



# Reducing basal nitrogen rate to improve maize seedling growth, water and nitrogen use efficiencies under drought stress by optimizing root morphology and distribution

Yin Wang<sup>a</sup>, Xinyue Zhang<sup>a</sup>, Jian Chen<sup>a</sup>, Anji Chen<sup>a</sup>, Liying Wang<sup>a</sup>, Xiaoying Guo<sup>a</sup>, Yali Niu<sup>a</sup>, Shuoran Liu<sup>a</sup>, Guohua Mi<sup>b</sup>, Qiang Gao<sup>a,\*</sup>

<sup>a</sup> Key Laboratory of Sustainable Utilization of Soil Resources in the Commodity Grain Bases in Jilin Province/College of Resources and Environmental Sciences, Jilin Agricultural University, Changchun 130118, Jilin, China

<sup>b</sup> Center for Resources, Environment and Food Security, College of Resources and Environmental Science, China Agricultural University, Beijing 100193, China

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## ABSTRACT

Frequent spring droughts and nitrogen (N) fertilizer overuse limit rain-fed maize production in Northeast China. However, the interactions between water-stress and N rates on maize seedling growth and root development remain elusive. In this study, a pot experiment was conducted with maize grown under three N rates (N omission, low and high N rates, i.e., N0, LN and HN) and severe water-stress (SS), moderate water-stress (MS) and well-watered (WW) conditions for 2-week from 4-leaf stage. Dry biomass (DM), leaf rolling performance, water use efficiency (WUE), N fertilizer use efficiency (NUE), root morphology and distribution were evaluated. It was showed that soil water levels and N rates had significant individual and interactive effects on most measured parameters. The dual stress of drought and N deficiency severely limited maize growth and N uptake. Nitrogen fertilization improved maize growth, N uptake and WUE under water-stress. However, compared with HN treatment, LN treatment had less water consumption, higher leaf relative water content and less leaf rolling symptom both under the MS and SS conditions. The reason is that LN enhanced root growth and elongation with more fine roots, especially in deep soil. Notably, maize plants in MS-LN treatment had an optimal root distribution characterized by higher root length density ( $0.30 \text{ cm cm}^{-3}$ ), larger and deeper penetration scale throughout the soil layers, and thus showed fewer drought responses and obtained the highest WUE ( $4.1 \text{ g L}^{-1}$ ) and NUE (17.2%) among all the water-stress treatments. In contrast, HN limited root growth and extension in deep soil and in turn increased water and N depletion, resulting in more severe leaf rolling and lower WUE and NUE. Therefore, reducing basal N rate is recommended to optimize root growth, morphology and distribution at the seedling stage in rain-fed maize production, to enhance drought resistance and improve WUE and NUE.

## 1. Introduction

At the global scale, the food demand is predicted to double by 2050, due to the increasing rapidly population and great transitions in diet structure (Tilman et al., 2011). Maize (*Zea mays* L.) has overtaken wheat and rice and become the most important staple food crop in China and many other countries (FAO, 2017), playing an increasingly significant role in ensuring food security and socio-economic stability. The Northeast Plain (NEP) is the major maize production zone of China, almost 40% of the farmland is sown for maize cultivation, and

accounting for approximately 1/3 of the national total maize production. Most of the maize fields are under rain-fed cultivation in the NEP; therefore, seasonal drought stress has been the largest limitation to maize production in this region during recent decades, causing 33% maize yields loss and even with no harvests at maturity in the extreme drought years (Zhang, 2004; Zhang et al., 2011a; Yin et al., 2016).

In the NEP, maize yields are more sensitive to drought stress at the milky-mature stage than other growing stages (Guo et al., 2017). Nevertheless, the frequency of drought occurrence is highest at the seedling stage, which often limits maize emergence and seedling

*Abbreviations:* N, nitrogen; N0, N omission; LN, low N rate; HN, high N rates; SS, severe water-stress; MS, moderate water-stress; WW, well-watered; DM, dry biomass; WUE, water use efficiency; NUE, N fertilizer use efficiency; ET, evapotranspiration; PWC, plant water consumption; RWC, relative water content; RLD, root length density; RDMD, root DM density; SRL, specific root length; IW, irrigation water amount; SWh, soil water stored amount at harvest; R: S ratio, root: shoot ratio

\* Corresponding author.

E-mail address: [gyt9962@126.com](mailto:gyt9962@126.com) (Q. Gao).

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growth, and even inducing irreversible damage and final yield losses (Zhang et al., 2011a). Drought stress affects maize seedling growth via inducing various changes in morphology, physiology and chemistry in plants. In general, soil drought induced water and nutrient absorption reduction in root systems, leaf rolling and decreased leaf photosynthetic rate and stomatal conductance, further limits photosynthesis and assimilate translocation, and thus resulting in lower growth rate and plant dry biomass (DM) reduction (Banziger et al., 2000; Yin et al., 2016; Zhang et al., 2011b). In addition to drought stress, N fertilizer management is another important challenge in maize production of NEP. A single basal application of excessive N fertilizer has been increasingly favored by farmers in local maize production, due to the increasing labor shortages and inconvenient topdressings at later stage (Gao et al., 2012; Yin et al., 2014). However, overuse of N fertilizer at seedling stage may leads to excessive vegetative growth with a greater plant size and larger leaf area, thus increasing superfluous water and energy consumption and also inducing more N leaching and gaseous loss to the environment (Peng et al., 2012; Wang et al., 2014; Rudnick et al., 2017). In the context of global climate warming, the frequency of drought events will increase dramatically in most regions of the NEP because of increasing temperature and declining precipitation (Yin et al., 2016). Predictably, maize production will likely suffer more frequent and severe spring droughts in these regions, which may also have large impacts on plant growth and N utilization of maize seedling. Therefore, it is necessary to investigate appropriate water and N management strategies with adaption to drought stress at the seedling stage, to optimize maize vegetative growth and improve WUE and NUE.

Root system play a crucial role in plant growth and productivity, enabling plants to anchor themselves tightly in the soil substrate and to forage their environment for water and nutrients (Ristova and Busch, 2014). During the vegetative growth period, root development and architecture display considerable plasticity in response to the heterogeneous distribution of soil water and nutrients resources (Eapen et al., 2005; Yu et al., 2014), and also in turn have significant influences on water and nutrient acquisition and plant growth at late period (Hodge et al., 2009; Lynch, 1995). Many studies have found that more photosynthetic product is transported to root system both under drought and low N stress, aiming to improve root growth and enhance water and nutrient absorption via producing higher root length, more fine root and deeper root distribution in soil profile (Mu et al., 2015; Oikeh et al., 1999; Sharp and Davies, 1985; Trachsel et al., 2013). By conducting a controlled slight soil water deficit at the early growing period based on water saving irrigation technique, such as regulated deficit irrigation or alternate partial root-zone irrigation methods, maize seedling experiences stress training and enhances plant drought resistance, thus could be better adapted to later drought stress during the growing season, ultimately with more effective nutrient uptake, WUE and grain yield (Pandey et al., 2000; Kang et al., 2017; Wang et al., 2017). By optimizing N fertilizer management methods, maize seedling increase water and nutrient absorption via forming a strong and dense root system and deeper root distribution, and thereby improve plant growth and NUE, and also improve enhance plant resistance to drought stress (Muschiatti-Piana et al., 2018; Peng et al., 2012; Trachsel et al., 2013; Rudnick et al., 2017). Moreover, numerous studies have indicated the interactive effects of soil water and N management on maize growth, grain yield and N uptake at various stages (Bennett et al., 1989; Hokam et al., 2011; Wang et al., 2008; Yin et al., 2014). Significant water-N interactions on actual evapotranspiration (ET) and soil profile water extraction pattern were also observed in maize by Lenka et al. (2009). However, in several other studies (Chilundo et al., 2017; Gheysari et al., 2009; Rudnick et al., 2017; Wang et al., 2017), the interactions between irrigation and N management are not observed in maize growth and WUE, especially not in root morphology and distribution (Chilundo et al., 2017). As yet, it is less clear how the interaction between soil water and N management affects maize root growth and morphology in relation to plant drought resistance, soil water consumption, WUE and

NUE.

The objectives of this study are to investigate the interactive effects of soil water-stress and N rates on maize root morphology and distribution at the seedling stage, and explore the relationships between root morphology and drought resistance, WUE and NUE of maize plant. The results would provide important references for soil water-N management strategies in maize production of NEP and other rain-fed cropping regions.

## 2. Materials and methods

### 2.1. Experimental site and materials

The pot experiment was conducted from June to July 2016, under a large rain shelter with natural light and temperature conditions in an experimental greenhouse at the Key Laboratory of Sustainable Utilization of Soil Resources in the Commodity Grain Bases of Jilin Province, Jilin Agricultural University, Changchun, China (43°48'29"N, 125°24'50"E). This area has semi-humid continental monsoon climate and had average monthly temperatures of 16.8 °C in June and 22.1 °C in July during the experimental period, which were slightly higher than the long-term averages (1985–2015) of 15.6 °C in June and 20.9 °C in July.

The experimental soil type is black soil (i.e., mollisol, USDA soil taxonomy), with a clay loam texture, moderately high organic matter content and inherent fertility, has a pH of 6.6, total N of 1.7 g kg<sup>-1</sup>, Olsen-P of 13.8 mg kg<sup>-1</sup>, exchangeable K of 130.9 mg kg<sup>-1</sup>, soil organic matter content of 2.6 g kg<sup>-1</sup> and soil water content at field capacity of 21.4% (on the mass basis). The soil was naturally air-dried and sieved passing through 5 mm mesh before filling into experimental pots.

Maize plants (ZD958, a dominant hybrid in China) were grown in plastic pots (42 cm in diameter and 45 cm in height, Fig. 1) filled with 55 kg of air-dried soil per pot. The soil depth of each experimental pot was approximately 36 cm, which could fully meet the root system growth during the experimental period (Oikeh et al., 1999; Trachsel et al., 2013). Moreover, approximately 4-cm-thick clastic rocks were spread over the bottom of each pot with fine sand to prevent water-logging in the subsoil. In each pot, a maize seedling was planted in the middle, and three PVC tubes (2 cm in diameter) with holes were installed along the pot wall at equidistant intervals, to avoid surface soil hardening and to simulate the water status of the soil profile in field.

### 2.2. Experimental design and implementation

The experimental treatments included three N-fertilization rates and three soil water levels. This experimental plan yielded 9 treatments (i.e., 3 × 3), and each treatment was replicated four times, with a total of 36 pots.

The three N-fertilization rates included 0, 0.12 and 0.24 g N per kg dry soil, representing N-omission (N0), low N (LN) and high N (HN), respectively. In addition to the N supply, 0.15 g P<sub>2</sub>O<sub>5</sub>, 0.15 g K<sub>2</sub>O and 0.02 g ZnSO<sub>4</sub> per kg dry soil were applied to meet the maize nutrient requirement for successful vegetative growth. Nitrogen fertilizer was supplied as urea (46% N), and P and K were applied as triple superphosphate (46% P<sub>2</sub>O<sub>5</sub>) and muriate of potash (60% K<sub>2</sub>O), respectively. All the fertilizers were applied and mixed into the soil in powdered forms at the commencement of the experiment.

The three soil water levels were 30%, 50% and 70% of field capacity, representing severe water-stress (SS), moderate water-stress (MS) and well-watered (WW) conditions, respectively. Maize seeds were sown on 7 June 2016 with three sprouting seeds in each pot. One plant per pot at the middle of the pots was chosen for uniformity on 20 June at the V2 stage. The soil water regimes in all the pots were initiated on 29 June at the V4 stage and lasted 2 weeks to the V6-V7 stage. Before the soil water was controlled, the soil water regimes in all pots were

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