Shoot and root traits of summer maize hybrid varieties with higher grain yields and higher nitrogen use efficiency at low nitrogen application rates

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Breeding high-yielding and nitrogen-efficient maize (*Zea* mays L.) hybrid varieties is a strategy that could simultaneously solve the problems of resource shortages and environmental pollution. Methods. We conducted a 2-year field study using four nitrogen application rates (0, 150, 225, and 300 kg N hm^{-2}) and two maize hybrid varieties (ZD958 and QS101) to understand the plant traits related to high grain yields and high nitrogen use efficiency (NUE).We found that ZD958 had a higher grain yield and nitrogen accumulation in the shoots at harvest as well as a higher NUE at lower nitrogen application rates (0 and 150 kg hm^{-2}) than QS101. The grain yields and NUE were almost identical for the two hybrid varieties at nitrogen application rates of 225 and 300 kg N hm^{-2}. Compared with QS101, ZD958 had higher above-ground and below-ground biomass amounts, a deeper root distribution, longer root length, root active absorption area, greater grain filling rate, and higher photosynthetic NUE than QS101 at lower nitrogen application rates. Our results showed that ZD958 can keep maintain a higher grain yield at lower nitrogen rates in a similar manner to N-efficient maize hybrid varieties. The selection of hybrids such as ZD958 with a deeper root distribution and higher photosynthetic NUE can increase the grain yield and NUE under low nitrogen conditions.

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Abstract

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21 that could simultaneously solve the problems of resource shortages and environmental pollution.
22 Methods. We conducted a 2-year field study using four nitrogen application rates (0, 150, 225,
23 and 300 kg N hm^{-2}) and two maize hybrid varieties (ZD958 and QS101) to understand the plant
24 traits related to high grain yields and high nitrogen use efficiency (NUE).We found that ZD958
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26 NUE at lower nitrogen application rates (0 and 150 kg hm^{-2}) than QS101. The grain yields and
27 NUE were almost identical for the two hybrid varieties at nitrogen application rates of 225 and
28 300 kg N hm^{-2}. Compared with QS101, ZD958 had higher above-ground and below-ground
29 biomass amounts, a deeper root distribution, longer root length, root active absorption area,
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Introduction

36 Tester and Langridge (2010) predicted that the global demand for food will increase by 70% by
37 2050, and thus there is an urgent need to address the problem of resource shortages in order to
38 meet future human needs (Good, 2004). Nitrogen fertilizer is the largest input resource for

39 agricultural production and it greatly improves crop yields (Guo et al., 2010). At present, the
40 nitrogen fertilizer application rate far exceeds the appropriate range in China (Fang et al., 2010).
41 The high nitrogen fertilizer application rate and low nitrogen use efficiency (NUE) have caused
42 groundwater and air pollution in many regions of China (Cui et al., 2010; Zhang et al., 2016).
43 Therefore, environmental and resource constraints mean that further increases in agricultural
44 production should be achieved by increasing the NUE rather than higher nitrogen inputs
45 (Hawkesford, 2014).
46   In 2005, China accounted for 38% of the global nitrogen consumptions but it only has 9% of
47 the world's arable land (FAO, 2011). Nitrogen fertilization-induced increases in grain yields have
48 been decreasing each year in China. Thus, the agricultural NUE decreased from 25 kg kg^{-1}
49 during 1958–1962 to 8 kg kg^{-1} during 1997–2003, which was significantly below than the lower
50 limit of the internationally recognized agricultural NUE range (10–30 kg kg^{-1}) (Dobermann,
51 2005). The NUE can be improved by adjusting the fertilization method employed, period, or
52 types (Abbasi et al., 2013; Zhao et al., 2013). Ciampitti and Vyn (2012, 2013) concluded that
53 modern varieties obtain relatively higher NUE levels than old varieties. However, the modern
54 breeding process is usually conducted on fertile soil or soil with high nitrogen fertilizer inputs,
55 and breeders have mainly focused on nitrogen absorption by plants and grain yields (Berlin &
56 Gallais, 2000), whereas they have neglected the performance of hybrid varieties under low
57 nitrogen conditions.
58   The selection of N-efficient hybrid varieties (hybrids with higher grain yield under low N
59 condition (Wu et al., 2011)) plays a significant role in improving the NUE. Differences in
60 nitrogen uptake and utilization vary significantly among genotypes (Kant et al., 2011; Bingham
61 et al., 2012), which are reflected in the responses of different varieties to nitrogen application, as
62 well as in the distribution and utilization of nitrogen in crops (Gallais & Coque, 2005). However,
63 there is still no general conclusion regarding the characteristics of high grain yield and high NUE
64 plants under low nitrogen conditions.
65   Roots play a supporting and fixing role in the soil, but they are also important organs that
66 allow plants to capture water and nutrients from the soil (Lynch, 2013; White et al., 2013).
67 Previous studies of roots have investigated the possibility of further improving crop productivity
68 and the NUE. The growth and distribution of roots are determined by genetic characteristics, and
69 they are also influenced by environmental factors. The morphology of roots is closely related to
70 the acquisition of soil resources and the development of plant shoots (Mi et al., 2010; Lynch,
71 2013; Li et al., 2017). The effects of various factors mean that roots respond to nitrogen in
72 significantly different manners in field trials compared with culture experiments (Wang et al.,
73 2003; Tian et al., 2005). The effects of nitrogen application on the roots are inconsistent under
74 field conditions, where some studies suggest that nitrogen application can promote root growth,
75 whereas others indicate that it can inhibit the growth of the root system (Chen et al., 2015; Feng
76 et al., 2016). There are reports showing that several plant shoot traits could also be associated
77 with grain yield and NUE in selecting for improving grain yield under low nitrogen conditions
78 (Banziger & Lafitte, 1997; Haegele et al., 2013; Chen et al., 2016; Talabi et al., 2017), such as

79 leaf longevity (stay-green), anthesis-silking interval, leaf chlorophyll concentration, grain N
80 accumulation and also number of kernels per ear. Banziger et al. (2006) and Morosini et al.
81 (2017) suggested that the development of low-N tolerant and N-efficient genotypes can result in
82 significant breeding progress. However, there have been few systematic studies of the shoots and
83 roots on the NUE in maize under field conditions.
84   In this study, we determined the characteristics of the shoots and roots in maize with high
85 yield and high NUE under lower nitrogen application rates. These results obtained in this study
86 provide insights into the mechanisms responsible for high grain yields and high NUE in maize,
87 thereby providing a theoretical basis to allow breeders to improve the NUE without reducing the
88 potential grain yield.

Materials and Methods

Field experiments

91 Field experiments were conducted at the Agricultural Experimental Station of Northwest
92 Agricultural and Forestry University (34°21′N, 108°10′E, 454.8 m altitude), Shaanxi province,
93 northwest China during the maize growing season (June to October) of 2014 and 2015. The soil
94 at the experimental site was loam soil with the following chemical properties in the top 60 cm
95 soil layer at the start of the experiment: organic matter content, 14.3 g kg^{-1}; total N, 1.09 g kg^{-1};
96 Olsen-P, 9.4 mg kg^{-1}; and NH$_4$OAc-K, 127 mg kg^{-1}.
97   The experimental design was a randomized block with three replications. Each plot comprised
98 one row with a length of 8 m and a row spacing of 60 cm, and the plants were spaced 25 cm
99 apart. Two high-yielding maize varieties currently used for local production were selected as the
100 typical contemporary maize hybrids: Zhengdan958 (ZD958: N-efficient hybrid) and
101 Qiangsheng101 (QS101: N-inefficient hybrid). And the two maize hybrids have the same growth
102 period. Plots were planted manually with two seeds per hill on June 17, 2014, and June 15, 2015.
103 They were thinned to obtain the desired plant population (67500 pl. ha) at V3. The date of
104 harvest was October 14, 2014 and October 15, 2015. Fertilizer N was sourced from urea (46%
105 N), evenly split in the fractions of 1/2 at pre-sowing and side-banded deep (5cm) into the soil on
106 the sowing rows of 1/2 at twelve-leaf stage. In addition, 150 kg phosphorus (P$_2$O$_5$) hm^{-2} as
107 calcium superphosphate (P$_2$O$_5$ 16%) and 150 kg potassium (K$_2$O) hm^{-2} as potassium sulfate
108 (K$_2$O 45%) were applied at pre-sowing. Irrigation was applied when the remaining 50 % of urea
109 (N 46 %) was applied. Plots were kept free of weeds, insects, and diseases according to standard
110 practices. Weather data were obtained from a local weather station approximately 100 m from
111 the experimental field. The daily mean precipitation and temperature data during the two
112 growing seasons are presented in Figure 1. During the two growing seasons, the effective
113 accumulated temperatures were 1617°C and 1565°C in 2014 and 2015, respectively, and the total
114 precipitation levels were 217 mm and 299 mm.

Sampling and measurements

Net photosynthetic rate (Pn) and photosynthetic nitrogen use efficiency (PNUE)

118   At anthesis from 10:00 to 12:00, three representative plants were selected from each plot and the
119   Pn values of the ear leaves were measured with a portable infrared gas exchange-based
120   photosynthesis analyzer system LI-6400 (LI-COR, Lincoln, NE, USA) while avoiding the midrib
121   (prevent leaf chamber leakage), which was coupled with a standard red/blue LED broadleaf
122   cuvette (6400-02B, LI-COR), under a consistent controlled light intensity of 1300 mmol m^{-2} s^{-1}.
123   After obtaining the photosynthesis measurements, the leaves were harvested to determine the
124   leaf area. The veins were then removed and the remaining parts were mixed and heated at 105°C
125   for 30 min, before drying to constant weight at 70°C. After determining the dry weight, the
126   sample was ground into powder and the nitrogen concentration was obtained using the Kjeldahl
127   method. Based on the leaf dry weight, leaf area, and nitrogen concentration, we calculated the
128   leaf specific nitrogen (SLN (g N m^{-2}) = leaf nitrogen content (g) /leaf area (m^2)) and PNUE
129   ((μmol CO$_2$.g^{-1} N s^{-1}) = Pn of ear leaves (μmol m^{-2} s^{-1}) /SLN (g N m^{-2})) (Sinclair and Horie,
130   1989).

Root length, dry weight and active absorption area

132   At anthesis, three representative and adjacent maize plants were selected in each plot the shoots
133   were collected and the root system was excavated to a depth of 60 cm, where it was divided into
134   three layers at every 20 cm using the soil profile method. In order to minimize any sampling and
135   measurement errors, each root system was excavated from an area of 0.15 m^2 (length = 0.6 m,
136   width = 0.25 m). The excavated roots were washed and enclosed in a plastic bag, before
137   scanning using a root scanner (with WinRHIZO scanning software). Then the dry weight was
138   determined after drying in an oven. Each scanned root image was processed using a root analysis
139   program (Regent Instruments Inc. WinRHIZO Pro 2007d) to obtain the root length in each layer
140   (Chen et al., 2015). The shoots were heated at 105°C for 30 min and then dried to constant
141   weight at 70°C before weighing. Root active absorption area were determined by methylene blue
142   dyeing method (Zhang et al., 1994).

Grain filling characteristics

144   During 2014 and 2015, beginning 4 days after anthesis, three tagged ears were sampled per
145   plot every 4 days until the grain reached physiological maturity. The grain in each row was
146   selected and stripped, before counting the total number of kernels removed from each ear. The
147   kernels were dried until constant weight in an oven at 75°C and then weighed with a balance.

Shoot biomass and grain yield

149   At maturity (when black layer formation was complete in all plants), the grain yield was
150   determined using plants from four undisturbed rows, where the plants on each side of the plot
151   were discarded to avoid border effects, and grain yield was expressed at 14% moisture.. Twenty
152   plants in each plot were used to determine the kernel number and 100-grain dry weight. The dry
153   weights of shoots from each plant were determined by oven-drying to constant weight at 75°C.
154   The plant nitrogen concentration was determined with the micro-Kjeldahl method (as described
155   by Nelson and Sommers (1973)), distillation, and titration to calculate the shoot N uptake.The
156   NUE was calculated according to the methods described by Xue et al. (2013) as well as the
157   following.

158 Agronomic efficiency of nitrogen fertilizer (AEN, kg kg^{-1}) = (grain yield with nitrogen
159 application – grain yield without nitrogen application) /amount of nitrogen applied
160 Nitrogen partial factor productivity (PFPN, kg kg^{-1}) = grain yield /amount of nitrogen applied
161 Nitrogen utilization efficiency (NUtE, kg kg^{-1}) = grain yield /nitrogen uptake by shoots at
162 maturity

Statistical analysis

164 The effects of treatments (hybrid variety and nitrogen application rate) and their interactions
165 were compared with analysis of variance (ANOVAs) for related traits performed in 2014 and
166 2015 using SPSS 18.0 software (SPSS Inc., Chicago, IL).. Multiple comparisons were performed
167 using Duncan's multiple range test and differences were considered statistically significant at P
168 < 0.05.
169 We analyzed the kernel dry weight dynamics using the logistic equation (Gu et al., 2001) in
170 Eq. (1):

171 $$W = \frac{A}{(1 + Be^{-Ct})^{\frac{1}{D}}},$$

172 Where W is the measured kernel dry weight (mg), t is the number of days after anthesis, A is
173 the final kernel weight, B is the initial value parameter, and C is the growth rate parameter. We
174 calculated the following grain filling characteristic parameters (Wang et al., 2014).
175 Days required to reach the maximum grain filling rate (Tmax, d) (2)

176 $$Tmax = \frac{\ln B}{C}$$

177 Grain weight at the maximum grain filling rate (Wmax, mg) (3)

178 $$Wmax = \frac{A}{2}$$

179 Maximum grain-filling rate (Gmax, mg grain^{-1} day^{-1}) (4)

180 $$Gmax = C \times Wmax - \frac{C \times Wmax^2}{A}$$

181 Final grain filling time (time taken for the grain weight to reach 99% (T0.99, days)) (5)

182 $$T0.99 = \frac{\ln B + 4.59512}{C}$$

183 Logistic equation for calculating the grain weight when t = T0.99 (W1, mg)   (6)

184 $$W1 = \frac{A}{(1 + Be^{-CT0.99})^{\frac{1}{D}}}$$

185 Average grain filling rate (Gave, mg grain^{-1} day^{-1}) (7)

186 $$Gave = \frac{W1}{T0.99}$$

187 The curves of the kernel dry weight dynamics were performed using SPSS 18.0 (SPSS Inc.,
188 Chicago, IL).

Results

Variance analysis

191   Table 1 shows the grain yield, yield components, shoot nitrogen uptake, and NUE in both years.
192   The hybrid varieties, nitrogen application rate, and their interactions had significant effects ($P <$
193   0.05) on the different variables (except for the kernel number). Measurements of other indicators
194   with similar results are not presented.

Grain yield and nitrogen use efficiency (NUE)

196   As shown in Table 2, at lower nitrogen application rates (N0 and N150), the grain yields were
197   significantly higher for ZD958 than QS101 at the same N rate ($P < 0.05$) There were no
198   significant differences ($P \geq 0.05$) in the grain yields from both hybrid varieties under normal
199   nitrogen application rates and higher application rates (N225 and N300) in both years. The grain
200   yield was higher at lower nitrogen rates with ZD958, which was mainly attributed to higher 100-
201   grain weight rather than the number of kernels compared with QS101 (Table 2).
202   Similar to the grain yield, the shoot nitrogen uptake, NUtE, and PFPN were significantly
203   higher for ZD958 than QS101 at lower nitrogen application rates. These indicators did not differ
204   significantly between the two hybrid varieties at N225 and N300 (Table 3). The AEN values
205   were similar for the two hybrids under N150. ZD958 had lower AEN values than QS101 under
206   the N225 and N300 treatments, mainly because QS101 had a much lower grain yield than that
207   ZD958 with nil nitrogen application. Thus, at low nitrogen rates, ZD958 with high grain yield
208   and NUE defined as an N-efficient maize hybrid, whereas QS101 with low grain yield and NUE
209   was defined as an N-inefficient maize hybrid. This confirms the choice of both hybrids.

Shoots and roots

211   Figure 2 shows the shoot and root biomasses and root/shoot ratios at anthesis. At lower nitrogen
212   application rates, the shoot and root biomasses were significantly higher with ZD958 than
213   QS101. These parameters did not differ significantly between the two hybrid varieties at N225
214   and N300 in both years.
215   At maturity, the shoot biomass was significantly greater with ZD958 than QS101 under the N0
216   and N150 treatments. Under N225 and N300, the shoot biomass did not differ significantly
217   between the two hybrid varieties. At the same nitrogen rate, there was no significant difference
218   in the root/shoot ratio between the two varieties. The root/shoot ratio increased initially and then
219   decreased as the nitrogen application rate increased for the same hybrid variety.
220   Figure 3-5 show the root dry weights, root lengths and root active absorption area for the two
221   hybrid varieties under different nitrogen application rates in both years. Under the N0 and N150
222   treatments, the root dry weight and root length in the 0–60 cm soil depth were significantly
223   higher for ZD958 than QS101. The root dry weight and root length in the 0–60 cm soil depth did
224   not differ significantly between the two hybrid varieties under the N225 and N300 treatments,
225   and they also did not differ significantly in the same hybrid between the N225 and N300
226   treatments. Moreover, the differences in the root dry weight and root length in the 0–20 cm soil
227   depth were not significant between the two hybrid varieties under all of the nitrogen rates,
228   whereas they differed significantly in the 20–60 cm soil depth. Thus, the differences between the
229   two hybrid varieties under the N0 and N150 treatments were related to differences in the deeper
230   roots. The root dry weight and root length increased as the nitrogen application rate increased,

231   and then decreased. Compared with the N225 treatment, the root dry weight and root length in
232   the 0–60 cm soil depth were significantly different in the N300 treatment because the root dry
233   weight in the 20–60 cm soil depth was significantly lower compared with that in the N225
234   treatment for both hybrid varieties (Fig. 3 and 4). Similar to root dry weight and root length, root
235   active absorption area of QS101 in the 20–40 cm and 40–60 cm soil layers decreased were
236   significantly lower than those of ZD958 under N0 and N150 treatments (Fig.3-5).

Leaf specific nitrogen (SLN), net photosynthetic rate (Pn), and
photosynthetic nitrogen use efficiency (PNUE)

239   The SLN, Pn, and PNUE results are shown in Figure 5. Under the N0 and N150 treatments, SLN
240   was significantly higher for ZD958 than QS101, but there were no significant difference between
241   the two hybrid varieties under N225 and N300. Similar to SLN, the Pn and PNUE values were
242   significantly higher for ZD958 than QS101 under the N0 and N150 treatments. The differences
243   between the two hybrid varieties were not significant for Pn and PNUE in N225 and N300. SLN
244   and Pn tended to increase with the nitrogen application rate, whereas PNUE exhibited a
245   decreasing trend (Fig. 6).

Grain filling characteristics

247   Table 4 shows the grain filling parameters for the two hybrid varieties under different nitrogen
248   application rates. Under N0 and N150 treatments, ZD958 exhibited a higher maximum grain
249   filling rate (Gmax, mg grain^{-1} d^{-1}), average grain-filling rate (Gave, mg grain^{-1} d^{-1}), and kernel
250   weight at the maximum grain filling rate (Wmax, mg) than QS101. QS101 required more days to
251   reach the maximum grain filling rate (Tmax, d) than ZD958.

Discussion

253   Many studies have shown that the application of nitrogen fertilizer can improve the
254   photosynthetic capacity, nitrogen content, total biomass, and grain yield but decrease the NUE
255   (Peng et al., 2016; Hammad et al., 2017). Similar to previous studies, our results showed that the
256   application of N300 increased the grain yield by 2–15% compared with N150. The PFPN value
257   under the N300 treatment was decreased by 42–49% compared with the N150 treatment in both
258   hybrid varieties (average of two years). Previous studies have rarely reported details of the plant
259   traits related to high grain yields and high NUE levels in maize hybrid varieties under low
260   nitrogen application rates.
261   It is well known that the NUE can be simply defined as the increase in the amount of nitrogen
262   that plants obtain from soil, or it can be defined as the more efficient use of absorbed nitrogen
263   (Garnett et al., 2009). Breeding hybrid varieties with both traits (especially under nitrogen-
264   deficient conditions) will be beneficial for agricultural sustainability (Garnett et al., 2009; Kant
265   et al., 2011). In this study, ZD958 obtained a higher yield than QS101 at low nitrogen rates (N0
266   and N150) and it absorbed more nitrogen from the soil while maintaining a higher NUE. These
267   results indicate the possibility of developing maize hybrid varieties with both higher nitrogen
268   uptake efficiencies and higher NUE levels. The grain yield increases of hybrid varieties with
269   high-yielding and high NUE range from 8% to 10%, and they can reduce the application of
270   nitrogen fertilizer by 16%, thereby demonstrating that it is feasible to cultivate new high yield

271   and high NUE maize varieties (Chen et al., 2013). Adu et al. (2018) also suggested maize grain
272   yield could increase under low-N supply at the same time maintaining the grain yield potential
273   under high-N conditions is achievable when NUE genotypes are adopted.

274   Most previous studies of the NUE in maize were conducted in potting conditions and few
275   studies investigated the shoot and root systems in hybrid varieties under field conditions. The
276   growth of plant shoots is closely associated with the size of the root system. Larger roots are
277   often beneficial for absorbing nutrients, thereby leading to a higher shoot biomass. The size and
278   morphology of the roots affect the absorption of nutrients, and the root length is an important
279   indicator of the effectiveness of root interception to acquire nitrogen. Thus, genotypes that
280   exhibit greater nitrogen uptake have a large root system, especially longer roots (Li et al., 2017;
281   Duan, 2019). In this study, under the low nitrogen conditions, we observed that the N-efficient
282   maize hybrid ZD958 had longer roots, higher root dry weights and root active absorption area
283   than the N-inefficient maize hybrid QS101 at lower N rates, which was mainly attributable to the
284   longer roots and higher root dry weight in the 20–60 cm soil depth. These findings suggest that
285   the N-efficient maize hybrid had a greater capacity for growing longer roots, which was reflected
286   by the aboveground performance in terms of its greater shoot biomass and grain filling rate
287   compared with QS101. A variety with a greater capacity for growing longer roots can maximize
288   the capture of nitrogen from the soil (Garnett et al., 2009; Mi et al., 2010), and we found that the
289   nitrogen content and shoot biomass of ZD958 was significantly higher than that of QS101.
290   Studies also have shown that the deep root environment of the soil was relatively stable, which
291   helps to enhance the buffering capacity to the adverse soil environment (Wasson et al., 2012),
292   improve the stress resistance, delay root senescence, and maintain root nutrient and water supply
293   to the shoot, obtain high grain yield (Saengwilai et al., 2014).Therefore, we conclude that under
294   low nitrogen conditions, larger shoot and root biomasses as well as higher grain filling rates will
295   contribute to better grain yields, and thus higher NUE levels for N-efficient maize hybrids.

296   The supply of nitrogen affects the growth of roots. Numerous studies have investigated the
297   effects of the nitrogen supply on the growth of maize roots under field conditions (Tian et al.,
298   2006; Wu et al., 2011; Liu et al., 2017). Nitrogen application has a positive effect on root growth
299   (Liu et al., 2009; York et al., 2015) but also adverse effects when provided in excess (Tian et al.,
300   2008; Chen et al., 2015). Root growth has a parabolic linear relationship with the nitrogen
301   application rate and excessive nitrogen application limits the growth of the root system (Feng et
302   al., 2016). Our results are similar to those obtained in previous studies. The length and dry
303   weight of the maize roots increased with the nitrogen application rate, but the length of the roots
304   decreased in the N300 treatment (Fig. 3 and 4), where this difference occurred mainly in the 20–
305   60 cm soil depth. The root system was obviously shallower in the N300 treatment than the N225
306   treatment and it was mostly distributed in the upper part of the soil. The root length and root dry
307   weight were also small, especially the root length in the N300 treatment. The same trend was
308   observed for both genotypes. This could explain why the maize grain yield did not increase
309   under higher nitrogen conditions.

310   Previous studies showed that N-efficient maize varieties obtained higher PNUE values
311   (Echarte et al., 2008; Chen et al., 2014). Similar to previous studies, we determined differences
312   in SLN and Pn between the two hybrid varieties. PNUE can be expressed as the ratio of Pn
313   relative to SLN. At the low nitrogen rate, ZD958 had higher SLN and Pn as well as PNUE
314   values, which indicated that the higher PNUE was attributed to a higher Pn value. A higher
315   PNUE can increase the grain filling rate and plant biomass, but also the NUE. The physiological
316   mechanism responsible for the higher PNUE in N-efficient maize hybrids under low nitrogen
317   conditions is not clear. However, it is possible that hybrid varieties with higher PNUE values can
318   allocate less nitrogen to their non-photosynthetic components than hybrids with lower PNUE
319   values (Hikosaka, 2004; Mu et al., 2016). In this study, we only investigated two varieties that
320   differed significantly in terms of their performance under low nitrogen conditions. The selection
321   of varieties was limited and thus it would be useful to select more varieties for further
322   assessments.

Conclusions

324   In this study, low nitrogen application rates substantially reduced shoot and root growth, nitrogen
325   uptake, and the grain yield in maize. The N-efficient maize hybrid variety obtained a higher
326   grain yield, greater shoot nitrogen uptake to the shoots from the soil, and a higher NUE at low
327   nitrogen application rates. Higher root and shoot biomasses, a deeper root distribution, longer
328   root length, root active absorption area, and a higher leaf PNUE are beneficial for the grain yield
329   and NUE. Thus, it is possible to obtain a high grain yield and NUE in maize under low nitrogen
330   conditions. The plant traits comprising a higher PNUE and deeper root distribution can be used
331   as selection criteria in breeding programs to select N-efficient hybrid varieties for growth in low
332   nitrogen conditions.

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Analysis-of-variance of grain yield, yield components, shoot nitrogen uptake and nitrogen use efficiency of maize between/among hybrid variety and nitrogen application rates in 2014 and 2015.

ns Not significant at the P = 0.05 level. * Significant at the P = 0.05 level. ** Significant at the P = 0.01 level. ‡ Significant at the P = 0.1 level. AEN, agronomic efficiency of nitrogen

fertilizer (kg kg^{-1}); PFPN, nitrogen partial factor productivity (kg kg^{-1}); NUtE, nitrogen

utilization efficiency (kg kg^{-1})

Year	Source of variation	Grain yield(t hm^{-2})	100-dry grain weight(g)	Grain number(ear^{-1})	Shoot nitrogen uptake(kg hm^{-2})	AEN	PFPN	NUtE
2014	Hybrid variety (H)	**	**	ns	**	*	*	**
	N rates(N)	**	**	**	**	**	**	**
	H*N	*	*	ns	‡	*	*	*
2015	Hybrid variety(H)	*	*	ns	**	*	*	**
	N rates (N)	**	**	**	**	**	**	**
	H*N	‡	‡	ns	*	*	*	*

Grain yield and yield components of maize hybrid varieties under four nitrogen application rates in 2014 and 2015.

N rate	Hybrid variety	2014			2015		
		100-dry grain weight (g)	Grain number (ear^{-1})	Grain yield (t hm^{-2})	100-dry grain weight (g)	Grain number (ear^{-1})	Grain yield (t hm^{-2})
N0	QS101	28.5c	417.56c	7.36c	27.73d	428.67d	7.71c
N150	QS101	28.65c	488.81ab	8.44b	30.97b	496.45b	8.74b
N225	QS101	31.34a	506.22ab	9.65a	32.64a	523.49ab	10.34a
N300	QS101	31.17a	502.81ab	9.76a	32.28a	495.3b	10a
N0	ZD958	30.02b	456.95cb	8.45b	29.34c	461.73c	8.69b
N150	ZD958	31.74a	523.87a	9.57a	32.51a	519.96ab	9.78a
N225	ZD958	31.44a	508.64ab	9.66a	32.68a	558.13a	10.26a
N300	ZD958	31.58a	544.07ab	9.72a	32.87a	518.93ab	10.06a

Shoot nitrogen uptake and nitrogen use efficiency of two maize hybrid varieties under four nitrogen application rates in 2014 and 2015.

AEN, agronomic efficiency of nitrogen fertilizer (kg kg^{-1}); PFPN, nitrogen partial factor

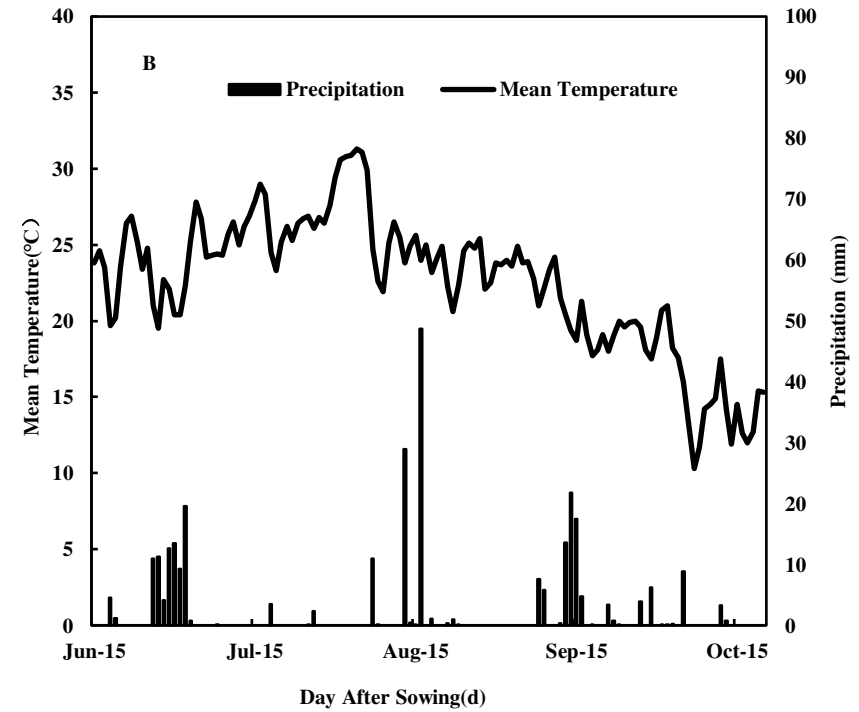
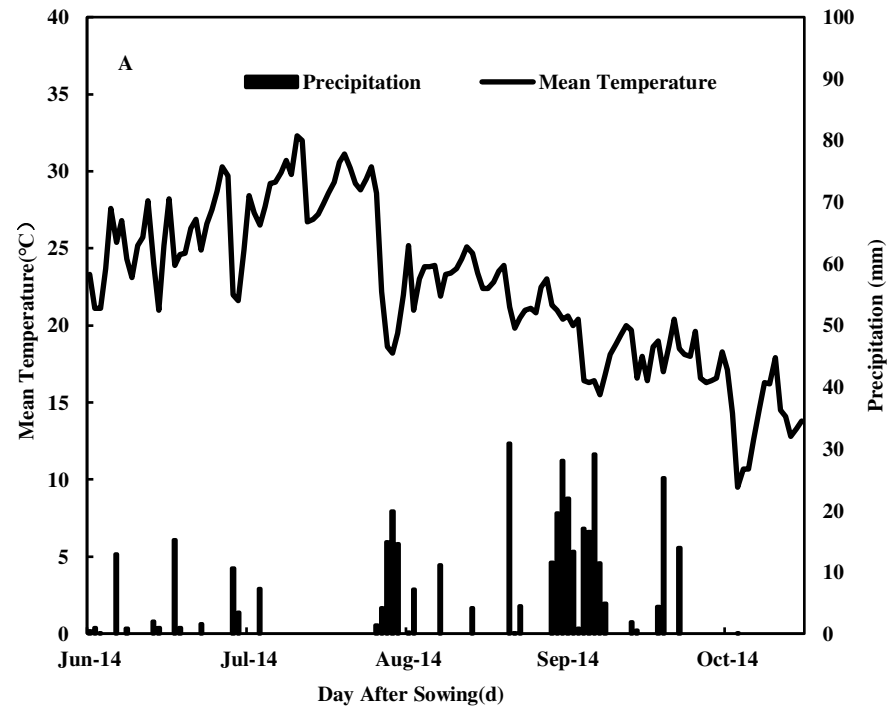
productivity (kg kg^{-1}); NUtE, nitrogen utilization efficiency (kg kg^{-1})

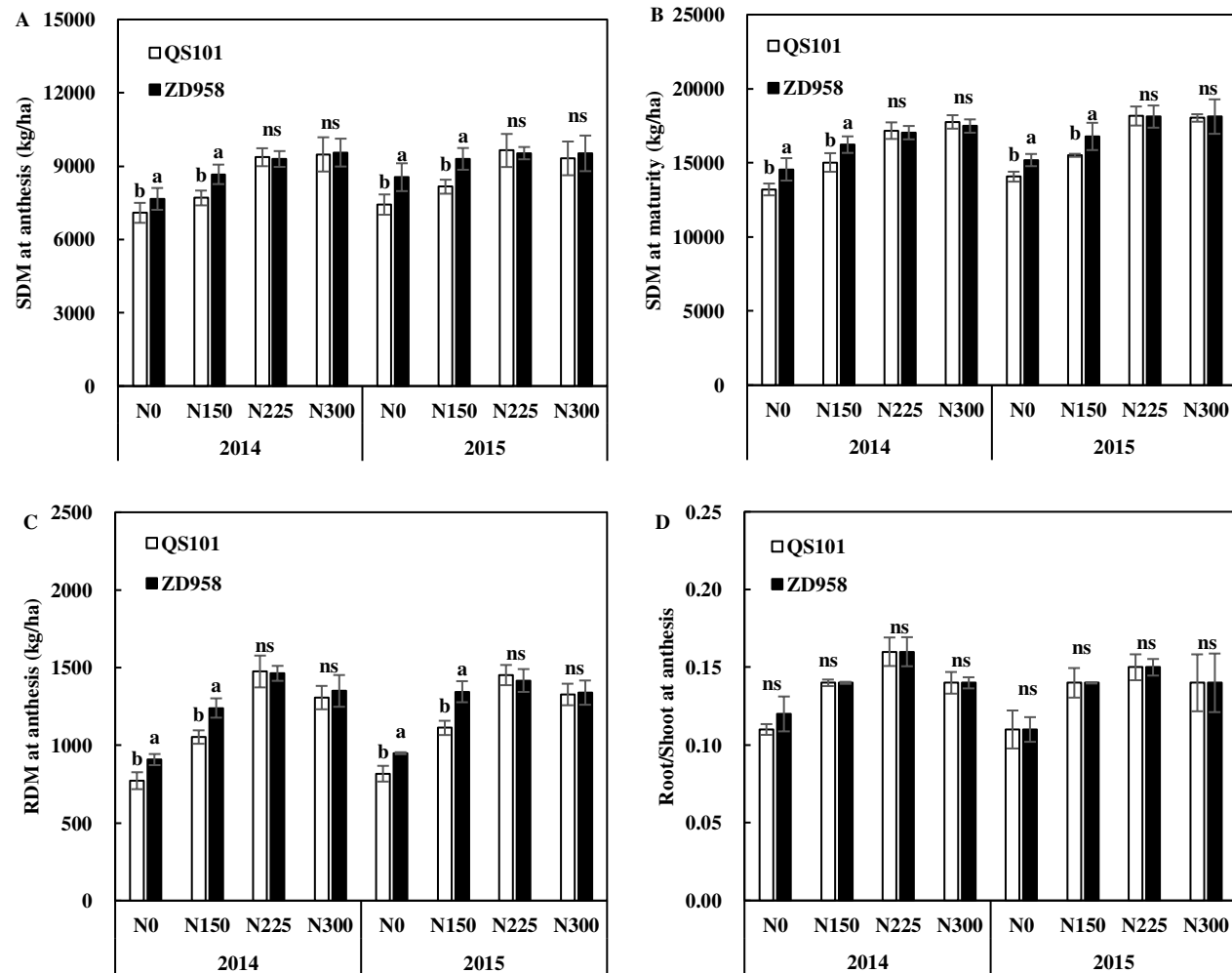
		2014				2015			
N rate	Hybrid variety	Shoot N uptake(kg hm^{-2})	AEN	PFPN	NUtE	Shoot N uptake(kg hm^{-2})	AEN	PFPN	NUtE
N0	QS101	115f	-	-	54.91b	117f	-	-	56.73b
N150	QS101	150d	7.18b	56.27b	48.47d	148d	6.86b	58.25b	50.66d
N225	QS101	190b	10.14a	42.87c	43.71e	186b	11.69a	45.95c	47.8e
N300	QS101	206a	7.99b	32.54c	40.89e	189a	7.65b	33.34c	45.42e
N0	ZD958	121e	-	-	59.78a	126e	-	-	59.19a
N150	ZD958	156c	7.46b	63.8a	52.65c	157c	7.26b	65.17a	53.65c
N225	ZD958	191b	5.35b	42.91c	43.39e	184b	7b	45.6c	47.94e
N300	ZD958	205a	4.22c	32.4c	40.84e	191a	4.57c	33.52c	45.19e

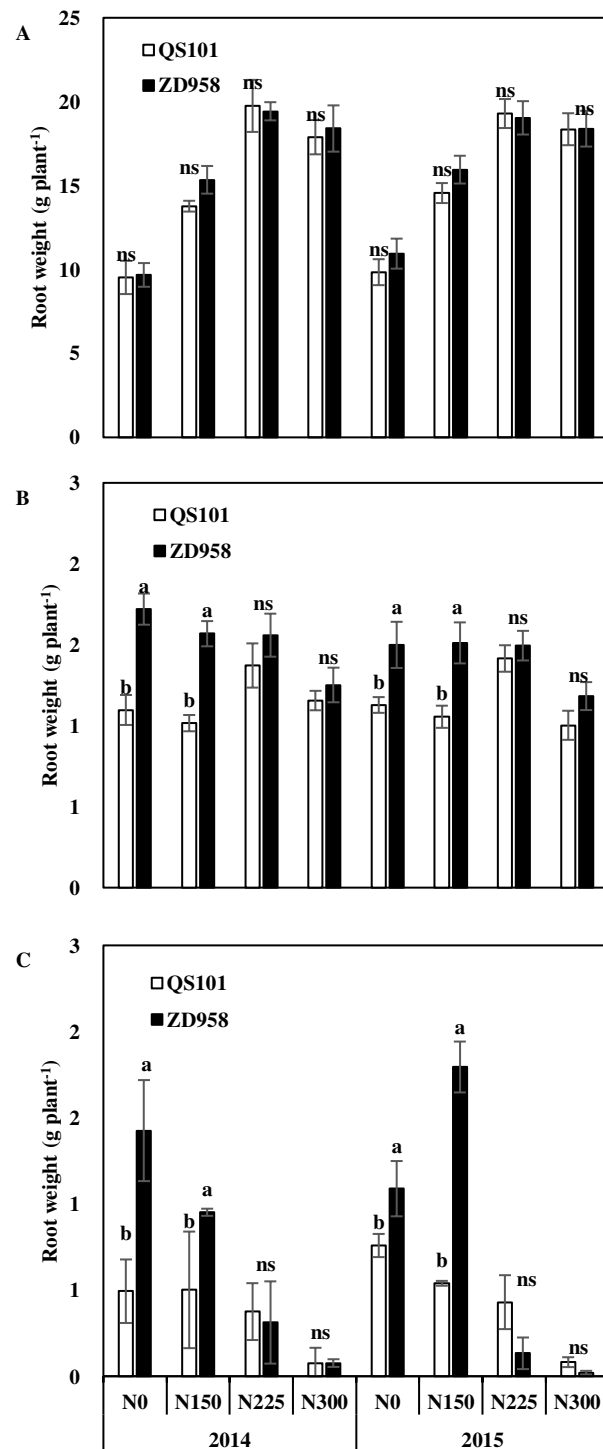
Characteristic parameters of two maize hybrid varieties under four nitrogen application rates during grain-filling stage in 2014 and 2015.

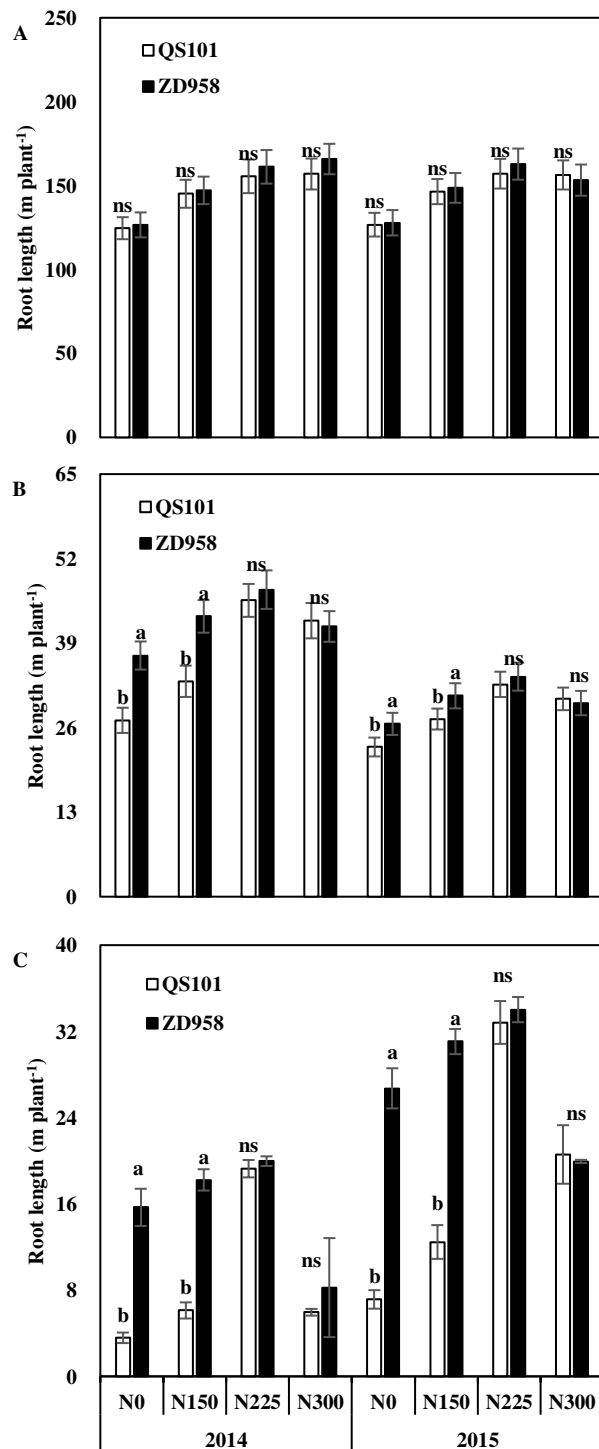
N0-QS, N150-QS, N225-QS, and N300-QS represent hybrid variety QS101 under 0,150,225, and 300 kg hm^{-2} nitrogen application rates respectively; N0-ZD, N150-ZD, N225-ZD, N300-ZD represent hybrid variety ZD958 under 0,150,225, and 300 kg hm^{-2} nitrogen application rates respectively. Tmax, the days needed for reaching the maximum grain filling rate (d); Wmax, the grain weight at the maximum grain filling rate (mg); Gmax, the maximum grain-filling rate (mg grain^{-1} d^{-1}); T0.99, the final grain filling time (the time of grain weight reaches 99%, d) ; Gave, the average grain-filling rate (mg grain^{-1} d^{-1})

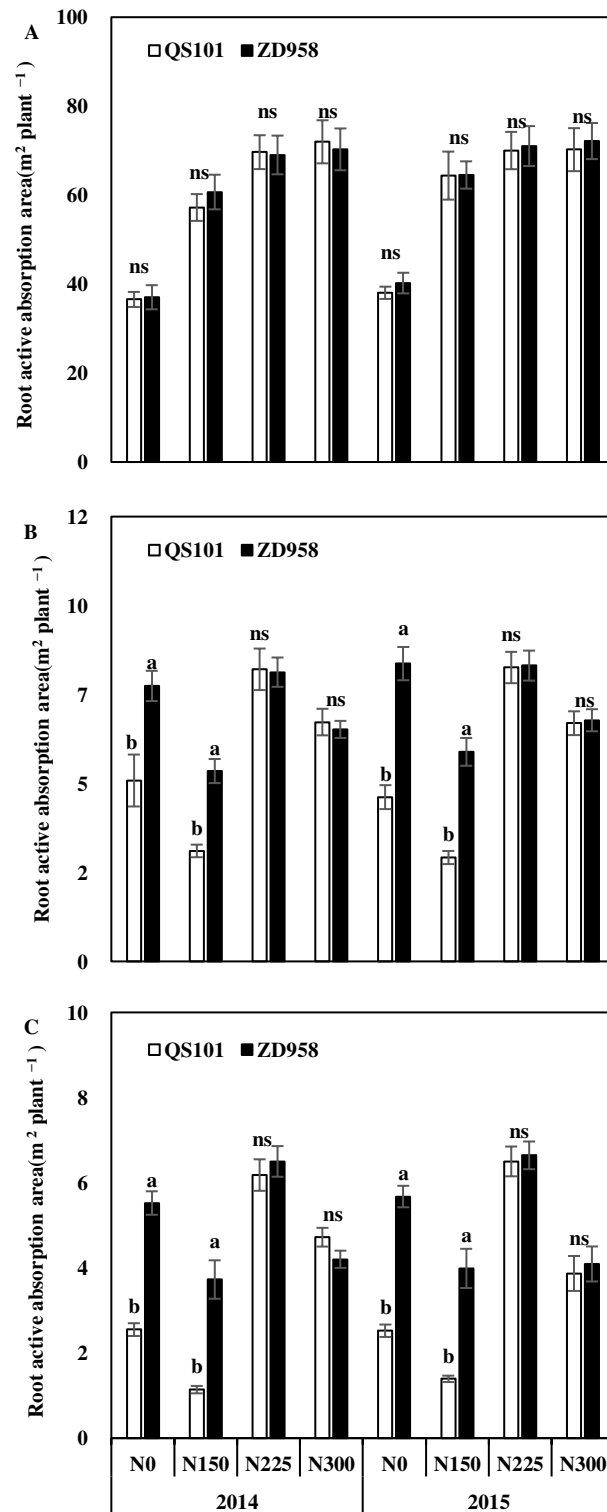
Year	Parameters	N0-QS	N150-QS	N225-QS	N300-QS	N0-ZD	N150-ZD	N225-ZD	N300-ZD
2014	A	21.85	25.72	28.09	28.46	25.44	28.15	28.22	28.38
	B	108.90	97.59	104.93	123.51	59.33	61.62	60.40	68.62
	C	0.21	0.20	0.20	0.19	0.19	0.19	0.19	0.19
	Tmax	22.59	22.95	23.51	24.95	21.07	21.32	21.85	22.43
	Gmax	1.13	1.28	1.39	1.37	1.23	1.36	1.32	1.34
	Wmax	10.92	12.86	14.05	14.23	12.72	14.07	14.11	14.19
	Gave	0.48	0.55	0.60	0.58	0.56	0.62	0.60	0.60
	T0.99	44.72	45.97	46.72	48.75	44.79	45.10	46.33	46.80
2015	A	22.95	26.99	31.49	31.52	26.56	29.86	31.25	31.13
	B	64.47	62.79	68.15	79.76	42.08	44.72	44.32	50.17
	C	0.19	0.18	0.18	0.18	0.18	0.18	0.18	0.18
	Tmax	22.36	22.78	23.41	24.94	20.79	21.08	21.65	22.25
	Gmax	1.07	1.23	1.42	1.38	1.19	1.35	1.37	1.37
	Wmax	11.48	13.49	15.75	15.76	13.28	14.93	15.62	15.57
	Gave	0.48	0.56	0.64	0.61	0.57	0.63	0.65	0.64
	T0.99	47.02	48.06	48.88	51.12	46.34	46.58	47.89	48.36

Daily mean temperature, precipitation during the maize growing seasons in 2014 (A) and 2015 (B) at Yangling District, Shaanxi Province, China.

Shoot dry matter (SDM) at anthesis (A) and maturity (B), root dry matter (RDM, C) and root/shoot at anthesis (D) of maize under various nitrogen rates in 2014 and 2015.

Vertical bars represent ± standard error of the mean (n =3) where these exceed the size of the symbol. Different lowercase letters above the column indicate statistical significance at the P = 0.05 level within the same N rate, and ns means not significant at the P = 0.05 level.

Root dry weight of maize in 0–20 cm (A), 20-40 cm (B) and 40-60 cm (C) soil layer at anthesis of two hybrid variety under various nitrogen (N) application rates in 2014 and 2015.

Data are averages observed for three replications. Vertical bars represent ± standard error of the mean (n =3) where these exceed the size of the symbol. Different lowercase letters above the column indicate statistical significance at the P = 0.05 level within the same N rate, and ns means not significant at the P = 0.05 level.

Root length of maize in 0–20 cm (A), 20-40 cm (B) and 40-60 cm (C) soil layer at anthesis of two hybrid variety under various nitrogen (N) application rates in 2014 and 2015.

Data are averages observed for three replications. Vertical bars represent ± standard error of the mean (n =3) where these exceed the size of the symbol. Different lowercase letters above the column indicate statistical significance at the P = 0.05 level within the same N rate, and ns means not significant at the P = 0.05 level.

Root active absorption area of maize in 0–20 cm (A), 20-40 cm (B) and 40-60 cm (C) soil layer at anthesis of two hybrid variety under various nitrogen (N) application rates in 2014 and 2015.

Data are averages observed for three replications. Vertical bars represent ± standard error of the mean (n =3) where these exceed the size of the symbol. Different lowercase letters above the column indicate statistical significance at the P = 0.05 level within the same N rate, and ns means not significant at the P = 0.05 level.

Specific leaf nitrogen (SLN, A), net photosynthetic rate (Pn, B) and photosynthesitic nitrogen use efficiency (PNUE, C) of two maize hybrid varieties under four nitrogen rates at anthesis in 2014 and 2015.

Vertical bars represent ± standard error of the mean (n =3) where these exceed the size of the symbol. Different lowercase letters above the column indicate statistical significance at the P = 0.05 level within the same N rate, and ns means not significant at the P = 0.05 level.