

Article

# Nitrogen Split Application Can Improve the Stalk Lodging Resistance of Maize Planted at High Density

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**Abstract:** The decrease of maize stalk quality is an important reason for stalk lodging during the grain filling stage. In the present study, a maize cultivar was planted at densities of 7.5, 9.0, 10.5, 12.0, and  $13.5 \times 10^4$  plants  $\text{ha}^{-1}$  and subjected to nitrogen application rates of 0, 270, 360, and 450  $\text{kg ha}^{-1}$  (denoted as N0, N270, N360, and N450). The stalk breaking force, mechanical strength, carbohydrate content, and nitrogen content of basal internodes were determined to study the effects of nitrogen application rate on the stalk lodging resistance of maize under different planting densities with integrated watering and fertilization using drip irrigation. At densities of 7.5 to  $10.5 \times 10^4$  plants  $\text{ha}^{-1}$ , the stalk breaking force, rind penetration strength (RPS), and crushing strength (CS) of the basal internode decreased first and then increased with increasing nitrogen application rate, with the lowest values obtained for the N270 treatment. Meanwhile, at planting densities of  $12.0 \times 10^4$  plants  $\text{ha}^{-1}$  and above, the stalk breaking force, RPS, and CS increased with increasing nitrogen application rate. The basal internode dry weight per unit length (DWUL) and total N content increased with increasing nitrogen application rate. The breaking force was significantly positively correlated with the DWUL and mechanical strength of the basal internode. The RPS showed a positive linear correlation with the contents of cellulose, lignin, and total N of the third internode. Under the split application of water and fertilizer, the maize stalk total dry matter and contents of cellulose, lignin, and total nitrogen increased with increasing nitrogen fertilization rate during the grain filling stage at high planting density, so the stalk lodging resistance improved.

**Keywords:** nitrogen fertilizer; planting density; drip irrigation; lodging resistance; stalk strength

## 1. Introduction

Maize lodging results in reduced yield [1,2], reduces the efficiency of mechanical harvesting, and increases harvesting costs [3–5]. Lodging includes root lodging and stalk lodging, with the latter causing higher loss of maize yield than the former [6]. Stalk lodging occurs most frequently at the basal internodes above the soil surface during grain filling [7,8]. This is because during the filling stage of maize, ear weight gradually increases, thus raising the maize plant's center of gravity. At the same time, carbohydrates in the stalk are transported to the grain, which reduces stalk quality and increases the ease of stalk breakage. Under dense planting conditions, the radiation quantities are low in the

lower part of the canopy and leaf aging accelerates [9], which causes stalk quality to decline and the stalk lodging rate to increase in the filling stage [10]. Therefore, improving the stalk quality of maize in the filling stage under dense planting conditions is an important measure that can be taken to improve its lodging resistance.

Lodging resistance in maize has been related to some of its morphological characteristics, such as plant height; diameter and length of the basal internode; rind thickness and unit length dry weight of the basal section; stalk breaking strength and crushing strength; stalk diameter, weight, and density; and rind penetrometer resistance [11–13]. Lodging resistance has also been related to cell wall structural components such as lignin and cellulose [14,15]. The breaking force of maize stalks can be used to determine the varieties with higher resistance to stalk breakage and a lower risk of stalk lodging in the field [16]. The basal internodes of maize stalks play a vital role in lodging resistance [17,18]. Measures of the mechanical strength of basal internodes, such as the rind penetration strength (RPS), crushing strength (CS), and bending strength (BS), are important factors affecting the stalk mechanical resistance [1] and have been shown to be significantly negatively correlated with the rate of stalk lodging in the field [19,20]. Cellulose and lignin are the main components of cell walls and play an important role in the mechanical strength of the stalk and accordingly in its ability to resist stalk lodging, while soluble carbohydrate also has an important effect on the plumpness and mechanical strength of stalks [21].

Stalk lodging resistance is affected by many factors, including genotype, environment, and cultivation practices [17,22,23]. Previous studies were conducted at low and medium plant density, while high plant density decreased the duration of internode thickening and dry matter accumulation, which caused the diameter and dry weight per unit length to decline, resulting in lodging [10]. N supply can significantly improve the stalk quality and decrease the risk of stalk lodging [24]. However, high nitrogen (N) and phosphorus (P) content can increase the elongation rate and length of the basal internode and significantly reduce the cellulose content of maize stalks [25,26], thereby decreasing stalk strength and increasing the lodging rate. Meanwhile, it was found that drip irrigation combined with reduced irrigation and slow-release N fertilizer effectively promoted maize rooting and increased yield [27], while splitting N into three applications proved to be a better strategy for all of the selected winter and summer cereals [28]. However, it was seldom mentioned how nitrogen application affected stalk lodging resistance under the conditions of close planting and multiple nitrogen fertilizer applications with irrigation.

The planting density and yield of maize are high in Northwest China; in this region, integrated mulch drip irrigation and fertilization is one of the key technologies used to increase planting density and yield. In recent research by our team [29], it was found that pure nitrogen application rates of 360 kg ha<sup>-1</sup> of nitrogen at 12.0 plants m<sup>-2</sup> obtained the highest grain yield (21.5–21.6 t ha<sup>-1</sup>) and economic return (USD 3399.7–3440.3 ha<sup>-1</sup>) and a relatively high nitrogen partial factor productivity (59.7–60.1 kg kg<sup>-1</sup>) and nitrogen agronomic efficiency (23.7–25.1 kg kg<sup>-1</sup>). However, it has not yet been clarified how the nitrogen application and plant density affect stalk lodging resistance of maize under the case of applying extreme doses of fertilization at extremely high sowing density. Therefore, we hypothesized that nitrogen split application can improve the stalk lodging resistance of maize planted at high density. To test this hypothesis, maize was planted under the split application of the conditions of water and fertilizer at different planting densities and with different nitrogen fertilizer treatments. The aim of this study was to explore the influences of nitrogen application rates on stalk lodging resistance at various planting densities and then to explain the potential mechanism. The differences in stalk mechanical strength and carbohydrate and N contents were determined for different treatments in order to determine the effect of nitrogen application rate and planting density on the lodging resistance of stalks. The results provide theoretical reference and a practical basis for the high-density cultivation and lodging resistance of maize under mulch drip irrigation.

## 2. Materials and Methods

### 2.1. Experimental Station

The experiment was conducted between 2017 and 2018 at the Experimental Demonstration Base of the Xinjiang Qitai Farm (43°50' N, 89°46' E; altitude: 1020 m a.s.l.) of the Crop Science Institute of the Chinese Academy of Agricultural Sciences. In this area, the rainfall is scarce, the soil is barren, and the light resources are sufficient. Precipitation and air temperature were measured by the Watch Dog Weather Station data loggers (Spectrum Technologies, Inc., Washington, DC, USA) at the experiment site. Selected monthly weather conditions during the experiment and their historical averages are shown in Table 1.

**Table 1.** Precipitation and air temperature during the 2017 and 2018 growing seasons.

Month	Precipitation (mm)			Average Temperature (°C)			Maximum Temperature (°C)			Minimum Temperature (°C)		
	2017	2018	2007–2016 Average	2017	2018	2007–2016 Average	2017	2018	2007–2016 Average	2017	2018	2007–2016 Average
May	42.7	59.5	26.7	17.2	13.7	17.0	23.6	21.0	24.9	10.7	6.5	9.3
June	72.2	6.2	25.0	21.0	20.9	21.8	27.3	27.7	29.3	14.9	13.9	14.2
July	0.9	15.9	35.5	24.3	21.9	23.2	30.7	28.4	31.1	17.9	15.2	15.5
August	18.9	88.0	28.5	20.5	21.1	21.7	27.1	27.8	30.4	14.4	14.8	13.9
September	13.5	30.9	17.8	14.9	12.7	15.5	22.4	20.6	24.6	8.1	5.9	8.1
October	27.8	28.1	15.7	5.2	6.4	7.8	12.6	13.9	16.4	−0.7	0.5	1.9
Total/average	176.0	228.6	137.3	17.2	16.1	17.8	24.0	23.2	26.1	10.9	9.5	10.5

The two-year trial was conducted at two different experimental fields in the same area with the same soil properties. The soil in the fields was sandy loam with 13.6% clay, 39.2% silt, and 47.2% sand. The alkali-hydrolyzed nitrogen was tested by the alkaline hydrolysis diffusion method [30], the available phosphorus in soils was tested by extraction with sodium bicarbonate [31], the available potassium was assessed with a modified sodium tetraphenylboron method [32], the organic matter content of the soil was determined by potassium dichromate volumetric method coupled with an external heating method [33], and the soil pH was determined by a PHS-3E model pH meter (Shanghai Instrument Science Instrument Co., Ltd., Shanghai, China). The determined soil nutrient contents are shown in Table 2.

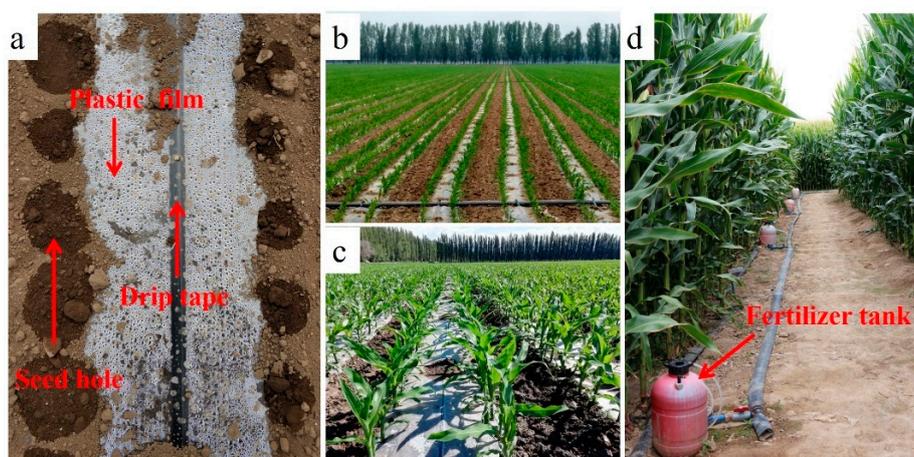
**Table 2.** Soil nutrient contents in the experimental field.

Year	Alkali-Hydrolyzed Nitrogen (mg kg <sup>−1</sup> )	Available Phosphorus (mg kg <sup>−1</sup> )	Available Potassium (mg kg <sup>−1</sup> )	Organic Matter (g kg <sup>−1</sup> )	pH
2017	81.5	65.9	147.3	14.3	8.0
2018	73.4	59.1	93.8	11.8	7.9

### 2.2. Experimental Design

The maize variety studied was Xianyu 335. A two-factor experimental design was used, and three repetitions were performed. Three different planting densities were used in 2017, namely 7.5, 10.5, and 13.5 × 10<sup>4</sup> plants ha<sup>−1</sup>, and some trends in these plant densities were found. Therefore, we reduced the density gradient from 3.0 × 10<sup>4</sup> to 1.5 × 10<sup>4</sup> plants ha<sup>−1</sup> in 2018. Five planting densities were used in 2018, namely 7.5, 9.0, 10.5, 12.0, and 13.5 × 10<sup>4</sup> plants ha<sup>−1</sup>. The planting density used by local farmers was 9.0 × 10<sup>4</sup> plants ha<sup>−1</sup>, and the highest grain yields were obtained at 12.0 × 10<sup>4</sup> plants ha<sup>−1</sup> [29]. In order to explore the trend of stalk lodging resistance of maize under the treatments of high nitrogen application and high plant density, four nitrogen fertilization levels were set in both years, namely no nitrogen fertilizer (N0) and 270, 360, and 450 kg ha<sup>−1</sup> of pure nitrogen (N270, N360, and N450,

respectively). N360 is the N application rate used by local farmers. Wide—narrow row planting (70 cm + 40 cm) and integrated plastic-film mulch with surface drip irrigation and fertilization were employed, and each nitrogen treatment was applied to separate plots with no interaction. A joint planter was used to synchronize these procedures so that drip tape was followed by plastic film with holes punched for seedling growth [34]. Seeds were planted along each row and covered with thin soil (Figure 1a). The plastic film was transparent with a width of 70 cm and a thickness of 0.01 mm. The area of each plot was 88 m<sup>2</sup> (length: 10 m; width: 8.8 m), and each treatment included three replications. Before sowing, 108 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> and 37.5 kg ha<sup>-1</sup> K<sub>2</sub>O were applied in treatment N0, and 36 kg ha<sup>-1</sup> N, 108 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>, and 37.5 kg ha<sup>-1</sup> K<sub>2</sub>O were applied in treatments N270, N360, and N450. In the latter three treatments, the remainder of the nitrogen dressing was applied with equal ratio in the V9 (ninth leaf), V12 (twelfth leaf), VT (tasseling), R2 (blister stage), and R3 (milk stage) stages by drip irrigation (Figure 1d). The total irrigation amount was the optimal amount (540 mm), as determined in a previous study on drip irrigation with plastic-film mulching systems in arid regions [34]. One day after sowing, 15 mm of water was applied to assure uniform, rapid germination (Figure 1a). To prevent late lodging and to harden seedlings, no irrigation was applied from sowing to 60 days after sowing. Chemical control (DA-6 Ethephon, China Agrotech, Shanxi, China) was applied at 600 mL ha<sup>-1</sup> in the V8–V10 period of maize growth. The 2,4-D butyl ester emulsion was used to weed before the seedlings were planted; phoxim granule was used to prevent and control corn borers, mancozeb wettable powders was used to control leaf spot diseases; and pyridaben wettable powders were used to control corn aphids, leafhopper and red spider. In addition, mechanical weeding was conducted three times in the prophase of maize growth.



**Figure 1.** Field image of seeding and nitrogen dressing method. Figure (a) showing the relative positions of row spacing, drip tape, and plastic film. (b–d) are pictures of the field during the experiment.

### 2.3. Measurements and Methods

#### 2.3.1. Stalk Breaking Force

Three maize plants were randomly selected from each plot at the filling stage (20 days after silking). Then, using a YYD-1 stalk strength instrument (Zhejiang Top Instrument Co., Ltd., Hangzhou, China), the stalk breaking force (SBF) was measured by pushing the plant over at the ear position. The thrust direction was always perpendicular to the direction of the stalk during the measurement [16]. Before the measurement, the root soil was solidified to avoid root lodging.

#### 2.3.2. Mechanical Strength of Basal Internodes

The third, fourth, and fifth internodes were excised from the stalk of each plant in each treatment. Rind penetration strength (RPS) of the basal third internode was determined with a stalk strength

tester. The stalk strength test probe with a cross-sectional area of 1 mm<sup>2</sup> was vertically applied to the middle of the basal internode, and the value of RPS was displayed on the screen of this tester [17]. By using a stalk strength test probe with a cross-sectional area of 1 cm<sup>2</sup>, the crushing strength (CS) was determined as the mechanical strength of the basal fourth internode. The bending strength (BS) of the fifth internode was measured using a mechanical three-point bend test [19]. The above measurements were carried out on the long axis of the fifth internode, and the test probe was moved downwards slowly with a constant speed during the measurement [35].

### 2.3.3. Carbohydrate and Total Nitrogen Contents of Basal Internodes

The length of every internode was measured with a ruler, after which the internodes were dried at 80 °C in a forced-draft convection oven to a constant weight and then weighed. Dry weight per cm was calculated by dividing the dry weight of the internode by its length, using the following formula: dry weight per unit length (DWUL, mg cm<sup>-1</sup>) = dry weight (g)/length (cm) × 1000.

The third internode samples of all treatments in 2018 were preserved. The dry internode samples were crushed, and then the crushed samples of each treatment were screened over a 1 mm mesh screen. The total nitrogen content was measured using a Hanon-K9840 Automatic Kellogg's Nitrogen Meter (Shandong Haineng Scientific Instrument Co., Ltd., Dezhou, China) [36], and the cellulose and lignin contents were measured using a fiber system (A200i, ANKOM, Macedon, NY, USA) and a filter bag as required by the manufacturer's instructions [37]. The per-unit-length contents of structural carbohydrate and total nitrogen in the basal internodes were calculated as follows: per-unit-length content (structural carbohydrate or total nitrogen) (mg cm<sup>-1</sup>) = DWUL (mg cm<sup>-1</sup>) × content (cellulose, lignin, or total nitrogen) percentage (%).

### 2.4. Data Analysis

Data analyses were conducted with SPSS 21.0 software (IBM Inc., Amonk, NY, USA). Statistical analysis was preceded by tests for normality and homogeneity of variances. The variances of the data were first calculated within years and then compared to assess the homogeneity of the variances. Planting density, nitrogen application rate, and their interaction effect were tested using univariate ANOVA. Planting density and nitrogen application rate were treated as fixed factors, and replication was considered a random effect. Differences were judged by the least significant differences test using a 0.05 level of significance. Pearson's correlations were calculated to determine the relationship between the RPS and the cellulose, lignin, and total nitrogen contents of the third internode. Figures were plotted using the SigmaPlot 14.0 software (Systat Software, Inc., San Jose, CA, USA).

## 3. Results

### 3.1. Stalk Breaking Force

There were significant differences in planting densities and nitrogen application rates between the two years, and the interaction of density and nitrogen application rate reached a significant level in 2018. With increasing planting density, the breaking force of maize stalks decreased (Figure 2). At planting densities of 7.5 to 10.5 × 10<sup>4</sup> plants ha<sup>-1</sup>, with increasing nitrogen application rate, the breaking force first decreased and then increased, with the lowest breaking force being observed for the N270 treatment. At planting densities of 12.0 to 13.5 × 10<sup>4</sup> plants ha<sup>-1</sup>, the breaking force gradually increased with the amount of applied nitrogen. For treatment N0, the rate of decline in stalk breaking force with increasing planting density is the fastest among the different nitrogen application treatments. The stalk breaking force was lower in 2018 than in 2017 under the same fertilization treatment.

### 3.2. Stalk Mechanical Strength

Planting density and nitrogen application rate had significant effects on RPS, CS, and BS of basal internodes, and the interaction of density and nitrogen application rate reached a significant

level of influence on stalk mechanical strength. With increasing planting density, the RPS of the third internode, the CS of the fourth internode, and the BS of the fifth internode decreased gradually (Figure 3). At planting densities of  $7.5$  to  $10.5 \times 10^4$  plants  $\text{ha}^{-1}$  in 2017, the RPS and CS of the basal internode decreased first and then increased with increasing nitrogen application rate, with the lowest values of RPS and CS being obtained for the N270 treatment. At planting densities of  $12.0$  to  $13.5 \times 10^4$  plants  $\text{ha}^{-1}$ , RPS and CS gradually increased with increasing nitrogen application rate. At planting densities of  $7.5$  to  $9.0 \times 10^4$  plants  $\text{ha}^{-1}$ , RPS in 2018 and BS in both years decreased first and then increased with increasing nitrogen application rate, with the lowest value being observed for the N270 treatment. Meanwhile, at planting densities of  $10.5$  to  $13.5 \times 10^4$  plants  $\text{ha}^{-1}$ , BS increased with increasing nitrogen application rate.

### 3.3. DWUL and Carbohydrate Content of Basal Internodes

Planting density and nitrogen application rate had effects on DWUL of the basal internodes in two years, but interaction of density and nitrogen application rate did not significantly affect DWUL of the basal internodes in 2018. With increasing planting density, the DWUL of the third, fourth, and fifth internodes of the maize stalks gradually decreased (Figure 4). Under the same planting density, the average DWUL increased with increasing nitrogen application rate. At planting densities of  $7.5$  and  $12.0 \times 10^4$  plants  $\text{ha}^{-1}$ , the average DWUL did not differ significantly between nitrogen treatments, while at the other planting densities there were significant differences in the average DWUL between the four nitrogen application rates.

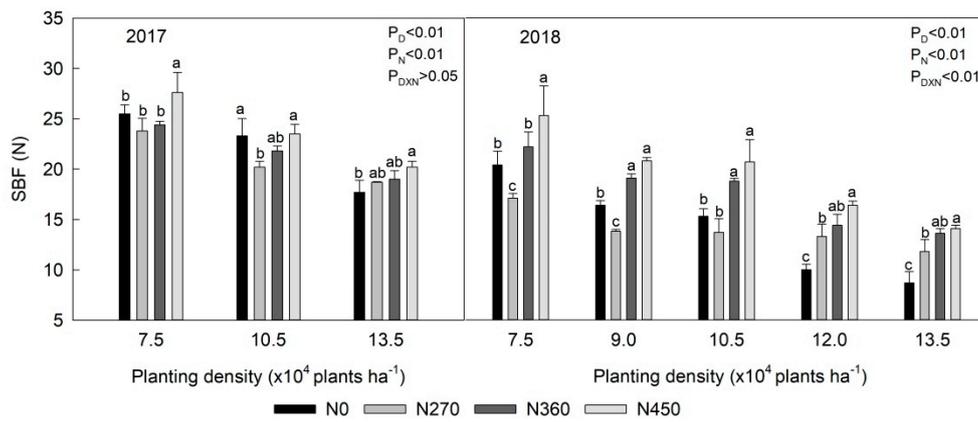
Planting density and nitrogen application rate had an effect on cellulose and lignin contents of the third basal internode of maize, and the interaction of density and nitrogen application rate had a significant effect on the contents of cellulose and lignin of the third basal internodes in both years. At a planting density of  $7.5 \times 10^4$  plants  $\text{ha}^{-1}$ , the cellulose and lignin contents of the third internode decreased first and then increased with increasing nitrogen application rate, with the lowest contents being observed for the N270 treatment (Figure 5). Meanwhile, at planting densities of  $9.0$  to  $13.5 \times 10^4$  plants  $\text{ha}^{-1}$ , the cellulose and lignin contents increased with increasing nitrogen application rate.

### 3.4. N Content of the Basal Internode

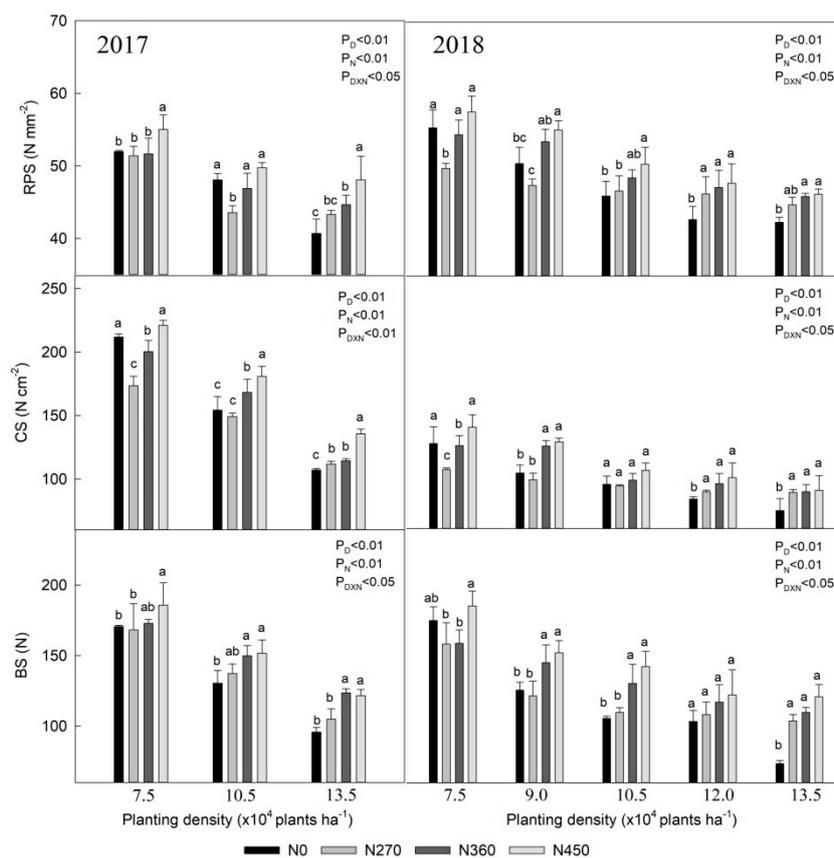
Planting density and nitrogen application rate had effects on the N content of the basal third internode of maize, and interaction of density and nitrogen application rate reached a significant level of influence on the N content of the basal third internode of maize. At the same planting density, the total N content of the third internode increased with increasing nitrogen application rate (Figure 6). For the N0 treatment, the N content showed a significant downward trend with increasing planting density, while there was no significant difference in N content at different densities for the other nitrogen treatments.

### 3.5. Related Analysis

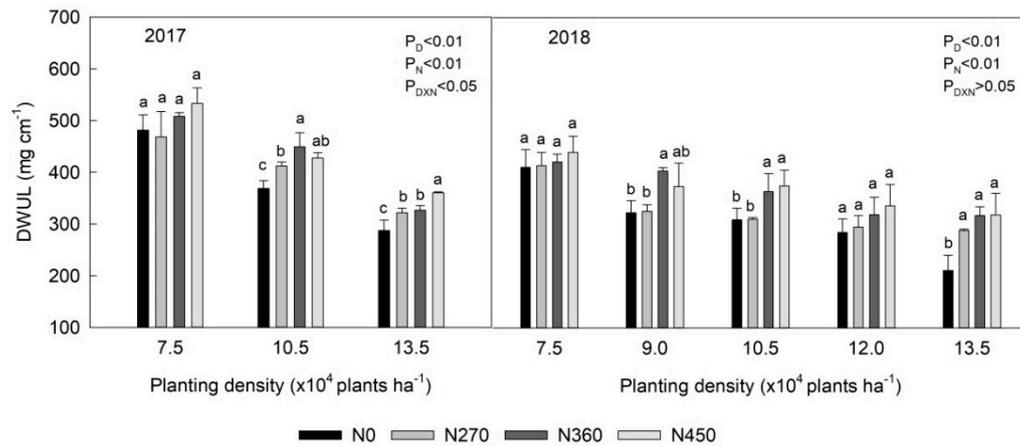
Significant positive correlations were observed between the maize stalk breaking force and the mechanical strength and DWUL of the basal internodes, while significant correlations were also observed between the DWUL and the RPS, CS, and BS of the basal internodes (Table 3). Moreover, the RPS of the third internode was positively linearly correlated with the per-unit-length cellulose content, lignin content, and total N content (Figure 7).



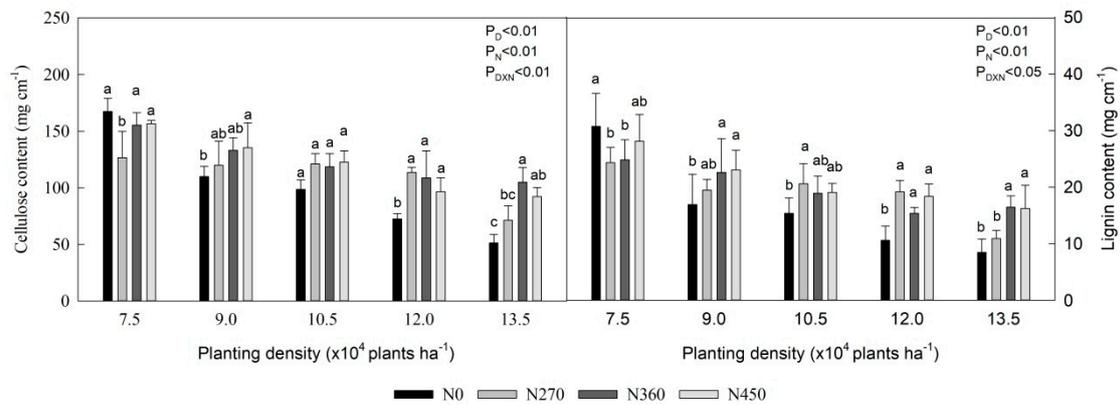
**Figure 2.** Effect of nitrogen application rate on the stalk breaking force of maize under drip irrigation for different planting densities. N0, N270, N360, and N450 indicate nitrogen application rates of 0, 270, 360, and 450 kg ha<sup>-1</sup>, respectively. Different letters indicate significant difference at the *p* < 0.05 level under the same planting density. P<sub>D</sub>, P<sub>N</sub> and P<sub>D</sub> × N stand for the *p*-value of variance analysis for stalk breaking force (SBF) of maize under the effects of planting densities, nitrogen application rates, and their interaction, respectively. The same notation is used in the following figures.



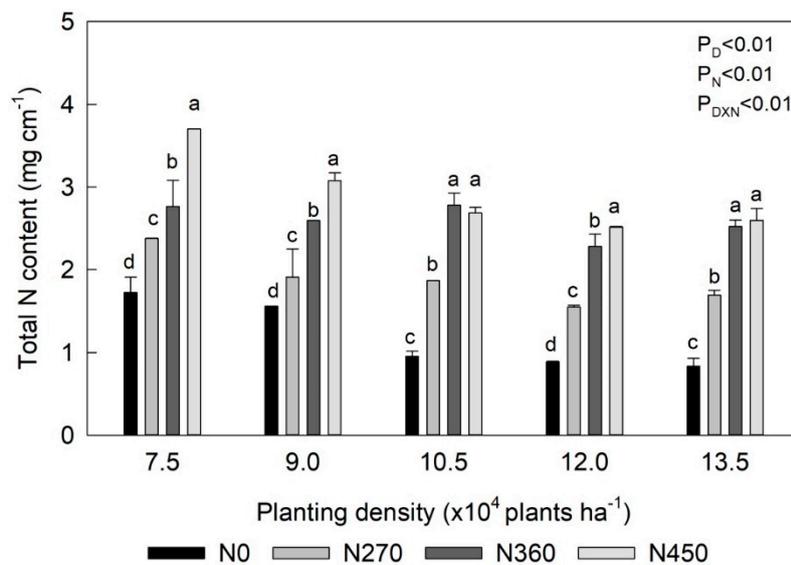
**Figure 3.** Effect of nitrogen application rate on the mechanical strength of maize stalks under drip irrigation at different planting densities. RPS: rind penetration strength; CS: crushing strength; BS: bending strength.



**Figure 4.** Effect of nitrogen application rate on the dry weight per unit length (DWUL) of the basal internodes of maize under drip irrigation at different planting densities.



**Figure 5.** Effect of nitrogen application rate on the cellulose and lignin contents of the third basal internode of maize under drip irrigation at different planting densities (year 2018).

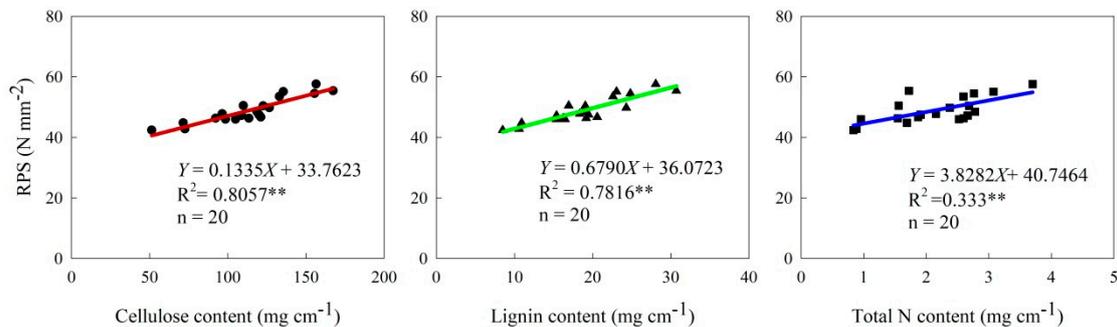


**Figure 6.** Effect of nitrogen application rate on the N content of the basal third internode of maize under drip irrigation for different planting densities (year 2018).

**Table 3.** Correlations between the stalk breaking force and the mechanical strength and dry weight per unit length (DWUL) of the basal internodes.

	Breaking Force	DWUL	RPS	CS
DWUL	0.896 **			
RPS	0.668 **	0.713 **		
CS	0.884 **	0.899 **	0.510 **	
BS	0.851 **	0.940 **	0.864 **	0.768 **

Note: \*\* indicates significant correlation at  $p < 0.01$ ,  $n = 32$ . DWUL: dry weight per unit length; RPS: rind penetration strength; CS: crushing strength; BS: bending strength.

**Figure 7.** Relationships between the rind penetration strength (RPS) and the cellulose, lignin, and total nitrogen contents of the third internode of maize under drip irrigation.

#### 4. Discussion

High-density planting is the most commonly used way to realize high yield and increase the efficiency of maize cultivation in Northwest China; however, the high risk of lodging under dense planting is an important factor which limits the increase of planting density and yield. Additionally, fertilizer is an important agricultural production substance and plays a very important role in food security. The application of nitrogen fertilizer can extend the functional period of the lower and middle leaves and improve the photosynthesis capacity of the maize population, especially for single leaves in the middle and late growth stages, which is highly conducive to increasing yield [38,39]. Previous research has found that the rate of maize lodging is significantly negatively correlated with stalk nitrogen content but is not strongly correlated with stalk phosphorus or potassium content [40]. In this study, SBF of maize was used as a comprehensive index to evaluate the stalk lodging resistance under different nitrogen fertilization treatments. The results show that with increasing planting density, the lodging resistance of maize stalks gradually decreased.

Previous studies reported that removal of ear at flowering increased the stalk quality [41]; thus, there was a competitive relationship between ear and stalk sinks [42]. At densities of  $7.5$  to  $10.5 \times 10^4$  plants  $\text{ha}^{-1}$ , the breaking force decreased first and then increased with increasing nitrogen application, with the lowest breaking force being observed for the N270 treatment. This result may be related to the distribution of nitrogen and carbohydrates in the plant. In the N0 (no nitrogen) treatment, the stalk nitrogen content was the lowest among all the treatments, and the differentiation of female ears was restricted, resulting in a smaller grain “sink” and the reduced transport of the dry matter from stalks to the grain in the filling stage, which led to a higher lodging resistance. However, in the N270 treatment, female ears developed normally, the total carbohydrate content in the stalk did not increase significantly, and the increased N content in the stalk was not conducive to cellulose and lignin synthesis, so the cellulose and lignin contents of stalks in the N270 treatment are lowest at a planting density of  $7.5 \times 10^4$  plants  $\text{ha}^{-1}$ . At higher nitrogen application amounts (treatments N360 and N450), the total carbohydrate content of stalks increased, which increased the stalk plumpness, thus improving the stalk lodging resistance. Under the high-density conditions of  $12.0$  to  $13.5 \times 10^4$  plants  $\text{ha}^{-1}$ , the low total carbohydrate content of the stalks was the most important

factor affecting the stalk mechanical strength. Increasing the nitrogen fertilization rate can prolong the functional period of the lower and middle leaves [43,44] and increase the total carbohydrate content of the stalks, thus enhancing the mechanical strength and lodging resistance of the stalks. Furthermore, increasing the nitrogen application rate at the V12 stage can significantly improve maize lodging resistance [45]. Liu et al. [46] showed that the flexural strength of maize stalks was negatively correlated with the lodging rate and that the amount of nitrogen fertilizer application could improve the lodging resistance or mechanical strength of the stalks. The results of the ANOVA analysis in this study show that the interaction effect between nitrogen fertilization rate and planting density reached a significant level for lodging indicators. Compared with the no-nitrogen treatment, the response of the maize stalks to the change in planting density in nitrogen application treatments is slow, and the stalk resistance is more sensitive to changes in planting density at higher nitrogen application rates; however, the sensitivity is still lower than that in the no-nitrogen treatment.

Carbohydrates are the material basis of mechanical strength in maize stalks, and the cellulose content per unit length of maize stalks explained 85% of the stalks' flexural strength [47]. In the late growth stage of maize, the dry matter content of stalks is directly related to the mechanical strength, with greater accumulation of stalk dry matter and lignin tending to be observed in lodging-resistant varieties [48]. In this study, the dry weight per unit length between the basal internodes in the maize filling stage was found to be positively correlated with the stalk mechanical strength and stalk breaking force. Cellulose, lignin, and total nitrogen contents were found to be positively correlated with internode RPS, consistent with the results of Ma et al. [49] This shows that the DWUL and cellulose, lignin, and total nitrogen contents of the basal internodes are the material basis of the stalk strength, and the increase of these parameters in the stalk in the filling stage is an important measure that can be taken to improve the lodging resistance.

The nitrogen application period has an important effect on the lodging resistance of maize stalks. The maize basal internode first rapidly thickens and elongates, followed by the rapid accumulation of dry matter, the rapid synthesis of structural components, and finally the increase of stalk strength [36]. At the V6 stage, the basal internodes grow from the bottom up, and a large amount of irrigation or nitrogen fertilizer being applied during this period will cause a rapid elongation of the basal internodes and material filling; consequently, stalk strength will not be increased in time, and the lodging resistance will therefore be reduced [49]. Additionally, high nitrogen levels are not conducive to the synthesis and accumulation of cellulose in maize stalks [26], and postponing nitrogen fertilization is therefore an important measure that can be taken to improve lodging resistance. In the traditional flooding irrigation mode in China, it is difficult to achieve nitrogen fertilizer topdressing in the late growth stage, and farmers typically perform heavy fertilization and watering before the seeding and V6 stage and do not fertilize during the filling stage. This heavy fertilization and watering during early growth stages and light fertilization and watering during late growth stages leads to the overgrowth of maize in the early growth stages and increases the risk of lodging in the late growth stage. Additionally, the fact that stalk material is transported to the grain in the filling stage results in a decline of the stalk material content. In China, integrated water–fertilizer technology is used to apply topdressing to maize in the late growth stages, which ensures that adequate amounts of nitrogen are available in all growth stages, thus improving the source–sink relationship of maize, preventing late-stage premature senescence due to lack of nitrogen, and consequently increasing the yield significantly [50,51]. A reasonable nitrogen application period can promote the growth of the maize stalk, significantly reduce the ratio of the length to diameter of the basal internodes, and improve the stalk lodging resistance. Applying fertilizer at the V6 stage results in a higher ratio of the internode length to diameter and should therefore be avoided in summer maize culture [45]. The results of previous research suggest that split nitrogen application with a small amount in seed manure or seeding fertilizer and at a high ratio in V12 stage fertilizer is beneficial to the robust growth of maize stalks and ears, thus promoting grain yield and stalk lodging resistance of the plant [45]. Therefore, by postponing nitrogen fertilization in drip irrigation systems, an increase in the amount of nitrogen application is beneficial in increasing the

cellulose, lignin, and total nitrogen contents between the basal internodes of the stalk during the filling stage and thus improving the stalk strength and lodging resistance.

In this study, soil foundation fertility was poor in the test fields, and this resulted in the stalk lodging resistance and grain yield increasing as different nitrogen fertilization rates increased. However, the effect of nitrogen on plant activity and grain yield decreased under long-term fertilization [52]. In addition, high doses of nitrogen compromise the soil–plant–atmosphere system, causing acidification of soil [53], and pollution of freshwater [54]. In arid and semiarid areas of Northwest China, the use of integrated watering and fertilization drip irrigation technology to postpone nitrogen application can maximize the yield and nitrogen efficiency of crops [29]. This will result in relatively low risks from emissions of greenhouse gases and nonpoint-source pollution. The effect of nitrogen application rate on environmental changes and sustainable agricultural systems using nitrogen split application with drip irrigation under long-term fertilization should be further clarified.

## 5. Conclusions

The use of integrated watering and fertilization drip irrigation technology to postpone nitrogen application and increase the nitrogen application rate in high-density planting during the filling stage increased the stalk contents of cellulose, lignin, and total nitrogen, which led to high stalk mechanical strength and lodging resistance. In conclusion, nitrogen split application can increase the maximum usable levels of nitrogen fertilization and plant density by mitigating the effects of high N doses and high seeding densities.

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## References

1. Flint-Garcia, S.A.; Jampatong, C.; Darrah, L.L.; McMullen, M.D. Quantitative trait locus analysis of stalk strength in four maize populations. *Crop Sci.* **2003**, *43*, 13–23. [[CrossRef](#)]
2. Bian, D.; Jia, G.; Cai, L.; Ma, Z.; Cui, Y. Effects of tillage practices on root characteristics and root lodging resistance of maize. *Field Crops Res.* **2015**, *185*, 89–96. [[CrossRef](#)]
3. Ma, D.; Xie, R.; Liu, X.; Niu, X.; Hou, P.; Wang, K.; Lu, Y.; Li, S. Lodging-Related stalk characteristics of maize varieties in China since the 1950s. *Crop Sci.* **2014**, *54*, 2805–2814. [[CrossRef](#)]
4. Pellerin, S.; Trendel, R.; Duparque, A. Relationship between morphological characteristics and lodging susceptibility of maize (*Zea mays* L.). *Agronomie* **1990**, *10*, 439–446. [[CrossRef](#)]
5. Kamara, A.Y.; Kling, J.G.; Menkir, A.; Ibikunle, O. Association of vertical root-pulling resistance with root lodging and grain yield in selected S-1 maize lines derived from a tropical low-nitrogen population. *J. Agron. Crop Sci.* **2003**, *189*, 129–135. [[CrossRef](#)]
6. Li, S.Y.; Wei, M.A.; Peng, J.Y.; Chen, Z.M. Study on yield loss of summer maize due to lodging at the big flare stage and grain filling stage. *Sci. Agric. Sin.* **2015**, *48*, 3952–3964.
7. Xue, J.; Xie, R.Z.; Zhang, W.F.; Wang, K.R.; Peng, H.; Bo, M.; Ling, G.; Shao-Kun, L.I. Research progress on reduced lodging of high-yield and-density maize. *J. Integr. Agric.* **2017**, *16*, 2717–2725. [[CrossRef](#)]
8. Brune, P.F.; Baumgarten, A.; McKay, S.J.; Technow, F.; Podhiny, J.J. A biomechanical model for maize root lodging. *Plant Soil* **2018**, *422*, 397–408. [[CrossRef](#)]
9. Wu, H.Y.; Zhang, Y.J.; Zhang, W.F.; Wang, K.R.; Jiang, C.D. Photosynthetic characteristics of senescent leaf induced by high planting density of maize at heading stage in the field. *Acta Agron. Sin.* **2019**, *45*, 248–255. [[CrossRef](#)]

10. Xue, J.; Gou, L.; Zhao, Y.; Yao, M.; Yao, H.; Tian, J.; Zhang, W. Effects of light intensity within the canopy on maize lodging. *Field Crops Res.* **2016**, *188*, 133–141. [[CrossRef](#)]
11. Remison, S.U.; Akinleye, D. Relationship between lodging, morphological characters and yield of varieties of maize (*Zea mays* L.). *J. Agric. Sci.* **1978**, *91*, 633–638. [[CrossRef](#)]
12. Esechie, H.A. Relationship of stalk morphology and chemical composition to lodging resistance in maize (*Zea mays* L.) in a rainforest zone. *J. Agric. Sci.* **1985**, *104*, 429–433. [[CrossRef](#)]
13. Abedon, B.G.; Darrah, L.L.; Tracy, W.F. Developmental changes associated with divergent selection for rind penetrometer resistance in the MoSCSSS maize synthetic. *Crop Sci.* **1999**, *39*, 108–114. [[CrossRef](#)]
14. Peiffer, J.A.; Flint-Garcia, S.A.; Leon, N.D.; McMullen, M.D.; Kaeppler, S.M.; Buckler, E.S. The genetic architecture of maize stalk strength. *PLoS ONE* **2013**, *8*, e67066. [[CrossRef](#)]
15. Bosch, M.; Mayer, C.D.; Cookson, A.; Donnison, I.S. Identification of genes involved in cell wall biogenesis in grasses by differential gene expression profiling of elongating and non-elongating maize internodes. *J. Exp. Bot.* **2011**, *62*, 3545–3561. [[CrossRef](#)]
16. Xue, J.; Gao, S.; Fan, Y.; Li, L.; Ming, B.; Wang, K.; Xie, R.; Hou, P.; Li, S. Traits of plant morphology, stalk mechanical strength, and biomass accumulation in the selection of lodging-resistant maize cultivars. *Eur. J. Agron.* **2020**, *117*, 126073. [[CrossRef](#)]
17. Xu, C.; Gao, Y.; Tian, B.; Ren, J.; Meng, Q.; Pu, W. Effects of EDAH, a novel plant growth regulator, on mechanical strength, stalk vascular bundles and grain yield of summer maize at high densities. *Field Crops Res.* **2017**, *200*, 71–79. [[CrossRef](#)]
18. Zhang, J.; Li, G.H.; Song, Y.P.; Liu, Z.H.; Yang, C.D.; Tang, S.; Zheng, C.Y.; Wang, S.H.; Ding, Y.F. Lodging resistance characteristics of high-yielding rice populations. *Field Crops Res.* **2014**, *161*, 64–74. [[CrossRef](#)]
19. Robertson, D.J.; Julias, M.; Lee, S.Y.; Cook, D.D. Maize stalk lodging: Morphological determinants of stalk strength. *Crop Sci.* **2017**, *57*, 926–934. [[CrossRef](#)]
20. Zhang, Y.; Liu, P.; Zhang, X.; Zheng, Q.; Chen, M.; Ge, F.; Li, Z.; Sun, W.; Guan, Z.; Liang, T. Multi-Locus genome-wide association study reveals the genetic architecture of stalk lodging resistance-related traits in maize. *Front. Plant Sci.* **2019**, *9*, 611. [[CrossRef](#)]
21. Chen, Y.; Chen, J.; Zhang, Y.; Zhou, D. Effect of harvest date on shearing force of maize stems. *Livest. Sci.* **2007**, *111*, 33–44. [[CrossRef](#)]
22. Hebert, Y.; Guingo, E.; Loudet, O. The response of root/shoot partitioning and root morphology to light reduction in maize genotypes. *Crop Sci.* **2001**, *41*, 363–371. [[CrossRef](#)]
23. Ma, D.L.; Xie, R.Z.; Niu, X.K.; Li, S.K.; Long, H.L.; Liu, Y.E. Changes in the morphological traits of maize genotypes in China between the 1950s and 2000s. *Eur. J. Agron.* **2014**, *58*, 1–10. [[CrossRef](#)]
24. Shi, D.Y.; Li, Y.H.; Zhang, J.W.; Liu, P.; Zhao, B.; Dong, S.T. Effects of plant density and nitrogen rate on lodging-related stalk traits of summer maize. *Plant Soil Environ.* **2016**, *62*, 299–306.
25. Rajkumara, R. Lodging in cereals—a review. *Agric. Rev.* **2008**, *29*, 55–60.
26. Li, H.C.; Li, L.A.; Wegenast, T.; Longin, C.F.; Xu, X.W.; Melchinger, A.E.; Chen, S.J. Effect of N supply on stalk quality in maize hybrids. *Field Crops Res.* **2010**, *118*, 208–214. [[CrossRef](#)]
27. Chilundo, M.; Joel, A.; Westrom, I.; Brito, R.; Messing, I. Response of maize root growth to irrigation and nitrogen management strategies in semi-arid loamy sandy soil. *Field Crops Res.* **2017**, *200*, 143–162. [[CrossRef](#)]
28. Zartash, F.; Qaiser, A.; Amna, K.; Sajjad, H.; Muhammad, A.; Ghulam, A.; Haseeb, Y.; Shahrish, N.; Muhammad, I.; Muhammad, S. Resource use efficiencies of C3 and C4 cereals under split nitrogen regimes. *Agron. J.* **2018**, *8*, 1–16.
29. Zhang, G.Q.; Shen, D.P.; Xie, R.Z.; Ming, B.; Xue, J.; Li, R.F.; Chen, J.L.; Wang, K.R.; Li, S.K. Optimizing planting density to improve nitrogen use of super high-yield maize. *Agron. J.* **2020**, 1–12. [[CrossRef](#)]
30. Page, A.L.; Miller, R.H.; Keeney, D.R. Methods of soil analysis, part 2: Chemical and microbiological properties. In *American Society of Agronomy and Soil Science Society of America*, 2nd ed.; Wisconsin: Madison, WI, USA, 1982; pp. 885–891.
31. Olsen, S.R.; Cole, C.V.; Watanabe, F.S. *Estimation of Available Phosphorus in Soils by Extraction with Sodium Bicarbonate*; US Department of Agriculture: Washington, DC, USA, 1954; Volume 939, p. 19.
32. Cox, A.E.; Joern, B.C.; Brouder, S.M.; Gao, D. Plant-available potassium assessment with a modified sodium tetraphenylboron method. *Soil Sci. Soc. Am. J.* **1999**, *63*, 902–911. [[CrossRef](#)]
33. Lu, R.K. *Analysis of Soil Agrochemicals*; China Agricultural Science and Technology Press: Beijing, China, 2000; pp. 106–107.

34. Zhang, G.Q.; Liu, C.W.; Xiao, C.H.; Xie, R.Z.; Ming, B.; Hou, P.; Liu, G.Z.; Xu, W.J.; Shen, D.P.; Wang, K.R.; et al. Optimizing water use efficiency and economic return of super high yield spring maize under drip irrigation and plastic mulching in arid areas of China. *Field Crop Res.* **2017**, *211*, 137–146. [[CrossRef](#)]
35. Robertson, D.; Smith, S.; Gardunia, B.; Cook, D. An improved method for accurate phenotyping of corn stalk strength. *Crop Sci.* **2014**, *54*, 2038–2044. [[CrossRef](#)]
36. Zheng, X.G.; Xin, R.Z. Determination of nitrogen content of fertilizer by automatic Kjeldahl apparatus. *Chem. Anal. Meterage* **2014**, *23*, 41–43.
37. Zhou, D.W.; Chen, J.; She, J.K.; Tong, J.; Chen, Y.X. Temporal dynamics of shearing force of rice stem. *Biomass Bioenergy* **2012**, *47*, 109–114. [[CrossRef](#)]
38. Muchow, R.C.; Sinclair, T.R. Nitrogen response of leaf photosynthesis and canopy radiation use efficiency in field-grown maize and sorghum. *Crop Sci.* **1994**, *34*, 721–727. [[CrossRef](#)]
39. Kim, S.H.; Sicher, R.C.; Bae, H.; Gitz, D.C.; Reddy, V.R. Canopy photosynthesis, evapotranspiration, leaf nitrogen, and transcription profiles of maize in response to CO<sub>2</sub> enrichment. *Glob. Chang. Biol.* **2006**, *12*, 588–600. [[CrossRef](#)]
40. Liu, Y.; Hou, P.; Xie, R.; Li, S.; Zhang, H.; Ming, B.; Ma, D.; Liang, S. Spatial adaptabilities of spring maize to variation of climatic conditions. *Crop Sci.* **2013**, *53*, 1693–1703. [[CrossRef](#)]
41. Beck, D.L.; Darrah, L.L.; Zuber, M.S. Effect of sink level on root and stalk quality in maize. *Crop Sci.* **1988**, *28*, 11–18. [[CrossRef](#)]
42. Xue, J.; Zhao, Y.; Gou, L.; Shi, Z.; Yao, M.; Zhang, W. How high plant density of maize affects basal internode development and strength formation. *Crop Sci.* **2016**, *56*, 3295–3306. [[CrossRef](#)]
43. Wang, S.; Han, X.R.; Zhan, X.M.; Yang, J.F.; Wang, Y.; Liu, Y.F.; Li, N. Effect of nitrogenous fertilizer levels on photosynthetic functions of maize ear leaves at grain filling stage. *J. Plant Nutr. Fertilizer* **2014**, *20*, 280–289.
44. Li, Q.; Ma, X.J.; Cheng, Q.B.; Dou, P.; Yu, D.H.; Luo, Y.H.; Yuan, J.C.; Kong, F.L. Effects of nitrogen fertilizer on post-silking dry matter production and leaves function characteristics of low-nitrogen tolerance maize. *Chin. J. Eco-Agric.* **2016**, *24*, 17–26.
45. Bian, D.H.; Liu, M.X.; Niu, H.F.; Wei, Z.B.; Xiong, D.U.; Cui, Y.H. Effects of nitrogen application times on stem traits and lodging of summer maize (*Zea mays* L.) in the Huang-Huai-Hai plain. *Sci. Agric. Sin.* **2017**, *50*, 2294–2304.
46. Liu, M.; Qi, H.; Zhang, W.J.; Zhang, Z.P.; Li, X.F.; Song, Z.W.; Yu, J.L.; Wu, Y.N. Effects of deep loosening and nitrogen application on anatomical structures of stalk and lodging in maize. *J. Maize Sci.* **2013**, *21*, 57–63.
47. Appenzeller, L.; Doblin, M.; Barreiro, R.; Wang, H.; Niu, X.; Kollipara, K.; Carrigan, L.; Tomes, D.; Chapman, M.; Dhugga, K.S. Cellulose synthesis in maize: Isolation and expression analysis of the cellulose synthase (CesA) gene family. *Cellulose* **2004**, *11*, 287–299. [[CrossRef](#)]
48. Wang, T.J.; Zhang, L.; Han, Q.; Zheng, F.X.; Wang, T.Q.; Feng, N.N.; Wang, T.X. Effects of stalk cell wall and tissue on the compressive strength of maize. *Plant Sci. J.* **2015**, *38*, 505–511.
49. Ma, F.Q.; Liu, X.G.; Wang, H.W.; Huang, C.L.; Wu, Y.J.; Hu, X.J.; Liu, Z.F. Stalk fiber quality traits and their correlations in maize. *Crops* **2014**, *4*, 44–48.
50. Feng, G.; Huang, C.L.; Xing, J.F. The research progress in lodging resistance of maize. *Crops* **2008**, *4*, 12–14.
51. Wang, Y.L.; Li, C.H.; Tan, J.F.; Zhang, X.; Liu, T.X. Effect of postponing N application on yield, nitrogen absorption and utilization in super-high-yield summer maize. *Acta Agron. Sin.* **2011**, *37*, 339–347. [[CrossRef](#)]
52. Gao, W.; Yang, J.; Ren, S.; Liu, H. The trend of soil organic carbon, total nitrogen, and wheat and maize productivity under different long-term fertilizations in the upland fluvo-aquic soil of North China. *Nutr. Cycl. Agroecosyst.* **2015**, *103*, 61–73. [[CrossRef](#)]
53. Guo, J.; Liu, X.; Zhang, Y.; Shen, J.; Han, X.; Zhang, W.; Christie, P.; Goulding, K.; Vitousek, P.M.; Zhang, F.S.; et al. Significant acidification in major Chinese croplands. *Science* **2010**, *327*, 1008–1010. [[CrossRef](#)]
54. Diaz, R.; Rosenberg, R. Spreading dead zones and consequences for marine ecosystems. *Science* **2008**, *321*, 926–929. [[CrossRef](#)] [[PubMed](#)]

