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Stomatal aperture rather than nitrogen nutrition determined water use efficiency of tomato plants under nitrogen fertigation

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ABSTRACT

Fertigation can improve water use efficiency (WUE) compared with conventional separate supply of water and fertilizers to plants. Yet the mechanisms underlying the improved WUE under fertigation remain largely elusive. Therefore, the impact of water and nitrogen (N) on leaf gas exchange, plant water relations, ABA signaling and WUE as well as leaf $\delta^{13}C$ and $\delta^{18}O$ were investigated in order to unravel how water and N modulate plant WUE. Results showed that reduced soil water regimes under N fertigation caused partial closure of stomata via decreased plant water status and intensified root-to-shoot ABA signaling, resulting in improved intrinsic WUE (WUE_i). Decreased soil water regimes increased plant WUE (WUE_p) and leaf $\delta^{13}C$, and the increased leaf $\delta^{13}C$ was due to reduced g_s and/or higher specific leaf N content enhanced photosynthetic capacity. Leaf $\delta^{18}O$ and $\delta^{13}C$ further indicated that the significant increase in leaf $\delta^{13}C$ under the reduced water regimes was caused primarily by reductions in g_s compared with N nutrition. Therefore, g_s rather than N nutrition predominated regulation of plant WUE under fertigation. Moderate soil water regimes with sufficient N supply are recommended for fertigation in terms of achieving high fresh fruit yield, WUE and nutrient uptake.

1. Introduction

Water and nitrogen (N) are the two most limiting factors for plant production globally. In order to increase yields, large amounts of fertilizers, especially N fertilizer, are applied to the soil each year. However, this leads to a dramatic reduction in N use efficiency (Tilman et al., 2002). Meanwhile, the arable land areas under irrigation are continuously increasing. This deteriorates the environment due to loss of N into water resources (Sebilo et al., 2013). To cope with these challenges, efficient management strategies are crucial for increasing water and N use efficiency towards agricultural sustainability (Quemada and Gabriel, 2016).

Fertigation has been widely used in crop production in many regions of the world. Compared with the supply of water and nutrients separately during conventional irrigation and fertilizer application, fertigation delivers water and nutrients simultaneously through irrigation systems to the vicinity of active roots, and this can facilitate water uptake and enhance the bioavailability of soil nutrients. Moreover, fertigation enables multiple applications of nutrients with required dosages during specific growing seasons in coordination with plant demand for more precise water and nutrient management (Bar-Yosef, 1999; Hebbar et al., 2004; Alva et al., 2008; Qin et al., 2016; Zhou et al., 2017). Thus, plant growth, yield, water and nutrient use efficiency under fertigation can be improved compared with conventional fertilizer application (Locascio et al., 1997; Singandhupe et al., 2003; Mahajan and Singh, 2006; Bhat et al., 2007; Badr et al., 2010). The advantages of fertigation over traditional fertilization methods also include uniformity of nutrient application, less nutrient loss through seepage or runoff, a reduction of soil compaction and mechanical damages to growing plants (Rapadopoulos, 1988; Asadi et al., 2002; Bryla and Machado, 2011).

The N effect under fertigation has received considerable attention. Klein et al. (1989) found that N concentration in the apple leaves was decreased significantly under low N fertigation at 50 kg ha^{-1} compared to other increased N treatments. Asadi et al. (2002) reported that N treatments at 150 and 200 kg ha^{-1} resulted in high yield of corn compared to low N at 100 kg ha^{-1} or no N treatment under N fertigation. Castellanos et al. (2013) observed highest yield and water use

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efficiency (WUE) of melon plants under N fertigation at 160 kg ha^{-1} compared with other varied N rates. Zhang et al. (2017) noted that the low N treatment during N fertigation at 110 kg ha^{-1} reduced the yield and WUE of winter wheat significantly, and they speculated that the yield differences were due to the significant reduction of photosynthetic rate (A_n) and stomatal conductance (g_s), however, the medium N treatment at 190 kg N ha^{-1} had similar yield and WUE as the high N treatment at 290 kg N ha^{-1}. Sinha et al. (2017); Mali et al. (2017) and Jayakumar et al. (2017) showed that high amount of fertilizers during fertigation increased the yield and WUE. However, the concomitant impact of water and N on WUE under fertigation in the aforementioned studies was not further investigated.

Plants exert some control over water loss from leaves via the narrowing of stomatal apertures, which is commonly observed in plants grown in drying soils. Such stress response is initiated and regulated by chemical and hydraulic signals. Root–derived abscisic acid (ABA), which is transported through the transpiration stream to the shoots, acts as early signals of soil drying (Blackman and Davies, 1985; Zhang and Davis, 1990; Tardieu et al., 1996; Sauter et al., 2001; Bahrun et al., 2002; Dodd et al., 1996; Liu et al., 2005; Dodd, 2005; Schachtman and Goodger, 2008). Hydraulic signals are produced when shoot water status decreases as a consequence of limited water uptake by roots (Chazen and Neumann, 1994; Comstock and Mencuccini, 1998). Water deficit induced root-to-shoot ABA signaling and decreased plant water status cause reductions in leaf expansion growth and stomatal opening (Gowing et al., 1990; Zhang and Davis, 1990; Davies and Zhang, 1991; Davies et al., 1994, 2002; Hartung et al., 2002; Wilkinson and Davies, 2002; Comstock, 2002), thereby WUE of plants is improved. In addition, previous studies showed that ABA may correlate with nitrogen in several plant species (Wang et al., 2010; Kiba et al., 2011 and literature cited therein).

Carbon isotopic composition ($\delta^{13}C$) provides a time-integrated measurement of plant WUE (Farquhar and Richards, 1984; Farquhar et al., 1989). There is a strong positive correlation between $\delta^{13}C$ and WUE in many crops including tomatoes (Martin and Thorstenson, 1988; Ellsworth et al., 2017). It is well established that the higher WUE and $\delta^{13}C$ are associated with a lower ratio between cell (C_i) and atmospheric (C_a) CO_2 concentrations (C_i/C_a). The decrease of C_i/C_a is due to the decrease of g_s or the enhancement of photosynthesis (A_n), or both (Condon et al., 2004). Oxygen isotopic composition ($\delta^{18}O$) reflects transpiration rates, as there is no further discrimination for the element during photosynthesis. After photosynthetic CO_2 assimilation, the $\delta^{18}O$ signal of water is transferred to plant tissues (Farquhar et al., 1998; Yakir, 1998; Barbour et al., 2000; Barbour, 2007). Increasing g_s results in less enrichment at the sites of evaporation within leaves caused by increased transpiration (Barbour, 2007). Therefore, $\delta^{18}O$ can aid our understanding of plant responses to water stress because the relative effect of g_s and photosynthetic capacity on changes in WUE can be separated (Farquhar et al., 1998; Barbour et al., 2000; Chaves et al., 2003).

Although the impact of N on plant growth, yield and N utilization under fertigation have been well documented, the mechanisms underlying the improved WUE and its modulation by water and N are still poorly understood. Therefore, the purpose of this study was to investigate the physiological responses of tomato plants subjected to different combinations of water and N treatment under fertigation and to explore the mechanisms regulating plant WUE. Specifically, we examined plant water relations, leaf gas exchange, ABA signaling, leaf $\delta^{13}C$ and $\delta^{18}O$, to determine the relations of these factors with plant WUE.

2. Materials and methods

2.1. Experimental setup

The experiment was conducted from April to July 2017 in a greenhouse with natural light and temperature-controlling equipment located at Institute of Environment and Sustainable Development in Agriculture, Chinese Academy of Agricultural Sciences (CAAS), Beijing, China. The photon flux density ranged from 450 to 800 μmol m^{-2} s^{-1}. The average day/night temperature was 27/22 °C during the experimental period. The soil was classified as sandy loam, having a pH of 7.6, total C of 9.0 g kg^{-1} and total N of 1.0 g kg^{-1}. The soil had a volumetric soil water content of 35% at pot water-holding capacity and of 11% at permanent wilting point. The pots used were 10.6 L (27.5 cm in diameter at the top edge, 20 cm in diameter at the bottom, 24 cm in depth). Before filling the pots, the soil was sieved passing through a 5 mm mesh. The pots were filled with 12.8 kg of air dried soil with a bulk density of 1.20 g dry soil cm^{-3}. The bottoms of the pots were perforated with small holes which allow free drainage. During the treatment period, there was no leaching from the pots. At the fifth leaf stage, tomato (*Lycopersicon esculentum* L., var. Qianxi) seedlings were transplanted into the pots. The soil water contents in the pots were monitored by a time domain reflectometer (TDR, MINITRASE, Soil Moisture Equipment Corp., CA, USA) with 17 cm probes installed in the middle area of the pots.

2.2. Fertigation treatments

Fertigation treatments comprised three levels of soil moisture and three N levels. The soil moisture levels consisted of 90%, 70% and 50% of soil water holding capacity, representing sufficiently, moderately and severely water-stressed conditions, which were denoted as WH, WM and WL, respectively. The N levels included low N (NL, 1.0 g N pot^{-1}), medium N (NM, 2.0 g N pot^{-1}) and high N (NH, 3.0 g N pot^{-1}). The experiment was a complete randomized design with four replicates in each treatment, and this yielded 36 pots in total. For N fertigation, 20% of the total applied N was used as basal fertilizer, which was mixed thoroughly with the soil before filling the pots. The remaining (80% of the total applied N) was delivered with irrigation water by fertigation in ten equal splits every five days on 0, 6, 11, 16, 21, 26, 31, 36, 41 and 46 DAT during the treatment period. Water was applied at 16:00 every day during the treatment period. NH_4NO_3 and $^{15}NH_4{}^{15}NO_3$ with 99% excess ^{15}N at 5% enrichment were used as N fertilizers. For all the pots, chemical fertilizers of P and K both at rate of 3.0 g pot^{-1} as KH_2PO_4 and K_2SO_4 were mixed into the soil to meet the macronutrient requirement for plant growth. Plant water use during the experimental period was calculated based on the amount of irrigation, TDR soil moisture measurements and the soil volume concerned. The water used for the irrigation was tap water with negligible concentrations of nutrients. The tomato plants were well-watered in the first 11 days after transplanting, and then the fertigation treatments were initiated. The fertigation treatment lasted 60 days.

2.3. Sampling, measurements and analyses

Leaf gas exchange including photosynthetic rate (A_n) and stomatal conductance (g_s) were measured weekly during the treatment period. Measurements were conducted at 9:00-11:00 am on the upper-canopy fully expanded leaves at the light intensity (PAR) of 1200 μmol m^{-2} s^{-1} and CO_2 concentration of 400 ppm with Li-6400 Portable Photosynthesis System (Li-6400, Li-Cor Biosciences, NE, USA). Data obtained from leaf gas exchange measurements were used for the calculation of intrinsic water use efficiency (WUE_i) as A_n/g_s.

At fruit maturity, plants were harvested on 60 days after onset of the fertigation treatment (DAT). Plant samples were divided into leaves, stem and fruits. Leaf area was measured with a leaf area meter (model 3050 A, Li-Cor Biosciences, NB, USA). Root segments (about 0.5 g) were excised from the root system in each pot. The root segments were tapped to remove adhering soil particles, briefly blotted with absorbent paper, and immediately placed and sealed inside a plastic sample cup (< 10 s) for root water potential (Ψ_r) measurement. The upper-canopy