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Full Length Article

Nitrogen Fertilization and Planting Density Effects on the Physiological Characteristics of Stem, Root Bleeding Sap and Lodging Resistance in Spring Maize

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Abstract

In this study, three nitrogen (N) fertilization rates (100, 200 and 300 kg ha^{-1}) and three planting densities (6.75, 8.25 and 9.75 million plants ha^{-1}) were explored for their effects on maize cellulose, hemicellulose and lignin contents including lignin synthesis enzyme activity. The results showed that with increasing N fertilization and planting density, the content of cellulose and hemicellulose in maize stalks increased, and the activity of the cinnamyl-alcohol dehydrogenase (CAD) improved which was beneficial to the synthesis of stem lignin. The N application rates and planting densities in each treatment increased IAA (auxin), GA (gibberellin) and CTK (cytokinin) endogenous hormone levels and decreased ABA (abscisic acid) levels. Correlation analysis showed that the cellulose content of stems was significantly negatively correlated with the lodging rate, indicating that the higher the cellulose content, the lower the lodging rate of stem. Under the N fertilizer and planting density treatments, there was a significant positive correlation between IAA, cellulose and hemicellulose contents and a significant positive correlation with lignin content, indicating that endogenous hormones promoted the quality of stem fibre. CTK and GA were significantly positively correlated with yield, indicating that endogenous hormone regulation affects maize yield. The maximum yield 9321.21 kg ha^{-1} was obtained with the combination of 8.25 million plants ha^{-1} and 200 kg nitrogen ha^{-1}(D2N2).

Keywords: Nitrogen fertilizer; Density; Stem and root; Lodging

Introduction

Maize is an important crop worldwide for grain, economic and feed production (Zhang *et al.* 2015; Kumar and Singh, 2017). Heilongjiang province is China's main maize producing area. In recent years, with the adjustment of the national planting structure, the area of maize in Heilongjiang province has been continuously reduced, with a reduction of 34 million ha in two years. With the continuous reduction of the maize planting area, the total maize production in Heilongjiang province reached 61.88 million tons, making outstanding contributions to China's food security and playing a pivotal role in China's food security (FAO 2017). At present, worldwide maize production is facing problems such as insufficient planting densities and excessive application of N fertilizer. Studies have shown that the planting density of maize in the United States is 85,000~100,000 plants ha^{-1} (Pei *et al.* 2017), whereas in Heilongjiang province, it is typically 45,000~60,000 plants ha^{-1}. Therefore, increasing the planting density can enhance the maize yield in China if optimum planting density exists (Shi *et al.* 2016; Ning *et al.* 2017). Exceeding the optimum planting density will not only inhibit the growth of maize per plant but also increase the risk of lodging, leading to difficulty in harvesting and decline in yield (Haegele *et al.* 2014).

Nitrogen (N) is a major factor restricting crop yields. Since the 19th century, the amount of N applied to maize under traditional cultivation has been approximately 130 kg ha^{-1}, and the application rate of N is currently twice these historic levels (Zhang *et al.* 2011). Excessive application of N fertilizer reduces the utilization rate and increases the risk of groundwater pollution (Ye *et al.* 2016). At the same time, such application increases the height of the crops, which causes the plant to stretch continuously at the base and results in plants which easily fall over. Excessive N fertilizer also accelerates its absorption and transport by crops, leading to premature ageing (Zhu *et al.* 2016).

Studies have shown that maize stems and roots have a "flow" function in the source-sink system, playing an important role in water and nutrient absorption, synthesis and transport (Wang *et al.* 2004). Endogenous hormones in the root system regulate the relationship between roots and shoots and play roles in improving crop quality. Previous studies have shown that the self-regulation of endogenous hormone levels in crops can regulate the growth and development of plants and the differentiation of tissues and organs (Kiba *et al.* 2011).

Cai *et al*. (2012) reported that appropriate planting density and reasonable N fertilizer application can facilitate the growth and development of maize. Density has a significant effect on the number of spikes per unit area of maize. The amount of nitrogen applied has a significant effect on the number of effective panicles and 100-grain weight of maize. Increasing the planting density or the application rate of N fertilizer decreased the lodging resistance of maize but increased the stem rate, grain weight and 100-grain quality (Deng *et al.* 2017; Piao *et al.* 2017).

Lodging is a serious obstacle to normal growth and yields in maize production. An annual loss of 5–25% of maize production is caused by lodging, and every 1% increase in lodging will cause a decrease of 108 kg•hm^{-2} in yield (Norboerg *et al.* 1988). Studies have shown that the lodging of maize stems is related to morphological indexes, such as plant height, ear height and length of internode elongation (Ma *et al.* 2014), and is closely related to stem cellulose, hemicellulose and lignin. When lodging occurs, the normal canopy structure of maize is destroyed, resulting in decreased photosynthesis and grain yield, reduced crop quality, and difficult harvesting (Li *et al.* 2017).

In previous studies, a series of experiments were performed on the effects of N fertilizer or planting density on the lodging of maize stems and the morphological shape and mechanical strength of stems (Gou *et al.* 2007; Bian *et al.* 2017; Xue *et al.* 2017; Yu *et al.* 2019). Research on the physiological indexes of maize stems, the composition of xylem sap and its relationship with stem lodging under different N fertilizers and planting densities has rarely been reported. The purpose of this study was to investigate the effects of a reasonable nitrogen application rate and planting density on the physiological characteristics of stems and root sap and the lodging resistance of spring maize stalks in Heilongjiang province and at the same time, the objective was to provide a theoretical and experimental basis for achieving lodging resistance and high yield of Heilongjiang spring maize with reasonable nitrogen fertilization and planting density.

Materials and Methods

Site description and weather data

This experiment was carried out at the A Cheng experimental base, Northeast Agricultural University, Heilongjiang province, China (45º 42’ N, 126º 36’ E). The soil was a typical black soil. It contained 28.35 g kg^{-1} organic matter, 1.55 g kg^{-1} total nitrogen, 24.92 mg kg^{-1} available nitrogen, 59.58 mg kg^{-1} available phosphorus and 219.5 mg kg^{-1} available potassium. Meteorological data during the maize growth cycle were provided by the Harbin Academy of Agricultural Sciences (Table 1). The test variety 'Nonghua 101' was provided by Beijing Golden Nonghua Seed Industry Technology Co., Ltd. The experiment was conducted using a randomized block design with two factors. The tested nitrogen treatments were 100 (N1), 200 (N2) and 300 kg ha^{-1} (N3) and the planting densities were 6.75 (D1), 8.25 (D2) and 9.75 (D3) million plants ha^{-1}. Before sowing, 100 kg ha^{-1} of phosphate fertilizer (superphosphate) and 100 kg ha^{-1} potassium fertilizer (potassium sulfate) were released as base fertilizer and applied to the ridge side (depth: 10 cm). The N fertilizer (urea, nitrogen content approximately 48%) was equally divided into two soil applications as base fertilizer before sowing and the other as top dressing before the ridge was closed.

Table 1: Daily mean values of the weather variables at the experimental site during the six months of the maize growing season in 2016 and 2017

Month	Average temperature (°C)		Precipitation (mm)		Sunshine (h)	
	2016	2017	2016	2017	2016	2017
April	8.0	17.6	15.2	74.1	219.10	246.9
May	16.0	22.7	106.8	27.5	183.00	282.5
June	20.1	25.2	206.1	49.5	238.10	244.8
July	24.3	30.6	44.2	16.9	246.20	302.7
August	23.2	28.6	31.7	54.4	283.70	204.8
September	17.1	23.5	70.3	35.8	152.40	211
Total	18.1	24.7	474.3	258.2	1322.50	1492.7

The test plot had 10 rows with 8 m in length and with row spacing of 65 cm and the area was 52 m^2. In the small section and the repeating section, a walkway with a width of 50 cm was arranged, and a protective buffer line with a width of 1 m was established around the plots. The other management measures were the same as those applied in high-yield fields. The trial was planted on April 25, 2016 and April 27, 2017 and harvested on September 28, 2016 and September 29, 2017 in the two study years.

Data collection and analyses

Stem lodging rate

At the time of harvesting, the central three rows of each treatment were selected and the number of lodgings was counted. The lodging rate is the ratio of the number of lodgings to the total number of plants.

Stem physiological index

In the elongation stage (July 5), the tasseling stage (July 25), the early filling stage (August 3) and the milk stage (August 24), a standard scrubbing method was used to determine the lignin, cellulose and hemicellulose content of the third internode of maize. This procedure was repeated three times for each indicator for each treatment and the average was taken.

Key lignin synthesis enzymes

The phenylalanine ammonia lyase (PAL) activity was determined (Heinzmann and Seitz 1974); the tyrosine ammonia lyase (TAL) activity was performed (Khan *et al.* 2003); the method for determining 4-coumaric acid: Co A ligase (4CL) activity was described (Knobloch and Hahlbrock, 1975) and the cinnamyl-alcohol dehydrogenase (CAD) activity was determined (Morrison *et al.* 1994).

Root wound fluid collection

Root wound fluid was collected during the elongation stage (July 5), tasseling stage (July 25), early filling stage (August 3), and milk stage (August 24). The collection time was from 5:00 pm to 5:00 a.m. the next day. A test tube was filled with moderately dry, absorbent cotton (approximately 2/3 of the volume of the finger tube). The plants were quickly cut with scissors at the 3rd stem section, the stems were rinsed with deionized water, the tubes were fixed on the residual stems with plastic wrap and collection was performed for 12 h.

Endogenous hormones

Three samples were selected from the top of the maize plant to the third stem section, frozen in liquid nitrogen for 30 min and stored in a -40°C refrigerator. The contents of auxin (IAA), gibberellin (GA), cytokinin (CTK) and abscisic acid (ABA) were determined by Shanghai Ji Ning Industrial Co., Ltd. with an enzyme-linked immunosorbent assay.

Yield

During the harvest stage, each group of 4 rows and 5 rows of each plot were selected as the actual harvest, and the whole spike was harvested to calculate the average single ear quality. Twenty uniform ears were selected to determine the average single ear quality. Ears were brought inside for air drying, and the number of ears per row, number of rows of grains and number of grains per ear were determined. After threshing, the moisture content of the grain and the 1000-grain weight were measured. Actual yield (kg ha^{-1}, 14% water content) = measured maize ear quality (kg)/measured area (m^2) × seed yield × 15 × 666.7 m^2 × (1 - grain moisture content)/0.86.

Data analysis

According to the analysis of variance, data were statistically analysed following standard methods using Microsoft Excel 2010 and SPSS 12.0. Differences between treatments were determined by a posteriori Tukey's test at $P < 0.05$.

Results

Cellulose contents

The lodging resistance of maize is related to the physiological characteristics of stem development. When the content of cellulose, hemicellulose and lignin in a unit volume of stem was high, the degree of lignification was high, the mechanical properties were good and the lodging rate was low. The cellulose content of the differently treated maize stems showed a curve with a single peak. As the growth period progressed, the cellulose content of the stem first increased and then decreased, reaching a maximum at the early filling stage (Fig. 1).

The cellulose content of the stem for D2N2 was lower than other treatments at the elongation stage. The cellulose content under the D1N3 and D2N2 treatments was significantly higher than under the other treatments during the tasseling stage and early filling stage. Compared with the D1N1 and D3N3, we found D1N3 treatment increased the cellulose content by 5.3%, 51.61% and 49.71%, 33.36% during the various stages, respectively. Compared to the D1N1 and D3N3 treatments, the D2N2 treatment increased the cellulose content by 0.39%, 45.39% and 36.14%, 9.69%, respectively. The maximum cellulose content at the tasseling stage was obtained with D1N2, and the maximum cellulose content values at the early filling stage and milk stage was obtained with D1N3 and D1N1, respectively.

Hemicellulose content

The change in hemicellulose content in stems treated with different N fertilizer rates was similar to cellulose contents. As the growth period progressed, the hemicellulose content of stems increased first and then decreased and the hemicellulose content of stems reached its maximum value at the early filling stage. Except during the early filling stage, the maximum hemicellulose content in the remaining periods was obtained with D1N1 (Fig. 2).

At the early filling stage, the maximum hemicellulose content of the stem was obtained with D1N3, and D1N2 and D2N1 that were 24.36, 23.12, 16.09 and 14.94% higher than D1N1 and D3N3, respectively. At the same planting density, the hemicellulose content decreased with increasing nitrogen application rates.

Lignin content

The lignin content of each treatment peaked at the early filling stage. During the tasseling stage, the lignin content of maize stems showed the trend D1N2>D3N3>D3N2, and the maximum value was obtained 44.87 mg g^{-1} for D1N2 treatment. In the early filling stage, the lignin content of maize stems in each treatment showed this trend D2N3> D1N2>D3N3, reaching a maximum of 50.05 mg g^{-1} under D2N3. The rest of the treatments did not show significant differences in lignin levels in these two periods (Fig. 3).

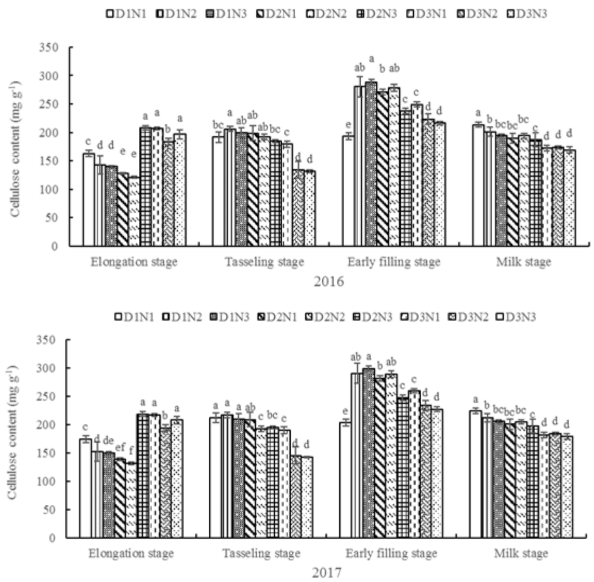

Fig. 1: Effects of N fertilizer and planting density on the cellulose content of maize

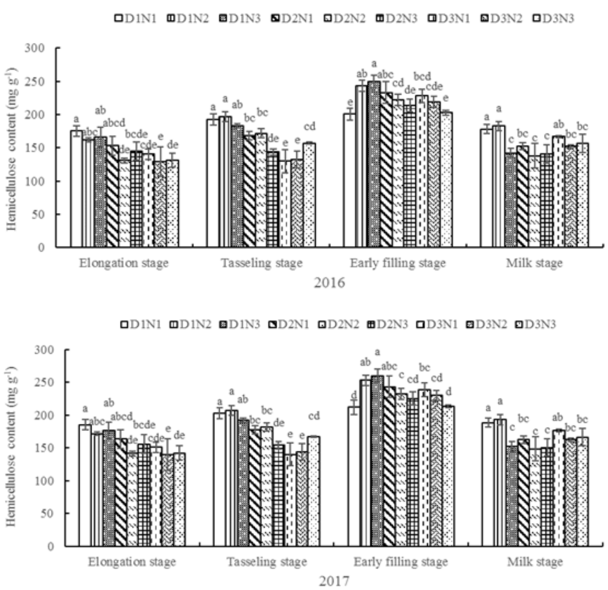

Fig. 2: Effects of N fertilizer and planting density on the hemicellulose content of maize

Key lignin synthesis enzymes activities of maize

As the growth period progressed, the PAL TAL and 4CL activity of the third internode of each treated maize plant gradually decreased. Except during the elongation stage, the D1N1 treatment did not result in activities that were significantly higher than other treatments. At the tasseling and the early filling stages, the PAL activity reached its maximum under the D1N3 treatment. The enzyme activity of each treatment showed this trend D1N3>D2N1>D2N2. In the milk stage, the difference in PAL activity between the treatments was not significant.

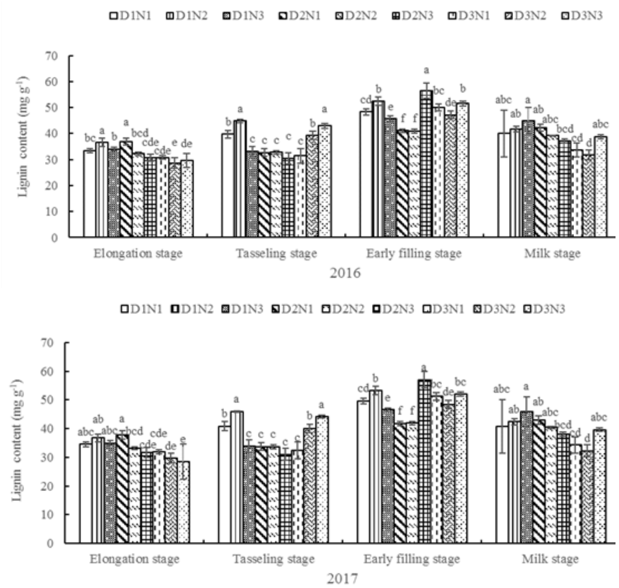

Fig. 3: Effects of N fertilizer and planting density on the lignin content of maize

The TAL activity of each treatment was lower than PAL activity. With advancing growth stage, the TAL activity of each treatment increased slightly at the early filling stage while the CAD activity decreased gradually, and each treatment increased the CAD activity during the milk stage. The treatments showed this trend D1N2>D1N3>D2N2 for CAD activity. The result indicated the CAD activity was significantly enhanced with increased planting density and decreased nitrogen fertilization, which promoted the synthesis of lignin. The 4CL activity of each treatment decreased rapidly after the elongation stage. At the early filling stage, the 4CL activity reached its maximum under D2N2 treatment, and this value was significantly higher than in the other treatments (Fig. 4).

Correlation analysis between lignin content and lignin synthetase activity

Correlation analysis showed that the lignin content of maize stems was significantly negatively correlated with the stem lodging rate. When the lignin content was high, the lodging rate of the maize stem was low and the lodging resistance was strong. There were significant positive correlations between lignin content and PAL activity, TAL activity and 4CL activity with correlation coefficients of 0.82, 0.52 and 0.78, respectively and were significantly negatively correlated with CAD activity (Table 2).

Endogenous hormones in root bleeding sap

The root system is an important site of endogenous hormone synthesis and transformation. The balance of hormone levels between roots and crowns is particularly important

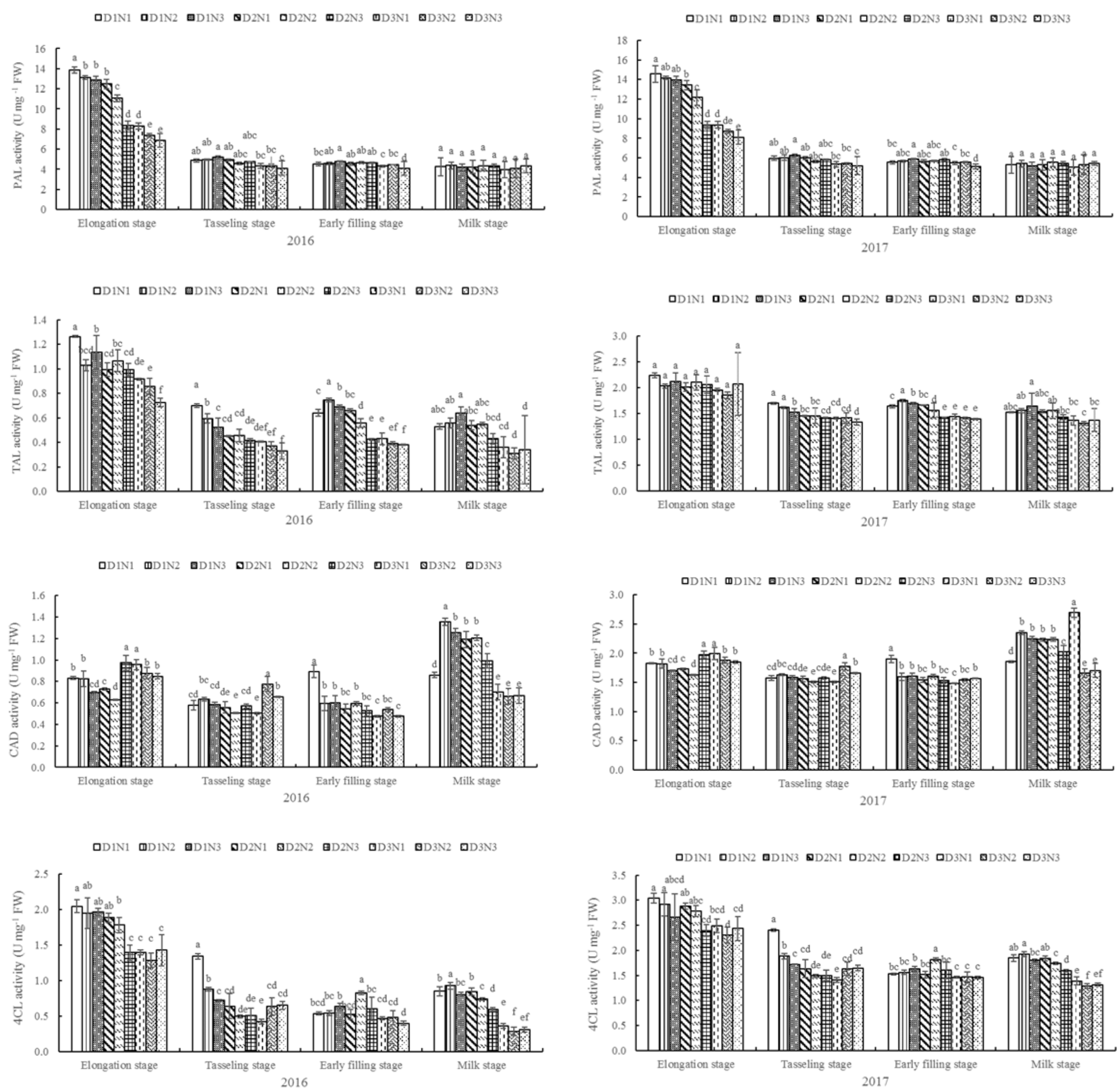

Fig. 4: Effects of N fertilizer and planting density on the activities of key lignin synthesis enzymes of maize, PAL, TAL, CAD and 4CL in the years 2016 and 2017

Table 2: Correlation analysis between lignin content and lignin synthetase activity in the elongation stage of maize

Year	PAL activity	TAL activity	CAD activity	4CL activity	Lodging percentage
2016	0.817**	0.507**	-0.437*	0.778**	-0.470*
2017	0.899**	0.310	0.470	0.823**	-0.491*

Note: * and ** indicate significance at 0.05 and 0.01 probability levels, respectively

for maize growth and morphogenesis. The four endogenous hormones IAA, ABA, GA and CTK exhibited different trends during the growth period (Table 3). The peak of IAA flow occurred during the early filling stage, the peak of GA and CTK flow occurred during the tasseling stage and the lowest value of ABA occurred during the early filling stage. Except for ABA, the trend of each hormone flow was similar under different treatments in different periods.

Taking the early filling stage as an example, at the same planting density level, the flow rates of IAA, GA and CTK increased significantly with increasing nitrogen application rates. The peak flow rate reached under the D2N2 treatment, which was 203.92, 168.13, 25.81 and 22.83%, as well as 68.16 and 22.14% higher than under D1N1 and D3N3, respectively.

Endogenous hormone ratios in root bleeding sap

Different N fertilizer density treatments not only affected the endogenous hormone flow in the root wound fluid of maize but also affected the ratio of endogenous hormones. Different N fertilizer treatments have different effects on the

Table 3: Effects of N fertilizer and planting density on endogenous hormones in root bleeding sap of maize

Year	Treatment	Elongation stage				Tasselling stage				Early filling stage				Milk stage			
		IAA	ABA	GA	CTK	IAA	ABA	GA	CTK	IAA	ABA	GA	CTK	IAA	ABA	GA	CTK
	D1N1	113.00±0.91e	14.82±0.06c	33.41±0.18g	132.89±2.97g	122.13±2.57g	12.21±0.15ab	45.53±3.88h	142.75±5.81e	134.55±3.69g	9.22±0.75b	40.29±0.14g	125.88±2.53g	124.05±2.57g	11.29±0.09c	20.99±0.75f	103.34±1.51d
	D1N2	130.96±3.66d	16.55±0.13a	36.99±0.47e	147.33±3.18ef	138.67±0.78f	12.16±0.67ab	53.62±3.24fg	188.23±3.63d	166.81±9f	8.93±0.33b	42.30±0.55e	174.06±2.53f	158.40±4.76e	12.59±0.1a	25.88±1.32de	146.42±6.06b
	D1N3	135.40±4.62d	14.68±0.05c	37.29±0.49e	149.31±4.7de	168.35±12.46e	12.69±0.05a	61.24±2.6de	200.06±6.87c	325.41±9.44d	12.91±1.87a	43.95±0.74d	182.91±1.94e	179.56±14.33d	12.37±0.04b	27.34±1.33cd	157.68±11.94b
	D2N1	138.57±5.07c	12.81±0.51d	38.36±0.25d	154.70±3.47cd	146.32±1.73f	12.56±0.11ab	57.30±2.03ef	187.49±8.05d	193.22±15.37e	6.19±1.06c	46.16±0.46c	188.20±1.7d	185.23±10.48d	11.08±0.14d	26.60±0.63de	148.64±6.63b
2016	D2N2	165.18±1.7a	12.28±0.08e	54.62±1a	170.60±3.14a	260.15±8.12a	11.24±0.06c	76.13±4.62a	242.75±3.71a	408.93±9.19a	5.06±0.34c	50.69±0.99a	211.68±1.54a	223.74±6.87a	11.22±0.04c	36.36±0.24a	192.00±8.15a
	D2N3	156.73±7.59ab	15.40±0.48b	39.30±0.7c	162.15±4.74b	226.86±6.95c	11.18±0.07c	69.40±3.21bc	221.04±4.48b	380.9±10.33ab	6.27±0.05c	48.94±0.48b	195.51±2.81c	211.12±3.45bc	12.63±0.03a	32.39±0.99b	183.95±10.25a
	D3N1	158.86±5.09ab	14.57±0.14c	42.23±0.32b	166.23±0.54ab	241.39±5.94b	12.27±0.12ab	71.57±1.99ab	235.79±5.88a	386.80±5.03ab	7.81±0.63b	49.37±0.33b	204.62±1.58b	216.91±3.95ab	12.41±0.05b	33.68±0.35b	189.73±6.59a
	D3N2	149.39±1.06b	12.67±0.04de	38.90±0.13cd	155.64±4.29c	207.85±9.14d	12.12±0.05b	65.38±2.65cd	210.65±2.67c	360.52±9.54c	8.93±0.8b	48.51±0.33b	196.66±2.03c	202.00±0.17c	11.28±0.08c	28.94±0.58c	178.70±10.76a
	D3N3	121.41±1.7d	12.47±0.08de	34.97±0.36f	142.59±2.3f	127.73±4.94g	11.24±0.52c	48.95±4.69gh	179.65±7.47d	152.51±2.05f	8.02±0.15b	41.27±0.24f	173.31±1.44f	143.31±0.78f	11.29±0.07c	25.09±1.88e	121.29±1.09c
	D1N1	122.93±1.44f	15.62±0.35c	34.69±0.12g	143.40±3.08f	133.44±2.75h	13.33±0.13ab	47.06±3.96h	150.50±5.3g	145.29±2.89g	10.39±0.83b	41.54±0.18f	136.26±2.56f	135.97±2.52f	12.53±0.13bc	22.24±0.54f	113.97±1.82d
	D1N2	140.51±1.61d	17.53±0.13a	38.07±0.48e	158.67±2.55de	151.15±2.5fg	13.30±0.57ab	54.98±3.12fg	197.93±2.54e	175.40±10.73f	10.08±0.38b	43.53±1.07e	185.26±2.03e	168.98±7.03d	13.83±0.11a	26.90±1.22de	156.79±5.07b
	D1N3	142.77±5.11cd	15.89±0.07c	38.43±0.44de	158.60±5.55de	176.81±9.4e	13.67±0.23a	62.32±2.53de	209.91±5.71cd	336.15±9.56d	13.92±1.7a	44.89±0.61d	193.84±1.66d	190.76±14.48c	13.73±0.2a	28.35±1.29cd	168.18±14.34b
	D2N1	150.33±5.81c	13.74±0.36d	39.49±0.27cd	165.12±3.46cd	156.83±2.78f	13.69±0.23a	57.87±0.54ef	199.55±5.56de	207.18±27.2e	7.83±0.86c	47.33±0.45c	196.24±2.97d	196.31±10.78c	12.38±0.39c	27.51±0.9de	160.60±7.45b
2017	D2N2	176.54±2.02a	13.33±0.13d	56.56±1.49a	180.26±5.69a	275.71±11.02a	12.61±0.24b	77.11±4.82a	247.60±9.97a	419.96±10.98a	6.25±0.32d	51.62±1.08a	222.71±2.05a	232.17±6.61a	12.50±0.13bc	37.62±0.3a	203.12±9.29a
	D2N3	169.83±6.58ab	16.50±0.43b	40.46±1.1c	172.15±4.51bc	233.75±8.72c	12.57±0.3b	68.62±3.16bc	228.75±7.07b	394.25±7.15b	7.46±0.26cd	49.82±0.31b	206.99±3.09c	221.49±4.72ab	13.72±0.12a	33.34±0.89b	194.30±10.06a
	D3N1	170.23±2.99ab	15.60±0.26c	43.50±0.65b	176.54±0.92ab	252.29±4.94b	13.62±0.2a	72.64±1.88ab	241.54±9.79a	398.86±5.92b	9.19±0.28b	50.15±0.33b	213.52±0.86b	226.92±7.03ab	13.79±0.18a	34.60±0.44b	202.34±8.69a
	D3N2	163.42±7.17b	13.72±0.1d	39.85±0.27c	166.24±5.07cd	217.39±6.25d	13.61±0.18a	66.47±3.13cd	214.56±2.54c	372.18±10.74c	10.38±0.62b	49.37±0.23b	206.63±2.18c	213.84±2.37b	12.93±0.48b	29.81±0.86c	188.47±11.08a
	D3N3	131.34±4.37e	13.54±0.07d	35.98±0.44f	154.80±5.86e	140.74±4.43gh	12.47±1.11b	50.04±4.43gh	185.87±5.02f	162.33±0.98fg	9.74±0.66b	42.32±0.27f	185.03±3.07e	154.28±4.03e	12.60±0.1bc	26.01±2.19e	133.79±0.41c

Note: Different lowercase letters after the same row show significant differences in the same period ($P < 0.05$). D1N1 (6.75 million plants ha^{-1}+100 kg N ha^{-1}), D1N2 (6.75 million plants ha^{-1}+200 kg N ha^{-1}), D1N3 (6.75 million plants ha^{-1}+300 kg N ha^{-1}), D2N1 (8.25 million plants ha^{-1}+100 kg N ha^{-1}), D2N2 (8.25 million plants ha^{-1}+200 kg N ha^{-1}), D2N3 (8.25 million plants ha^{-1}+300 kg N ha^{-1}), D3N1 (9.75 million plants ha^{-1}+100 kg N ha^{-1}), D3N2 (9.75 million plants ha^{-1}+200 kg N ha^{-1}), D3N3 (9.75 million plants ha^{-1}+300 kg N ha^{-1}). The same below

ratio of endogenous hormones, changing the balance between hormones. As fertilization rates increased, the ratios of IAA/ABA, GA/ABA, and CTK/ABA first increased and then decreased and both were peak at the initial stage of grain filling stage (Table 4).

At the same planting density level, the ratios of IAA/ABA, GA/ABA and CTK/ABA increased significantly with increasing nitrogen application rates. The D2 planting density and N1 nitrogen application treatments showed that the ratios of IAA/ABA, GA/ABA and CTK/ABA were 11.03–31.7, 2.4–7.6, and 12.09–31.05, respectively. Under N2 treatment, the ratios of IAA/ABA, GA/ABA, and CTK/ABA were 13.42–80.95, 3.24–10.05 and 13.89–41.93, respectively. Under N3 treatment, the ratios of IAA/ABA, GA/ABA and CTK/ABA were 10.31–60.75, 2.55–7.81 and 10.53–31.19, respectively.

Correlation analysis between endogenous hormones and fibre traits

Correlation analysis showed that IAA was significantly positively correlated with stem cellulose, hemicellulose and lignin. There was a significant negative correlation between ABA and stem cellulose, hemicellulose and lignin. There was no significant correlation between GA and CTK and stalk fibre traits. These results indicated that increasing IAA can promote the synthesis of stem cellulose, hemicellulose and lignin. Measures can be taken in production to increase the concentration of IAA, decrease the concentration of ABA, enhance stalk fibre traits, and reduce the stalk lodging rate (Table 5).

Yield and yield components

The maize yield increased significantly after treatment with N fertilizer density, reaching a maximum of 9321.21 kg ha^{-1} under D2N2 treatment, which was 5.14% and 59.01% higher than D1N1 and D3N3, respectively. The difference reached a significant level. In terms of factors, after the treatment of N fertilizer density, the effect on the number of ear rows was not significant, but the number of grains and 100-grain weight increased significantly (Table 6).

Correlation analysis between cellulose, hemicellulose, lignin content and lodging resistance of maize in milk stage

Correlation analysis showed that the cellulose content of maize stems was significantly positively correlated with the

Table 4: Effects of N fertilizer and planting density on endogenous hormones ratios in root bleeding sap of maize

Year	Treatment	Elongation stage			Tasseling stage			Early filling stage			Milk stage		
		IAA/ABA	GA/ABA	CTK/ABA	IAA/ABA	GA/ABA	CTK/ABA	IAA/ABA	GA/ABA	CTK/ABA	IAA/ABA	GA/ABA	CTK/ABA
	D1N1	7.59±0.11f	2.25±0.02e	8.97±0.24e	10.09±0.11f	3.73±0.36f	11.53±0.32e	14.67±1.46g	4.39±0.34de	13.72±1.27e	10.98±0.18f	1.86±0.08g	9.15±0.07f
	D1N2	7.89±0.2f	2.24±0.02e	8.90±0.23e	11.62±0.89e	4.43±0.48e	15.51±0.95d	18.68±0.58g	4.74±0.24d	19.52±0.97d	12.58±0.48e	2.06±0.09f	11.63±0.39e
	D1N3	9.10±0.34e	2.54±0.03d	10.17±0.34d	13.08±0.78d	4.82±0.2de	15.74±0.33d	25.49±2.98f	3.45±0.45e	14.38±2.25e	14.52±1.21d	2.21±0.1e	12.75±0.92d
	D2N1	11.03±0.32c	3.00±0.13b	12.09±0.57b	11.69±0.04e	4.56±0.12e	15.02±0.49d	31.70±4.76e	7.60±1.34b	31.05±5.84b	16.72±0.84c	2.40±0.07d	13.42±0.57d
2016	D2N2	13.42±0.18a	4.45±0.06a	13.89±0.22a	23.47±0.86a	6.78±0.44a	21.13±0.93a	80.95±4.27a	10.05±0.88a	41.93±2.71a	19.95±0.57a	3.24±0.02a	17.12±0.78a
	D2N3	10.31±0.71d	2.55±0.04d	10.53±0.11d	19.97±0.77b	6.21±0.3ab	19.70±0.22b	60.75±1.19b	7.81±0.05b	31.19±0.7b	16.71±0.26c	2.56±0.07c	14.56±0.83c
	D3N1	10.98±0.21c	2.90±0.02c	11.41±0.14c	19.68±0.32b	5.83±0.12bc	18.89±0.47b	49.75±4.12c	6.35±0.56c	26.33±2.43c	17.48±0.38bc	2.71±0.02b	15.29±0.56bc
	D3N2	12.07±0.45b	3.07±0.02b	12.28±0.36b	17.10±0.61c	5.40±0.2cd	17.11±0.51c	40.54±2.85d	5.46±0.53cd	22.15±2.18cd	17.90±0.14b	2.56±0.05c	15.83±0.86b
	D3N3	9.66±0.11e	2.81±0.03c	11.44±0.25c	11.46±0.84e	4.37±0.59e	15.97±0.94d	19.01±0.48g	5.14±0.12d	21.61±0.53d	12.69±0.02e	2.22±0.15e	10.74±0.03e
	D1N1	7.88±0.26f	2.22±0.05f	9.19±0.27e	10.01±0.15f	3.53±0.32e	11.29±0.29e	14.05±1.34f	4.01±0.33e	13.17±1.15e	10.86±0.3e	1.78±0.06h	9.10±0.23f
	D1N2	8.02±0.06f	2.17±0.04f	9.05±0.1e	11.38±0.57e	4.14±0.38d	14.90±0.68cd	17.39±0.52f	4.32±0.27de	18.40±0.92d	12.21±0.41d	1.94±0.1g	11.34±0.42de
	D1N3	8.98±0.3e	2.42±0.03e	9.98±0.31d	12.94±0.86d	4.56±0.13cd	15.35±0.22cd	24.33±2.28e	3.25±0.36f	14.07±1.81e	13.89±0.99c	2.07±0.08ef	12.25±1.09cd
	D2N1	10.94±0.41c	2.87±0.07bc	12.02±0.42b	11.46±0.15e	4.23±0.04d	14.58±0.34d	26.64±4.01e	6.10±0.66bc	25.31±3.25bc	15.85±0.64b	2.22±0.13de	12.98±0.71c
2017	D2N2	13.25±0.27a	4.24±0.09a	13.53±0.37a	21.86±0.82a	6.11±0.27a	19.62±0.45a	67.29±3.28a	8.28±0.59a	35.69±1.56a	18.57±0.55a	3.01±0.04a	16.26±0.9a
	D2N3	10.30±0.64cd	2.45±0.04e	10.43±0.08d	18.59±0.27b	5.46±0.34b	18.21±0.89b	52.89±1.07b	6.69±0.22b	27.79±1.39b	16.15±0.48b	2.43±0.08bc	14.16±0.61b
	D3N1	10.91±0.31c	2.79±0.02c	11.32±0.25c	18.52±0.16b	5.33±0.07b	17.73±0.63b	43.40±0.87c	5.46±0.18c	23.24±0.74c	16.45±0.34b	2.51±0.03b	14.67±0.46b
	D3N2	11.91±0.44b	2.90±0.04b	12.12±0.43b	15.97±0.25c	4.89±0.28bc	15.77±0.21c	35.89±1.21d	4.77±0.28d	19.94±1.09d	16.56±0.75b	2.31±0.03cd	14.58±0.7b
	D3N3	9.70±0.29d	2.66±0.03d	11.44±0.48c	11.35±1.18e	4.05±0.69de	14.97±1.14cd	16.71±1.08f	4.36±0.29de	19.04±0.97d	12.25±0.27d	2.07±0.18ef	10.62±0.08e

Note: Different lowercase letters after the same row show significant differences in the same period ($P < 0.05$). D1N1 (6.75 million plants ha^{-1}+100 kg N ha^{-1}), D1N2 (6.75 million plants ha^{-1}+200 kg N ha^{-1}), D1N3 (6.75 million plants ha^{-1}+300 kg N ha^{-1}), D2N1 (8.25 million plants ha^{-1}+100 kg N ha^{-1}), D2N2 (8.25 million plants ha^{-1}+200 kg N ha^{-1}), D2N3 (8.25 million plants ha^{-1}+300 kg N ha^{-1}), D3N1 (9.75 million plants ha^{-1}+100 kg N ha^{-1}), D3N2 (9.75 million plants ha^{-1}+200 kg N ha^{-1}), D3N3 (9.75 million plants ha^{-1}+300 kg N ha^{-1})

Table 5: Correlation Analysis between Endogenous Hormones and Fibre Traits

Year	Traits	IAA	ABA	GA	CTK
2016	Cellulose	0.530**	-0.591**	0.002	0.184
	Hemicellulose	0.456**	-0.599**	0.046	0.092
	Lignin	0.380*	-0.632**	-0.122	-0.021
2017	Cellulose	0.529**	-0.602**	-0.003	0.205
	Hemicellulose	0.460**	-0.616**	0.049	0.105
	Lignin	0.385*	-0.635**	-0.107	-0.002

Note: * and ** indicate significance at 0.05 and 0.01 probability levels, respectively

Table 6: Effects of N fertilizer and density on yield and yield components of maize

Year	Treatment	Ear rows	Row grains	Grain number per spike	100-grain weight (g)	Theoretic Yield (kg hm^{-2})
2016	D1N1	13ab	42.75a	555.75a	37.42b	8865.91a
	D1N2	14.5a	38ab	551a	36.69b	8293.77a
	D1N3	13ab	35.5ab	461.5ab	38.04b	8138.39ab
	D2N1	13.5ab	31b	418.5abc	41.98a	6966.23ab
	D2N2	12b	37.75ab	453ab	40.35a	9321.21a
	D2N3	13ab	37.5ab	487.5ab	40.69a	9184.83a
	D3N1	14ab	32b	448ab	37.88b	8998.32ab
	D3N2	13ab	31.25b	406.25bc	38.40b	8854.79ab
	D3N3	13ab	23.75c	308.75c	38.39b	5862.53b
2017	D1N1	15ab	32a	489.25a	33.39b	8732.68cd
	D1N2	16.5a	29.5ab	488.88a	35ab	8992.71cd
	D1N3	15ab	32.75a	466.25a	34.69ab	8249.50d
	D2N1	15.5ab	29.5ab	468a	33.26b	9912.66ab
	D2N2	14b	33.5a	469a	34.82ab	10831.57a
	D2N3	15ab	30.75ab	469.5a	32.8b	8973.68cd
	D3N1	16ab	30ab	495a	32.98b	9992.68ab
	D3N2	15ab	31ab	461.5a	34.87ab	9637.91bc
	D3N3	15ab	24.75b	380.75b	37.32a	6602.17e

Note: Different letters in the same column indicate significant differences ($P < 0.05$). D1N1 (6.75 million plants ha^{-1}+100 kg N ha^{-1}), D1N2 (6.75 million plants ha^{-1}+200 kg N ha^{-1}), D1N3 (6.75 million plants ha^{-1}+300 kg N ha^{-1}), D2N1 (8.25 million plants ha^{-1}+100 kg N ha^{-1}), D2N2 (8.25 million plants ha^{-1}+200 kg N ha^{-1}), D2N3 (8.25 million plants ha^{-1}+300 kg N ha^{-1}), D3N1 (9.75 million plants ha^{-1}+100 kg N ha^{-1}), D3N2 (9.75 million plants ha^{-1}+200 kg N ha^{-1}), D3N3 (9.75 million plants ha^{-1}+300 kg N ha^{-1})

hemicellulose and lignin contents and significantly negatively correlated with the lodging rate. This indicates that the stem cellulose and hemicellulose are closely related to the lodging resistance of the stem. When the cellulose and hemicellulose content is high, the maize stem has strong lodging resistance (Table 7).

Correlation analysis of root bleeding sap and its components with yield

There was a significant positive correlation between GA and CTK in the root wound fluid of maize during the four growth stages of maize. CTK (0.99**, 0.98**) had the greatest correlation with yield during the elongation stage and milk stage. GA (0.98**) had the highest correlation

Table 7: Correlation analysis between cellulose, hemicellulose and lignin contents and lodging resistance of maize in the milk stage

Year	Cellulose content	Hemicellulose content	Lignin content
2016	-0.804**	0.060	-0.375
2017	0.102	-0.803**	0.004

Note: * and ** indicate significance at 0.05 and 0.01 probability levels, respectively

Table 8: Correlation analysis of root bleeding sap and its components with yield

Year	Traits	IAA	ABA	GA	CTK
2016	Elongation stage	0.559	-0.186	0.802**	0.985**
	Tasseling stage	0.665	-0.342	0.978**	0.935**
	Early filling stage	0.641	-0.192	0.903**	0.801**
	Milk stage	0.504	-0.201	0.944**	0.983**
2017	Elongation stage	0.704*	-0.136	0.681*	0.674*
	Tasseling stage	0.650	0.237	0.656	0.545
	Early filling stage	0.504	-0.616	0.749*	0.453
	Milk stage	0.663	-0.092	0.609	0.618

Note: * and ** indicate significance at 0.05 and 0.01 probability levels, respectively

with yield during the tasseling stage. There was no significant correlation between IAA and ABA and yield (Table 8).

Discussion

The lignin, cellulose and hemicellulose in the stem are closely related to the lodging resistance (Jung *et al.* 2015). Kamran *et al*. (2018) found that high cellulose content increases the strength of the stem and enhances its lodging resistance. Zhang *et al*. (2019) found that extremely significant differences in stem lignin content among varieties with different lodging resistance. The lignin content of stems with strong lodging resistance is significantly higher than that of varieties that are prone to lodging. Barrière *et al.* (2010) showed a significant negative correlation between stem lignin content and actual lodging rates and a significant positive correlation between stem lignin content and flexural strength. Nitrogen fertilization and planting density treatment significantly increased the cellulose, hemicellulose and lignin content in maize internodes. The results showed that as the growth period progressed, the cellulose content in the stem first increased and then decreased, and each treatment showed maximum cellulose values at the early filling stage. The D1N3 and D2N2 treatments resulted in significantly higher cellulose contents than did the other treatments during the heading and the early filling stage. This result indicated that increasing nitrogen fertilization and establishing a reasonable planting density can increase the cellulose content in the stem, but a high planting density will reduce the cellulose content. The PAL, TAL, CAD and 4CL are key enzymes for lignin synthesis in grasses. Correlation analysis showed significant positive correlations between lignin content and PAL activity, TAL activity and 4CL activity (correlation coefficients were 0.817, 0.507 and 0.778, respectively), which were all significantly negatively correlated with CAD activity. Higher PAL, TAL and 4CL activities contribute to the synthesis and accumulation of lignin.

The inorganic ions and water absorbed by the roots from underground, in addition to supplying the growth and development of the roots, also flow to the shoots through the xylem. It is now known that IAA, ABA, GA3 and CTK can be synthesized in the roots and play an important role in regulating the growth of roots and information exchange between root and crown (Locher and Pilet, 1994). Tian *et al.* (2008) found that maize roots have a threshold for the concentration of IAA and that its high concentrations inhibit root growth. The study showed that endogenous hormones and yield were significantly correlated at each measurement period. The concentration of IAA in the wound fluid varied significantly with different nitrogen fertilization and planting density treatments. The results of this study showed that during the same growth period, the concentrations of IAA, GA3 and CTK first increased and then decreased, reaching maximum levels under the D2N2 treatment. Ma *et al.* (2019) and Chen *et al.* (2012) also showed that shows that there is a certain correlation between hormone content and lodging resistance of stem. The ABA concentration decreased first and then increased. Correlation analysis showed that IAA had a significant positive correlation with fibre content, such as cellulose, and ABA showed a significant negative correlation with the same, indicating that a high concentration of IAA can increase the cellulose content, thus increasing the lodging resistance of stems. In this study, the concentration of the IAA under the D2N2 treatment was the highest, the concentration of ABA was lowest and the lodging resistance was the best. Swarup *et al.* (2008) pointed out that the polar transport of auxin can regulate the expression of expansion and cell wall relaxant, increase the expansibility of cell wall rapidly and regulate cell growth. Our study also indirectly shows that auxin may promote the growth of cell wall in the later period of corn growth, and then improve the lodging resistance of corn stalk. The present study results areconsistent with previous results indicate that the N fertilizer rate and planting density in the D2N2 treatment are the most suitable for maize growth with good resistance to lodging and high yield.

Maize yield is associated with a variety of traits, most of which can be increased by increasing the planting density and increasing the number of effective spikes (Asim*et al.* 2013; Kir and Yavuz, 2019). Reasonable densification is a key strategy to achieve large-scale yield increases in spring maize in Heilongjiang province and previous studies have suggested that increasing planting density will result in a decrease in grain number per spike and 100-grain weight, and the number of kernels affected by environmental impacts will be greater (Cui *et al.* 2015). The results of this experiment showed that the effect on ear rows was not significant, but the number of grains and 100-grain weight increased significantly. Under the interaction between N fertilizer and planting density treatments, the density increased, the number of ear rows, the number of rows and the 100-grain quality all increased first and then decreased. Increasing the density increases the number of effective spikes, hence, the yield results showed this trend D2 > D3 > D1. As planting density increased, maize production first increased and then declined, indicating that excessive planting density leads to a decline in maize production. The analysis showed a significant positive correlation between GA3 and CTK and yield. Therefore, in addition to increasing maize yield by increasing planting density, high yields were achieved by appropriate increase in the concentrations of GA3 and CTK. In this experiment, the maximum yield of maize was obtained under D2N2 with 82,500 plants ha^{-1}, which is basically consistent with previous studies (Liu *et al.* 2012).

Conclusion

The cellulose content of stems was negatively correlated with the lodging rate in maize. Nitrogen application significantly increased the cellulose and hemicellulose contents of the stem. At the same time, the activity of CAD was significantly improved, the lignin content increased, and the stem lodging rate lowered; thus, the lodging resistance of maize was enhanced. Appropriate nitrogen fertilization and planting density treatment also significantly improved the endogenous hormone levels, which beneficially regulated the relationship between root and crown, affected the physiological activity of the aboveground parts, and ultimately increased the yield of maize. The maximum yield 9321.21 kg ha^{-1} was obtained with the combination of 8.25 million plants ha^{-1} and 200 kg nitrogen ha^{-1}(D2N2).

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