_2 of gas exchange in the flag leaves of wheat grown hydroponically at two NO_3^- supplies, and we measured the nitrate reductase activity and the contents of Rubisco, proteins, nitrate and several C and N metabolites, together with the transcript abundance of primary C and N metabolism genes in flag leaves and roots at both anthesis and early grain filling (EGF) stages.

2. Material and methods

2.1. Plant material and growth conditions

Durum wheat plants (*Triticum durum* Desf. cv. Regallo) were grown hydroponically under ambient (390 μmol mol^{-1}) and elevated (700 μmol mol^{-1}) CO_2 concentrations using controlled environment chambers maintained on a daily cycle of 16 h light/8 h dark with a day/night temperature and relative humidity of 20/15 °C and 65/70%, respectively. The irradiance was 400 μmol m^{-2} s^{-1} provided by a combination of blue- plus red-peak fluorescent lamps. Wheat seeds were germinated and grown under hydroponic conditions as described by [31]. Briefly, twelve days after sowing, 60 seedlings for each one of the two CO_2 concentrations studied were transferred to 12 plastic pots for hydroponic culture of 10 L capacity containing an aerated nutrient solution with a NO_3^- concentration of 5 mM provided by 3 mM KNO_3 and 1 mM $Ca(NO_3)_2$. The other macronutrients and micronutrients in the nutrient solution were supplied as 1 mM $MgSO_4$, 0.5 mM KH_2PO_4/K_2HPO_4, 0.1 mM KCl, 0.1 mM NaCl, 40 μM ortho–ortho EDDHA Fe chelate, 50 μM H_3BO_3, 10 μM $MnSO_4$, 1 μM $CuSO_4$, 1 μM $ZnSO_4$ and 0.5 μM Na_2MoO_4 with the pH adjusted to 5.6. Three weeks later, when the plants were at the tillering stage (Zadoks 27, [38]), half of the pots in each chamber received a nutrient solution with a NO_3^- concentration of 0.5 mM (LN, low nitrogen) by supplying 0.25 mM KNO_3 and 0.125 mM $Ca(NO_3)_2$, and the other half of the pots continued receiving a NO_3^- concentration of 5 mM (HN, high nitrogen). The additional concentrations of potassium and calcium required in the LN nutrient solution were supplied as 2.850 mM KCl and 0.875 mM $CaCl_2$. Each one of the growth chambers had six pots with five plants per pot for each NO_3^- concentration in a fully randomized design. The solution was renewed once a week for the first three weeks, twice a week for the next month, and three times a week thereafter. The pots were rotated once a week within each growth chamber to avoid the effects of intra-chamber environmental gradients, and the pH of the nutrient solution was adjusted to 5.6 daily with 1 M HCl.

To determine N uptake and carry out biochemical and transcriptional analyses, at two growth stages, anthesis and EGF (Zadoks 65 and 71, respectively), three hours after the beginning of the light period in the growth chamber the plants were incubated for 24 h in 2.5 L flasks filled with a fixed volume of 2.2 L of a fresh 0.5 or 5 mM NO_3^- nutrient solution (see [31]). The incubations were carried out under the same growth chamber light intensity, temperature, photoperiod and humidity conditions indicated above. There were four replicates, each consisting of one plant, for each level of N supply and $[CO_2]$ studied. Once the incubation period had ended, the flag leaves of the three older tillers of each incubated plant were cut and immediately plunged into liquid N under illumination. Thereafter, roots were detached from the shoots, rinsed twice in 1 mM $CaSO_4$ to remove the NO_3^- nutrient solution from the root surface and minimize NO_3^- efflux, and blotted dry on tissue paper. They were cut in half longitudinally, and one half