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Effect of the transgenerational exposure to elevated CO_2 on the drought response of winter wheat: Stomatal control and water use efficiency

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ABSTRACT

Climate change predicts more frequent drought spells along with an elevation in atmospheric CO_2 concentration ($e[CO_2]$). Although the responses of winter wheat (*Triticum aestivum* L.) plants to drought or a single generation exposure to $e[CO_2]$ have been well documented, the transgenerational effect of $e[CO_2]$ in combination of drought on stomatal behavior, plant water consumption and water use efficiency (*WUE*) have not been investigated. Seeds harvested from plants after two generations (2014–2015) continuously grown in ambient CO_2 ($a[CO_2]$, 400 $\mu mol\ L^{-1}$) and $e[CO_2]$ (800 $\mu mol\ L^{-1}$) were sown in 4 L pots, and the plants were grown separately in greenhouse cells with either $a[CO_2]$ or $e[CO_2]$. At stem elongation stage, in each of the cells half of the plants were subjected to progressive drought stress until all the plant available soil water was depleted, and the other half were well-watered and served as controls. The results showed that transgenerational exposure of the winter wheat plants to $e[CO_2]$ could attenuate the negative impact of drought stress on dry biomass (*DM*) and *WUE*. The modulations of multi-generational $e[CO_2]$ on leaf abscisic acid concentration, stomatal conductance, and leaf water status could have contributed to the enhanced *DM* and *WUE*. These findings provide new insights into the response of wheat plants to a future drier and CO_2-enriched environment.

1. Introduction

The responses of crop plants to elevated atmospheric CO_2 concentration ($e[CO_2]$) have been extensively researched (Ainsworth and Long, 2005; Long et al., 2004; Pazzaglia et al., 2016). Most of these studies, however, have focused on the response of a single generation of plant to $e[CO_2]$; the long-term response over multiple generations, which will most likely have the primary effect on plant performance in a future climate, have received little attention. In natural ecosystems, early studies have shown that transgenerational effects can result either from maternal environmental effects or from evolutionary responses to novel selection pressures and are important because they may alter the ultimate ecological impact of the environmental change (e.g., Lau et al., 2008). Similarly, in agro-ecosystem crop plants will also experience transgenerational $e[CO_2]$ in a future climate, and their performance may not be predicted based on single generational response, particularly if the crops are also subjected to other abiotic stress like drought. Therefore, for better predicting climate change impact on crop productivity, it is essential to understand the impact of maternal CO_2 growth environment on offspring's performance (Andalo et al., 1999; Lau et al., 2008).

Earlier studies have shown that plant response to $e[CO_2]$ can be modulated by the CO_2 environment that the maternal plant has experienced (Bezemer et al., 1998; Huxman et al., 1998a; Steinger et al., 2000; Schulte et al., 2002; Derner et al., 2004; Ward and Kelly, 2004; Lau et al., 2008; Bezemer and Jones, 2012). For instance, seedlings of the annual grass *Bromus madritensis* respond to $e[CO_2]$ by increasing their growth rate, but this increase is less when the parent plants was also grown at $e[CO_2]$ (Huxman et al., 1998b, 2001). On the contrary, in spring wheat (*Tritium aestivum* L.), Derner et al. (2004) reported that the plant did not respond to $e[CO_2]$ during the first generation, whereas plants that had been grown for two or three generations in $e[CO_2]$ produced more biomass than plants grown for two or three generations in ambient CO_2 ($a[CO_2]$) (Derner et al., 2004). The discrepancy between the transgenerational response of plants to $e[CO_2]$ could be due to the

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different plant species examined; these authors have proposed that difference in the seed size and quality harvested from the maternal plants, which were raised at different CO_2 environment maybe responsible for the growth responses of the offspring to $e[CO_2]$ (Huxman et al., 2001; Derner et al., 2004).

Along with increasing in atmospheric $[CO_2]$, global change predicts more frequent drought spells in future climate, and which will complicate the effect of $e[CO_2]$ on crop plants (Jiang et al., 2016; Xu et al., 2016). It is well know that $e[CO_2]$ leads to an increase in photosynthetic rate (A) but a reduction in stomatal conductance (g_s) (Ainsworth and Rogers, 2007), resulting in an increase of water use efficiency (*WUE*) (Pazzaglia et al., 2016). As g_s is more sensitive to drought than A, an increase of *WUE* is often seen in plants grown under moderate drought (Liu et al., 2005). Indeed, *WUE* tends to increase both under CO_2 enrichment and under drought, and a synergic interaction of the two factors may further increase *WUE* (Housman et al., 2006). The number of stomata on leaf epidermis is an important factor that determines g_s (Yan et al., 2012; Sun et al., 2014). $e[CO_2]$ is found to reduce stomatal density (*SD*) in different plant species including winter wheat (Woodward and Kelley, 1995). However, it has also been reported that, even though g_s was lowered, *SD* remained unchanged in species collected from a naturally enriched CO_2 spring which had exposed for generations to $e[CO_2]$ as compared to those collected from nearby control site (Bettarini et al., 1998). Zhou et al. (2013) found that, in a top-open chamber experiment, ten-year exposure to $e[CO_2]$ increases stomatal number of *Pinus koraiensis* and *P. sylvestriformis* needles. Thus, there is still no consensus regarding how $e[CO_2]$ affects *SD* and its implications in regulating *WUE*. *SD* is found to increase under moderate drought but decrease under severe drought (Xu and Zhou, 2008; Yan et al., 2012). Therefore, it is interesting to examine the interactive effect of drought and $e[CO_2]$ on *SD* and g_s, and which may provide further insights into the regulatory mechanisms of water availability and CO_2 environment effect on *WUE*.

It is well known that ABA plays an important role in regulating stomatal aperture of plants grown in drying soils, thereby controlling the transpiration rate of the plants (Liu et al., 2005). However, it remains elusive if drought-induced ABA accumulation in the leaf could be modulated by $e[CO_2]$ and synergistically affecting g_s. Furthermore, the $e[CO_2]$ will be persistently influencing crop performance in future climate whereas drought may occur occasionally; thus it is essential to take the transgenerational $e[CO_2]$ effect into account when studying crop drought stress responses. Therefore, the objective of this study was to investigate if transgenerational exposure to $e[CO_2]$ could further enhance *WUE* of wheat under drought stress and to explore the underlying mechanisms in terms of stomatal control of transpiration via modifying stomatal morphology and leaf ABA concentration.

2. Material and methods

2.1. Experimental setup

Seeds of winter wheat (*Triticum aestivum* L. var. Lianmai 6) harvested from plants after two generations (2014–2015) continuously grown in ambient CO_2 concentration ($a[CO_2]$, 400 μmol L^{-1}) and elevated CO_2 concentration ($e[CO_2]$, 800 μmol L^{-1}) were sown in 4 L pots (16.5 cm in height and 17 cm in diameter, with 4 drainage holes) filled with peat material (Sphagnum, 32% organic matter, pH = 5.6–6.4 and EC = 0.45 ms cm^{-1}) on February 15, 2016. The peat contained 50% of water (v/v) at full pot holding capacity and 15% at permanent wilting point, resulting in a total plant available water of 35% (i.e., 1.4 kg) in the pot. In the first generation, wheat plants were grown in $a[CO_2]$ and $e[CO_2]$ from February 15 to July 15 2014, and the second generation was grown from September 15 to January 31 2015. The environmental $[CO_2]$ was well controlled 24 h a day during the whole growth seasons in both generations. From sowing, the plants were grown separately in two greenhouse cells (6 m × 8 m), one with $a[CO_2]$ and another with $e[CO_2]$. The CO_2 enrichment was achieved by emission of pure CO_2 from a bottled tank, released in one point and distributed in the greenhouse cells through internal ventilation. The $[CO_2]$ concentration in the greenhouse cells was monitored every six seconds by a CO_2 Transmitter Series GMT220 (Vaisala Group, Helsinki, Finland). The climate conditions in the greenhouse were set at: day/night temperature 20/16 °C, photoperiod 16 h, relative humidity 70%, supplemental light 400 μmol m^{-2} s^{-1} supplied by sunlight plus meta-halide lamps.

At the 3rd leaf stage (i.e., Zadoks code 13, (Zadoks et al., 1974)), the seedlings were thinned to three plants per pot. At stem elongation stage (i.e., Zadoks code 26), in each cell half of the plants were subjected to progressive drought stress (D) until all the plant available soil water was depleted, and the other half were well-watered (W) and served as control. In total, there were eight treatments based on maternal CO_2 environment ($[CO_2]_m$), offspring CO_2 environment ($[CO_2]_o$) and water treatment (WT), and each with four replicates (i.e., 4 pots). A schematic illustration of the experimental design and treatments is shown in Fig. 1. The CO_2 concentration under $e[CO_2]$ and $a[CO_2]$ during the experiment were shown in Fig. 2.

2.2. Measurements

At the end of the drought treatment, i.e., 8 d after withholding watering, stomatal conductance (g_s) of the latest fully expanded leaf was measured using a leaf porometer (Decagon Devices, Pullman, WA, USA) on both adaxial and abaxial surfaces at 10:00–12:00 h. The sum of the g_s of the adaxial and abaxial was calculated. Midday leaf water potential (Ψ_l) was measured after g_s measurement with a pressure chamber (Soil Moisture Equipment, Santa Barbara, CA, USA) on the latest fully expanded leaf. After measuring Ψ_l, the leaf was divided into two parts, one for determining the relative water content (*RWC*) of leaf, and the other was packed in aluminum foil and frozen in liquid nitrogen for later analyzing ABA concentration. *RWC* was determined according to the protocol by Jensen et al. (2000). *RWC* was calculated as:

$$RWC = (FW - DW)/(TW - DW) \tag{1}$$

where *FW* is the leaf fresh weight, *DW* is the leaf dry weight and *TW* is the leaf turgid weight, which was obtained by weighting the leaf samples after full hydration by floating them on distilled water for 3 h under dim light and room temperature. The ABA concentration in the leaf samples was measured by an enzyme linked immunosorbent assay (ELISA) using a monoclonal antibody for ABA (AFRC MAC 252) according to Asch (2000).

Stomatal density (*SD*) was calculated by counting the number of stomata on images taken from both the abaxial and adaxial sides of the second latest fully expanded leaf with a Dino-Lite digital microscope(AM411 series with ver. 1.4.1, Vidy Precision Equipment Co. Ltd, Wuxi, China) without damaging the leaf. The image of calibration sample was also saved to calculate the actual area of the image using AxioVision SE64 software (Rel. 4.4.3, Carl Zeiss Microscopy, Jena, Germany) (Akhtar et al., 2015). The mean *SD* of the adaxial and abaxial surfaces was calculated.

Cumulative evapotranspiration (*ET*) of the plants during the WT treatment period was recorded by daily weighing the pots with a balance (Mettler-Toledo, Glostrup, Denmark), to calculate plant daily water use (for both W and D plants) and irrigation amount for the W plants. On d 8 after onset of WT treatment, plants were harvested and the leaves and stems were separated for