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REGULAR ARTICLE

Genotypic difference in the plasticity of root system architecture of field-grown maize in response to plant density

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Abstract
Aims To investigate genotypic differences in the plasticity of root system architecture in response to increasing planting density and understand how this plastic response affects grain yield.
Methods A two-year field study was conducted with eight maize hybrids and three planting densities (60,000, 75,000, and 90,000 plants per ha). High-throughput imaging system and an automatic analysis method with Root Estimator for Shovelomics Traits (REST) software were adopted to study root architecture. The coefficient of variation (CV) was determined to reflect the plastic response of the root traits at different planting densities.
Results Root size and root architecture varied with increasing plant density and among the genotypes. With increasing planting density, root biomass and root length per plant decreased. The average root opening angle in inter-row and intra-row directions (RA), average root maximal width in inter-row direction and intra-row directions (RMW), ratio of root opening angle between intra-row and inter-row directions (RatioRA), and ratio of root maximal width between intra-row and inter-row directions (RatioRMW) were also reduced. These results suggest that plants growing under high planting density have narrower root extension width, steeper root angle, and greater root distribution in inter-row direction. The CV of all the root traits between the neighboring plants increased with increasing plant density. Although significant genotype × planting density interactions occurred with most of the root traits, there was a quadratic correlation between grain yield and most of the root traits, especially at high planting density ($R^2 = 0.17 \sim 0.48$). There was a negative linear relationship between grain yield and the CV of root biomass, root length, RA, RMW, RatioRA, and RatioRMW ($R^2 = 0.21 \sim 0.37$). Among the eight hybrids, JQ202 had medium root size, more inter-row root distribution, and the smallest CV in root traits across three planting densities, and the highest grain yield. LY99, SR999 and DY39 had largest CV for root traits and the smallest grain yield.
Conclusions Genotypes with less variation in root size, medium root size, medium broad root system and more inter-row root distribution help to reduce root-to-root competition and tend to have higher yield at high planting density.

Keywords Genotypic plasticity · Inter-row · Intra-row · Root system architecture · Plant density · Maize

Abbreviations

D60000	60,000 plants per ha
D75000	75,000 plants per ha

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D90000	90,000 plants per ha
REST	Root Estimator for Shovelomics Traits
CV	Coefficient of variation
RA	Average root opening angle in inter-row and intra-row directions
RMW	Average root maximal width in inter-row and intra-row directions
RatioRA	Ratio of root opening angle between intra-row and inter-row directions
RatioRMW	Ratio of root maximal width between intra-row and inter-row directions.

Introduction

Root system architecture including root growth and spatial distribution plays a crucial part in soil resources acquisition, plant growth, and crop performance (deDorlodot et al. 2007; Lynch 1995; Rogers and Benfey 2015). Root biomass, root length and nodal root number collectively determine the root size. Root length plays an important role in the acquisition of water and nutrients (Asseng et al. 1998; Brück et al. 1992; Gahoonia and Nielsen 2004; Lynch and van Beem 1993; Wiesler and Horst 1994; Brady et al. 1995; Dunbabin et al. 2003a&b; Zhu and Lynch 2004; Liao et al. 2001). Nodal roots number, especially the roots grown from the aboveground node, are closely related to root lodging resistance (Stamp and Kiel 1992; Guigo and Herbert 1997; Liu et al. 2012). Root to shoot ratio indicates the percentage of root system for supporting shoot growth and yield formation (Anderson 1988). Root length density reflects the relative distribution of root length in the soil profile, which is closely related with the spatial availability of nutrients in the soil (Peng et al. 2012). The root angle and root maximal width determines the 3-dimensional root distribution in the soil (Colombi et al. 2015), therefore has an overall effect on nutrient and water uptake (Dathe et al. 2016; Dunbabin et al. 2003b; Hammer et al. 2009; Kato et al. 2006; Lynch 1995; Lynch and Brown 2001; Manschadi et al. 2006; Mi et al. 2010; Mi et al. 2016; Richardson et al. 2011; Singh et al. 2012; Zhu et al. 2005), as well as lodging resistance (Crook and Ennos 1994; Ennos et al. 1993; Guigo and Herbert 1997; Pinthus 1967).

Root growth is determined by endogenous genetic as well as environmental factors (Hébert et al. 1995; McCully 1995; McCully 1999; Rogers and Benfey 2015). Genotypic differences in axial roots number (Burton et al. 2013; Sanguineti et al. 1998), root length (Kumar et al. 2012; Manavalan et al. 2012; Tian et al. 2008), root surface area (Costa et al. 2002), root biomass (Kumar et al. 2012), nodal root angle (Chakravarty and Karmakar 1980; Jenison et al. 1981), root maximal width (Crook and Ennos 1994; Grift et al. 2011; Kato et al. 2006; Oyanagi et al. 1993; Singh et al. 2010; Kumar et al. 2012; Manavalan et al. 2012) and root nutrient uptake (Barber and Mackay 1986; Nielsen and Barber 1978) have been documented. Environmental factors, such as nitrogen availability (Chun et al. 2005; Gao et al. 2015; Gaudin et al. 2011; Tian et al. 2008; Wang et al. 2005), phosphorus supply (Hajabbasi and Schumacher 1994; Liu et al. 2004; Mollier and Pellerin 1999), water condition (Barber et al. 1988; Grzesiak et al. 1999) and soil temperature (Barber et al. 1988; Kaspar and Bland 1992; McMichael and Burke 1998) can also affect root growth.

Plant density tolerance in maize is defined as a cultivar's ability to maintain or improve yield performance with increasing planting density (Mansfield and Mumm 2014). Increasing the planting population per ha as well as enhancing individual grain yield performance are major approaches to increase grain yield per hectare in maize (Duvick 2005; Tokatlidis and Koutroubas 2004). Light interception per plant as well as the whole-plant photosynthesis is decreased with increasing planting density (Andrade et al. 1999; Edmeades and Daynard 1979; Tollenaar et al. 1992). The photosynthate allocation to roots is greatly reduced (Li et al. 2014; Poorter et al. 2016). Maize plants adjust the azimuth angle of leaves to adapt to supra-optimal plant density (Girardin 1992; Girardin and Tollenaar 1994; Maddonni et al. 2001; Maddonni et al. 2002). Breeders have tried to improve the aboveground traits, such as erectness of leaves, so that light interception per plant is improved and photosynthesis is enhanced (Buren et al. 1974; Carlone and Russell 1987; Chen et al. 2013; Fellner et al. 2006; Tollenaar and Lee 2002; Duvick 2005; Lee and Tollenaar 2007). However, less is known about how root traits should be improved for better adaptation to high planting density.

Root plasticity is the ability to exploit available resources by adjusting root growth and/or physiological activity (Huang and Eissenstat 2000). Root biomass, root length, and axial roots number are reduced under limited photosynthesis supply in maize (Chen et al. 2012b; Demotes-Mainard and Pellerin 1992; Hebert et al. 2001; Lambers and Posthumus 1980; Liu et al. 2012; Stamp and Kiel 1992; York et al. 2015). The smaller root system at high planting density may also contribute to less root-to-root competition between neighboring plants. Nevertheless, due to the variation of germination energy of seeds, soil condition (temperature, water availability etc.), as well as planting depth (Benjamin and Hardwick 1986; Nafziger et al. 1991), plant size inequality and non-uniform plant populations are observed. Based on size-asymmetric competition theory, larger plants usually have stronger competition ability than the smaller ones (Weiner et al. 2001). Competition between neighboring plants increased as plant density increased (Glenn and Daynard 1974; Maddonni and Otegui 2004; Rossini et al. 2011). Coefficient of variation (CV) is a parameter to quantify phenotypic variability and competitive ability of the individual plant (Edmeades and Daynard 1979). The CV of root size differs among genotypes (Costa et al. 2002), and variation is increased at high planting density (Bonan 1991; Edmeades and Daynard 1979; Tokatlidis et al. 2010; Tokatlidis and Koutroubas 2004). In principle, the CV of root traits can be taken as a parameter of root-to-root competition between neighboring plants. However, less is known about the change in CV of root traits with increasing plant density, how this change affects grain yield, and whether this change can be modified genetically. In the present study, a high-throughput imaging system with an automatic analysis method (REST software) (Trachsel et al. 2011; Lynch 2011), together with classic root scanning method and WinRHIZO software were adopted to answer the above questions.

Materials and methods

Experimental design

The study was conducted in Lishu (43°2′ N, 123°3′ E), Jilin Province in Northeast China in 2016 and 2017. The soil was a Black Soil, equivalent to typic Hapludoll in the USDA Soil Taxonomy system (Soil Survey Staff 1998). Chemical properties for the 0–20 cm soil layer in two years are shown in Table 1S. Precipitation and temperature during the growing seasons are shown in Fig. 1S. The amount of rainfall during the maize growing season was 655.4 mm and 478.4 mm in 2016 and 2017, respectively (Supplementary information Fig. 1S). In 2016, rainfall was relatively evenly distributed throughout the growing season, although little rainfall was recorded in early July. A severe spring drought occurred in 2017, 15.7 mm of water was applied by drip irrigation on 5 May to ensure seedling emergence.

The experiments were a split-plot design with three replicates (blocks), with hybrids as the main plots, planting density as sub-plots (Supplementary information Fig. 2S). According to grain yield performance with increasing planting densities in a pre-experiment, six maize hybrids were chosen in 2016, i.e. Xianyu335 (XY335), Nonghua101 (NH101), Liangyu99 (LY99), Shengrui999 (SR999), Liangyu918 (LY918) and Jinqing202 (JQ202). In 2017, two more hybrids, Zhengdan958 (ZD958) and Danyu39 (DY39) were added. JQ202, XY335, NH101 and ZD958 are more tolerant to high planting density compared to the others. The sub-plots were 25-m long, 7.2-m wide in 2016, and 20-m long, 7.2-m wide in 2017 (Supplementary information Fig. 2S). The pre-crop was rain-fed maize. Plots were plowed by subsoiling to a depth of 40 cm after harvesting to create a loose soil environment. According to the recommended soil nutrient management (Cui et al. 2008), 240 kg N ha^{-1}, 100 kg P_2O_5 ha^{-1} and 100 kg K_2O ha^{-1} were applied for all three plant density treatments to create a sufficient and equal nutrient environment. Considering the disturbance to the soil and root growth, no topdressing was applied. Instead, coated controlled release NPK compound fertilizers were used to satisfy the needs of plants during the whole growth period. The fertilizers were broadcasted before planting, and then the soil was rotary tilled to a depth of 12 cm.

Three planting densities were imposed, that is, 60,000, 75,000 and 90,000 plants ha^{-1}, which are abbreviated as D60000, D75000 and D90000, respectively. 75,000 plants per ha was an appropriate planting density for high-yield achievement locally, and therefore was treated as a control. 90,000 plants per ha was used to create a supra-optimal population. 60,000 plants per ha was intended to create a weak competition environment. The row widths were 0.6 m for all the three density treatments. The distance between plants within the row was 0.28 m (for D60000), 0.22 m (for D75000), and 0.18 m (for D90000), respectively. Maize was overseeded by hand on 30 April 2016, 1 May 2017, respectively. The plots were thinned to the designed planting density at V3 stage. Weeds in the plots were controlled using herbicides (atrazine and acetochlor). Pesticides were applied as needed to control insect populations.

Plant sampling and dry matter measurement

Because of the difference in phenological periods under three plant densities, at least 100 consecutive plants in the central rows of each plot were tagged at V4 in order to mark the physiological stage. The dates of VT were recorded when 60% of the tagged plants reached the stage (D'Andrea et al. 2008). At VT, fifteen successive plants from each plot were investigated. Stalks were cut exactly 25 cm above the soil surface to meet the requirements for root imaging. The inter-row direction and the intra-row direction were marked. The plants were separated into leaves, stalk (including the sheaths, tassel, husks, and either cobs at maturity or ear-shoot at silking) and grain. All plant samples were oven-dried at 105 °C for 30 min, and then dried at 70 °C to a constant weight, then weighed to obtain dry weight. Coefficient of variation (CV) of shoot biomass among 15 plants for each plot was calculated.

At maturity, two middle rows of plants (20 m in length × 1.2 m in width) per plot were harvested to measure grain yield per plot (Chen et al. 2014). Grain moisture was determined and grain yield was standardized to 14% moisture.

Root sampling and analysis

Method I: Root system architecture by Shovelomics

Root sampling Ninety percent of maize root growth occurs in the 0–35 cm topsoil (Dwyer et al. 1996; Peng et al. 2010). To analyze the three-dimensional distribution (vertical, within-row and inter-row direction) of the root system architecture, a modified monolith method (Böhm 1979) was applied (Fig. 1). After cutting off the shoots at silking stage, a soil volume centered around the plant root (distance between plants × distance between rows × depth) of 28 cm × 60 cm × 35 cm (D60000), or 22 cm × 60 cm × 35 cm (D75000), or 18 cm × 60 cm × 35 cm (D90000) for each root system was excavated using shovels. The roots of fifteen successive plants were taken in each plot (Fig. 1). The excavated root crowns were shaken briefly to remove a large fraction of the soil adhering to the root crown. Afterwards, root crowns were soaked into the water for 12 h, detergent was added to the water to help to separate roots from the remaining soil particles. Each root was put into a mesh bag and washed using a water gun (YLQ3721-90A, Shanghai Yili Electric Appliance Co., Ltd) at an appropriate pressure which had been tested beforehand to avoid damage. The roots were kept in water until further analysis.

Fig. 1 A monolith method for studying the 3-dimensional distribution of the root system. Root samples were taken from consecutive plants. A soil volume of 28 cm × 60 cm × 60 cm centering plant root at D60000

Imaging tent and camera information The imaging tent was constructed using aluminum bars connected with plastic joints and covered with a black fabric, which is made of reflective fabric inside (DEEP, Shanghai Meinuo Photographic Equipment Co., Ltd) (Fig. 2). The dimensions of the tent are 80 × 80 × 80 cm. A twenty-four mega-pixel digital camera (Sony ILCE-5100 L, Sony, Tokyo, Japan) was placed on the top center of the tent at 80 cm above the ground and a 25 mm fixed focal length was set. The imaging plane was at the bottom of the tent. Root crowns was placed on the imaging plane facing the camera which included: the root crown was first fixed to capture a picture of the intra-row direction, then the root crown was 90° overturned in the clockwise direction to capture another picture of the inter-row direction. Two brightness-adjustable LED flashlights (DEEP, Shanghai Meinuo Photographic Equipment Co., Ltd., Shanghai, China) illuminated the root systems from both sides to create an optimal environment. A label was placed in the field of view of the camera for sample identification. A 20 mm fixed diameter marker (Feiyue model design Co., Ltd., Taian, China) was also placed in the field of view of the camera for calculating the dimension of picture later. The whole set up was controlled by a smart mobile phone through the PlayMemories Mobile software (PlayMemories Mobile software, Sony, Tokyo, Japan). The picture size at the focal plane was 51.7 × 52.4 cm resulting in a pixel size of 0.13 mm. An aperture value of 5.0 and an exposure time of 1/60 s allowed for optimal image quality with minimized background noise. Uniform and fixed illumination settings were done during

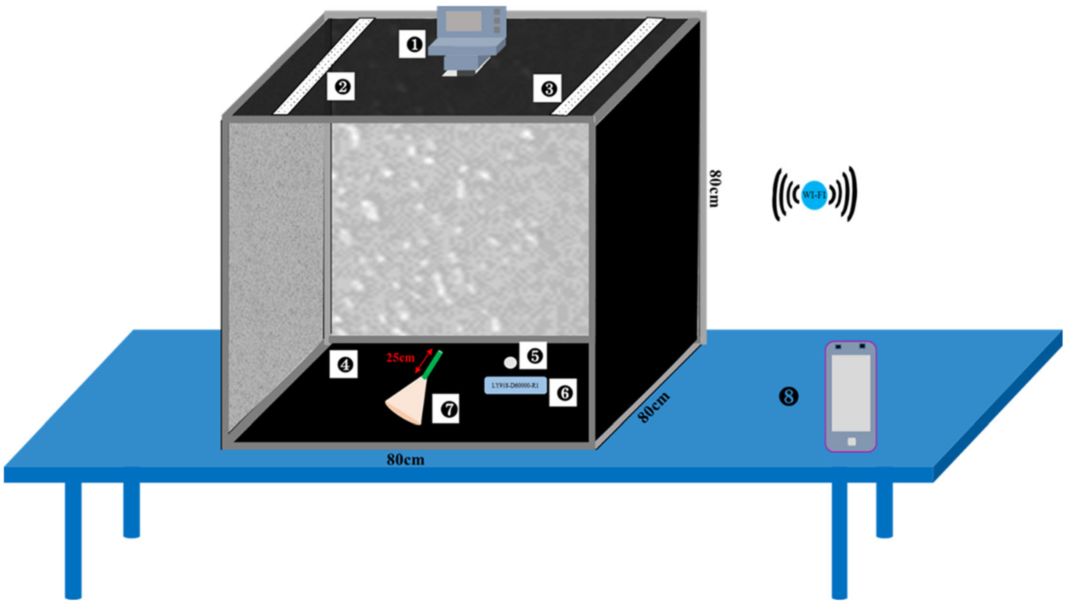

Fig. 2 The system for taking root images. Root imaging tent is cuboid-shape (80 × 80 × 80 cm) with a black fabric outside which made of reflective fabric inside. Part (1): Digital camera; Part (2) and Part (3): Two brightness-adjustable LED flashlights; Part (4): Imaging plane with black fabric; Part (5): A 20 mm disk marker in diameter; Part (6): Label for sample identification; Part (7): Root crown with 25-cm stalk; Part (8): Smart mobile phone connected to digital camera through PlayMemories Mobile software

the entire process to optimize image quality. Finally, images were recorded and stored as JPEG files (Fig. 3a).

Image processing with REST Before processing the images with REST software, images were renamed at first according to the labels. Then Adobe Photoshop CS6 software (Adobe Systems Incorporated, California, USA) was used to crop off the label part. The size of each cropped image was calculated according to the size of marker.

Cropped images were analyzed using Root Estimator for Shovelomics Traits (REST, version 1.0.1) software (Institute of Agricultural Science, ETH Zurich, Switzerland) in MatLab 7.12 (The Mathworks, Natick Massachusetts, United States) (Colombi et al. 2015). Major steps were as follows: parameters such as width of image and the related options were typed into the panel and soil surface was marked manually according to the user's

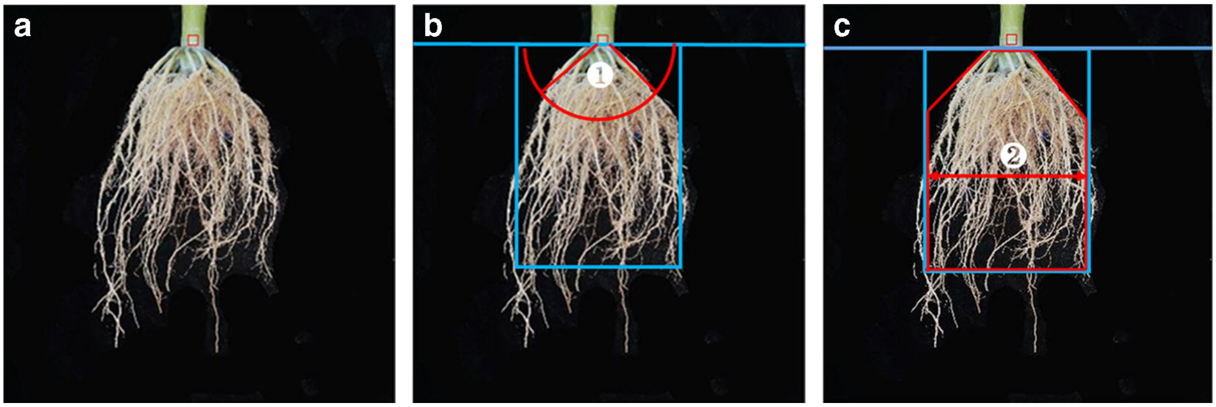

Fig. 3 Image processing with Root Estimator for Shovelomics Traits (REST). a, a root image; b, 90% of the region of interest (root system after correction for outstanding roots, the blue box); a 10 cm arc in diameter was virtually drawn from the middle of the stalk, root opening angle (①) was defined as the angle between left and right outermost root; c, a red convex hull was drawn, and root maximal width (②) was determined by the maximal width of convex hull

manual for REST. Results included root opening angle, and root maximal width.

In order to reduce errors due to single roots standing out of the bulk root stock, 5% of root pixels from the lower edge were discarded on the horizontal axis, and 2.5% of root pixels on each side were discarded on the vertical axis. The following analysis was based on the images comprising 90% of pixels classified as roots (Fig. 3, the blue box). A 10 cm arc in diameter was virtually drawn from the middle of the stalk when software was running. The angle between left and right outermost root was defined as root opening angle (Fig. 3b, ①). A convex hull was drawn when software was running, and root maximal width (Fig. 3c, ②) was determined by the maximal width of the convex hull. Detailed instructions could be obtained from user's manual for Root Estimator for Shovelomics Traits (REST, version 1.0.1).

After imaging, the number of the primary root, seminal roots and each whorl of nodal roots was counted and then the root samples were dried at 70 °C to obtain dry weight. Coefficient of variation (CV) of root biomass among 15 plants for each plot was calculated.

Method II: Root morphological traits by WinRhizo

To analyze root morphological traits, at VT, a soil volume centered around maize roots (distance between plants × distance between rows × depth) of 28 cm × 60 cm × 35 cm (D60000), or 22 cm × 60 cm × 35 cm (D75000), or 18 cm × 60 cm × 35 cm (D90000) for each root system was excavated using shovels. The roots of ten successive plants were taken in each plot. Root clumps were soaked in water for 12 h, detergent was added to the water to help to separate roots from soil particles. Then the roots were washed under low pressure with a water gun (YLQ3721-90A, Shanghai Yili Electric Appliance Co., Ltd). A garden pruner was used to cut off the primary root, seminal roots and each whorl of nodal roots. The number of roots was counted and then the roots were frozen until further analysis.

For analyzing root morphological traits, each axial root was scanned (Epson V700, Indonesia) at 600 dots per inch resolution. While scanning, the root sample was placed in a glass rectangular dish (200 mm × 150 mm) with a layer of water about 4- to 5- mm deep to untangle and minimize root overlap. When necessary, the roots were separated into subsamples to fit the rectangular dish. Images were recorded and stored as JPEG files. The scanned images were analyzed using the software WinRHIZO 5.0 (Regent Instruments Ins., Quebec City, Canada) to obtain the root length (Shao et al. 2018). Root length density was obtained by dividing root length by the soil volume. After scanning, the roots were dried at 70 °C to obtain dry weight.

Statistical analysis

Analysis of variance was performed using the General Linear Model (GLM) procedure in SPSS 18.0 (SPSS Inc., Chicago, Illinois, USA). The following statistical model is used:

$$Y = \mu + \text{Block} + \text{Hybrid} + \text{Block} \times \text{Hybrid} + \text{Density} + \text{Hybrid} \times \text{Density} + \text{Block} \times \text{Hybrid} \times \text{Density}$$

With Y is the response variable, u is the overall mean. Block, Hybrid and Density indicates the fixed effect of block, main-plot, and sub-plot, respectively. Block × Hybrid indicates the main-plot error. Density × Hybrid means the interaction between hybrid and density treatment. Block × Hybrid × Density indicates the sub-plot error. Differences were compared using the Tukey HSD test at the 0.05 and 0.01 levels of probability. The coefficients of determination (R^2) for the relationships between root traits and grain yield, CV of root traits and grain yield, CV of root biomass and CV of shoot biomass at VT, CV of root biomass and CV of harvest index were calculated using SPSS 18.0 (SPSS Inc., Chicago, Illinois, USA), and the model with the highest R^2 was selected. Fig. 1S was constructed using Microsoft Excel (Microsoft Corporation, Redmond, Washington, USA). Figures 4, 5, 6, 7, 8 and 9, and Figs. 3S to 5S were constructed using GraphPad Prism 5 (GraphPadSoftware Inc., San Diego, California, USA).

Results

Genotypic differences in grain yield at different planting densities

Genotypic differences, as well as the interaction of hybrid × density were found to have a significant impact on grain yield (Tables 1 and 2). Grain yield was the same at D60000 and D75000, and decreased at D90000 in 2016. In 2017, grain yield was reduced with increased planting density, possibly due to the dry weather (Fig. 4).

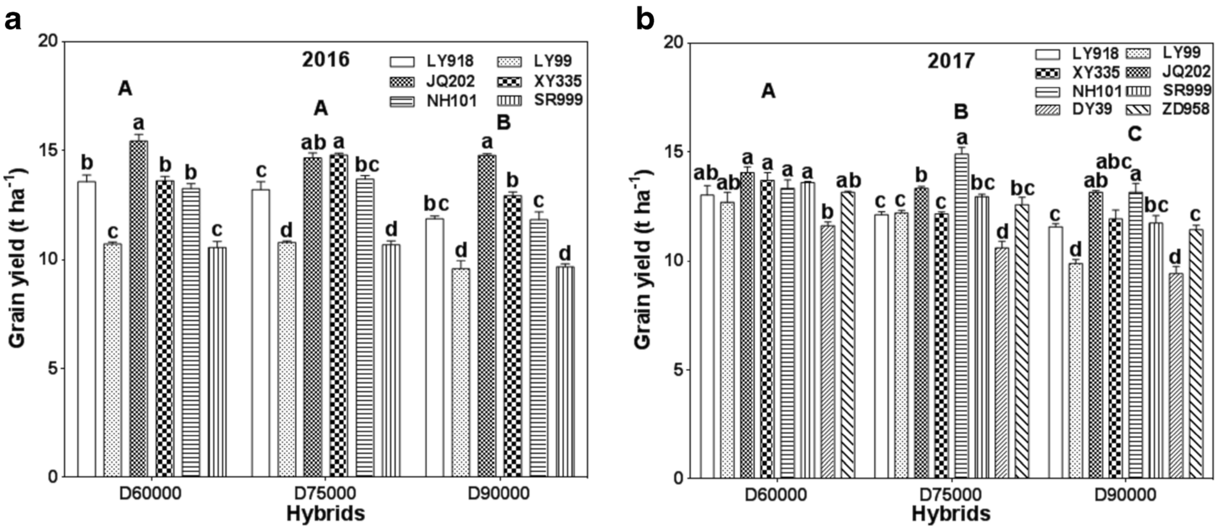

Fig. 4 Genotypic differences in grain yield at different plant densities for two years. Bars denote the SE of the mean ($n = 3$). Different lowercase letters represent significant differences among hybrids under the same planting density ($P < 0.05$). Different capital letters represent significant differences among planting densities across all the hybrids ($P < 0.05$). Grain yield was standardized to 14% moisture

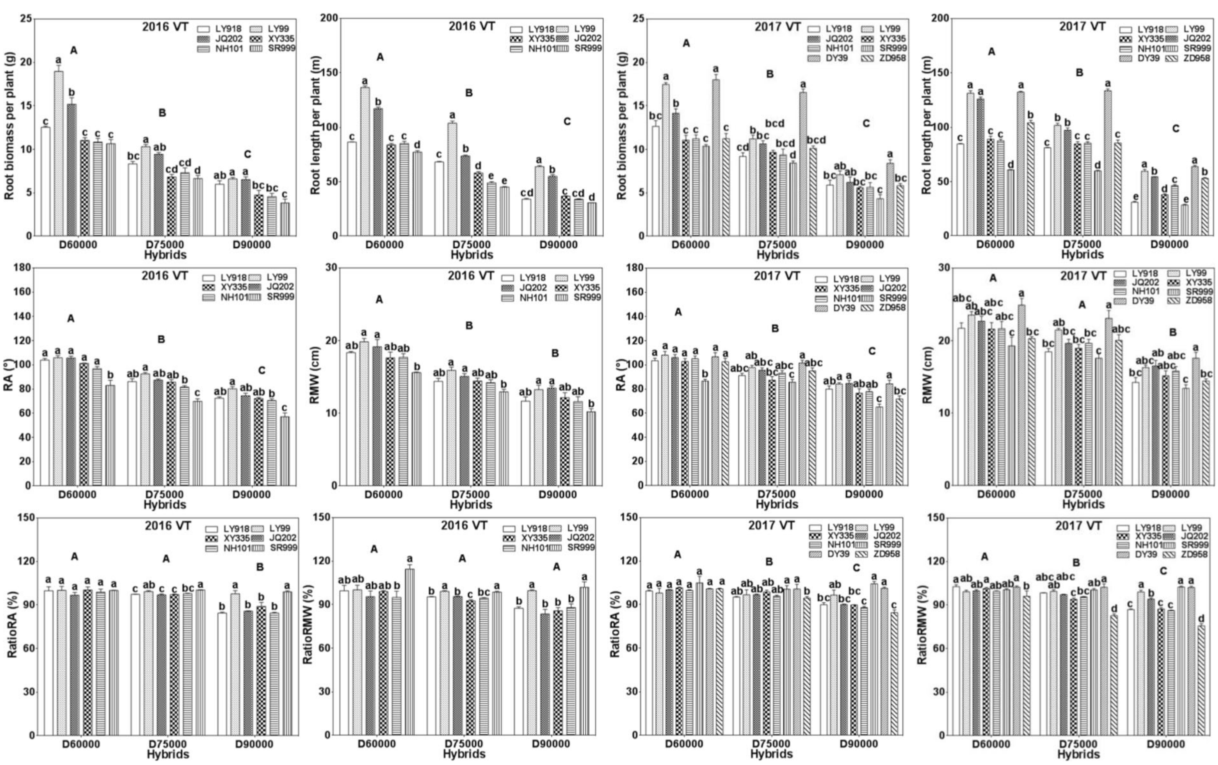

Fig. 5 Genotypic differences in Root biomass, Root length per plant, Root angle (RA), Root maximal width (RMW), RatioRA, and RatioRMW at three plant densities for two experimental years. Bars denote the SE of the mean ($n = 3$). Different lowercase letters represent significant differences among hybrids under the same planting density ($P < 0.05$). Different capital letters represent significant differences among planting densities across all the hybrids ($P < 0.05$)

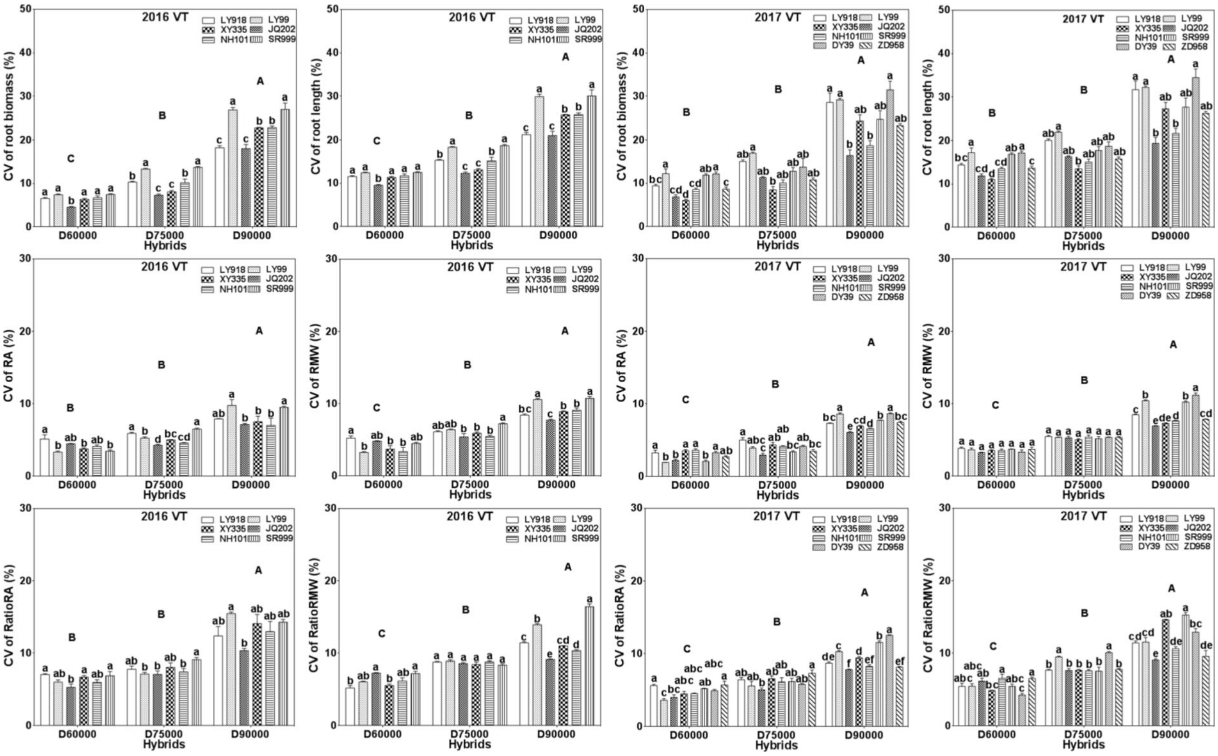

Fig. 6 Genotypic differences in the CV of root biomass, CV of root length, CV of root angle, CV of root maximal width, CV of RatioRA, and CV of RatioRMW at three plant densities for two experimental years. Bars denote the SE of the mean (n = 3). Different lowercase letters represent significant differences among hybrids under the same planting density (P < 0.05). Different capital letters represent significant differences among planting densities across all the hybrids (P < 0.05)

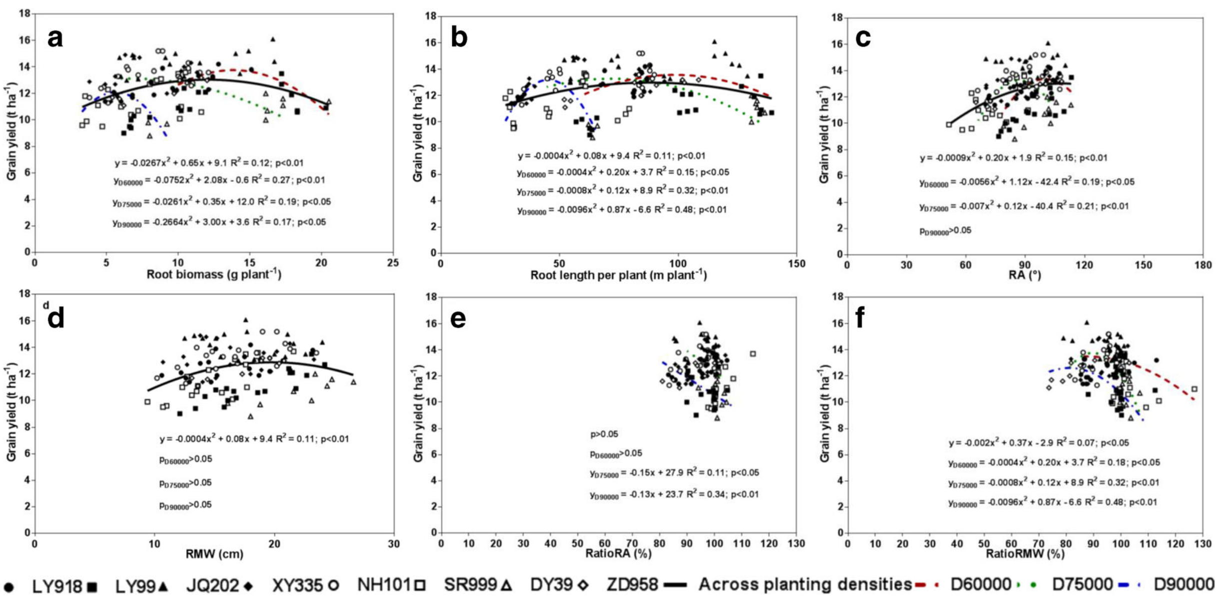

Fig. 7 Correlation between grain yield and Root biomass (a), Root length per plant (b), Root angle (c), Root maximal width (d), RatioRA (e), RatioRMW (f) under different planting densities. Correlation analysis was conducted across planting densities and at each planting density. Data of 2016 and 2017 were pooled for correlation analysis. Each point represents the value of each repetition of different treatment

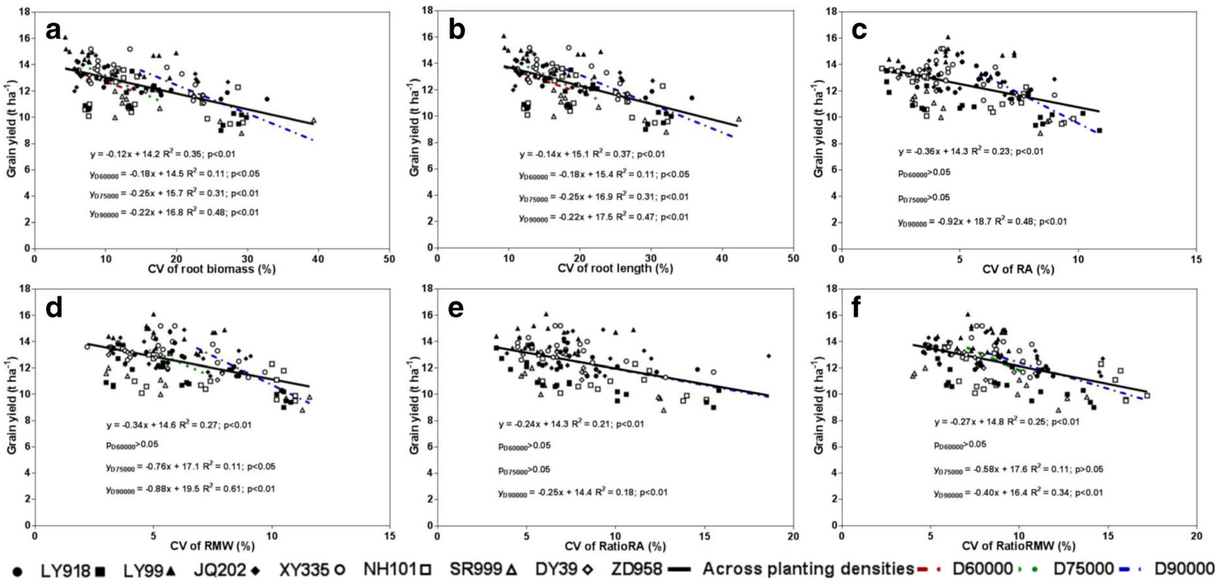

Fig. 8 Correlation between grain yield and CV of root biomass (a), CV of root length (b), CV of root angle (c), CV of root maximal width (d), CV of RatioRA (e), CV of RatioRMW (f) under different planting densities. Correlation analysis was conducted across planting densities and at each planting density. Data of 2016 and 2017 were pooled for correlation analysis. Each point represents the value of each repetition of different treatment

In 2016, across three planting densities, JQ202 produced highest grain yield, followed by XY335, LY918 and NH101. LY99 and SR999 had lowest grain yield. XY335 had highest grain yield under D75000. Compared to D75000, grain yield of LY918, LY99, XY335, NH101 and SR999 was reduced by 10, 11, 13, 14 and 10%, respectively, at D90000, with no effect on grain yield of JQ202.

In 2017, JQ202 produced highest grain yield at D60000, followed by XY335, SR999, NH101, ZD958 and LY918. LY99 and DY39 had lowest grain yield. At D75000, NH101 had highest grain yield, followed by JQ202 and SQ999. ZD958, LY99, XY335 and LY918 had lower grain yield, and DY39 had lowest grain yield. At D90000, NH101 and JQ202 had highest grain yield, followed by XY335, SR999, ZD958 and LY918. LY99 and DY39 had lowest grain yield. At D90000, compared to D75000, grain yield of LY918, LY99, JQ202, XY335, NH101, SR999, DY39 and ZD958 decreased by 5, 19, 1, 2, 12, 9, 11 and 9, respectively.

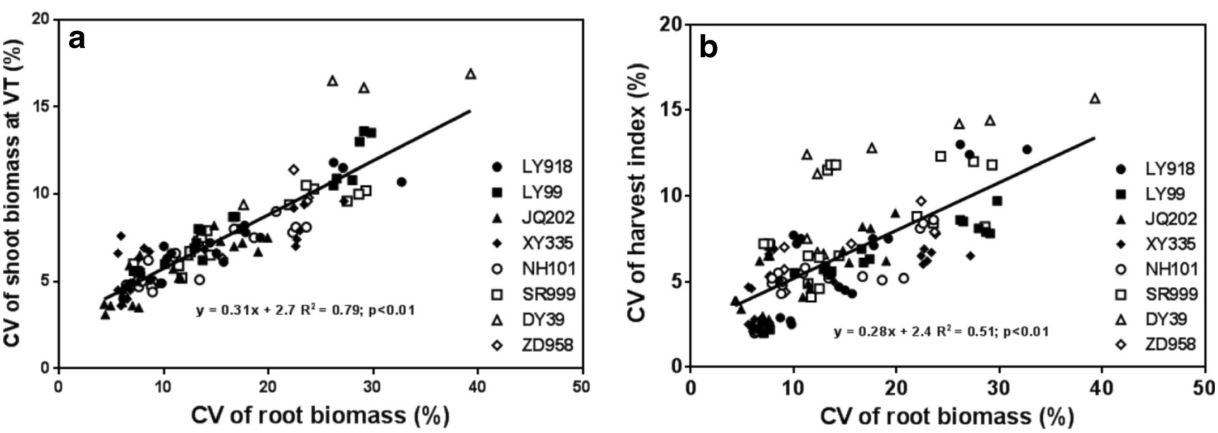

Fig. 9 Correlation between the CV of root biomass and the CV of shoot biomass at VT (a) and the CV of harvest index (b). Data across different planting densities in two experimental years were pooled for the analysis. Each point represents the value of each repetition of different treatments

Table 1 The parameters and their abbreviations examined in the study

Abbreviation	Description
D60000	60,000 plants per ha
D75000	75,000 plants per ha
D90000	90,000 plants per ha
REST	Root Estimator for Shovelomics Traits
CV	Coefficient of variation, %
RA	Average root opening angle in inter-row and intra-row directions, °
RMW	Average root maximal width in inter-row and intra-row directions, cm
RatioRA	Ratio of root opening angle between intra-row and inter-row directions, %
RatioRMW	Ratio of root maximal width between intra-row and inter-row directions, %

Genotypic differences in root traits at different planting densities

Root biomass, root length per plant, RA, RMW, RatioRA, and RatioRMW were reduced with increasing planting density across hybrids and experimental years (Fig. 5). Total nodal root number, root length density, and root to shoot ratio were also reduced (supplementary information Fig. 3S). These results indicate smaller root size, narrower root extension width, steeper root angle, and more root distribution in inter-row direction at higher planting density.

There was a significant interaction of hybrid × density on root biomass (Table 2). Across three plant densities and two years, DY39 and LY99 had largest root biomass, followed by JQ202, LY918 and ZD958. NH101, XY335 and SR999 had the smallest root biomass.

The interaction effect of hybrid × density was significant on root length (Table 2). In 2016, across three plant densities, LY99 had longest root length, followed by JQ202, XY335 and LY918. SR999 and NH101 had shortest root length. In 2017, across three plant densities, DY39 and LY99 had longest root length, followed by JQ202, ZD958, NH101, XY335 and LY918. SR999 had shortest root length.

Across three planting densities and two years, DY39, LY99 and JQ202 had largest RA and RMW, followed by LY918, NH101, XY335 and ZD958 with medium values, while SR999 had the least RA and RMW.

In 2016, no significant differences in RatioRA were found at D60000. At D75000 and D90000, SR999 and LY99 had larger RatioRA than JQ202, XY335, NH101 and LY918. In 2017, at both D60000 and D75000, there was no significant difference in RatioRA among hybrids. At D90000, SR999, DY39 and LY99 had largest RatioRA, followed by JQ202, LY918, XY335 and NH101 with medium RatioRA values, while ZD958 had the smallest RatioRA values.

In 2016, across all planting densities, LY99 and SR999 had larger RatioRMW than LY918, XY335, JQ202 and NH101. In 2017, there was no significant difference among hybrids at D60000. At D75000 and D90000, DY39, SR999 and LY99 had largest RatioRMW, followed by LY918, JQ202, XY335, NH101 with medium RatioRMW values, while ZD958 had the smallest RatioRMW values.

Genotypic differences in the CV of root traits at different planting densities

CV of root biomass, CV of root length, CV of RA, CV of RMW, CV of RatioRA, and CV of RatioRMW all increased with increasing planting density across hybrids and experimental years (Fig. 6). CV of total nodal root number, CV of root length density, CV of root to shoot ratio were also increased with increasing planting density (supplementary information Fig. 4S).

There was a significant interaction of hybrid × density on the CV of root biomass (Table 2). In 2016, SR999 and LY99 had largest CV of root biomass across three plant densities, followed by NH101, XY335 and LY918, while JQ202 had the smallest CV for root biomass. In 2017, across three plant densities, DY39, LY99, SR999 and LY918 had largest CV of root biomass, followed by XY335 and ZD958. NH101 and JQ202 had the smallest CV for root biomass.

The interaction of hybrid × density on CV of root length was significant (Table 2). In 2016, SR999 and LY99 had largest CV of root length across three plant densities, followed by NH101, XY335 and LY918. JQ202 had the smallest CV for root length. In 2017, across three plant densities, DY39, LY99, SR999 and LY918 had largest CV of root length, followed by XY335 and ZD958. NH101 and JQ202 had lowest CV of root length.

The interaction of hybrid × density on CV of RA was significant (Table 2). In 2016, no significant differences were found for CV of RA at D60000. At D75000 and D90000, SR999, LY99 and LY918 had largest CV of RA, followed by NH101 and XY335. JQ202 had smallest CV of RA across three plant densities. In 2017, at D60000 and

Table 2 Summary of split-plot ANOVA results (F-values) for the parameters examined in the study

Year	Source	DF	Grain yield (t ha^{-1})	Root length per plant (m)	Root biomass per plant (g)	RA (°)	RMW (cm)	RatioRA (%)	RatioRMW (%)
2014	Block (B)	2	0.50^{ns}	0.89^{ns}	2.27^{ns}	1.42^{ns}	0.69^{ns}	0.65^{ns}	1.34^{ns}
	Hybrids (H)	5	241.37**	1138.73**	57.79**	58.98**	43.72**	11.76**	14.48**
	B × H	10	0.71^{ns}	0.61^{ns}	1.32^{ns}	2.63*	0.70^{ns}	1.39^{ns}	0.59^{ns}
	Density (D)	2	42.55**	2927.35**	639.68**	972.87**	508.82**	100.85**	13.60**
	H × D	10	3.18*	29.92**	11.06**	1.34^{ns}	0.64^{ns}	7.59**	1.55^{ns}
2015	Block (B)	2	2.13^{ns}	1.58^{ns}	1.69^{ns}	1.21^{ns}	0.42^{ns}	1.06^{ns}	0.01^{ns}
	Hybrids (H)	7	42.44**	437.66**	59.48**	17.86**	42.88**	7.50**	67.32**
	B × H	14	1.07^{ns}	1.18^{ns}	0.99^{ns}	1.32^{ns}	1.22^{ns}	1.66^{ns}	0.76^{ns}
	Density (D)	2	77.40**	3146.33**	447.79**	335.89**	570.49**	40.80**	82.28**
	H × D	14	4.68**	39.36**	6.43**	2.33*	1.92^{ns}	4.25**	11.17**

Year	DF	CV of RMW (%)	CV of RatioRA (%)	CV of RatioRMW (%)	CV of shoot biomass at VT (%)	CV of harvest index (%)
2014	2	1.58^{ns}	1.20^{ns}	1.26^{ns}	2.26^{ns}	1.84^{ns}
	5	26.49**	6.29**	120.15**	36.71**	461.6**
	10	0.95^{ns}	0.89^{ns}	0.53^{ns}	3.17*	1.89^{ns}
	2	992.38**	193.09**	1142.31**	951.6**	3165.19**
	10	19.24**	2.30*	56.92**	10.24**	42.57**
2015	2	0.33^{ns}	0.38^{ns}	1.95^{ns}	3.68^{ns}	1.62^{ns}
	7	101.79**	45.12**	15.98**	77.10**	288.65**
	14	0.43^{ns}	0.63^{ns}	1.13^{ns}	0.87^{ns}	1.16^{ns}
	2	2501.27**	945.60**	1207.91**	589.37**	974.41**
	14	39.53**	24.09**	41.89**	14.42**	66.38**

Block, Hybrids and Density are abbreviated B, H and D, respectively, in the interaction terms. ns: not significant ($P > 0.05$); *, **: significant at $P < 0.05$ and $P < 0.01$, respectively

D75000, NH101, XY335, LY918 and DY39 had larger CV of RA than the other hybrids. At D90000, LY99, SR999 and DY39 had largest CV of RA, followed by LY918, XY335, ZD958 and NH101 with medium CVs, while JQ202 had the smallest CVs for RA.

In 2016, at D60000, LY918, JQ202 and SR999 had larger CV of RMW than the other hybrids. At D75000 and D90000, SR999 and LY99 had largest CV of RMW, followed by NH101, XY335 and LY918, while JQ202 had the smallest CV values for RMW. In 2017, no significant genotypic differences were found for CV of RMW at D60000 and D75000. At D90000, DY39, LY99 and SR999 had largest CV of RMW, followed by LY918, ZD958, NH101 and XY335, while JQ202 had the smallest CV for RMW.

In 2016, at D60000 and D75000, LY918, SR999 and XY335 had larger CV of RatioRA than the other hybrids. At D90000, LY99 had largest CV of RatioRA, and JQ202 had the smallest CVs for RatioRA. In 2017, at D60000 and D75000, ZD958, XY335 and LY918 had larger CV of RatioRA than other hybrids. At D90000, DY39, LY99 and SR999 had largest CV of RatioRA, followed by LY918, ZD958, NH101 and XY335, while JQ202 had the smallest CVs for RatioRA.

In 2016, at D60000 and D75000, no significant differences were found for CV of RatioRMW. At D90000, SR999, LY99 and LY918 had largest CV values of RatioRMW, while JQ202 had the smallest values. In 2017, at D60000, no significant differences were found for CV of RatioRMW. At D75000 and D90000, DY39 and LY99 had larger CV of RatioRMW than other hybrids.

Correlation between grain yield and the root traits

Across genotypes, planting densities and years, there was a weak quadratic correlation ($R^2 = 0.07\sim0.15$) between grain yield and either root biomass, root length, RA, RMW or RatioRMW (Fig. 7). However, under high planting density (D75000 and D90000), the R^2 value of the quadratic correlation between grain yield and either root length (Fig. 7b), RatioRA (Fig. 7e, linear correlation), or RatioRMW (Fig. 7f) became much stronger ($R^2 = 0.17 \sim 0.48$).

Correlation between grain yield and the CV of the root traits

Across genotypes, planting densities and years, there was a negative linear correlation between grain yield and either CV of root biomass ($R^2 = 0.35$, $p < 0.01$), CV of root length ($R^2 = 0.37$, $p < 0.01$), CV of RA ($R^2 = 0.23$, $p < 0.01$), CV of RMW ($R^2 = 0.27$, $p < 0.01$), CV of RatioRA ($R^2 = 0.21$), or CV of RatioRMW ($R^2 = 0.25$, $p < 0.01$) (Fig. 8a-f).

Planting density significantly affected the correlation between grain yield and root traits (Fig. 8, Supplementary information Fig. 5S). As plant density increased, the value of R^2 for the correlation between grain yield and all the root traits increased.

Correlation between the CV of root growth and either shoot growth or harvest index

Across genotypes, planting densities and years, there was a positive linear correlation between CV of root biomass and either CV of shoot biomass at VT ($R^2 = 0.79$, $p < 0.01$; Fig. 9a) or CV of harvest index ($R^2 = 0.51$, $p < 0.01$; Fig. 9b).

Discussion

Due to the serious shadowing under high plant density, light conditions are poor and photosynthesis is weakened, which results in a sharp decline in plant growth (Andrade et al. 1993; Tetio-Kagho and Gardner 1988). Meanwhile, shoot-to-root allocation of photosynthates is reduced as planting density increased (Demotes-Mainard and Pellerin 1992; Hebert et al. 2001; Lambers and Posthumus 1980; Poorter et al. 2016). In maize, the poor investment of photosynthate into the roots leads to overall reduction in root growth, as indicated by the reduced root to shoot ratio, root biomass, root length, root length density, and total nodal root number (Liu et al. 2012; Shao et al. 2018) (Fig. 5). In barley, the competition for light resource resulted in a reduction in the number of tillers and nodal roots, however, the nodal root number per tiller are not much affected (Hecht et al. 2018). Hecht et al. (2016) reported that the lower investment of carbon into roots is partly compensated by increased specific root length and shallow rooting. Due to increased plant-to-plant competition for light resource, the variation among plant growth in a canopy increases, and the degree of variation is closely related to the population yield performance in maize (Sarlangue et al. 2007; Tollenaar and Wu 1999). In the present study, it was found that the CVs of all the root traits investigated were enhanced (Fig. 6), indicating stronger competition between the roots of the neighboring plants. Ecologically, high competiveness between individuals is helpful for a species to thrive in a natural habitat; however, high competiveness between neighboring plants of crops at field level might be harmful for grain yield production per unit area (Donald 1968; Grace and Tilman 2012; Hecht et al. 2016; Wilson 1988; Zhang et al. 1999). For example, genotypes adapted to high planting density usually have erect leaves and smaller tassels (Duvick 2005), traits which are beneficial to reduce competition for light between the neighboring plants. However, the genotypic differences in the plasticity of root system architecture of field-grown maize in response to plant density, and the relationship between root competitiveness and grain yield per unit area are not well investigated.

Based on the quadratic relationship between grain yield and root size, as indicated by root biomass and root length (Fig. 7), it can be speculated that both oversized and undersized root systems could cause yield reductions per ha. Genotypes like DY39 and LY99 with oversized root systems may aggravate root-to-root competition, and lead to lower grain yield, especially at D90000 (Fig. 4). On the other hand, genotypes like SR999 with undersized root systems may limit support for shoot growth, acquisition of water and nutrients (Lynch 2013), also resulting in lower grain yield (Fig. 4). Genotypes like LY918, XY335, ZD958 and NH101 reduced root size with increasing planting density, which may contribute to higher grain yield at D90000 (Fig. 4). JQ202 had relatively balanced root size across the three planting densities, which may partially explain its high yield across all planting densities (Fig. 4).

Root architecture, as reflected by RA, RMW, RatioRA and RatioRMW, can exert great impact on water and nutrient acquisition efficiency and therefore on grain yield. Flat RA and broad RMW were crucial for exploring a wide growth space, and promoting nutrient and water uptake (Hammer et al. 2009; Lynch and Brown 2001; Richardson et al. 2011; Singh et al. 2012; Zhu et al. 2005). However, a large root width also implies wide horizontal extension (Liu et al. 2009), which may aggravate root-to-root competition under limited space, thus generating a negative impact on grain yield. In practice, the distance between rows is larger than the distance within plants. Reduced RatioRA and RatioRMW indicated a distinct root performance, which might help to reduce root-to-root competition in the intra-row direction and maximize root growth in inter-row direction. From the perspective of root carbon economics, less RatioRA indicated steeper RAIntra than RAInter, which was good for coordinating root growth under limited space, weakening root-to-root competition, and reducing the metabolic costs caused by root competition (Lynch 1995). From the point of root ecology, the difference of root system arrangement in intra-row and inter-row directions may be illustrated by the identity recognition, which is helpful to lessen root-to-root competition and obtain grain yield production (Chen et al. 2012a; File et al. 2012; Murphy et al. 2017; Weiner 2003; Zhu and Zhang 2013). In this study, a quadratic relationship was found between grain yield and RA, RMW, and RatioRMW (Fig. 7), confirming the viewpoints mentioned above. Among the genotypes, as planting density increased, broader RA and RMW, and less sensitivity of RatioRA and RatioRMW might be accounted for by poor grain yield performance of LY99 and DY39 (Fig. 4). Over-steep RA and over-narrow RMW, as well as the smaller sensitivity of RatioRA and RatioRMW, might cause reductions in grain yield of SR999 (Fig. 4). JQ202, ZD958, NH101, LY918 and XY335 kept medium broad RA and RMW through reducing RatioRA and RatioRMW, optimizing root growth in both intra-row and inter-row directions, thus improving density-tolerant ability, and producing higher grain yield (Fig. 4). In fact, steeper roots in the topsoil might not be good for root-lodging resistance (Crook and Ennos 1993; Crook and Ennos 1994; Ennos 1991), and therefore might reduce grain yield. Serious root lodging happened in LY918 and SR999 under D90000 in 2016 when there was abundant rain before silking.

A uniform plant population is essential to get high productivity in maize (Sarlangue et al. 2007; Tollenaar and Wu 1999; Tokatlidis and Koutroubas 2004). Due to plant-to-plant competition for limited light resources, plant size inequality between neighboring plants could be aggravated as plant density increased (Glenn and Daynard 1974; Maddonni and Otegui 2004; Rossini et al. 2011). The parameter CV is used to quantify phenotypic variability and competitive ability of individual plant (Edmeades and Daynard 1979). Increased CV of shoot biomass under high planting density has been reported in maize (Bonan 1991; Edmeades and Daynard 1979; Glenn and Daynard 1974; Ipsilandis and Vafias 2005; Maddonni and Otegui 2006; Muldoon and Daynard 1981; Tollenaar and Wu 1999; Tokatlidis and Koutroubas 2004). In our study, we found that the CV of root biomass, CV of root length, CV of RA, CV of RMW, CV of RatioRA and CV of RatioRMW were all increased as plant density increased (Fig. 6). The CV of these root traits was strongly and negatively correlated with grain yield (Fig. 8). The value of R^2 for this correlation was much higher than that for the correlation between root size and grain yield, indicating that the variation of root traits played a greater effect on grain yield than the average root size. Interestingly, the CV of root biomass was strongly and positively correlated with the CV of shoot biomass, and the CV of harvest index (Fig. 9), suggesting that increased variation of root growth could result in variation of shoot growth and finally the variation in ear/grain development, or vice versa. Among genotypes, JQ202 had the smallest CV of root traits across three planting densities, which may explain its highest grain yield (Fig. 4). NH101 and XY335 had medium-high CV of root traits at low density, but less increase in CV of root traits with increasing planting density, thus producing high grain yield at D90000 (Fig. 4). LY99, SR999 and DY39 had largest CV of root traits across three planting densities, and therefore the smallest grain yield (Fig. 4).

In conclusion, root size, root architecture and the variation of root traits between neighboring plants play great roles on grain yield. The variation in root biomass for neighboring plants was positively correlated with the variation in shoot biomass and harvest index. Genotypes with less variation in root size, medium root size, medium broad root system and more inter-row root distribution help to reduce root-to-root competition and tend to have higher yield at high planting density.

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