



Growth performance and antioxidative response in bread and durum wheat plants grown with varied potassium treatments under ambient and elevated carbon dioxide



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ABSTRACT

It is predicted that atmospheric carbon dioxide (CO₂) level will double by the end of the current century. Although the fertilization effect of CO₂ on plant growth is well documented, studies that investigate plant nutritional requirements under elevated CO₂ are scarce. Potassium (K) is an essential plant nutrient with prominent roles in key physiological processes. Aim of this work, was to determine the effects of K deficiency on plant growth as affected by elevated CO₂ and how antioxidant defense systems (activities of SOD, CAT, POX, APX, GR, MDHAR, DHAR, lipid peroxidation and total antioxidant activity) respond to K deficiency under ambient (a-CO₂: 400 ppm) or elevated (e-CO₂: 900 ppm) atmospheric CO₂ conditions in durum (*Triticum durum* cv. Sarıçanak-98) and bread wheat (*Triticum aestivum* cv. Adana-99). Plants were grown in hydroponics with sufficient (1850 μM), low (60 μM) or deficient (20 μM) K and under a-CO₂ or e-CO₂. As expected, e-CO₂ promoted biomass production with adequate K supply, however in low and deficient K plants biomass was either not affected or even decreased by e-CO₂. It was observed that low or deficient supply of K induced oxidative stress, but e-CO₂ had no significant impact on antioxidative response of plants and thus could not alleviate detrimental effects of K deficiency. Under K deficiency, CAT activity decreased in both species but this decrease was accompanied with increases in POX and APX which may be for adapting to the changing environment. In general, responses in antioxidant defense enzymes were linked to K nutritional status of plants rather than e-CO₂ conditions.

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1. Introduction

The current estimated world population of 7.3 billion is expected to rise to 11.2 billion by the end of this century (UN-ESA, 2015). Many people, particularly those living in developing countries, are suffering from nutrient deficiency-related disorders, therefore, in addition to meeting the ever increasing global food demand, there is also an urgent need to enhance the nutritional quality of food crops (FAO, 2015). It is widely accepted that a combination of agronomic and genetic approaches is required to simultaneously enhance yield and quality of crop plants. Recently adverse effects of climate change on crop production have been increasingly reported in various regions of the world (Parry et al.,

2004; Kang et al., 2009; DaMatta et al., 2010; Godfray et al., 2010; IPCC, 2014). Accordingly, the requirement of adequate and balanced nutrition of crop plants to alleviate detrimental effects of global climate change has been frequently emphasized by many researchers highlighting the importance of plant nutrition research in addressing global food safety and security issues (Lynch and St. Clair, 2004; Cakmak, 2005; DaMatta et al., 2010; Tausz et al., 2013; St. Clair and Lynch, 2010) and it is clear that the adverse effects of climate change (increasing temperatures, rising CO₂ levels and increased biotic and abiotic stresses) will continue to be challenging for both farmers and researchers during the next few decades.

Anthropogenic greenhouse gas (GHG) emissions are the causes of climate change and carbon dioxide is the main GHG. It is predicted that until the end of this century atmospheric CO₂ will be varying between 430 and 480 ppm or 720–1000 ppm in the best

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and worst case scenarios respectively (IPCC, 2014). For a variety of C_3 species it has been reported that e- CO_2 levels enhance plant growth and yield. This is mainly due to enhanced availability of CO_2 in the vicinity of RuBisCO resulting in increased CO_2 fixation efficiency and decreased photorespiration (Reddy et al., 2010; Dippery et al., 1995; Long et al., 2004). However “this CO_2 fertilization effect” has been mostly demonstrated under optimum environmental conditions with sufficient nutrients and the knowledge on combined effects of e- CO_2 and mineral nutrient deficiencies is scarce and mostly focusing on P and N.

K is an essential nutrient with vital roles in survival and productivity of plants. It has very important roles in establishing cell turgor and maintaining cell ionic balance, activation of many enzymes including ones involved in photosynthesis, phloem and xylem transport, and biosynthesis of proteins, lipids and ascorbate (Marschner, 2012; Cakmak, 2005; Maathuis, 2009; Hafsi et al., 2014). Under severe K deficiency organelles such as chloroplasts and mitochondria are damaged, because K is essential for efficient operation of electron transfer chain in these organelles (Mengel, 2007). ROS produced due to insufficient electron transfer capacity especially under high light play a major role in occurrence of damage and cause disruptions in photosynthetic metabolism (Cakmak, 2005).

ROS are by products of metabolism and are produced in steady state in every aerobic organism. However, under environmental fluctuations including nutrient deficiency, due to loss of balance in different metabolic processes an increase in production of ROS is frequently observed (Mittler, 2002; Suzuki et al., 2012). Previous studies provide evidence for modulation of antioxidant defense and cellular redox status under K deficiency (Tewari et al., 2004, 2007; Hafsi et al., 2011). In these works, it has been shown that activity of superoxide dismutase (SOD), key enzyme in detoxification of $O_2^{\cdot-}$, is induced by K deficiency. Moreover, these works demonstrate that other components of antioxidant defense such as catalase (CAT), peroxidase (POX) and ascorbate peroxidase (APX), which take role in scavenging of H_2O_2 , are also induced by K deficiency. In addition to enzymatic antioxidants, contents of non-enzymatic antioxidants such as ascorbate and glutathione and activities of enzymes related to these molecules such as glutathione reductase (GR), monodehydroascorbate reductase (MDHAR) and dehydroascorbate reductase (DHAR) are also increased by K deficiency to support antioxidant defense (Tewari et al., 2007; Hafsi et al., 2011). These works are focused on sole effect of K deficiency on oxidative damage and antioxidant defense, however, regulation of antioxidant defense remains to be elucidated in plants grown in e- CO_2 environments and at the same time deficiency of K.

The aim of this work was to test hypothesis whether detrimental effects of K deficiency on plant growth would be worse with e- CO_2 and to elucidate how antioxidative defense systems respond to K deficiency under a- CO_2 and e- CO_2 conditions. Considering its role in human and animal nutrition as a staple food crop as well as the inhibitory effect of e- CO_2 on photorespiration of C_3 plants, two different wheat species *Triticum durum* cv. Sariçanak-98 and *Triticum aestivum* cv. Adana-99 were chosen for this investigation as the biological model. Plants were grown under a- CO_2 (400 ppm) and e- CO_2 (900 ppm) concentrations with adequate, low and deficient levels of K in nutrient solution. After twenty days of growth, changes in biomass production, nutrient concentration (K, Cu, Zn, Fe, Mn) and antioxidative responses (activities of SOD, CAT, POX, APX, GR, MDHAR, DHAR, lipid peroxidation and total antioxidant activity) were determined to address the effects of elevated carbon dioxide (e- CO_2 : 900 ppm) in combination with K deficiency on plant growth and antioxidant defense.

2. Materials and methods

2.1. Growth conditions and treatments

Durum wheat (*Triticum durum* cv. Sariçanak-98) and bread wheat (*Triticum aestivum* cv. Adana-99) plants were grown in aerated nutrient solution with adequate (1850 μM), low (60 μM) and deficient (20 μM) levels of K and under ambient (a- CO_2 : 400 ppm) and elevated (e- CO_2 : 900 ppm) CO_2 conditions in controlled plant growth chambers (light intensity: 450 $\mu mol m^{-2} sec^{-1}$; day/night period: 16/8 h; temperature: 24/20 °C, relative humidity: 65/75%). The low and deficient K levels were chosen by preliminary experiments to provide a dose-dependent and significant reduction in biomass production whereas the adequate K level ensured sufficient K supply for optimum plant growth without symptoms of K deficiency (necrosis and chlorosis of the oldest leaf), but with a sufficient level of shoot K concentration reported for wheat at early vegetative stage (Reuter and Robinson, 1997).

Seeds were germinated in perlite moistened with saturated calcium sulfate solution. Following germination (for five days) seedlings were transferred to 2.7L hydroponics culture pots. Except for the K treatments mentioned above, all plants received the following basal nutrient solution composition: 1000 μM $MgSO_4$, 4000 μM $Ca(NO_3)_2$, 1 μM $ZnSO_4$, 1 μM $MnSO_4$, 1 μM H_3BO_3 , 0.2 μM $CuSO_4$, 0.01 μM $(NH_4)_6Mo_7O_{24}$, 0.01 μM $NiCl_2$ and 100 μM Fe-EDTA. Adequate K plants received 750 μM K_2SO_4 , 100 μM KCl and 250 μM KH_2PO_4 , low K plants received 30 μM K_2SO_4 , 50 μM $CaCl_2$ and 125 μM $Ca(H_2PO_4)_2$, 215 μM $CaSO_4$ and deficient K plants received 10 μM K_2SO_4 , 50 μM $CaCl_2$, 125 μM $Ca(H_2PO_4)_2$ and 215 μM $CaSO_4$.

Different concentrations of K_2SO_4 , KCl and KH_2PO_4 were used for creating K deficiency. Adequate concentrations of $CaCl_2$, $Ca(H_2PO_4)_2$ and $CaSO_4$ was supplied for K deficiency plants for complementing Cl^- , PO_4 and SO_4 . The nutrient solutions were replenished every three days to compensate plant uptake. Plants were harvested 15 days after transfer to nutrient solution as shoot and root samples.

2.2. Shoot and root biomass production and analysis of nutrients

Shoot and root biomass production of plants were determined following drying of samples at 80 °C until a constant weight. Shoot concentrations of K, Cu, Zn, Fe, Mn were determined by inductively coupled plasma optical emission spectrometry (Vista-Pro Axial, Varian Pty Ltd, Mulgrave, Australia) following acid digestion in a closed vessel microwave system (MARSXpress, CEM Corporation).

2.3. Lipid peroxidation

Lipid peroxidation levels (determined as thiobarbituric acid reactive substances (TBARS)) were measured in a bulk of first and second oldest leaves according to Heath and Packer (1968).

2.4. Total antioxidant activity

Total antioxidant activity levels were measured from third and fourth oldest leaves together, according to Prieto et al. (1999), based on reduction of molybdenum. Following extraction with absolute ethanol, 3 mL of reaction solution containing 0.6 M H_2SO_4 , 28 mM Na-phosphate and 4 mM ammonium molybdate was added to samples. After incubation for 90 min in 95 °C water bath, samples were cooled down to room temperature. Then, absorbance of samples was read at 695 nm against L-ascorbic acid.

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