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## Durum wheat grain yield and quality under elevated CO<sub>2</sub> : first results of a free air carbon dioxide enrichment (FACE) experiment

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**Abstract.** Free-air CO<sub>2</sub> enrichment (FACE) experiments the study of the effects of elevated [CO<sub>2</sub>] on plants and ecosystems grown under natural conditions without enclosure. Here the results of the first harvest 2011/2012 on the aboveground biomass production, grain yield and grain quality of 12 durum wheat (*Triticum turgidum* var. *durum*) genotypes grown under FACE conditions. These genotypes are representative of the durum wheat breeding history in Italy. The free-air system, installed in the experimental farm of the Genomics Research Centre of CRA in Fiorenzuola d'Arda, allows the study of the effect of increased atmospheric CO<sub>2</sub> mixing ratios, expected for the mid of the 21<sup>st</sup> century on crop yield and quality. The results showed an increase in biomass and grain yield and a decrease in grain crude nitrogen content due to elevated CO<sub>2</sub>. Moreover, high genetic variability was observed for all of these traits within the genotypes.

Keywords. Free Air Carbon Dioxide Enrichment (FACE) – Genetic diversity – Grain yield – Grain quality.

## Rendement et qualité des grains de blé dur sous CO, élevé : les premiers résultats d'une expérience d'enrichissement en dioxyde de carbone à l'air libre (FACE)

**Résumé.** L'enrichissement en  $CO_2$  à l'air libre (FACE) permet d'étudier expérimentalement les effets de la hausse du  $[CO_2]$  dans l'atmosphère sur les plantes et les écosystèmes cultivés en plein champ et à l'air libre. Nous allons illustrer les résultats de la première récolte 2011/2012 sur la production de biomasse aérienne, le rendement en grain et la qualité du grain de 12 génotypes de blé dur (Triticum turgidum var. durum) cultivés dans des conditions FACE. Ces génotypes sont représentatifs de l'histoire de la sélection du blé dur en Italie. Le système à l'air libre, installé dans la ferme expérimentale du Centre de recherche en génomique du CRA à Fiorenzuola d'Arda, permet d'évaluer l'effet de l'augmentation des rapports de mélange du  $CO_2$  dans l'atmosphère, annoncée pour le milieu du siècle, sur le rendement et la qualité des cultures. Les résultats ont montré une augmentation de la biomasse et du rendement en grains et une diminution de la teneur en azote brut du grain liées à la concentration accrue de  $CO_2$ . En outre, une forte variabilité génétique a été observée pour l'ensemble de ces caractères entre les génotypes.

**Mots-clés.** Enrichissement en dioxyde de carbone à l'air libre (FACE) – Diversité génétique – Rendement en grain – Qualité du grain.

#### I – Introduction

Continued growth of the world population, motorization and industrialization is resulting in an increased emission of greenhouse gases, especially  $CO_2$ , from combustion of fossil fuels, industrial processes, and deforestation. Based on the reports by the IPCC (Intergovernmental Panel on Climate Change, 2007), atmospheric  $CO_2$  concentration is continuously rising (Balouchi *et al.*, 2009). The measurements at the Mauna Loa station (Hawaii) show an increase from below 320 ppm at the start of the measurements in 1958 to 393 ppm in 2011. Further increases are expected during the 21<sup>st</sup> century due to continued use of fossil fuels, leading to estimated concentrations around 550 ppm for the mid-century. The rising atmospheric  $CO_2$  levels are a

cause of the ongoing anthropogenic climate warming and thus a matter of concern with respect to global change.

CO<sub>2</sub> is the main source of organic carbon of living beings. Plant photosynthesis fixes and reduces CO<sub>2</sub>, incorporating the carbon into biomolecules. The direct causes of the instantaneous increase in C3 photosynthesis with elevation of CO<sub>2</sub> are two properties of the primary carboxylase of C3 photosynthesis: ribulose-1,5-bisphosphatecarboxylase/oxygenase (RuBisCo). The enzyme catalyzes the carboxylation of ribulose-1,5-bisphosphate (RubP) with CO<sub>2</sub> to yield two molecules of 3-phosphoglyceric acid (3PGA). First, RuBisCo is not saturated at present levels of atmospheric CO<sub>2</sub>, and so elevated CO<sub>2</sub> increases the velocity of carboxylation and net photosynthesis. In addition, CO<sub>2</sub> is a competitive inhibitor of the oxygenation reaction, which leads to photorespiratory release of CO<sub>2</sub> (Long et al., 2006; Ainsworth and Rogers, 2007) and so elevated CO<sub>2</sub> reduces the rate of oxygenations. Thus, the historical rise in atmospheric CO<sub>2</sub> as well as the expected further increases during the coming decades have the potential to lead to increased carbon assimilation by C3 photosynthesis. Many studies on the effects of elevated CO, on C3 plant photosynthesis and growth have demonstrated a stimulation of photosynthetic production and, subsequently, growth, although not always as high as expected on the base of the enzyme kinetic properties of RuBisCo. The photosynthetic capacity is often reduced after long-term exposure to elevated CO<sub>2</sub>, a phenomenon known as down-regulation (Arp, 2006).

Change in biomass in response to  $CO_2$  enrichment has been reported to vary with species and a persistent increase of biomass production during growth in elevated  $CO_2$  was observed. Besides carbon fixation, also the plant tissue chemistry of nitrogen is greatly affected by atmospheric  $CO_2$  enrichment. In particular, the most commonly reported effect is a decrease in the dry mass concentration of N (Nm). It was reported that the mean value of Nm decreased by 14% in above-ground tissues and 9% in roots, reaching a 12.9% decrease for leaves in free-air carbon dioxide enrichment (FACE) experiments and 14% for seeds (Taub and Wang, 2008). For wheat, barley and rice, the reduction in grain protein concentration was 10-15% of the value at ambient  $CO_2$  (Taub *et al.*, 2008). This effect, known as growth dilution, leads to reduced concentration of protein with increased yield. Such decrease in Nm can have important implications for plant physiological processes and for food chains, as well as on the performance of insect herbivores and can affect herbivore population dynamics.

Many research efforts to understand how plants and ecosystems will respond to rising atmospheric CO, have been undertaken. The primary effects on plants of elevated CO, have been well documented and include reduction in stomata conductance (gs) and transpiration, improved water-use efficiency (WUE), higher rates of photosynthesis (A), and increased light-use efficiency. The majority of these conclusions have come from studies of individual species grown in closed controlled environments. While the conclusions from these experiments form the basis for the knowledge of plant physiological responses to elevated CO<sub>2</sub>, there are serious limitations to using enclosure systems when studying the effects of elevatedCO, on plants. Chambers also are limited in size and may have limited capacity to allow investigators to follow trees and crops to maturity within an experimental facility. Large-scale free-air CO<sub>2</sub> enrichment (FACE) experiments allow the exposure of plants to elevated CO<sub>2</sub> under natural and fully open-air conditions. FACE technology uses no confinement structures, rather an array of vertical or horizontal vent pipes to release jets of CO<sub>2</sub>-enriched air or pure CO<sub>2</sub> gas at the periphery of vegetation plots. FACE relies on natural wind and diffusion to disperse the CO<sub>2</sub> across the experimental area. More recent field studies have employed a FACE technique in which pure CO<sub>2</sub> gas is released as high-velocity jets from emission tubes (through numerous small perforations) positioned horizontally at the periphery of a FACE octagon (Miglietta et al., 2001). FACE design allows good temporal and spatial control of CO<sub>2</sub> concentrations throughout crop canopies and forest plantations (Long and Ainsworth, 2005).

There is now a pressing need to understand more about long-term adaptation and genetic changes in future  $CO_2$  concentrations, particularly for adaptive traits that are relevant to plant productivity and ecological characteristics that determine survival, fitness, yield and interaction with pathogens. Genetic variability in this response needs to be characterized to identify the most promising genotypes for breeding of new varieties that optimally exploit elevated levels of atmospheric  $CO_2$ .

A FACE experiment was conceived to study the effects of elevated  $CO_2$  on growth, yield and grain quality of 12 durum wheat (*Triticum turgidum* var. *durum*) genotypes that were products of the durum wheat breeding history in Italy. Genetic variability and GxE interactions under ambient/ elevated  $CO_2$  are been observed for plant development and growth, canopy-related traits, yield, yield components, quality traits and metabolite composition. Furthermore, eco-physiological analyses are carried out to describe the physiological mechanisms modified in response to elevated  $CO_2$  and flag leaf samples are collected for transcriptomic (RNAseq) and metabolomic studies, and grain for quality. The analyses of the samples collected during the first growing season (2012) have been partially completed and the presentation illustrates the first main findings and summarizes the expected impact of increased atmospheric  $CO_2$  on yield response and quality in durum wheat.

#### **II – Material and Methods**

Twelve durum wheat genotypes (Triticum turgidum var. durum) were grown within the FACE facility of the Genomics Research Centre of the Consiglio per la Ricerca e sperimentazione in Agricoltura (CRA-GPG) at Fiorenzuola d'Arda (44.927°N, 9.893°E) applying a split-plot design with FACE and control octagons distributed at random within the experimental field (4 FACE, 4 controls). The single FACE and control systems contained two blocks (northern and southern side) with plots (1.32 x 2.2 m) for the 12 genotypes as sub-plots. The genotypes include modern high-yielding varieties (Simeto, Ciccio, Claudio, Anco Marzio, Saragolla), varieties with high protein content (Svevo, Aureo), varieties with a prominent role in Italian durum wheat breeding (Cappelli, Creso, Ofanto) and two lines of the Ofanto x Cappelli mapping population (RIL11 and RIL28). Sowing at optimal sowing time (October 19th 2011) was assured by a pre-harrowing irrigation due to dry soil conditions. The CO<sub>2</sub> mixing ratio for the FACE treatment target was fixed at 570 ppm representing a value within the upper range of scenarios for the mid Century atmospheric mixing ratio. Carbon dioxide sensors are located in the centre of the octagons and of the anemometers on top of the control units. The readings of the CO, concentration, wind speed and velocity are used to calculate the level of the fumigation and the sectors in which CO<sub>2</sub> is released. The readings of CO<sub>2</sub> sensors as well as the variables describing the fumigation are transferred to a central server within the institute via fibre glass cables. FACE treatment was started on November 16th, 2011 and stopped when leaves were senescent at June 14th, 2012. The experiment was performed according to standard local agronomic practice and with the objective to avoid major pests and diseases. The plots were fertilised with application of an N:P:K fertiliser at pre-seeding and two top-dressings with ammonium nitrate for a total of 149 kg N ha<sup>-1</sup>. At final harvest (July 2<sup>nd</sup>, 2012) 1.5 linear meters per plot were harvested for determination of yield components. Subsequently, the whole plots were harvested manually and aboveground biomass and grain yield were determined. Grain nitrogen content determined with the Kjeldahl method, and grain crude protein content calculated as 5.7\*N.

Figure 1. Two of the durum wheat FACE octagons in April 2012 with the  $CO_2$  tank in the background.

### **III – Results and Discussion**

The traits related to growth showed a more vigorous development under elevated  $CO_2$  (Badeck *et al.*, 2012). Flag leaf light-saturated photosynthesis was higher under elevated  $CO_2$  when measured at growth conditions and stomata conductance substantially reduced leading to an increased instantaneous water use efficiency (+30 to 60%) at the leaf level (Badeck *et al.*, in press). The increased leaf level water use efficiency (WUE) was not fully compensated by the higher transpiring surface area, as evidenced by measurements of soil water content in the uppermost 6 cm of soil (data not shown), that showed a slightly higher water content in the elevated  $CO_2$  treatment.

A good growing season led to high yield in the ambient as well as the elevated  $CO_2$  treatments. The average grain dry mass yield was 7.91 t DM ha<sup>-1</sup> under ambient  $CO_2$  and the FACE treatment increased the average yield significantly (p<0.01) to 8.91 t DM ha<sup>-1</sup>. Total aboveground dry biomass at harvest was 18.5 t DM ha<sup>-1</sup> under ambient  $CO_2$  and increased (p<0.01) to 21.6 t ha<sup>-1</sup> under FACE. There is a considerable and statistically significant genetic variability of yield as well as of the  $CO_2$  response of yield. Grain dry matter yield was increased between 4.4% (var. Ciccio) and 20.4% (RIL28) (Fig.2). The average increase in grain yield of durum wheat by 12.6% due to elevated  $CO_2$  was similar to the medium effect of 14.4% reported by Ainsworth and Long (2005) for bread wheat based on a meta-analysis of five FACE experiments. The variability among the durum wheat cultivars (+4.4 to 20.4%) filled a substantial part of the confidence range found for the effect in bread wheat (-1.6 to +33.1%). The increase in grain yield of durum wheat was mainly due to increased numbers of tillers, while the number of grains per spike and the thousand kernel weight (TKW) changed only marginally (Fig.3).

Averaged across all genotypes, the crude grain protein content decreased by 7.0% for plants grown in elevated  $CO_2$  relative to the controls with a substantial variation between genotypes (-2.2% for Ofanto to -10.8% for Aureo).

Figure 2. Graph describing the grain yields of 12 genotypes of durum wheat grown in the FACE octagons (E enrichment red bars) and in atmospheric  $CO_2$  concentration (A ambient blue bars).



Figure 3. Effect of elevated  $CO_2$  on grain yield and yield components. The box plots show the distribution of genotype means.

### **IV – Conclusions**

Atmospheric  $CO_2$  content elevated to 570 ppm lead to a stimulation of grain yield in durum wheat that is comparable to results obtained on bread wheat, whereas crude grain protein content decreased indicating potential losses in grain quality. Substantial between genotype variability in the yield and quality response to elevated  $CO_2$  hints to genetic variability that can be exploited for selection of varieties best suited for the mid-century atmospheric  $CO_2$  content.

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