INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
20F–135/2021/25–1–43–51
DOI: 10.17957/IJAB/15.1636
http://www.fspublishers.org

*Full Length Article*

# Planting Density and Variety Modulated Root and Leaf Characteristics to Improve Grain Yield of Spring Maize

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*Received 28 July 2020; Accepted 13 August 2020; Published 10 December 2020*

## Abstract

To better understand the accumulation and transport of substances under different planting densities, the adaptation of maize root and leaf in response to increasing planting densities was investigated. In this two-year filed study, three maize varieties, Fumin108 (FM), Xianyu335 (XY) and Dika159 (DK), were sown under three different planting densities: 15,000 ($D_1$), 60,000 ($D_2$) and 90,000 plants ha$^{-1}$ ($D_3$) during 2018 and 2019. Increase in planting density gradually increased leaf area index along with reduced leaf area and net photosynthetic rate of individual leaves. In the 0–20 cm soil layer, the average root dry matter decreased by 55.88 and 80.92%, and the average root number decreased by 31.18 and 38.71% under $D_2$ and $D_3$, respectively, compared with $D_1$. With increase in planting density, yield and dry matter per plant of maize gradually decreased while yield and dry matter per ha was increased with increase in $D_1$-$D_2$ density and then flattened in $D_2$-$D_3$ density. Compared with $D_1$, two-year average yield per plant was decreased by 34.10 and 51.87% under $D_2$ and $D_3$, respectively. The difference in the number of roots of XY, FM and DK were not significant, so change in variety did not alleviate the decrease in the number of roots. At higher planting densities (above $D_2$), the increase in density did not increase per ha grain yield. In conclusion, the suitable plant density was about 60,000 plants ha$^{-1}$ to harvest more yield of spring maize while density higher than that reduced leaf area and photosynthesis per plant. Moreover, leaf area, root number and net photosynthesis per plant was higher in lower planting density coupled with overall less yield on ha basis and thus seemed wastage of soil nutrients and light resources. 

**Keywords:** Grain yield; Leaf source; Maize variety; Planting density; Root source

## Introduction

One half of the increase in maize (*Zea mays* L.) production has been attributed to improved fertilizers, farmland management, and cultivation techniques, while the other half increase has been attributed to heterosis (Yang *et al.* 2019). However, 35 to 40% of the increase in maize yield has been due to genetic improvement in China. Improved cultivation techniques and field management models have played a major role in improving maize production in China (Dai 2000). Among them, increasing planting density is one of the key management practices. Increasing the planting density usually increases maize grain yield until an optimum number of plants per unit area is reached (Duvick 2005; Turgut *et al.* 2010). However, after reaching the optimum density, the grain yield decreases as the density increases (Zhang *et al.* 2019). With increasing planting density per plant yield and biomass decreases (Maddonni and Otegui 2006). Therefore, determining the optimal planting density will facilitate the early realization of high-yield maize cultivation. High-density and ultra-high-density planting helped achieve higher maize yields (Zhang *et al.* 2019). Tokatlidis and Koutroubas (2004) conducted field experiments and argued that increase in modern maize yield is dependent on an increase in density rather than an increase in yield per plant. The source-sink ratio of maize varies with planting density, and the coordination between source and sink organs is directly related to crop yield. Source and sink are closely linked to each other; size of source and its ability to accumulate and distribute substances directly affect sink formation and enrichment (Oorbessy *et al.* 2016). To explore the effect of source

**To cite this paper:** Yang Q, X Shao, Y Cao, Y Lv, Z Liu, W Xu, L Wang, Y Wang (2021). Planting Density and variety modulated root and leaf characteristics to improve grain yield of spring maize. *Intl J Agric Biol* 25:43–51

organs on the coordinated growth of source and sink under different planting densities is conducive to identify ways to increase maize yield.

Sources are organs that synthesize and provide nutrients for plant growth. There are three types of sources: leaf sources, stem and sheath sources, and root sources. The former two are the photosynthetic sources and the latter is the nutrition source of crops. Leaves are the main source organs, and about 95% of the grain yield comes from organic compounds, such as carbohydrates and proteins, synthesized via photosynthesis (Fang *et al.* 2018). Within a certain range, the photosynthetic intensity of crops positively correlates with leaf area index (LAI) (Yan *et al.* 2019). Therefore, the amount of green leaf area significantly affects leaf photosynthetic capacity, which in turn determines crop dry matter accumulation and grain yield (Jiang *et al.* 2000). Reasonable utilization of group light energy is the basis of dry matter accumulation, and the flatness of maize leaves is an important criterion to measure the quality of maize itself. Leaf is the main source organ in maize, where the topmost leaf is compact, and the bottommost leaf is flattened to help absorb more light energy. An increase in group leaf area was partly due to the increase in density; larger group leaf area helped achieve high yield (Liu *et al.* 2000). Therefore, understanding in source-sink relationship is important to improve yield in maize.

The development of roots, an important organ that absorbs nutrients and water, is closely related to the growth of aboveground parts and the formation of grain "sink" (Santiago *et al.* 2019). Grain yield formation stage is a critical stage for plant nutrient absorption. Nitrogen (N) absorbed by plants after silking accounts for more than 60% of the total nitrogen absorbed during the entire growth period. Nitrogen absorbed is related to higher dry matter accumulation efficiency and an abundant supply of root assimilation during the filling period. As plant density increases, the interaction between roots of the neighboring plants has a greater influence on grain formation. Any impact on dry matter distribution and nutrient absorption significantly affects the change in yield (Yang *et al.* 2020). Studies have positively correlated root biomass with green leaf area (Ogawa *et al.* 2005). Further studies on the effects of root interaction on resource distribution, capture, and utilization during grain formation are necessary. This will help breeders to develop high-yielding maize and agronomists to efficiently use resources to increase yield.

Due to the difficulty in sampling and determination of root system, studies have so far focused on yield and photosynthetic performance to evaluate the effects of sources on physiological characteristics of maize. However, research on the interaction between roots of the same variety in a group and its effect on resource distribution and mineral absorption and utilization is relatively less. To fill this knowledge gap, this experiment was conducted to evaluate the influence of leaf source and root source on yield of divergent spring maize varieties under high-medium-low planting density.

## Materials and Methods

### Experimental site

The experiment was conducted at the Jilin Academy of Agricultural Sciences, Qian'an County (N: 45°01, E: 124°02). The area was located in a semi-arid region with a continental monsoon climate in the mid-temperate zone, sufficient light and heat resources, and an average frost-free period of 146 days. The maize growing seasons in 2018 and 2019 (May 13 to October 8, 2018; May 12 to October 9, 2019) had total precipitation of 407.90 and 506.60 mm, a daily average temperature of 21.09 and 20.53°C and an effective accumulated temperature of 1656.55 and 1592.10°C, respectively.

### Experimental design

In this two-year field study, three maize varieties [Fumin108 (FM), Xianyu335 (XY) and Dika159 (DK)] were sown under three different planting densities i.e., 15,000 ($D_1$), 60,000 ($D_2$), 90,000 plants ha$^{-1}$ ($D_3$) during 2018 and 2019. Wide and narrow row planting (70 cm, 40 cm) was adopted, and the soil was covered with degradable plastic film. Experiment was conducted under randomized complete block design with factorial arrangement. Each treatment was composed of three replicates with net plot size of 20 m × 10 m. All the plots were supplied with nitrogen (N, 280 kg ha$^{-1}$), phosphorus ($P_2O_5$, 123 kg ha$^{-1}$), and potassium ($K_2O$, 127 kg ha$^{-1}$). Total phosphorus (P), potassium (K) fertilizers, and half of nitrogen (N) fertilizer were applied at pre-sowing, and the remaining N fertilizer was top-dressed at six-leaf stage (V6). Irrigation was carried out on all test points to ensure that the water is non-restrictive. Recommended pesticides available in market were sprayed to control pests and diseases while weeds were controlled manually.

### Analysis of soil samples

Samples of soil from the surface layer (0–20 cm) soils were collected at random in triplicate at maturity. The soil was divided into 2 sub-samples after sieving it to < 5 mm. A part of the sample was used to determine the composition of soil N ($NO_3^-$-N and $NH_4^+$-N) and the soil water content using a standard gravimetric method, whereas the other part was air-dried for analysis of total N. The moisture content of soil was dried at 105°C to a constant weight. $NH_4^+$-N and $NO_3^-$-N contents were measured by AAIII continuous flow auto-analyzer. The organic matter content of the soil was determined by the potassium dichromate oxidation-colorimetric method (China Soil Science Association Agricultural Chemistry Committee 1983). The total N content of the soil was determined with a Hanon K9860 Kjeldahl analyzer (Lu 2000).

### Measurement of plant parameters

The green leaf length and width of three plants with different treatments were measured at twelfth-leaf stage (V12), tasseling stage (VT), 20 days after flowering (R20), and 40 days after flowering (R40).

Leaf area index (LAI) = leaf area per plant ($m^2$) × number of plants per unit land area (plant) / land area ($m^2$). At twelfth-leaf stage (V12), tasseling stage (VT), and 20 d (R20) after flowering, different parameters including photosynthetic rate (Pn), stomatal conductance (Gs), transpiration rate (Tr), and intercellular $CO_2$ concentration (Ci) were measured using a portable LI-6400 photosynthesis meter around 10 noon on a sunny day. Three replicates were maintained per treatment. Chlorophyll fast-phase fluorescence kinetic parameters were measured using a Handy PEA (Hansatedi Company) at twelfth-leaf stage (V12), tasseling stage (VT), and 20 days after flowering (R20), and three replicates were maintained per treatment.

Maize plants were sampled at tasseling (VT) and physiological maturity (R6) (Han *et al.* 2014; Jia *et al.* 2018a). The dry matter accumulation was determined after drying the plant parts at 80°C to a constant weight. The nitrogen content of the plant was determined by an AAIII continuous flow analyzer (Yang *et al.* 2019). All the ears in the middle 3 rows of each plot were harvested at physiological maturity used to determine grain yield and yield components, which including kernel number, and 1000-kernel weight. The kernels were separated from the cob by hand and air dried to determine the yield, which was expressed at 14% moisture content.

### Statistical analysis

The data were prepared using Sigma Plot 10.0 and Microsoft Excel 2010. DPS 15.10 software was used to perform two-way analysis of variance (ANOVA) and means were separated using Duncan's New Multiple Range (DMNR) test at a probability level of 0.05. Moreover, Microsoft Excel program was used for graphical presentation of data.

## Results

### Effects of variety and planting density on maize leaf source

Leaf area index of maize was significantly different between varieties and planting densities (Table 1). The leaf area index of each treatment reached the maximum at tasseling (VT) and then gradually decreased. As the planting density increased from low ($D_1$) to high ($D_3$), leaf area index gradually increased while leaf area per plant decreased. Under $D_1$ planting density, there was little difference in leaf area index among varieties; however, the difference in leaf area index among varieties was significant at $D_2$ and $D_3$ densities (Table 1). The maize variety XY showed intolerance to densities. Compared with other varieties, the leaf area index of XY decreased as the density increase (Table 1).

At tasseling and maturity, planting density had a significant effect on leaf nitrogen content of a single plant. Leaf nitrogen content of a single plant of maize gradually decreased as the density increased (Table 2). At the tasseling stage, compared with $D_1$ (1.50 g), the two-year average maize leaf nitrogen content of $D_2$ and $D_3$ decreased by 9.4 and 35.3%, respectively (Table 2). At maturity, compared with $D_1$ (0.77 g), the two-year average maize leaf nitrogen content of $D_2$ and $D_3$ decreased by 37.3 and 51.0%, respectively (Table 2).

The single leaf net photosynthetic rate decreased with increase in density at twelfth-leaf stage (V12), VT, and 20 days after flowering (R20) stage. Compared with $D_1$, the average net photosynthetic rate of $D_2$ and $D_3$ decreased by 7.5 and 12.1%, respectively, at the V12 stage and decreased by 1.0 and 44.5%, respectively, at the VT stage (Table 3). At the V12 and VT stage, the effects of variety, density, and variety × density on net photosynthetic rate were significant (Table 4). The effects of variety and variety × density on net photosynthetic rate reached a significant level after 20 days of flowering (Table 4). The effect of density on net photosynthetic rate first increased and then decreased with growth. At the V12 and R20 stages, GS, Ci, and Tr showed no significant differences between the three densities. During the VT stage, GS, Ci, and Tr decreased as the density increase (Table 3).

The maximum fluorescence (Fm') under light-adapted state at twelfth-leaf stage (V12) first increased and then decreased with increase in density, except for XY at $D_3$ (Table 5). The Fm' and actual photochemical efficiency (ΦPSII) under photoadaptation at the VT stage first increased and then flattened with increase in density. The electron transfer rate (ETR) increased with increase in density at VT stage and was significantly different between the three densities (Table 5). The Fm', ΦPSII, and ETR in the light-adapted state at 20 days after flowering (R20) increased with increase in density; however, the differences between the three densities were not significant (Table 5). Density had a significant effect on the ΦPSII at the VT stage (Table 4).

The maximum variable fluorescence (Fv) and maximum fluorescence (Fm) in the dark-adapted state increased with increase in density at V12, VT, and R20 (Table 6). Significant differences were observed in the maximum photochemical efficiency (Fv/Fm) between low density ($D_1$) and higher densities ($D_2$ and $D_3$) during V12 and R20 stages, and the difference between $D_2$ and $D_3$ was not significant. These findings indicate that within a certain density range, the maximum photochemical efficiency gradually increased with increase in density and then flattened (Table 6). The effect of density on the maximum photochemical efficiency (Fv/Fm) was significant during the V12, VT, and R20 stages (Table 4).

**Table 1:** Effect of planting density on LAI of three spring maize varieties in 2019

| Varieties | Planting densities | V12 | VT | R20 | R40 |
|---|---|---|---|---|---|
| Xianyu335 | $D_1$ | 1.03e | 1.04c | 0.89c | 0.70c |
| | $D_2$ | 4.13c | 4.54b | 4.18b | 3.48b |
| | $D_3$ | 5.42b | 5.71a | 5.19a | 4.25a |
| Fumin108 | $D_1$ | 1.02e | 1.14c | 0.98c | 0.77c |
| | $D_2$ | 3.66d | 4.51b | 4.03b | 3.24b |
| | $D_3$ | 5.71ab | 5.89a | 5.41a | 4.41a |
| Dika159 | $D_1$ | 1.11e | 1.03c | 0.90c | 0.70c |
| | $D_2$ | 3.68d | 4.29b | 3.76b | 3.06b |
| | $D_3$ | 5.86a | 5.56a | 5.01a | 4.11a |

Values followed by different small letters in the same column are significantly different from each other at $P \leq 0.05$

LAI= Leaf area index; V12= Twelfth-leaf stage; VT= Tasseling stage; R20= 20 days after flowering; R40= 40 days after flowering; $D_1$ = 15000 plants ha $^{-1}$; $D_2$ = 60000 plants ha $^{-1}$; $D_3$ = 90000 plants ha $^{-1}$

**Table 2:** Effect of planting density on leaf nitrogen content of three spring maize varieties

| Varieties | Planting densities | VT (g) | | R6 (g) | |
|---|---|---|---|---|---|
| | | 2018 | 2019 | 2018 | 2019 |
| Xianyu335 | $D_1$ | 1.31bc | 1.58ab | 0.84a | 0.79ab |
| | $D_2$ | 0.83d | 1.48ab | 0.48bc | 0.52ce |
| | $D_3$ | 1.03bd | 1.05c | 0.32c | 0.30ef |
| Fumin108 | $D_1$ | 1.42b | 1.69ab | 0.62ab | 0.83a |
| | $D_2$ | 1.98a | 1.51ab | 0.49bc | 0.75ac |
| | $D_3$ | 0.99bd | 0.84c | 0.38c | 0.59bd |
| Dika159 | $D_1$ | 1.28bd | 1.72a | 0.79a | 0.74ac |
| | $D_2$ | 0.96bd | 1.39b | 0.45bc | 0.20f |
| | $D_3$ | 0.94cd | 0.98c | 0.31c | 0.36df |

Values followed by different small letters in the same column are significantly different from each other at $P \leq 0.05$

VT= Tasseling stage; R6= Physiological maturity; $D_1$ = 15000 plants ha $^{-1}$; $D_2$ = 60000 plants ha $^{-1}$; $D_3$ = 90000 plants ha $^{-1}$

**Table 3:** Effect of planting density on photosynthetic parameters of leaves of three maize varieties at different stages in 2019

| Stage | Varieties | Planting densities | Pn $(m^2 s)^{-1}$ | Gs mol $(m^2 s)^{-1}$ | Ci mmol $mol^{-1}$ | Tr mmol $(m^2 s)^{-1}$ |
|---|---|---|---|---|---|---|
| V12 | Xianyu335 | $D_1$ | 42.14ab | 0.350cd | 113.53b | 7.77ab |
| | | $D_2$ | 43.01a | 0.435bc | 122.24ab | 7.76ab |
| | | $D_3$ | 45.63a | 0.691a | 160.38a | 8.90a |
| | Fumin108 | $D_1$ | 44.94a | 0.509b | 137.44ab | 8.18a |
| | | $D_2$ | 45.58a | 0.680a | 159.72a | 8.89a |
| | | $D_3$ | 37.27bc | 0.365cd | 120.83ab | 7.13ab |
| | Dika159 | $D_1$ | 44.80a | 0.521b | 143.39ab | 8.04ab |
| | | $D_2$ | 33.26c | 0.284d | 138.37ab | 7.31ab |
| | | $D_3$ | 33.06c | 0.293d | 115.66b | 6.26b |
| VT | Xianyu335 | $D_1$ | 9.39ab | 0.451ab | 20.81ab | 10.87a |
| | | $D_2$ | 8.52b | 0.376bc | 19.13bc | 9.81ab |
| | | $D_3$ | 5.50cd | 0.203ef | 18.93bc | 6.42ef |
| | Fumin108 | $D_1$ | 10.54a | 0.487a | 17.13cd | 10.73a |
| | | $D_2$ | 8.92ab | 0.366cd | 16.18cd | 8.99bc |
| | | $D_3$ | 4.88cd | 0.213ef | 16.95cd | 6.33f |
| | Dika159 | $D_1$ | 6.40c | 0.286de | 23.68a | 7.65de |
| | | $D_2$ | 8.64b | 0.339cd | 14.84d | 8.21cd |
| | | $D_3$ | 4.23d | 0.155f | 19.20bc | 4.97g |
| R20 | Xianyu335 | $D_1$ | 19.64bc | 0.319a | 63.87d | 3.84ab |
| | | $D_2$ | 15.35c | 0.232bd | 68.89d | 3.19bc |
| | | $D_3$ | 28.24a | 0.241bc | 94.63c | 4.09a |
| | Fumin108 | $D_1$ | 27.36a | 0.175d | 131.08a | 2.57c |
| | | $D_2$ | 30.58a | 0.287ab | 112.53b | 3.90ab |
| | | $D_3$ | 26.13ab | 0.205cd | 131.31a | 3.15bc |
| | Dika159 | $D_1$ | 29.69a | 0.240bc | 125.92a | 3.54ab |
| | | $D_2$ | 27.16a | 0.262ac | 114.04b | 3.33ac |
| | | $D_3$ | 31.71a | 0.289ab | 107.77b | 4.10a |

Values followed by different small letters in the same column are significantly different from each other at $P \leq 0.05$

V12= Twelfth-leaf stage; VT= Tasseling stage; R20= 20 days after flowering; Pn= Net photosynthetic rate; Gs= Stomatal conductance; Ci= Intercellular $CO_2$ concentration; Tr= Transpiration rate; $D_1$ = 15000 plants ha $^{-1}$; $D_2$ = 60000 plants ha $^{-1}$; $D_3$ = 90000 plants ha $^{-1}$

## Effect of variety and planting density on maize roots

With increase in plant density, both root dry matter and root number gradually decreased (Table 7). In the 0–20 cm soil layer, compared with $D_1$ (43.95 g), the average root dry weight of the tasseling stage (VT) and maturity (R6) under $D_2$ (17.74 g) and $D_3$ (8.79 g) decreased by 59.3 and 83.2%, respectively. Compared with $D_1$ (94), the average number of roots of VT and R6 under $D_2$ (64) and $D_3$ (56) decreased by 32.1 and 40.8%, respectively. Compared with $D_1$ (0.59 g), the average root nitrogen content of VT and R6 under $D_2$ (0.21 g) and $D_3$ (0.10 g) decreased by 64.2 and 83.9%, respectively (Table 7). The effects of variety, density, and variety × density on root dry weight were significant at VT and R6 stages. Density showed a significant effect on the number of maize roots at VT and R6 stages (Table 4).

In the 0–20 soil layer, the soil nitrate nitrogen ($NO_3^-$) and ammonium nitrogen ($NH_4^+$) under $D_1$ were more than $D_2$ and $D_3$ densities at the maturity stage of both years (Table 8). Compared with $D_1$ (3.72 mg $kg^{-1}$), the two-year average soil $NH_4^+$ content of $D_2$ and $D_3$ decreased by 9.08 and 19.25%, respectively. However, $NO_3^-$ content first decreased and then increased as the density increased. Compared with $D_1$ (18.40 mg $kg^{-1}$), the two-year average soil $NO_3^-$ content of $D_2$ and $D_3$ decreased by 52.3 and 35.3%, respectively (Table 8). Compared with $D_1$ (1.70 g $kg^{-1}$), the two-year average of total nitrogen content of $D_2$ and $D_3$ decreased by 18.41 and 8.72%, respectively. Compared with $D_1$ (3.5%), the two-year average soil organic matter of $D_2$ and $D_3$ was decreased by 12.5 and 5.2%, respectively (Table 8).

## Effect of variety and planting density on dry matter accumulation and yield of maize

At the VT and R6 stages, dry matter per plant decreased significantly with increase in planting density while dry matter per ha first increased and then flattened (Table 9). At the VT stage, the two-year average dry matter per plant under $D_2$ and $D_3$ decreased by 26.72 and 44.27%, respectively, compared with $D_1$. At the R6 stage, the two-year average dry matter per plant under $D_2$ and $D_3$ decreased by 36.90 and 54.30%, respectively, compared with $D_1$. At the VT stage, the two-year average dry matter per ha of $D_2$ and $D_3$ increased by 198.81 and 238.83%, respectively, compared with $D_1$. At the R6 stage, the two-year average dry matter per ha under $D_2$ and $D_3$ increased by 152.4 and 174.2%, respectively, compared with $D_1$ (Table 9). At R6 stage, year and variety showed significant effects on dry

**Table 4:** Statistical summary of Pn, ΦPSII, Fv / Fm, root dry weight, number of roots, dry matter per plant, dry matter per ha, yield per ha and HI of three maize varieties grown under different planting densities

| Measurement index | Origin of variance | V12 | | VT | | R20 | | R6 | |
|---|---|---|---|---|---|---|---|---|---|
| | | F value | p value | F value | p value | F value | p value | F value | p value |
| Pn | Variety (A) | 12.9035 | 0.0005 | 7.8798 | 0.0041 | 11.5948 | 0.0008 | - | - |
| | Density (B) | 7.4639 | 0.0051 | 48.3917 | 0.0000 | 2.8546 | 0.0871 | - | - |
| | A×B | 8.3261 | 0.0008 | 4.2014 | 0.0163 | 3.6701 | 0.0264 | - | - |
| ΦPSII | Variety (A) | 2.2355 | 0.1393 | 1.0235 | 0.3817 | 0.9230 | 0.4175 | - | - |
| | Density (B) | 1.1532 | 0.3405 | 14.5188 | 0.0003 | 3.4550 | 0.0566 | - | - |
| | A×B | 0.5257 | 0.7184 | 1.0334 | 0.4204 | 0.4235 | 0.7895 | - | - |
| Fv / Fm | Variety (A) | 3.6883 | 0.0482 | 0.4142 | 0.6677 | 0.4442 | 0.6490 | - | - |
| | Density (B) | 7.7151 | 0.0045 | 25.2743 | 0.0000 | 8.7284 | 0.0027 | - | - |
| | A×B | 0.9034 | 0.4852 | 0.5006 | 0.7357 | 1.0177 | 0.4277 | - | - |
| Root dry weight | Variety (A) | - | - | 20.0982 | 0.0000 | - | - | 5.6183 | 0.0142 |
| | Density (B) | - | - | 131.349 | 0.0000 | - | - | 78.3639 | 0.0000 |
| | A×B | - | - | 13.2659 | 0.0001 | - | - | 1.6680 | 0.2064 |
| Number of roots | Variety (A) | - | - | 2.4197 | 0.1207 | - | - | 9.3944 | 0.0020 |
| | Density (B) | - | - | 20.8556 | 0.0000 | - | - | 36.2259 | 0.0000 |
| | A×B | - | - | 0.6874 | 0.6111 | - | - | 0.7429 | 0.5766 |
| Dry matter per plant | Year (A) | - | - | 0.012 | 0.913 | - | - | 23.827 | 0.0000 |
| | Variety (B) | - | - | 1.445 | 0.250 | - | - | 12.041 | 0.0001 |
| | Density (C) | - | - | 125.48 | 0.000 | - | - | 332.81 | 0.0000 |
| | A×B | - | - | 0.303 | 0.741 | - | - | 4.3528 | 0.0207 |
| | A×C | - | - | 3.346 | 0.047 | - | - | 3.7819 | 0.0329 |
| | B×C | - | - | 1.423 | 0.247 | - | - | 0.7560 | 0.5611 |
| | A×B×C | - | - | 7.523 | 0.000 | - | - | 1.5199 | 0.2183 |
| Dry matter per ha | Year (A) | - | - | 2.4835 | 0.1243 | - | - | 11.471 | 0.002 |
| | Variety (B) | - | - | 0.8165 | 0.4505 | - | - | 11.020 | 0.000 |
| | Density (C) | - | - | 426.811 | 0.0000 | - | - | 257.773 | 0.000 |
| | A×B | - | - | 3.2535 | 0.0509 | - | - | 1.251 | 0.299 |
| | A×C | - | - | 8.4501 | 0.0010 | - | - | 1.003 | 0.377 |
| | B×C | - | - | 2.5349 | 0.0580 | - | - | 1.960 | 0.123 |
| | A×B×C | - | - | 5.6637 | 0.0013 | - | - | 0.391 | 0.813 |
| Yield per ha | Year (A) | - | - | - | - | - | - | 45.5231 | 0.0000 |
| | Variety (B) | - | - | - | - | - | - | 1.9575 | 0.1568 |
| | Density (C) | - | - | - | - | - | - | 145.2296 | 0.0000 |
| | A×B | - | - | - | - | - | - | 3.7212 | 0.0346 |
| | A×C | - | - | - | - | - | - | 0.7767 | 0.4679 |
| | B×C | - | - | - | - | - | - | 3.0202 | 0.0311 |
| | A×B×C | - | - | - | - | - | - | 2.3357 | 0.0752 |
| HI | Year (A) | - | - | - | - | - | - | 2.9833 | 0.0932 |
| | Variety (B) | - | - | - | - | - | - | 1.0152 | 0.3731 |
| | Density (C) | - | - | - | - | - | - | 4.0032 | 0.0275 |
| | A×B | - | - | - | - | - | - | 13.3025 | 0.0001 |
| | A×C | - | - | - | - | - | - | 5.9217 | 0.0062 |
| | B×C | - | - | - | - | - | - | 10.2722 | 0.0000 |
| | A×B×C | - | - | - | - | - | - | 5.5553 | 0.0015 |

V12= Twelfth-leaf stage; VT= Tasseling stage; R20= 20 days after flowering; R6= Physiological maturity; Pn= Net photosynthetic rate; ΦPSII= Actual photochemical efficiency; Fv/Fm= Maximum photochemical efficiency; HI= Harvest index

matter per plant and dry matter per ha. Density significantly affected dry matter accumulation per plant and dry matter per ha at VT and R6 stages (Table 4).

At the maturity stage (R6), the two-year average yield per ha under $D_2$ and $D_3$ increased by 73.27 and 79.91%, respectively, compared with D1 (Table 10). Compared with $D_1$, the two-year average grains per ear under $D_2$ and $D_3$ was decreased by 2.6 and 10.9%, respectively (Table 10). Compared with $D_1$, the two-year average ears per unit area under $D_2$ and $D_3$ increased by 74.3 and 133.0%, respectively. Compared with $D_1$, the two-year average 1000-kernel weight under $D_2$ and $D_3$ was decreased by 2.8 and 13.9%, respectively (Table 10). Compared with $D_1$, the two-year average harvest index under $D_2$ and $D_3$ was increased by 3.9 and 5.1%, respectively (Table 10). The effects of year and density on yield per ha were significant while the effect of variety was not significant at the maturity stage. The effects of year, variety, and density on yield per plant were significant at the maturity stage. Density had a significant effect on harvest index (Table 4).

## Discussion

Planting density is an important factor that improves root and canopy conditions and affects the group photosynthetic system, and increase density is the easiest way to improve yield, because within a certain period of time, it is difficult to increase the crop's yield potential through breeding (Xu *et al.* 2017). Root is the nutrient source while leaf is the photosynthetic source of the crop. These two sources are

**Table 5:** Effect of planting density on fluorescence and light response index of plant leaves of three maize varieties in 2019

| Varieties | Planting densities | V12 | | | VT | | | R20 | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Fm' | ΦPSII | ETR | Fm' | ΦPSII | ETR | Fm' | ΦPSII | ETR |
| Xianyu335 | $D_1$ | 84.33a | 0.833a | 3.50b | 63b | 0.775bc | 2.39e | 85.00ac | 0.43ab | 1.54b |
| | $D_2$ | 88.67a | 0.857a | 3.78ab | 100a | 0.854a | 2.75d | 97.33ac | 0.51ab | 1.82ab |
| | $D_3$ | 57.00cd | 0.793a | 3.67ab | 117a | 0.866a | 3.76a | 108.00a | 0.54a | 2.10a |
| Fumin108 | $D_1$ | 43.67d | 0.795a | 3.51b | 61b | 0.752c | 2.68d | 82.67bc | 0.42ab | 1.50b |
| | $D_2$ | 64.67bc | 0.803a | 3.71ab | 97a | 0.837a | 3.11c | 91.33ac | 0.45ab | 1.71ab |
| | $D_3$ | 63.00bc | 0.792a | 3.78ab | 108a | 0.856a | 3.77a | 92.67ac | 0.45ab | 1.80ab |
| Dika159 | $D_1$ | 77.33ab | 0.817a | 3.62ab | 74b | 0.813ab | 3.13bc | 73.67c | 0.38b | 1.42b |
| | $D_2$ | 93.00a | 0.875a | 4.04a | 103a | 0.842a | 3.36b | 94.67ac | 0.49ab | 1.89ab |
| | $D_3$ | 91.67a | 0.850a | 3.99ab | 106a | 0.851a | 3.76a | 98.67ab | 0.52a | 2.09a |

Values followed by different small letters in the same column are significantly different from each other at $P \leq 0.05$

V12= Twelfth-leaf stage; VT= Tasseling stage; R20= 20 days after flowering; Fm'= Maximum fluorescence; ΦPSII = Actual photochemical efficiency; ETR = Electron transfer rate; $D_1$ = 15000 plants ha $^{-1}$; $D_2$ = 60000 plants ha $^{-1}$; $D_3$ = 90000 plants ha $^{-1}$

**Table 6:** Effect of planting density on fluorescence dark response index of plant leaves of three maize varieties in 2019

| Varieties | Planting densities | V12 | | | VT | | | R20 | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Fv | Fm | Fv/Fm | Fv | Fm | Fv/Fm | Fv | Fm | Fv/Fm |
| Xianyu335 | $D_1$ | 64.00df | 68.33d | 0.832c | 72b | 86bc | 0.840b | 51.00bc | 94.67b | 0.54c |
| | $D_2$ | 71.00cd | 83.00cd | 0.855bc | 102a | 114a | 0.895a | 64.33ab | 109.33ab | 0.59ab |
| | $D_3$ | 82.33bc | 92.67bc | 0.888ab | 116a | 129a | 0.901a | 72.00a | 117.33a | 0.61a |
| Fumin108 | $D_1$ | 49.33f | 67.00d | 0.830c | 64b | 74c | 0.854b | 53.67bc | 96.33b | 0.56bc |
| | $D_2$ | 65.00de | 78.00cd | 0.834c | 99a | 110a | 0.899a | 62.33ac | 107.00ab | 0.58ac |
| | $D_3$ | 95.00ab | 110.67ab | 0.860bc | 107a | 119a | 0.899a | 62.33ac | 108.67ab | 0.57ac |
| Dika159 | $D_1$ | 53.67ef | 66.67d | 0.832c | 72b | 84bc | 0.861b | 50.33c | 94.00b | 0.54c |
| | $D_2$ | 97.33a | 108.67ab | 0.895ab | 95a | 106ab | 0.893a | 59.67ac | 103.67ab | 0.57ac |
| | $D_3$ | 109.67a | 120.33a | 0.912a | 107a | 118a | 0.902a | 68.67a | 114.67a | 0.60ab |

Values followed by different small letters in the same column are significantly different from each other at $P \leq 0.05$

V12= Twelfth-leaf stage; VT= Tasseling stage; R20= 20 days after flowering; Fv = Maximum variable fluorescence; Fm = Maximum fluorescence; Fv/Fm = Maximum photochemical efficiency; $D_1$ = 15000 plants ha $^{-1}$; $D_2$ = 60000 plants ha $^{-1}$; $D_3$ = 90000 plants ha $^{-1}$

**Table 7:** Effect of planting density on root dry weight, root number and nitrogen content of 20 × 20 × 20 cm volume in 2019

| Varieties | Planting densities | VT | | | R6 | | |
|---|---|---|---|---|---|---|---|
| | | Root dry weight (g) | Number of roots | Nitrogen content (g) | Root dry weight (g) | Number of roots | Nitrogen content (g) |
| Xianyu335 | $D_1$ | 23.57c | 87a | 0.48b | 35.99b | 78b | 0.47a |
| | $D_2$ | 13.28de | 58bc | 0.18cd | 14.08cd | 51d | 0.15b |
| | $D_3$ | 5.90f | 48c | 0.08d | 8.00d | 48d | 0.07b |
| Fumin108 | $D_1$ | 38.29b | 97a | 0.54b | 53.11a | 101a | 0.65a |
| | $D_2$ | 20.75c | 76ab | 0.27c | 20.62c | 62bd | 0.22b |
| | $D_3$ | 10.56df | 59bc | 0.12cd | 12.40cd | 55d | 0.14b |
| Dika159 | $D_1$ | 55.76a | 95a | 0.78a | 56.94a | 105a | 0.63a |
| | $D_2$ | 17.86cd | 59bc | 0.21cd | 19.80cd | 77bc | 0.24b |
| | $D_3$ | 5.99ef | 64bc | 0.06d | 9.91cd | 59cd | 0.10b |

Values followed by different small letters in the same column are significantly different from each other at $P \leq 0.05$

VT= Tasseling stage; R6= Physiological maturity; $D_1$ = 15000 plants ha $^{-1}$; $D_2$ = 60000 plants ha $^{-1}$; $D_3$ = 90000 plants ha $^{-1}$

**Table 8:** Effect of planting density on soil nutrient status of 0-20 cm soil layer at maize maturity stage

| Varieties | Planting densities | $NH_4^+$-nitrogen (mg kg$^{-1}$) | | $NO_3^-$-nitrogen (mg kg$^{-1}$) | | Total nitrogen (g kg$^{-1}$) | | Soil organic matter (%) | |
|---|---|---|---|---|---|---|---|---|---|
| | | 2018 | 2019 | 2018 | 2019 | 2018 | 2019 | 2018 | 2019 |
| Xianyu335 | $D_1$ | 2.23cd | 6.77a | 21.53a | 27.63a | 1.61ab | 2.34a | 2.97bd | 5.41a |
| | $D_2$ | 2.88a | 4.20bc | 13.37d | 10.79c | 1.36b | 1.30a | 3.39ab | 3.00b |
| | $D_3$ | 1.93cd | 4.36bc | 19.89b | 7.04d | 1.45ab | 1.58a | 3.46a | 3.66ab |
| Fumin108 | $D_1$ | 1.93cd | 4.83b | 19.77b | 13.53b | 1.76a | 1.40a | 3.01ad | 3.31ab |
| | $D_2$ | 2.33bc | 3.90cd | 5.24f | 8.16d | 1.31b | 1.46a | 2.81cd | 3.27b |
| | $D_3$ | 2.02cd | 3.43d | 5.77ef | 12.79b | 1.42ab | 1.42a | 2.81cd | 3.32ab |
| Dika159 | $D_1$ | 2.68ab | 3.85cd | 17.28c | 10.62c | 1.69ab | 1.41a | 3.26ac | 2.94b |
| | $D_2$ | 1.80d | 4.16c | 6.49e | 8.23d | 1.50ab | 1.40a | 2.65d | 3.17b |
| | $D_3$ | 1.86d | 4.04cd | 13.54d | 12.49b | 1.48ab | 1.97a | 2.78d | 3.79ab |

Values followed by different small letters in the same column are significantly different from each other at $P \leq 0.05$

$D_1$ = 15000 plants ha $^{-1}$; $D_2$ = 60000 plants ha $^{-1}$; $D_3$ = 90000 plants ha $^{-11}$

indispensable and contribute differently to yield (Liu *et al.* 2018). Increase in density causes a series of changes in the root and leaf sources of maize plants that help adapt to changes in the external environment. Studies have pointed out that nitrogen uptake in plants is determined by the size of the root system. Longer root system increases surface

**Table 9:** Effect of planting density on plant dry matter accumulation at flowering and mature stages in three spring maize varieties

| Year | Varieties | Planting densities | VT Dry matter per plant (g) | R6 Dry matter per plant (g) | VT Dry matter per ha (kg ha$^{-1}$) | R6 Dry matter per ha (kg ha$^{-1}$) |
|---|---|---|---|---|---|---|
| 2018 | Xianyu335 | $D_1$ | 235.47a | 592.52a | 3438.33e | 8887.75c |
| | | $D_2$ | 119.70d | 403.59c | 7302.00d | 24215.60a |
| | | $D_3$ | 137.45cd | 271.01de | 11976.40a | 24391.20a |
| | Fumin108 | $D_1$ | 187.27b | 497.93b | 2839.33e | 7468.95c |
| | | $D_2$ | 163.60bc | 298.54d | 10148.00bc | 17912.20b |
| | | $D_3$ | 112.80d | 225.49e | 10551.00bc | 20294.10b |
| | Dika159 | $D_1$ | 188.27b | 471.97b | 2747.33e | 7079.60c |
| | | $D_2$ | 158.17bc | 325.72d | 9661.33c | 19543.20b |
| | | $D_3$ | 121.90d | 224.53e | 11259.00ab | 20208.00b |
| 2019 | Xianyu335 | $D_1$ | 199.33ab | 595.27a | 2990.00c | 8929.05c |
| | | $D_2$ | 175.33bc | 389.43b | 10520.00a | 23366.00ab |
| | | $D_3$ | 117.00d | 305.89cd | 10530.00a | 27530.40a |
| | Fumin108 | $D_1$ | 228.33a | 629.19a | 3425.00c | 9437.80c |
| | | $D_2$ | 149.67c | 351.80bc | 8980.00b | 21108.20b |
| | | $D_3$ | 102.00d | 268.02d | 9180.00ab | 24121.80ab |
| | Dika159 | $D_1$ | 208.00a | 597.45a | 3120.00c | 8961.70c |
| | | $D_2$ | 147.33c | 359.30bc | 8840.00b | 21557.80b |
| | | $D_3$ | 102.33d | 250.47d | 9210.00ab | 22542.60b |

Values followed by different small letters in the same column are significantly different from each other at $P \leq 0.05$
VT= Tasseling stage; R6= Physiological maturity; $D_1$ = 15000 plants ha$^{-1}$; $D_2$ = 60000 plants ha$^{-1}$; $D_3$ = 90000 plants ha$^{-1}$

**Table 10:** Effect of planting density on yield and related traits of spring maize varieties

| Year | Varieties | Planting densities | Number of grains per ear | Ears per unit area (ear ha$^{-1}$) | 1000-grainweight (g) | Grain yield (kg ha$^{-1}$) | HI (%) |
|---|---|---|---|---|---|---|---|
| 2018 | Xianyu335 | $D_1$ | 568ab | 29000cd | 395.87a | 6660.59de | 50.47c |
| | | $D_2$ | 558ac | 58500b | 388.84a | 12774.28ab | 60.30ab |
| | | $D_3$ | 423d | 85000a | 336.60b | 12849.23ab | 59.91ab |
| | Fumin108 | $D_1$ | 551abc | 36500c | 347.83b | 6899.54d | 62.62a |
| | | $D_2$ | 594a | 59000b | 343.18b | 12269.09ac | 57.30b |
| | | $D_3$ | 502bc | 81000a | 318.80bc | 13263.52a | 56.97b |
| | Dika159 | $D_1$ | 567ab | 26500d | 339.92b | 5327.13e | 46.02d |
| | | $D_2$ | 543ac | 59000b | 342.52b | 10916.48c | 58.59b |
| | | $D_3$ | 489cd | 82500a | 293.58c | 11594.72bc | 57.83b |
| 2019 | Xianyu335 | $D_1$ | 591a | 41026d | 410.39a | 9668.53c | 50.52d |
| | | $D_2$ | 572ab | 66667c | 391.01ab | 14892.06ab | 59.21ac |
| | | $D_3$ | 487c | 73504bc | 347.93cd | 12920.24b | 58.92ac |
| | Fumin108 | $D_1$ | 523ac | 44444d | 417.19a | 9237.41c | 59.37ac |
| | | $D_2$ | 574ab | 64957c | 377.40b | 13366.73b | 53.42cd |
| | | $D_3$ | 516bc | 83761ab | 326.98d | 13671.51b | 56.59bd |
| | Dika159 | $D_1$ | 489c | 41026d | 410.35a | 7842.52c | 65.43a |
| | | $D_2$ | 531ac | 66667c | 409.65a | 13509.27b | 58.70ac |
| | | $D_3$ | 508bc | 90598a | 372.18bc | 16254.92a | 61.08ab |

Values followed by different small letters in the same column are significantly different from each other at $P \leq 0.05$
$D_1$ = 15000 plants ha$^{-1}$; $D_2$ = 60000 plants ha$^{-1}$; $D_3$ = 90000 plants ha$^{-1}$

area of the root, which helps the plant to absorb more nitrogen (Zhu *et al.* 2016; Jia *et al.* 2020). The change in planting density changed the environmental conditions of maize at various growth stages. This affected growth and development of the root system, which in turn promoted nitrogen absorption, assimilation, and distribution in maize (Shi *et al.* 2016; Jia *et al.* 2018). In this study, increase in planting density of maize reduced the number of roots and the dry weight of roots. The replacement of varieties in cultivation did not alleviate the reduction in number of roots. Increase in planting density also reduced the soil nitrate nitrogen and ammonium nitrogen residues, which improved the nutrient use efficiency. Soil nitrate content had the highest nutrient use efficiency at medium planting density ($D_2$). Higher the density, higher the nutrient use efficiency; however, this theory holds true only within a certain density range. Nitrogen content of the root system clearly indicates that the increase in density reduced the absorption and utilization of nutrients per plant in maize. In the planting density range of $D_1$–$D_2$, the nutrient utilization efficiency of a single plant decreased, whereas of the group improved. At higher densities (above $D_2$), the number of roots significantly reduced and nutrient absorption and utilization by a single plant got restricted, which resulted in a decrease in nutrient utilization efficiency of the group.

Root system of a plant influences growth and development of the aboveground parts. In maize, the moisture and nutrient absorption capacity of the root system depends on the size and distribution in the soil and on the photosynthetic supply from aboveground parts. In turn, the root system provides the inorganic nutrients required for leaf growth and photosynthesis (Lu *et al.* 2017). Studies have found close interaction between roots and leaves of maize. Leaf area is closely related to root dry weight and total root absorption area (Chilundo *et al.* 2017; Liu *et al.* 2018). Additionally, leaf number and photosynthetic capacity are important parameters to determine yield (Zhang *et al.* 2017). In this experiment, with increase in density, plant leaf area and photosynthesis and fluorescence decreased, which resulted in a decrease in dry matter accumulation. Increase in density resulted in smaller leaves with lesser surface area for photosynthesis, the main source of material accumulation. Increase in density decreased stomatal conductance and intercellular $CO_2$ concentration, which significantly reduced the photosynthetic rate per unit time. Additionally, the decrease in leaf nitrogen content affected the leaf photosynthetic rate, which decreased plant dry matter accumulation. Significant difference was observed in dry matter per ha accumulation between $D_1$ and $D_2$ and not between $D_2$ and $D_3$. This finding indicates that in the low to medium density range (D1–D2), the increase in density reduced photosynthesis and dry matter accumulation of each plant; however, it increased dry matter accumulation of the group. At higher planting densities (above $D_2$), photosynthesis of a single plant played a major role in dry matter per ha accumulation, and therefore, the difference in dry matter per ha accumulation with increase in density was insignificant.

Planting density is one of the important factors that influence grain yield in maize and use of an optimal planting density is the best way to obtain high yield (Nyakudya and Stroosnijder 2014). In our study, medium ($D_2$) planting density significantly increased the yield per ha compared to low planting density ($D_1$). Medium ($D_2$) and high ($D_3$) planting densities showed no significant difference between each other in grain yield. These findings indicate that within the range $D_1$–$D_2$, increase in density significantly increased maize yield; however, further increase in density in the range $D_2$–$D_3$ did not increase the yield. In maize, number of ears per unit area is the main factor that contributes to yield increase. In the present study, in the range $D_1$–$D_2$, grains per ear and 1000-kernel weight remained almost the same; however, ears per unit area increased significantly with increase in density. In the range $D_2$–$D_3$, the differences in grains per ear, 1000-kernel weight, and ears per unit area were significant. The effects of grains per ear and 1000-kernel weight on yield may have predominated in the range $D_2$–$D_3$. Although ears per unit area increased, grains per ear and 1000-kernel weight decreased significantly with increase in density in the range $D_2$–$D_3$ with no increase in yield. Increase in density increased harvest index in the density range $D_1$–$D_2$. However, at low density, the proportion of total grains in total dry matter was relatively small and the transfer of photosynthetic products to the grains was low. These products remained concentrated in the stalks and leaves and resulted in waste of photosynthetic products. Therefore, increase in density increased harvest index. However, no significant increase was observed in the harvest index with increase in planting density from $D_2$ to $D_3$. Therefore, the planting density should be increased considering the local conditions.

In the current study, we studied the changes in root system in the 0–20 cm soil layer; however, there is a lack of research on deeper roots. In future, we will have to systematically explore the effects of variety and density on microbial diversity and nutrient absorption and utilization in the deep root soil.

## Conclusion

Increase in planting density reduced the root number and root dry weight of individual plants and all three varieties showed similar decrease in root number, which limited soil nutrient absorption and utilization. Increase in planting density weakened individual plant photosynthetic ability, while increased population dry matter accumulation. In conclusion, all three maize varieties harvested higher grain yield under planting density of 60,000 plants $ha^{-1}$ and density lower than that could cause wastage of soil and light resources.

## Acknowledgment

We acknowledge the financial supports of the National Key Research and Development Program of China under Grant No. 2016YFD0300103 and the National Natural Science Foundation of China under Grant No. 31701349.

## Author Contributions

Lichun Wang and Yongjun Wang conceived and designed the experiments; Qinglong Yang performed the experiments; Qinglong Yang, Xiwen Shao and Wenhua Xu analyzed the data; Yujun Cao, Yanjie Lv and Zhiming Liu contributed reagents/materials/analysis tools; Qinglong Yang wrote the paper.

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