Europ. J. Agronomy 63 (2015) 55–61

Contents lists available at ScienceDirect

European Journal of Agronomy

journal homepage: www.elsevier.com/locate/eja

Genetic improvement of root growth increases maize yield via enhanced post-silking nitrogen uptake

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ARTICLE INFO

Article history:
Received 30 April 2014
Received in revised form
20 November 2014
Accepted 21 November 2014

Keywords:
Root length density
Root surface area
Post-silking N-uptake
Grain yield
Introgression line

ABSTRACT

Root breeding has been proposed as a key factor in the “second green revolution” for increasing crop yield and the efficient use of nutrient and water resources. However, few studies have demonstrated that the genetic improvement of root characteristics directly contributes to enhancing nutrient-use-efficiency in crops. In this study, we evaluated the contribution of root growth improvement to efficient nitrogen (N) acquisition and grain yield under two different N-levels in a 3-year field experiment. We used two near-isogenic maize testcrosses, T-213 (large-root) and T-Wu312 (small-root), derived from a backcross of a BC_4F_3 population from two parents (Ye478 and Wu312) with contrasting root size. We found that the root length density, root surface area, and dry weight at the silking stage were 9.6–19.5% higher in T-213 compared with the control T-Wu312. The root distribution pattern in the soil profile showed no significant differences between the two genotypes. The overall increase in root growth in T-213 enhanced post-silking N uptake, which increased grain yield by 17.3%. Correspondingly, soil nitrate concentrations in the >30 cm soil layer were reduced in T-213 under the high N treatment. These positive effects occurred under both adequate and inadequate N-supply and different weather conditions. Our study provides a successful case that increasing root size via genetic manipulation contributes directly to efficient N-uptake and higher yield.

1. Introduction

Nitrogen (N) fertilizer application has been a major driving force in crop production. However, nitrogen use efficiency (NUE) is estimated to be only 33% in cereals (Raun and Johnson, 1999). Increased NUE, (i.e., increased yield with less N input) has been an essential step in sustainable agriculture. Root architecture and size play a fundamental role in nutrient and water uptake (Mi et al., 2010; Lynch, 2013; White et al., 2013). For example, in the soil profile shallow roots are advantageous for phosphorus uptake (Lynch and Brown, 2008). Yet for N-uptake, deeper roots in the soil profile can reduce nitrate-leaching (Wiesler and Horst, 1993). Chun et al. (2005) found the root size was positively correlated to post-silking N-uptake. In a solution culture system, root growth was positively correlated to N uptake and above-ground biomass (Wang et al., 2004; Tian et al., 2006). However, the complex relationship between root and shoot growth suggests that the correlation between root growth and nutrient uptake may be explained by greater shoot-growth potential. Therefore, the direct contribution of improved root growth to enhanced nutrient-use-efficiency in maize remains unclear.

Molecular and genetic studies have indicated that root traits are controlled by multi-genes. Some quantitative trait loci (QTLs) controlling maize root growth relative to nutrient uptake have been identified (Tuberosa et al., 2003; Zhu et al., 2005; Ribaut et al., 2007), but the extent of genetic modification of root traits affecting nutrient acquisition in maize is unclear. Near-isogenic inbred lines (NILs) are a powerful tool for studying the function of certain plant traits (Harrison et al., 2004). We have developed a root specific NIL population using two parent types, Ye478 and Wu312 with large and small sized roots, respectively (Wang et al., 2004; Liu et al., 2011). In crossing the NILs with the same tester inbred line 178, a total of 220 testcrosses were obtained where testcross T-213 was identified as having a larger root size compared with T-Wu312, the cross between the recurrent parent Wu312 and 178. These two genotypes were used to evaluate the contribution of

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http://dx.doi.org/10.1016/j.eja.2014.11.009
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improved root growth with N acquisition, dry matter accumulation and yield formation at two N-levels under field conditions over a 3-year period.

2. Materials and methods

2.1. Maize genotypes

In China, the donor parent Ye478 with its large root system is a popular inbred line and is the parent of more than 50 commercial maize hybrids. The recurrent parent, Wu312 with its small root system was derived from an unknown inbred line (Wang et al., 2004; Tian et al., 2006). Using these two parents, a BC4F3 backcross population containing 220 lines was developed using the small-root Wu312 as the recurrent parent (Liu et al., 2011). Following this, 220 testcrosses were generated by crossing these lines with 178, a parent of the popular Chinese commercial hybrid ND108. In a preliminary selection experiment using a solution culture system, two of the testcrosses, T-213 (L-213 × 178) and T-Wu312 (Wu312 × 178), were found to have similar shoot dry weights but significantly different root sizes at the seedling stage. The total root length of T-213 was larger than T-Wu312 (Wu, 2011). The female parent of T-213, L213, had 14 chromosomal inserts from the donor parent Ye478 and was theoretically 85.4% isogenic to the recurrent parent (Wu312) based on the molecular marker test using 143 SSR markers (Cai et al., 2012). In these inserted chromosomal fragments, three QTLs associated with vertical root pulling resistance (Liu et al., 2011) and total nodal root length (Cai et al., 2012) were identified (Table 1S).

2.2. Experimental conditions and fertilization

The field experiments were conducted in 2009–2011 on typical Ustochrept soils at the Shangzhuang Experimental Station, China Agricultural University, Beijing (116°11′N, 40°8′E). At the beginning of the experiment the chemical characteristics of the first 30 cm of the soil profile were: total N 0.83 g kg^{-1}, Olsen-P 7.63 mg kg^{-1}, NH_4OAc-K 76.3 mg kg^{-1}, organic matter 11.5 g kg^{-1}, pH(H_2O) 8.0. The experimental fields were irrigated and plowed before sowing, with 135 kg P_2O_5 ha^{-1} (superphosphate [$Ca(H_2PO_4)_2{\cdot}H_2O$]), 75 kg K_2O ha^{-1} (potassium sulfate), 30 kg $ZnSO_4$ ha^{-1} applied as the base fertilizer. There were two N application levels, high N (HN) and low N (LN). In the HN treatment, a total of 240 kg N ha^{-1} was applied, with 90 kg N ha^{-1} as the main component and a side-dressing of 90 and 60 kg N ha^{-1} (V6 and V12 growth-stages, respectively). In the LN treatment, no N was applied except in the 2010 experiment where, 120 kg N ha^{-1} was applied as a base fertilizer.

The test-cross maize hybrid T-213 and T-Wu312 were sown on May 14, 2009, May 11, 2010, and May 10, 2011. The plots were over-sown with hand planters and then thinned at the seedling stage to 60,000 plant ha^{-1}. The experiment was a randomized block design with four replicates, with each plot 8.5 m long and 4.5 m wide. The distance between rows and plants was 50 cm and 33 cm, respectively. Plots were kept free of weeds, insects and disease. The weekly precipitation, mean daily temperature and sunshine hours are shown in Fig. 1S. The mean precipitation was 197, 308, and 530 mm (2009–2011, respectively). In the spring of 2009, irrigation was used to ensure normal plant growth because of a severe drought. No irrigation was applied in the other two years. In 2010, high temperatures occurred during the anthesis and silking stages with the highest recorded temperature around 34.5 °C. These adverse weather conditions may have had a severe negative impact on pollination and early grain development. In 2011, rain occurred at the silking and early grain filling stages.

2.3. Dry matter (DM) and N accumulation

At silking and physiological maturity, four consecutive plants per plot were cut at soil surface height and dried to a constant weight at 65 °C. Silking stage was determined when 50% of the ears in a plot attained silking. The physiological maturity stage was determined when the black layer was visible at the grain base in 50% of the ears. Dry samples were weighed and ground to powder and N concentration was determined using the semi-micro-Kjeldhal method.

2.4. Root sampling and measurements

After the shoots were sampled at the silking stage, two consecutive roots per plot were excavated within a soil volume of 50 cm × 33 cm × 60 cm (length, width, and depth, respectively). The soil columns were divided into six 10-cm deep layers. Roots were cleaned of soil using a banister brush. Debris and weeds were hand-sorted from the maize roots during washing. After washing, root samples were stored at −20 °C until analysis. The roots were floated in water in a transparent plastic tray (20 cm × 15 cm) and scanned with a scanner (Epson1600, India) which allows lightening from both sides (Cai et al., 2012; Chen et al., 2014). The entire root system of a plant was scanned. Large root samples that could not fit into the tray were divided in order to avoid overlapping during scanning. The scanned images were analyzed for total root length using WinRHIZO (version Pro 5.0, Canada). The roots were then dried and weighed. Root length densities (RLD) were calculated by dividing the root length by the volume of the soil core.

2.5. Measurement of soil nitrate nitrogen

At harvest, four soil samples per plot were taken using an auger. In 2009 and 2010, soil samples to a depth of 120 cm at 30 cm increments were taken. In 2011, soil samples were taken to a depth of 60 cm at 10 cm increments. Soil samples were extracted using 0.01 mol L^{-1} $CaCl_2$ and analyzed for $NO_3{}^-$–N using the continuous flow analysis (TRAACS 2000, Bran and Luebbe, Norderstedt, Germany).

2.6. Grain yield

At physiological maturity, two rows of plants were harvested to assess yield. The number of ears was recorded. The grain was dried and weighed. Grain yield was standardized to 14% moisture content.

2.7. Statistical analysis

All the data across genotypes and environments were pooled for analysis of variance using a GLM with three factors (genotype, N-level, and years) (SAS/STAT, SAS Institute, 1988). The differences were compared using the least significant difference (LSD) test. All figures were constructed using GraphPad Prism 5 (GraphPad Software Inc., 2007) and Microsoft Excel 2010(Microsoft Inc., 2010).

3. Results

3.1. Grain yield, harvest index, and biomass accumulation

There were significant differences in grain yield between genotypes, N levels, and years (Table 1). Across N treatments and years, the grain yield of T-213 increased by 17.3% compared with T-Wu312. The genotype × N, year × nitrogen, and genotype × N × year interaction had a significant effect on maize grain yield. The grain yield of T-213 was higher than that of T-Wu312