agronomy

Article

Effects of Plant Growth Regulators and Nitrogen Management on Root Lodging Resistance and Grain Yield under High-Density Maize Crops

Ning Sun [1,†], Xifeng Chen [2,†], Hongxiang Zhao [1], Xiangmeng Meng [1] and Shaofeng Bian [1,*]

[1] Jilin Academy of Agricultural Sciences, Changchun 130033, China
[2] Agricultural College, Jilin Agricultural University, Changchun 131008, China
* Correspondence: bianshaofeng@cjaas.com; Tel.: +86-1590-4428-080
† These authors contributed equally to this work.

check for updates

Citation: Sun, N.; Chen, X.; Zhao, H.; Meng, X.; Bian, S. Effects of Plant Growth Regulators and Nitrogen Management on Root Lodging Resistance and Grain Yield under High-Density Maize Crops. *Agronomy* **2022**, *12*, 2892. https://doi.org/10.3390/agronomy12112892

Academic Editor: Domenico Ronga

Received: 17 October 2022
Accepted: 14 November 2022
Published: 18 November 2022

Abstract: Lodging is one of the main factors causing yield loss of maize under high-density planting conditions. Root lodging as an important lodging type has received little attention. Plant growth regulators (PGRs) and nitrogen fertilizer can coordinate the relationship between root lodging and yield. This two-year field experiment was conducted with two nitrogen levels of N225 (225 kg ha^{-1}) and N300 (300 kg ha^{-1}) at a high planting density (90,000 plants ha^{-1}) during the maize growth season from 2019 to 2020. Plant growth regulator (Yuhuangjin, the mixture of 3% DTA-6 and 27% ethephon) was sprayed at the V8 stage. The results showed that PGRs significantly decreased plant height, improved root distribution and dry weight, enhanced photosynthetic rate and activities of photosynthetic carboxylase in ear leaves, and improved root bleeding sap and root activities after the silking stage. N225 combined with PGRs reduced the occurrence of root lodging and was conducive to photosynthate accumulation and root nutrient supply; it coordinated root regulation and morphological and physiological shoot functions, and played a crucial role in reducing root lodging and improving maize yield.

Keywords: root lodging; root-bleeding sap; endogenous hormone; photosynthetic characters; yield

1. Introduction

Low planting density of maize in Northeast China leads to a low average yield per unit area. Therefore, increasing planting density is the key factor to achieve high and stable yield of maize crops [1–3]. However, when the planting density exceeds a certain range, the population structure of maize changes, plant height, ear height and internode length increase, and the stalk becomes thin and delicate [4–6]. Too much density can also cause the root system to be smaller and thinner: the number of aerial roots and total roots decreases significantly, the angle between aboveground nodal roots and the ground increases significantly, and the dry weight ratio of the root system in the topsoil layer increases, while that in the deep soil layer decreases [7,8]. The above causes an increase in the lodging rate and decrease in grain quality and yield of maize [9,10]. This is accompanied by an increase in competition between root and canopy under high-density conditions, resulting in disharmony between source and sink, and hindering material and energy transfer between root and canopy. Many tillage methods such as intertillage and subsoiling are often used to improve the surface soil environment, promote root downscaling, reduce root crowding at the surface layer, and regulate the root quality of a high-density population [11]. However, the effect is greatly affected by soil fertility, variety characteristics, inter-seasonal rain, and heat distribution, which seriously restrict the application and popularization of the technology.

Plant growth regulators (PGRs) have great advantages in many methods of population structure optimization. It has been previously shown that spraying PGRs during the five to nine leaf stages could significantly decrease internode length and plant height, increase stem diameter at the base internode, and enhance lodging resistance of maize [12–14], resulting in significantly increased grain production. At present, most of the studies on PGRs focus on the above-ground part, such as plant morphology, while the research on the root system is relatively less [15]. As a tall stalk crop, maize has a strong root system, which can not only provide sufficient water and nutrients for its aboveground growth, but also effectively support its tall growth aboveground and prevent lodging [16–18]. There are few reports on whether PGRs can change root–shoot architecture to prevent root lodging.

The rational operation of nitrogen fertilizer is the focus of crop management in current agricultural production. As the nutrient element most demanded by maize, proper nitrogen application is conducive to regulating crop growth and development, improving photosynthetic performance, and achieving high quality and yield [19]. In the northeast plain of China, the agricultural system is heavily dependent on nitrogen application, and the yield is increased by a large amount of nitrogen application [20]. Excessive application of nitrogen fertilizer reduces nitrogen use efficiency, increases the lodging risk of maize, and causes yield loss [21].

Based on the above analysis and our previous studies, we hypothesized that the PGRs under appropriate nitrogen levels and varieties could reduce the risk of root lodging and increase the yield of maize. To verify this hypothesis, we established a 2-year field maize experiment in the Northeast China Plain, located in a semi-humid area, to examine the effects of PGRs and nitrogen fertilizer levels on (1) plant agronomic characters and photosynthetic characteristics of ear leaf; (2) root morphological characters, root activity, and endogenous hormone in root bleeding sap; and (3) root lodging resistance and grain yield. This paper will offer a basis for further increasing planting density, reducing lodging, reducing fertilizer application, and achieving a high yield of spring maize in northeast China.

2. Materials and Methods

2.1. Experimental Site

The field experiment was conducted in 2019 and 2020 at the Field Experimental Station of Jilin Academy of Agricultural Sciences at Huadian Country, Jilin Province, China (42.58° N, 126.44° E). The soil type at the experimental site is alluvial soil, the contents of organic matter, total N, available P, and available K in the 0–20 cm soil layer were 18.42 g kg^{-1}, 1.86 g kg^{-1}, 25.53 mg kg^{-1}, and 171.78 mg kg^{-1}, respectively. The climatic conditions of this region are humid and cool areas. The weather conditions of 2019 and 2020 are shown in Figure 1.

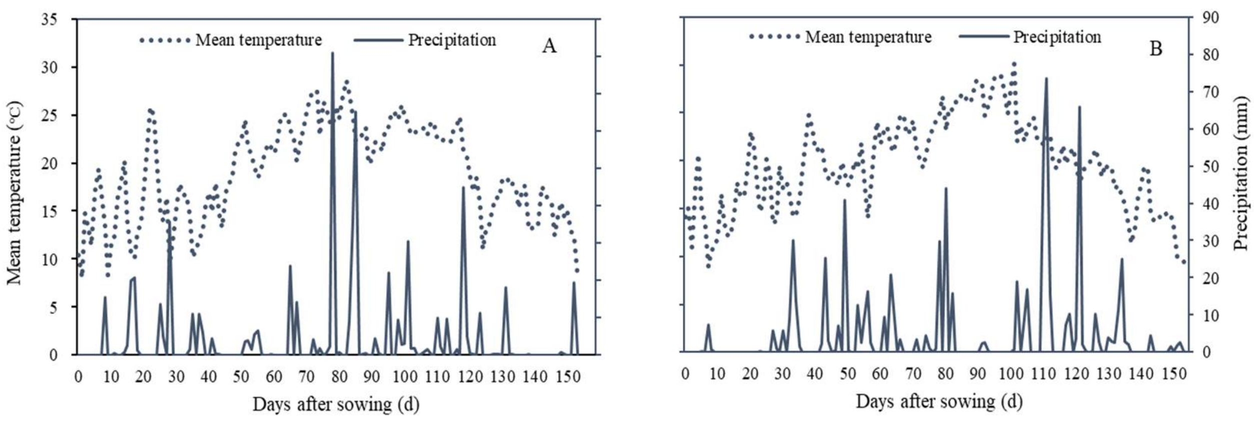

Figure 1. Daily mean temperature and daily precipitation in 2019 (**A**) and 2020 (**B**) growing seasons.

2.2. Experimental Design

The tested maize (*Zea mays* L.) varieties were Dika159 (DK159) and Xianyu335 (XY335), which are two of the main high-yield cultivars used in local production. Yuhuangjin (the mixture of 3% DTA-6 and 27% ethephon,) was used as the plant growth regulator (provided by Haolun Co., Ltd., Fujian, China), PGRs treatment (PGR) and water control treatment (CK) were set. Nitrogen fertilizer application was carried out in two levels: 225 kg hm^{-2} (N225 in Figures 2–4 and Tables 1–4) and 300 kg hm^{-2} (N300 in Figures 2–4 and Tables 1–4). The experiment was laid out as a re-split trial design, the main plot treatment was variety, nitrogen fertilizer level was in the sub-plot, and the PGRs treatment was randomly distributed in the sub-plot. The length of the test plot was 10 m, the row spacing was 0.6 m, and the area was 10 rows, with 3 replicates.

The experiment was sown on 27 April 2019 and 28 April 2020, and harvested on 27 September 2019 and 26 September 2020, respectively. Half of the nitrogen fertilizer and 120 kg hm^{-2} P_2O_5 and 130 kg hm^{-2} K_2O were used at seed sowing, and the other half of nitrogen was applied at the jointing stage. PGR was sprayed at a rate of 450 L hm^{-2} at the V8 stage. We sprayed PGR and water (CK) evenly onto the surface of the maize leaves using knapsack sprayers between 4:00 p.m. and 6:00 p.m.

2.3. Equipment and Methodology

2.3.1. Stalk Traits

Stalk traits were measured at 10 days after silking. Ten maize plants were randomly selected and cut at the ground in each plot. The plant height (from the ground to the top of the plant), the ear height (from the ground to the first ear-bearing node of the plant), and the center of gravity (from the ground to the fulcrum of the balance location) were measured with a ruler. The ear height coefficient was calculated using Equation (1):

$$\text{Ear height coefficient (\%)} = \text{ear height}/\text{plant height} \times 100 \quad (1)$$

2.3.2. Photosynthetic Characters of Ear Leaf

Photosynthetic characters of ear leaf were measured at 10 days and 30 days after silking. Five representative plants were selected from each treatment. The net photosynthetic rate (Pn) was measured by photosynthetic instrument (Li-Cor 6400, LI-COR Biosciences, Lincoln, NE, USA) from 10:00 a.m. to 12:00 a.m. with red and blue light source leaf chamber under 1500 μmol m^{-2} s^{-1} of artificial light and the CO_2 concentration in the leaf chamber was set at 400 umol mol^{-1}. Leaf photosynthetic enzyme activity including ribulose-1,5-bisphosphate carboxylase (RuBPCase) and phosphoenolpyruvate carboxylase (PEPCase) activities was measured according to the method of Li Hesheng [22] and Qi qige [23], respectively.

2.3.3. Root Morphological Traits, Root Dry Weight, and Root Activity

Root morphological traits were measured at 10 days after silking. Roots were obtained by profile sample method [24]; five plants were selected and 0–40 cm soil and roots were excavated in a horizontal area with a radius of 20 cm from the center of the maize plant. The roots in the soil layer were washed with water to remove impurities. The total number of roots were counted manually. Afterwards, the root crowns were placed on a plate covered with graph paper. The root length, maximum horizontal distribution width (A), and the minimum horizontal distribution width (B) of roots under 20 cm below the ground were measured. Root breadth area was calculated using Equation (2):

$$\text{Root breadth area (cm}^2) = \pi \times A/2 \times B/2 \quad (2)$$

The roots were put into the oven and dried at 75 °C until a constant weight was reached and then the dry weight material was weighted.

Root TTC reducing activity was measured at 10 days and 30 days after silking. The roots were obtained by the above profile method, washed with clean water, and the root activity was determined by TTC reduction method [25].

2.3.4. The Bleeding Sap Rate and Endogenous Hormones Flow

Root-bleeding sap collection was carried out according to the method of Chen [26]. On a sunny morning from 8:00 a.m. to 11:00 a.m. at 10 days and 30 days after silking, the ground part of 5 maize plants was cut off at 8 cm above the ground, then covered with a centrifuge tube containing degreasing cotton and secured with plastic wrap to collect the root-bleeding sap. The centrifuge tubes were collected after three hours, and the weight was measured [27]. The bleeding sap rate was calculated as the weight increase in the centrifuge tube per hour per plant (g h^{-1} $plant^{-1}$). The root bleeding sap was stored in a refrigerator at −80 °C and used for the subsequent determination. Endogenous hormones flow was measured by indirect enzyme-linked immunosorbent assay method (ELISA, [28]).

2.3.5. Root Lodging Rate and Grain Yield

The number of lodged plants were counted in each plot before harvest. If the plant leaned more than 30° with straight and unbroken culms from the vertical axis, we classified it as root lodged [29]. The root lodging rate in each plot was calculated using Equation (3):

$$\text{Root lodging rate (\%)} = \text{number of root lodged plants/total number of plants} \times 100 \quad (3)$$

At R6 stage, the yield was measured by randomly collecting ten ears from the middle four rows in each plot. The yield was determined with a fixed 14% grain water content.

2.4. Statistical Methods

The mean value was compared by the analysis of variance (ANOVA) to analyze the significant differences between samples with different treatments ($p < 0.05$). The interaction effect between variety, N level, and PGRs treatment was analyzed using the general linear model (GLM). The effects of variety, N level, PGRs treatment, and their interactions were taken as fixed factors and effects of field replicate as random. Year effects on different parameters were not analyzed due to the large difference in root lodging rates between two years. Correlations between different parameters were conducted with Pearson's correlation. All the above statistical analyses were performed by SPSS 19.0 procedures (SPSS Inc., Chicago, IL, USA). A redundancy analysis (RDA) was determined by CANOCO 5 software package to confirm vital factors that contributed to the root lodging state. The figures were created in Microsoft Excel 2010.

3. Results

3.1. Agronomic Characteristics of Maize Stalk

Variety (V), N rate (N), and PGRs treatment (P) had significant effects on plant height, ear height, and center of gravity height in both years. PGRs treatment also had significant effects on the ear height coefficient. V × P had significant effects on plant height in both years and V × P also had significant effects on the center of gravity height in 2020 (Table 1). Compared with N225, the plant, ear, center of gravity height, and ear height coefficient of N300 were higher by 2.84%, 6.65%, 3.57%, and 4.09% in 2019 and by 3.17%, 5.61%, 8.46%, and 1.97% in 2020, respectively. Compared with the water treatment, PGR decreased the plant, ear, center of gravity height, and ear height coefficient by 6.91%, 18.98%, 14.47%, and 13.04% in 2019, and 5.91%, 15.70%, 15.83%, and 10.61% in 2020.

Table 1. Effects of PGRs and nitrogen fertilizer on the agronomic characteristics of maize stalk.

Variety	N Rates ($kg \cdot hm^{-2}$)	Treatments	Plant Height (cm)	Ear Height (cm)	Center of Gravity Height (cm)	Ear Height Coefficient (%)
2019						
DK159	N225	PGR	280 d	120 d	106 e	43.0 d
		CK	318 c	152 b	128 c	47.8 b
	N300	PGR	292 d	130cd	114 d	44.7 c
		CK	323 c	165 ab	137 b	51.0 a
XY335	N225	PGR	329 bc	140 c	129 c	42.6 d
		CK	340 ab	170 ab	141 ab	50.0 a
	N300	PGR	338 ab	148 b	124 c	43.8 d
		CK	350 a	177 a	147 a	51.4 a
Variety (V)			***	***	***	ns
N rates (N)			*	*	*	ns
PGRs (P)			***	***	***	***
V × N			ns	ns	ns	ns
V × P			**	ns	ns	ns
N × P			ns	ns	ns	ns
V × N × P			ns	ns	ns	ns
2020						
DK159	N225	PGR	303 e	128 d	104 e	42.4 c
		CK	333 c	154 ab	138 b	46.4 b
	N300	PGR	311 d	133 bc	119 d	42.6 c
		CK	343 bc	163 a	141 b	47.6 a
XY335	N225	PGR	339 bc	141 d	126 d	41.6 d
		CK	351 ab	165 a	140 b	47.2 a
	N300	PGR	353 ab	151 cd	135 c	42.7 d
		CK	361 a	174 a	156 a	48.2 b
Variety (V)			***	***	***	ns
N rates (N)			***	*	***	ns
PGRs (P)			***	***	***	***
V × N			ns	ns	ns	ns
V × P			***	ns	*	ns
N × P			ns	ns	ns	ns
V × N × P			ns	ns	ns	ns

Different lowercase letters represent significant differences among treatments ($p < 0.05$); ns means non-significant; *, **, and *** indicate significance at $p < 0.05$, < 0.01, and < 0.001 probability levels, respectively.

3.2. Photosynthetic Characteristics of Ear Leaves

According to Table 2, Variety (V), N rate (N), and PGRs treatment (P) had significant effects on the net photosynthetic rate (Pn), RuBPCase, and PEPCase activity of ear leaf at 10 days (T1) and 30 days (T2) after silking. V × N had significant effects on PEPCase at T2 and Pn at T1, respectively, in 2019 and 2020. V × P had more significant correlations in Pn and RuBPCase than in the PEPCase in two years. N × P and V × N × P had significant effects on PEPCaes at T1 in 2020. Pn decreased with the increase in nitrogen fertilizer. Pn under N200 was higher by 9.43–18.34% than N300 and PGR significantly increased the Pn at 10 days after silking (T1) and 30 days after silking (T2). Compared with the water treatment, PGR increased Pn by 9.58% and 11.45% in 2019 and 2020. The RuBPCase and PEPCase activity decreased with the increase in nitrogen fertilizer; RuBPCase and PEPCase activity under N225 was higher by 4.87–9.05% and 5.53–10.99% than N300, respectively. PGR significantly increased the RuBPCase and PEPCase activity at the T1 and T2 stages. Compared with the water treatment, PGR increased RuBPCase and PEPCase activity by 6.33%, 9.56%, and 14.50%, 9.87% in 2019 and 2020. Under the combined effects of nitrogen fertilizer and chemical control, Pn, RuBPCase, and PEPCase activity of PGR treatment under N225 were higher than that under other treatments.

Table 2. Effects of PGRs and nitrogen fertilizer on the photosynthetic characteristics of ear leaves.

Variety	N Rates ($kg \cdot hm^{-2}$)	Treatments	Pn ($\mu mol \cdot m^{-2} \cdot s^{-1}$)		RuBPCase Activity ($\mu molprotein \cdot h^{-1} \cdot mg^{-1}$)		PEPCase Activity ($\mu molprotein \cdot h^{-1} \cdot mg^{-1}$)	
			T1	T2	T1	T2	T1	T2
2019								
DK159	N225	PGR	66.4 a	49.5 a	169.9 a	131.0 a	95.0 a	68.9 a
		CK	60.7 c	46.6 b	158.8 bc	117.5 cd	87.3 b	65.9 b
	N300	PGR	63.5 b	43.2 c	163.8 ab	120.7 bc	90.7 b	65.8 b
		CK	57.4 d	39.2 d	148.9 de	115.3 de	81.5 cd	60.6 cd
XY335	N225	PGR	61.8 bc	47.7 ab	153.0 cd	123.3 b	83.5 c	61.0 c
		CK	56.9 d	43.1 c	143.7 ef	118.6 cd	75.8 ef	55.5 e
	N300	PGR	54.3 e	39.5 d	145.0 e	112.6 e	78.5 de	58.2 d
		CK	49.5 f	36.1 e	138.7 f	107.2 f	72.6 f	49.7 f
Variety (V)			**	**	***	**	*	***
N rates (N)			***	**	**	***	**	***
PGRs (P)			***	***	***	***	**	***
V × N			ns	ns	ns	ns	ns	*
V × P			*	ns	**	ns	ns	ns
N × P			ns	ns	ns	ns	ns	ns
V × N × P			ns	ns	ns	ns	ns	ns
2020								
DK159	N225	PGR	63.7 a	44.2 a	154.8 a	131.9 a	90.1 a	59.4 a
		CK	56.7 cd	39.9 b	142.1 cd	116.2 bc	82. 0c	56.9 b
	N300	PGR	58.5 bc	38.8 bc	145.5 bc	118.9 b	86.0 b	56.0 b
		CK	52.8 e	34.9 de	137.3 e	103.8 d	75.5 d	49.8 cd
XY335	N225	PGR	59.6 b	40.9 b	149.1 b	117.5 bc	81.7 c	52.0 c
		CK	54.4 de	36.4 cd	142.9 cd	103.4 d	75.0 d	47.3 de
	N300	PGR	52.0 e	35.2 de	138.9 e	111.6 c	77.2 d	47.2 e
		CK	45.9 f	32.2 e	134.8 f	95.7 e	72.8 e	41.3 f
Variety (V)			***	**	***	**	***	**
N rates (N)			**	**	***	**	***	**
PGRs (P)			***	***	***	***	***	***
V × N			*	ns	ns	ns	ns	ns
V × P			ns	*	*	ns	ns	ns
N × P			ns	ns	ns	ns	*	ns
V × N × P			ns	ns	ns	ns	*	ns

Different lowercase letters represent significant differences among treatments ($p < 0.05$); T1 represents 10 days after silking; T2 represents 30 days after silking; ns means non-significant; *, **, and *** indicate significance at $p < 0.05$, < 0.01, and < 0.001 probability levels, respectively.

3.3. The Root Characteristics of Maize

Variety (V), N rate (N), and PGRs treatment (P) had significant effects on total root number (TRN), root length (RL), root breadth area (RBA), and root dry weight (RDW) in both years (Table 3). N × P had significant effects on RBA in 2019. Nitrogen fertilizer and PGR treatment affected the root morphological characters of DK159 and XY335. Compared with N225, the total root number (TRN), root length (RL), root breadth area (RBA), and root dry weight (RDW) of DK159 under N300 decreased by 8.38%, 12.32%, 5.17%, and 10.46%, and those of XY335 decreased by 6.48%, 12.47%, 7.04%, and 11.71%, respectively. PGR treatment significantly affected the root morphological characteristics. Compared with water treatment, the mean values of TRN, RL, RBA, and RDW of DK159 under PGR across both N rates increased by 11.97%, 11.81%, 7.01%, and 13.64%, and those of XY335 increased by 12.46%, 11.79%, 7.57%, and 12.72%, respectively.

Table 3. Effects of PGRs and nitrogen fertilizer on the root characteristics of maize.

Varieties	N Rates ($kg \cdot hm^{-2}$)	Treatments	Total Root Number	Root Length (cm)	Root Breadth Area (cm^2)	Root Dry Weight (g)
2019						
DK159	N225	PGR	59 a	1769a	494 a	28.1 a
		CK	55 b	1584 bc	472 bc	24.8 b
	N300	PGR	56 b	1575 bc	482 ab	25.0 b
		CK	48 de	1362 de	440 de	20.7 d
XY335	N225	PGR	55 b	1625 b	453 cd	25.2 b
		CK	50 cd	1473 cd	427 e	22.9 c
	N300	PGR	53 bc	1397 d	438 de	21.6 cd
		CK	46 e	1254 e	392 f	18.2 e
Variety (V)			***	***	***	***
N rates (N)			***	***	***	***
PGRs (P)			***	***	***	***
V × N			ns	ns	ns	ns
V × P			ns	ns	ns	ns
N × P			ns	ns	*	ns
V × N × P			ns	ns	ns	ns
2020						
DK159	N225	PGR	60 a	1688 a	488 a	26.6 a
		CK	54 bc	1540 b	460 b	24.4 bc
	N300	PGR	56 b	1487 b	463 b	25.1 ab
		CK	49 e	1345 c	429 c	22.3 de
XY335	N225	PGR	52 cd	1535 b	452 b	24.9 ab
		CK	48 e	1362 c	431 c	21.5 de
	N300	PGR	50 de	1376 c	420 c	22.5 cd
		CK	43 f	1219 d	388 d	20.4 e
Variety (V)			***	***	***	***
N rates (N)			***	***	***	***
PGRs (P)			***	***	***	***
V × N			ns	ns	ns	ns
V × P			ns	ns	ns	ns
N × P			ns	ns	ns	ns
V × N × P			ns	ns	ns	ns

Different lowercase letters represent significant differences among treatments ($p < 0.05$); ns means non-significant; * and *** indicate significance at $p < 0.05$ and < 0.001 probability levels, respectively.

3.4. The Root Activity of Maize

According to Figure 2, Variety (V), N rate (N), and PGRs treatment (P) had significant effects on root activity. N × P had significant effects on root activity at T1 in 2019. The root TTC reducing activity at T2 was higher than that at T1 of both two maize varieties. Root activity decreased with the increase in nitrogen fertilizer; root activity under N225 was higher by 14.63–33.66% than N300; and PGR significantly increased the root activity at both 10 days and 30 days after silking. Compared with the water treatment, PGR increased root activity by 24.36% and 23.60% in 2019 and 2020. Under the combined effects of nitrogen fertilizer and chemical control, root activity under N225 and PGR was higher than that under other treatments.

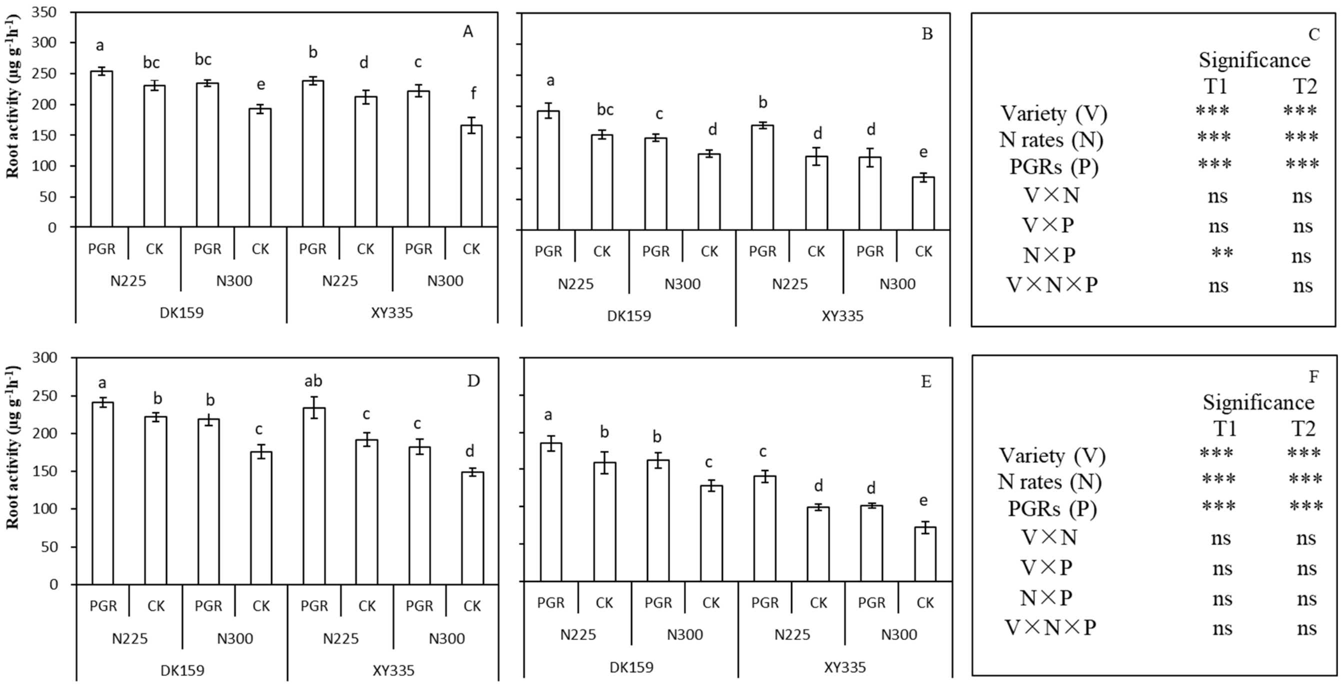

Figure 2. Effects of PGRs and nitrogen fertilizer on the root TTC reducing activity at 10 days after silking (**A**,**D**) and 30 days after silking (**B**,**E**) in 2019 and 2020. Effects of Variety (V), N rates (N), PGRs (P), and their interaction effects in 2019 (**C**) and 2020 (**F**) were listed (T1 represents 10 days after silking; T2 represents 30 days after silking). The vertical bars denote the mean ± the standard error of the mean; ns means non-significant; ** and *** indicate significance at $p < 0.01$ and < 0.001 probability levels, respectively. Different lowercase letters represent significant differences among treatments ($p < 0.05$).

3.5. The Root Bleeding Sap

According to Figure 3, Variety (V), N rate (N), and PGRs treatment (P) had significant effects on root bleeding sap (RBS). N × P had significant effects on RBS at T1 in 2019. V × N and V × P had significant effects on RBS at T2 in 2020. RBS at 30 days after silking was higher than that at 10 days after silking of both maize varieties. RBS decreased with the increase in nitrogen fertilizer; RBS under N225 was higher by 5.92–10.74% than N300; and PGR significantly increased the RBS at both stages. Compared with the water treatment, PGR increased RBS by 14.55% and 12.63% in 2019 and 2020. Under the combined effects of nitrogen fertilizer and chemical control, RBS under N225 was higher than that under other treatments.

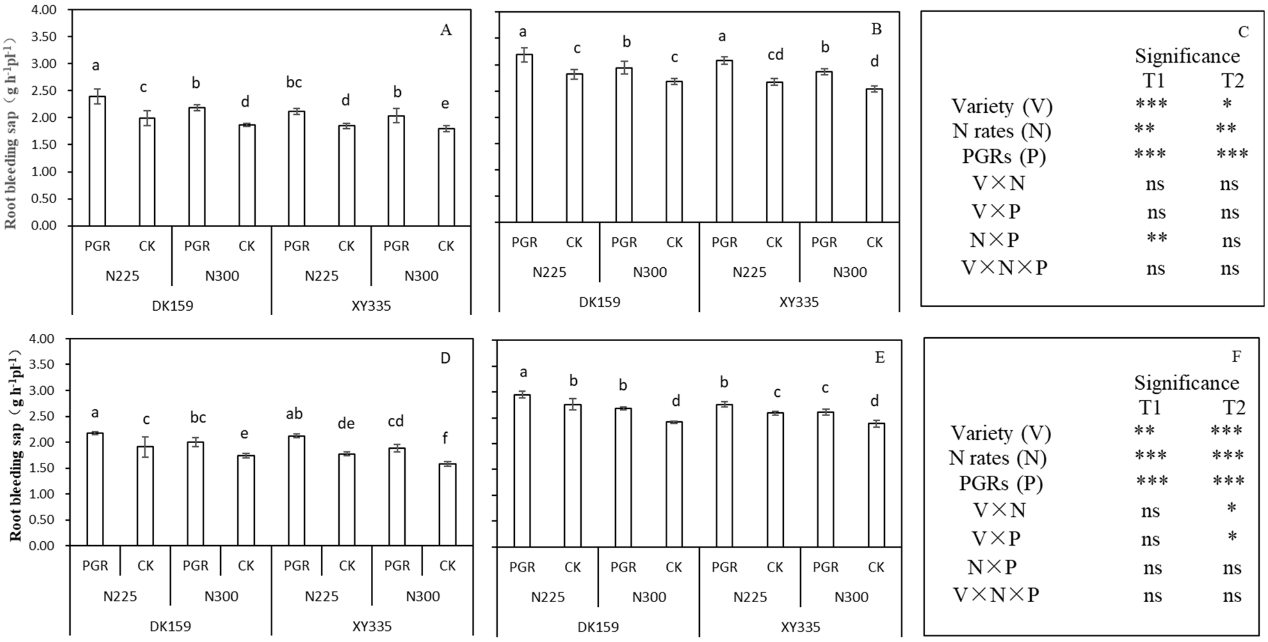

Figure 3. Effects of PGRs and nitrogen fertilizer on root bleeding sap at 10 days after silking (**A**,**D**) and 30 days after silking (**B**,**E**) in 2019 and 2020. Effects of Variety (V), N rates (N), PGRs (P), and their interaction effects in 2019 (**C**) and 2020 (**F**) were listed (T1 represents 10 days after silking; T2 represents 30 days after silking). The vertical bars denote the mean ± the standard error of the mean; ns means non-significant; *, **, and *** indicate significance at $p < 0.05$, < 0.01 and < 0.001 probability levels, respectively. Different lowercase letters represent significant differences among treatments ($p < 0.05$).

3.6. The Endogenous Hormones of Maize Root Bleeding Sap

Variety (V) and PGR treatment (P) had significant effects on IAA, GA, and CTK, while N rate (N) had significant effects on GA and CTK, and V × P had significant effects on plant height in both years. V × N had significant effects on IAA in 2019, N × P had significant effects on GA in 2019 and 2020, and V × N × P had significant effects on GA in 2019 (Table 4). The flow of IAA, GA, and CTK of root bleeding sap was different between the two cultivars (Table 4). As the nitrogen fertilizer of DK159 increased, the flow of IAA increased by 5.21%, and the flow of GA and CTK decreased by 9.58% and 13.36%, but the endogenous hormones of XY335 all decreased in varying degrees. After PGR treatment, the endogenous hormones of DK159 and XY335 under N225 and N300 were all changed. Taking the N300 as an example, the IAA flow of DK159 was increased by 77.49% and the GA and CTK were decreased by 30.80% and 42.21%. The IAA flow of XY335 was increased by 70.92% and the GA and CTK were decreased by 28.19% and 56.94%.

Table 4. Effects of PGRs and nitrogen fertilizer on the endogenous hormones of root bleeding sap.

Variety	N Rates (kg·hm^{-2})	Treatments	IAA (μL h^{-1}pl^{-1})	GA (μL h^{-1}pl^{-1})	CTK (μL h^{-1}pl^{-1})	GA/IAA	CTK/IAA	CTK/GA
2019								
DK159	N225	PGR	514.8 b	198.9 d	594.7 a	0.39 d	1.16 b	3.01 b
		CK	291.1 c	309.7 a	425.2 c	1.07 a	1.47 a	1.38 e
	N300	PGR	542.3 ab	188.1 de	518.8 b	0.35 d	0.96 d	2.79 c
		CK	305.5 c	271.8 b	364.8 d	0.89 b	1.20 b	1.34 f
XY335	N225	PGR	556.9 a	137.8 f	548.6 b	0.25 e	0.99 c	3.98 a
		CK	320.7 c	292.6 ab	399.7 c	0.91 b	1.25 b	1.37 e
	N300	PGR	538.0 ab	162.6 ef	516.1 b	0.30 e	0.96 d	3.18 b
		CK	314.8 c	226.5 c	328.8 e	0.72 c	1.04 c	1.45 d
Variety (V)			*	***	**	***	**	***
N rates (N)			ns	**	***	***	***	**
PGRs (P)			***	***	***	***	***	***
V × N			*	ns	ns	ns	ns	ns
V × P			ns	ns	ns	ns	ns	**
N × P			ns	***	ns	***	ns	**
V × N × P			ns	*	ns	ns	ns	*
2020								
DK159	N225	PGR	572.4 b	222.9 cd	614.9 a	0.39 d	1.08 b	2.78 b
		CK	329.9 d	349.5 a	437.8 c	1.06 a	1.34 a	1.25 e
	N300	PGR	614.1 ab	212.4 d	536.4 b	0.35 d	0.87 c	2.56 c
		CK	350.8 cd	306.3 b	375.8 de	0.90 b	1.11 b	1.23 e
XY335	N225	PGR	660.9 a	154.2 e	567.7 b	0.23 e	0.86 c	3.73 a
		CK	400.3 c	330.6 ab	411.8 cd	0.83 b	1.03 b	1.25 e
	N300	PGR	618.2 ab	181.7 de	533.9 b	0.29 e	0.86 c	2.98 b
		CK	383.5 cd	255.6 c	338.8 e	0.67 c	0.88 c	1.33 d
Variety (V)			*	***	*	**	**	*
N rates (N)			ns	**	**	*	*	*
PGRs (P)			***	***	***	***	**	***
V × N			ns	ns	ns	ns	ns	ns
V × P			ns	ns	ns	ns	ns	*
N × P			ns	**	ns	ns	ns	ns
V × N × P			ns	ns	ns	ns	ns	ns

Different lowercase letters represent significant differences among treatments ($p < 0.05$); ns means non-significant; *, **, and *** indicate significance at $p < 0.05$, < 0.01, and < 0.001 probability levels, respectively; IAA: 3-Indoleacetic acid; GA: gibberellin; CTK: cytokinin.

Variety (V), N rate (N), and PGR treatment (P) had significant effects on GA/IAA, CTK/IAA, and CTK/GA, V × P had significant effects on CTK/GA in both years. N × P had significant effects on GA/IAA and CTK/GA in 2019 and V × N × P had significant effects on CTK/GA in 2019 (Table 4). PGR treatment had different degrees of influence on the ratio of endogenous hormones. The GA/IAA ratio and CTK/IAA ratio were decreased by 56.72–72.29% and 2.27–21.62%; the CTK/GA ratio was increased by 108.13–198.40%. PGR altered the balance between the endogenous hormones.

3.7. Yield and Lodging Rate

In 2020, strong wind weather occurred at the end of August, so the lodging rate in 2020 was significantly higher than that in 2019 (Figure 4). Variety (V), N rate (N), and PGR treatment (P) had a more significant effect on the root lodging rate. N rates had no significance on grain yield, which was associated with decreased yield due to lodging. In both years, the root lodging rate (RLR) under PGR was lower than that under CK. Compared with CK, the RLR of DK159 and XY335 in 2019 were reduced significantly by 50.17% and 61.93.%. Those of DK159 and XY335 in 2020 were reduced significantly by 55.59% and 46.52%. RLR increased with the increase in nitrogen fertilizer and RLR under N225 was lower by 21.9–22.4% than N300.

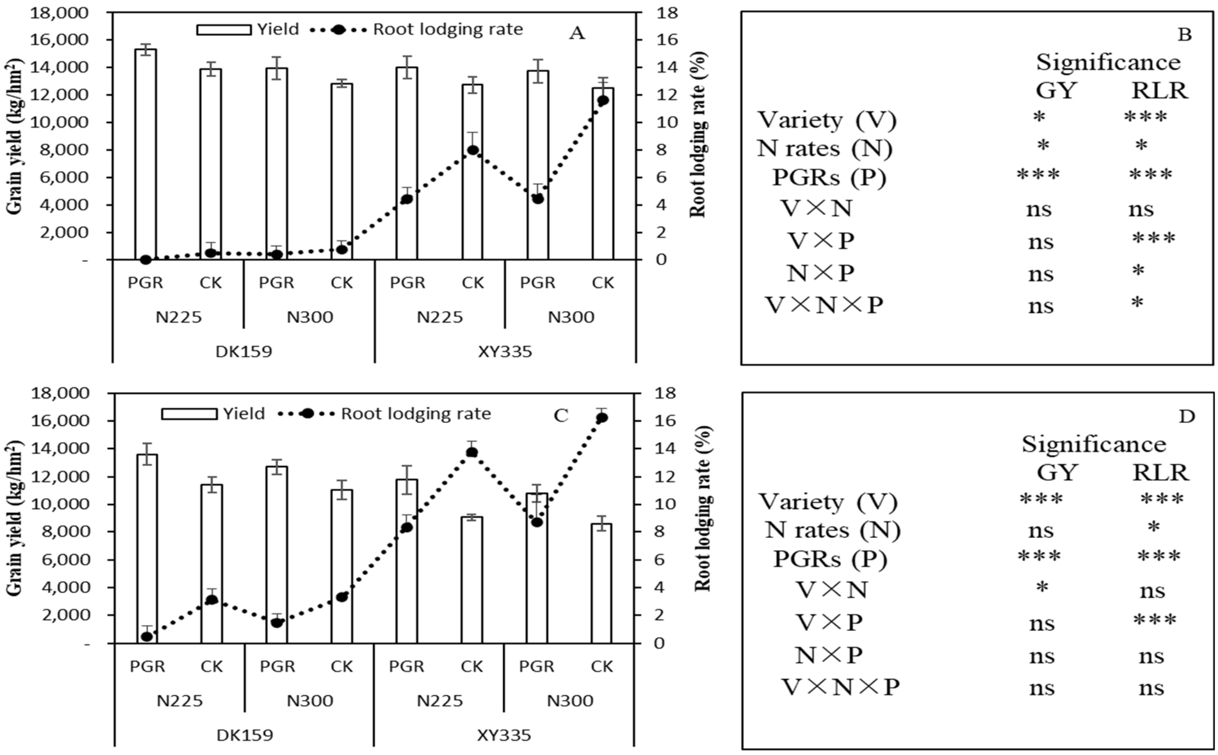

Figure 4. Effects of PGRs on grain yield and root lodging rate in 2019 (**A**) and 2020 (**C**). Effects of Variety (V), N rates (N), PGRs (P), and their interaction effects in 2019 (**B**) and 2020 (**D**) were listed (GY represents grain yield; RLR represents root lodging rate). The vertical bars denote the mean ± the standard error of the mean; ns means non-significant; * and *** indicate significance at $p < 0.05$ and <0.001 probability levels, respectively.

The yield of DK159 and XY335 decreased by 5.81% and 3.65% with the increase in nitrogen fertilizer, respectively (Figure 3). After PGR treatment, the yield of the two cultivars increased in varying degrees. Taking N225 as an example, the yield of DK159 and XY335 under PGR increased by 10.28% and 10.09%, respectively, in 2019; those of DK159 and XY335 increased by 19.00% and 25.62%, respectively, in 2020.

3.8. Correlation Analysis

The relationships among the grain yield, root lodging rate, and root–shoot traits of maize in 2019 and 2020 were examined by RDA. According to the results (Figure 5), the root lodging rate (RLR) was positively correlated with the plant height (PH), ear height (EH), center of gravity height (CGH), and ear height coefficient (EPC). Conversely, the root lodging rate was negatively correlated with total root number (TRN), root length (RL), root breadth area (RBA), and root dry weight (RDW). The grain yield (GY) was negatively correlated with RLR, PH, EH, CGH, and EPC. However, GY was positively correlated with TRN, RL, RBA, and RDW. The RDA showed that all shoot–root variables evaluated significantly explained the variation of root lodging and grain yield in 2019 and 2020, respectively. In particular, the root lodging rate had significant correlations with PH and RBA. In 2019, PH and RBA were vital factors that contributed to more than 80% of the total variation. In 2020, PH and RBA were vital factors that contributed to more than 85% of the total variation.

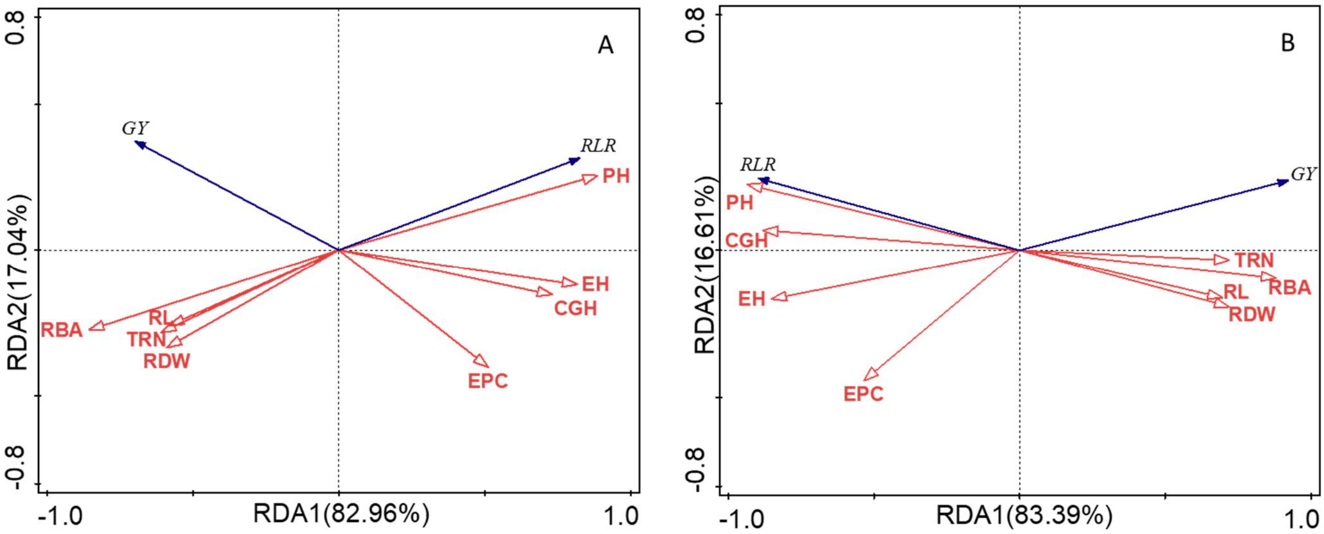

Figure 5. The redundancy analysis (RDA) used to identify the relationships among the grain yield, root lodging rate, and the maize shoot-root traits. (**A**) The relationships between grain yield, root lodging rate (blue arrows), and the maize shoot-root traits (red arrows) in 2019; (**B**) the relationships between grain yield, root lodging rate (blue arrows), and the maize shoot-root traits (red arrows) in 2020 (GY: grain yield; RLR: root lodging rate; PH: plant height; EH: ear height; CGH: center of gravity height; EPC: ear height coefficient; TRN: total root number; RL: root length; RBA: root breadth area; RDW: root dry weigh).

There was a significant or extremely significant positive correlation between the GA/IAA ratio and the shoot agronomic traits (Table 5). We also found that CTK/GA ratio was negative and significantly correlated with the shoot traits but was positive and significantly correlated with the root traits (Tables 5 and 6).

Table 5. Pearson's correlation coefficients and their statistical significance of endogenous hormones ratio and plant agronomic traits.

	Plant Height	Ear Height	Center of Gravity Height	Ear Height Coefficient
GA/IAA	0.459 *	0.653 **	0.606 **	0.788 **
CTK/IAA	−0.051	0.242	0.263	0.431 *
CTK/GA	−0.595 **	−0.693 **	−0.584 **	−0.826 **

Correlation coefficient values with "*" means significant difference ($p < 0.05$) and with "**" means very significant difference ($p < 0.01$).

Table 6. Pearson's correlation coefficients and their statistical significance of endogenous hormones ratio and root physical traits.

	Total Root Number	Root Length	Root Breadth Area	Root Dry Weight
GA/IAA	−0.277	−0.295	−0.265	−0.338
CTK/IAA	−0.090	0.167	−0.074	0.116
CTK/GA	0.616 **	0.494 *	0.428 *	0.511 *

Correlation coefficient values with "*" means significant difference ($p < 0.05$) and with "**" means very significant difference ($p < 0.01$).

Both photosynthetic index of ear leaf, root activity, and root bleeding sap were significantly positive correlated with grain yield of maize. Root lodging rate was not significantly correlated with yield in the slight-lodging year of 2019. In the serious-lodging year of 2020, photosynthetic index of ear leaf, root activity, and root bleeding sap were also significantly positively correlated with grain yield of maize, but root lodging rate was significantly negatively correlated with yield. Root activity and root bleeding sap had more significant correlations in the slight-lodging year but the photosynthetic index of ear leaf had more significant correlations in the serious-lodging year (Table 7).

Table 7. Pearson's correlation coefficients and their statistical significance of grain yield and ear leaf and root indicators.

	Pn	RuBPCase	PEPCase	Root Activity	Root Bleeding Sap	Root Lodging Rate
Grain yield in 2019	0.846 **	0.894 **	0.869 **	0.924 **	0.953 **	−0.669
Grain yield in 2020	0.862 **	0.905 **	0.940 **	0.914 **	0.847 **	−0.912 **

Correlation coefficient values with "**" mean very significant difference ($p < 0.01$).

4. Discussion

4.1. Effects of PGRs and Nitrogen Fertilizer on Root Lodging Resistance

At present, maize production relied on increasing the population number to obtain high yield [30]. The increase in planting density led to changes in light, temperature, water, nutrients, and space conditions required for maize growth. On the one hand, the plant was affected by shade effect: the leaf area, chlorophyll content and photosynthetic rate decreased, the plant was prone to premature senescence and death, and the photosynthetic capacity and assimilate accumulation capacity decreased. On the other hand, the activity, absorption capacity, and synthesis capacity of the root system decreased. The combined effect of the two factors resulted in a decrease in single plant quality and an increase in lodging risk in the high-density population, which was not conducive to the realization of a high yield of maize.

There are two kinds of maize lodging. In addition to stem lodging, root lodging is also a common disaster in maize production, especially under the climate and natural environment in Northeast China. Root quality is one of the key factors that cause plant lodging and yield decline under high-density conditions. Specific performance was as follows: with the increase in the density, the layer number, root total number, root length, root dry weight, root volume, root surface area, and active absorption area of maize root decreased, and as the growth period advanced, the decline was more and more obvious [8,31]. Rational application of nitrogen fertilizer can significantly increase the root dry weight, root length, and root absorption area of maize [32]. Plant growth regulators can increase root absorption capacity by improving root morphology [33]. At the same time, it is beneficial to maintain the function of the aboveground growth and prevent the occurrence of lodging.

Zhang et al. [34] indicated root lodging resistance was positively correlated with stem lodging resistance in maize. However, Xue et al. [35] showed that there was no correlation between root and stem lodging resistances. In this study, RDA analysis showed that root lodging rate was not only significantly positively correlated with root morphology traits but also significantly negatively correlated with plant stalk agronomic traits; plant height (PH) and root breadth area (RBA) were the most important factors that affected root lodging. Synergistic regulation of root–shoot traits is of great significance for root resistance.

4.2. Effects of PGRs and Nitrogen Fertilizer on Photosynthetic Traits of Plants

Photosynthesis is the physiological basis of crop growth, development, and yield formation. The improvement of photosynthetic capacity plays an important role in increasing grain yield. Increasing the planting density is beneficial to increase the light interception rate of the population and improve the photosynthetic capacity of the population, but excessive density would accelerate leaf senescence and reduce the photosynthetic capacity of the population [36]. Nitrogen fertilizer is an important factor regulating crop photosynthesis. The rational application of nitrogen fertilizer can take advantage of the appropriate planting density to give full play to the population advantages for photosynthetic production [37]. Nitrogen application could increase NR and GS activities in ear leaves and delay the decline in chlorophyll content in leaves after anthesis, significantly increase leaf area index and leaf photosynthetic rate, and provide the basis for high yield [38]. PGRs treatment significantly increased chlorophyll content and photosynthetic carboxylase activity in leaves and significantly increased photosynthetic rate [39]. In this study, maize yield was significantly or extremely significantly positively correlated with Pn, RuBP carboxylase, and PEP carboxylase activities in ear position leaves. The Pn, RuBP carboxylase, and PEP carboxylase decreased with the increase in nitrogen application rate. PGRs significantly increased the net photosynthetic rate and photosynthetic carboxylase activity in leaves. The results of this study also showed that nitrogen application of 225 kg·hm^{-2} at high density and PGRs treatment could improve the activity of maize leaf source, prolong the duration of photosynthetic function of maize leaf, and lay the foundation for the improvement of maize yield.

4.3. Effects of PGRs and Nitrogen Fertilizer on Root Traits and Root Bleeding Sap

Rational application of nitrogen fertilizer could significantly increase root dry weight and root length, coordinate the production, accumulation, and transport of photosynthetic substances between root, leaf, and grains, and increase grain yield [40]. Roots played an important role in water and nutrient absorption and lodging resistance during the whole growth period of maize, and the number of roots and bending resistance were higher under the nitrogen application rate of 120 kg·hm^{-2} than without nitrogen application [41]. In this study, the total root number (TRN), root length (RL), root breadth area (RBA), and root dry weight (RDW) of DK159 and XY335 decreased with an increase in the application of nitrogen fertilizer. PGRs increased the above root indexes in different degrees and effectively improved the root traits of maize under high density, which played a positive role in preventing lodging and premature senescence. Nitrogen application of 225 kg·hm^{-2} and PGRs treatment effectively promoted root growth and development, which was beneficial to improve lodging resistance and coordinate the functional balance between root and aboveground parts, so as to provide a guarantee for the improvement of yield.

The hormone system is the most important information system in plants. As a signal substance produced by plants themselves, the hormone system participates in the regulation of plant growth and development. Roots not only produce endogenous hormones (CTK, ACC) but also receive endogenous hormones (IAA, GA, etc.) produced from the aboveground plant and transported to the root system. The interaction pathway is mainly realized through bleeding sap. Therefore, plants can regulate the root–canopy function by regulating root bleeding flow and endogenous hormone content [42]. Studies show that root hormones not only play an important role in regulating root growth, development, and function, but also act as signal carriers to participate in regulating the growth, development, and metabolism of the aboveground plant. Nitrogen had a great influence on the intensity of root bleeding sap and insufficient nitrogen application significantly reduced the intensity of root bleeding sap in each period, thus leading to a significant decline in root absorption capacity, insufficient dry matter accumulation, and reduced yield [43]. PGR affected plant growth and development through external application of plant growth regulators to regulate endogenous hormone content of plants [44]. The flow of three endogenous hormones in root bleeding was measured in this experiment, the results indicate that with the increase in nitrogen fertilizer, the flow of IAA increased, GA and CTK decreased for DK159, while the flow of IAA, GA, and CTK of XY335 decreased. The endogenous hormone change may be related to the variety characteristics. Under the condition of high density, the population was closed, the canopy light and temperature conditions deteriorated, and the competition between nutrients and water in the rhizosphere intensified, resulting in the internal physiological metabolism of the plant disorder. Furthermore, the individual plant quality decreased and then affected the production and transport of endogenous hormones, resulting in the change in endogenous hormone content in the root system. After PGRs treatment, the flow of IAA and CTK significantly increased, while the flow of GA significantly decreased. These changes promoted plant growth and increased root quality, which was of great significance to yield under high-density conditions.

Plant physiological activities are regulated by a variety of endogenous hormones and it is not the absolute content of certain hormones but the relative content of various hormones that determines the physiological effects [45]. In this study, Pearson's correlation analysis showed that the GA/IAA ratio was significantly positively correlated with the stem agronomic traits; the CTK/GA ratio was significantly negatively correlated with the stem traits but was significantly positively correlated with the root traits. The change in the ratio of endogenous hormones in the maize root system changes the balance level of hormones, and the balance level is different in different treatments, which may be related to the influence of PGR on the synthesis period, synthesis site, and transport route of hormones.

4.4. Effects of PGRs and Nitrogen Fertilizer on Grain Yield

Maize grain yield increased with the increase in nitrogen application rate, but when the nitrogen application rate reached a certain level, the yield showed a downward trend [46]. PGRs improved ear characters and increased the yield of maize [47]. Otie [48] studied the effects of multiple chemical control agents and different nitrogen application rates on maize growth and yield and the results showed that maize yield was high under the combined effect of 180 $kg \cdot hm^{-2}$ nitrogen fertilizer and chemical control. In this study, the average yield of maize in 2020 was significantly lower than that in 2019, which was related to strong wind weather in the test area in late-August 2020, which was in the maize filling period. PGRs treatment significantly reduced root lodging rate and increased yield. Under the nitrogen application rate of 225 $kg \cdot hm^{-2}$ and PGRs treatment, the maximum yield was 15,306.34 $kg \cdot hm^{-2}$. It should be pointed out that the root lodging rate of XY335 was higher between the two varieties; in both years, the difference in the root lodging rate between DK159 and XY335 was extremely significant. It can be seen that the negative correlation between the yield and the root lodging rate is significant, which is not only due to the difference between varieties but is also related to the fact that root lodging was more likely to be caused by the humid and rainy climate in the test location.

5. Conclusions

N225 application in combination with PGRs significantly decreased the height of plants and improved root distribution and dry weight. The change in stem and root morphology was effectively affected by the balance levels of endogenous hormones and reduced the occurrence of root lodging. N225 combined with PGRs obviously enhanced the photosynthetic rate and activities of photosynthetic carboxylase in leaves and improved root bleeding sap and root activities after the silking stage. As a result, it was conducive to photosynthate accumulation and root nutrient supply and provided a material basis for yield formation. Therefore, nitrogen fertilizer and PGRs management might be an appropriate approach in high-density planting of maize production because this approach can coordinate and regulate root and shoot morphological and physiological functions and plays a crucial role in reducing root lodging and improving maize yield.

Author Contributions: N.S.: conceptualization, data collection, data analysis, methodology, software, writing original draft. X.C.: data collection, data interpretation, methodology, software, writing original draft. H.Z.: literature search, data analysis, writing original draft. X.M.: study design, revising & editing draft. S.B.: methodology, resources, study design, writing & editing draft. All authors have agreed to take responsibility for the accuracy and authenticity of the whole research content. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Basic Scientific Research Funds Project of Jilin Province, China (KYJF2021ZR020), the National Maize Industry Technology System of China (CARS-02-50), and the Agricultural Science and Technology Innovation Project-Innovation Team of Jilin Province, China (CXGC2021TD003).

Data Availability Statement: Not applicable.

Acknowledgments: The authors would like to thank the anonymous reviewers for their valuable comments and suggestions, which substantially improved the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Tokatlidis, I.S.; Koutroubas, S.D. A review of maize hybrids' dependence on high plant populations and its implications for crop yield stability. *Field Crops Res.* **2004**, *88*, 103–114. [CrossRef]
2. Tollenaar, M.T.; Lee, E.A. Yield potential, yield stability and stress tolerance in maize. *Field Crops Res.* **2002**, *75*, 161–169. [CrossRef]
3. Yang, J.Z.; Chen, M.L.; Zhang, H.S. Meta-Analysis of the relationship between maize crop yield and plant density from 1950s to 2000s in China. *Sci. Agric. Sin.* **2013**, *46*, 3562–3570.
4. Li, S.T.; Bian, D.H.; He, L.; Wang, D.M.; Zheng, X.M.; Cui, Y.H. Lodging characteristics of summer maize and chemical regulation research progresses preventing lodging in the north China plain. *J. Maize Sci.* **2018**, *26*, 95–101.
5. Boomsma, C.R.; Santini, J.B.; Tollenaar, M.; Vyn, T.J. Maize morphophysiological responses to intense crowding and low nitrogen availability: An analysis and review. *Agron. J.* **2009**, *101*, 1426. [CrossRef]
6. Liu, T.N.; Gu, L.M.; Dong, S.T.; Zhang, J.W.; Liu, P.; Zhao, B. Optimum leaf removal increases canopy apparent photosynthesis, 13C-photosynthate distribution and grain yield of maize crops grown at high density. *Field Crops Res.* **2015**, *170*, 32–39. [CrossRef]
7. Chen, Y.L.; Wu, Q.P.; Chen, X.C.; Chen, F.J.; Zhang, Y.J.; Li, Q.; Yuan, L.X.; Mi, G.H. Root growth and its response to increasing planting density in different maize hybrids. *Plant Nutr. Fertil. Sci.* **2012**, *18*, 52–59. [CrossRef]
8. Li, N.; Huo, Z.X.; Li, J.M.; Wu, P.B.; Duan, L.S.; Li, Z.H. Effects of density on agronomic, root traits and yield of maize with different plant types. *Maize Sci.* **2008**, *16*, 98–102.
9. Guo, S.L.; Chen, N.N.; Qi, J.S.; Yue, R.Q.; Han, X.H.; Yan, S.F.; Lu, C.X.; Fu, X.L.; Guo, X.H.; Tie, S.G. Study on the relationship between yield and lodging traits of maize under different planting densities. *Maize Sci.* **2018**, *26*, 71–77.
10. Li, S.Y.; Ma, W.; 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.
11. Wang, X.B.; Hou, H.P.; Zhou, B.Y.; Sun, X.F.; Ma, W.; Zhao, M. Effect of strip subsoiling on population root spatial distribution of maize under different planting densities. *Asta Agron. Sin.* **2014**, *40*, 2136–2148. [CrossRef]
12. Zhang, Q.; Zhang, L.Z.; Evers, J.; Werf, W.V.D.; Zhang, W.Q.; Duan, L.S. Maize yield and quality in response to plant densityand application of a novel plant growth regulator. *Field Crops Res.* **2014**, *164*, 82–89. [CrossRef]
13. Fan, H.C.; Gu, W.R.; Yang, D.G.; Yu, J.P.; Po, L.; Zhang, Q.; Zhang, L.G.; Yang, X.H. Effect of chemical regulators on physical and chemical properties and lodging resistance of spring maize stem in Northeast China. *Acta Agron. Sin.* **2018**, *44*, 909–919. [CrossRef]
14. Ahmad, I.; Kamran, M.; Ali, S.; Bilegjargal, B.; Cai, T.; Ahmad, S.; Meng, X.P.; Su, W.N.; Liu, T.N.; Han, Q.F. Uniconazole application strategies to improve lignin biosynthesis, lodging resistance and production of maize in semiarid regions. *Field Crops Res.* **2018**, *222*, 66–77. [CrossRef]
15. Kamran, M.; Ahmad, I.; Wang, H.; Wu, X.; Xu, J.; Liu, T.; Ding, R.; Han, Q. Mepiquat chloride application increases lodging resistance of maize by enhancing stalk physical strength and lignin biosynthesis. *Field Crops Res.* **2018**, *224*, 148–159. [CrossRef]
16. Herder, G.D.; Isterdael, G.V.; Beeckman, T.; Smet, I.D. Theroots of a new green revolution. *Trends Plant Sci.* **2010**, *15*, 600–607. [CrossRef]
17. Garnett, T.; Conn, V.; Kaiser, B.N. Root based approaches to improving nitrogen use efficiency in plants. *Plant Cell Environ.* **2009**, *32*, 1272–1283. [CrossRef]
18. Lynch, J.P. Steep, Cheap and deep: An ideotype to optimize water and N acquisition by maize root systems. *Ann. Bot.* **2013**, *112*, 347–357. [CrossRef]
19. Li, C.H.; Liu, K.; Zhou, S.M.; Luan, L.M. Response of photosynthesis to ecophysiobgial factors of summer maize on different fertilizer amounts. *Acta Agron. Sin.* **2002**, *28*, 265–269.
20. Liu, P.; Dong, S.T.; Li, S.K.; Zhang, J.W. Efficient utilization of nitrogen in high-yielding maize. *Sci. Agric. Sin.* **2017**, *50*, 2232–2237. [CrossRef]
21. Ye, D.L.; Zhang, Y.S.; Al-Kaisi, M.M. Ethephon improved stalk strength associated with summer maize adaptations to environments differing in itrogen availability in the North China Plain. *J. Agric. Sci.* **2016**, *154*, 960–977. [CrossRef]
22. Li, H.S. *Principles and Techniques of Plant Physiological and Biochemical Experiments*; Higher Education Press: Beijing, China, 2005; pp. 134–140. ISBN 978-7-04-008076-6.
23. Qi, Q.G.; Li, K.; Li, G.; Li, C.Y.; Cao, G.J. Effects of nitrogen nutrient level on leaf carbon metabolism of spring maize. *Agric. Sci. Technol.* **2010**, *38*, 9973–9974, 9978.

24. Holanda, F.; Mengel, D.; Paula, M.; Carvaho, J.; Bertoni, J. Influence of crop rotations and tillage systems on phosphorus and potassium stratification and root distribution in the soil profile. *Commun. Soil Sci. Plant Anal.* **1998**, *29*, 2383–2394. [CrossRef]
25. Zhao, S.J.; Shi, G.A.; Dong, X.C. *Plant Physiology Experiment Instruction*; China Agricultural Science and Technology Press: Beijing, China, 2002; pp. 30–40. ISBN 978-7-10-921288-6.
26. Chen, F.J.; Mi, G.H.; Liu, J.A.; Zhang, F.S. Differences of nitrogen forms in xylem bleeding fluid of maize inbred lines and its relationship with nitrogen efficiency. *Sci. Agric. Sin.* **1999**, *32*, 43–48.
27. Wang, H.; Xu, R.R.; Li, Y.; Yang, L.Y.; Shi, W.; Liu, Y.J. Enhance root-bleeding sap flow and root lodging resistance of maize under a combination of nitrogen strategies and farming practices. *Agric. Water Manag.* **2019**, *224*, 105742. [CrossRef]
28. He, Z.P. *Experimental Guidance for Chemical Control of Crops*; Beijing Agricultural University Press: Beijing, China, 1993; pp. 10–15. ISBN 978-7-81-002841-7.
29. Sezegen, B.; Carena, M.J. Divergent recurrent selection for cold tolerance in two improved maize populations. *Euphytica* **2009**, *167*, 237–244. [CrossRef]
30. Zhao, M.; Li, J.G.; Zang, B.; Dong, Z.Q.; Wang, M.Y. Compensation mechanism for tapping potential of crop high yield. *J. Crops* **2006**, *32*, 1566–1577.
31. Guan, J.H.; Guo, X.Y.; Liu, Y.; Liu, K.L.; Wang, J.H.; Guo, X.D. Spatial distribution dynamics of maize root dry weight under different densities. *Maize Sci.* **2007**, *4*, 105–108, 118.
32. Zhang, Y.; Qin, H.D.; Wu, L.M.; Zhang, J.; Li, Z.; Huang, M.; Jiang, L.G. Growth characteristics and the effect of nitrogen application on the maize root. *J. China Agric. Univ.* **2014**, *19*, 62–70.
33. Zhang, X.J.; Cai, J. Application effect of maize special regulator Yuhuangjin on summer maize. *Anhui Agric. Sci.* **2007**, *13*, 88.
34. Zhang, P.; Gu, S.C.; Wang, Y.Y.; Yang, R.M.; Yan, Y.; Zhang, S.; Sheng, D.C.; Wang, P.; Huang, S. Morphological and mechanical variables associated with lodging in maize (*Zea mays* L.). *Field Crop Res* **2021**, *269*, 108178. [CrossRef]
35. 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]
36. Lv, L.H.; Tao, H.B.; Zhang, Y.J.; Zhao, M.; Zhao, J.R.; Wang, P. Canopy structure and photosynthesis traits of summer maize under different planting densities. *Acta Agron. Sin.* **2008**, *34*, 447–455.
37. Chen, Z.H.; Fan, L.Y.; Li, C.F. Study on regulation technology of spring maize density fertilizer. *J. Maize Sci.* **1996**, *4*, 57–59.
38. 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-anthesis matter production and leaf function characteristics of different maize cultivars with low nitrogen tolerance. *Chin. J. Eco-Agric.* **2016**, *24*, 17–26. [CrossRef]
39. Sun, X.F.; Ding, Z.S.; Hou, H.P.; Ge, J.Z.; Tang, L.Y.; Zhao, M. Characteristics of light and matter production and changes of carbon and nitrogen content in different spring maize varieties after anthesis. *Acta Agron. Sin.* **2013**, *39*, 1284–1292. [CrossRef]
40. Qi, D.L.; Wu, X.; Hu, T.T. Effects of nitrogen application on maize root growth, yield and nitrogen utilization. *Sci. Agric. Sin.* **2014**, *47*, 2804–2813. [CrossRef]
41. Cheng, S.; Li, P.C.; Liu, Z.G.; Zhao, L.F.; Mi, G.H.; Yuan, L.X.; Chen, F.J. Effects of density and nitrogen fertilizer on nodal root number of maize hybrids. *J. Plant Nutr. Fertil.* **2016**, *22*, 1118–1125.
42. Shi, X.D.; Liu, Y.F.; Wen, Z.Q.; Wang, W.W. Research progress of plant root bleeding sap. *Anhui Agric. Sci.* **2006**, *34*, 2043–2045.
43. Li, H.L.; Sun, Y.Y.; Qu, J.L.; Wei, C.Q.; Sun, G.H.; Zhao, Y.T.; Chai, Y.S. Effects of nitrogen application rate on morphological and physiological characteristics of roots of Northeast japonica rice. *Chin. J. Rice Sci.* **2012**, *26*, 723–730. [CrossRef]
44. Li, C.Z.; Li, C.J. Ridge-furrow with plastic film mulching system decreases the lodging risk for summer maize plants under different nitrogen fertilization rates and varieties in dry semi-humid areas. *Field Crops Res.* **2021**, *263*, 108056. [CrossRef]
45. Wei, X.T.; Zhang, M.C.; Zhang, Y.; Li, Z.H.; Duan, L.S. Effects of ethephon on internode elongation and endogenous hormones in different genotypes of maize. *Chin. J. Pestic. Sci.* **2011**, *13*, 475–479.
46. Cai, H.G.; Yuan, J.C.; Liu, J.Z.; Yan, X.G.; Zhang, H.X.; Liang, Y.; Ren, J. Nitrogen demand and optimum nitrogen application rate of spring maize under high density planting. *Sci. Agric. Sin.* **2017**, *50*, 1995–2005. [CrossRef]
47. Huang, G.M.; Liu, Y.R.; Guo, Y.L.; Peng, C.X.; Tan, W.M.; Zhang, M.C.; Li, Z.H.; Zhou, Y.Y.; Duan, L.S. A novel plant growth regulator improves the grain yield of high-density maize crops by reducing stalk lodging and promoting a compact plant type. *Field Crops Res.* **2021**, *260*, 107982. [CrossRef]
48. Otie, V.; Ping, A.; John, N.M. Interactive effects of plant growth regulators and nitrogen on corn growth and nitrogen use efficiency. *J. Plant Nutr.* **2016**, *39*, 597–1609. [CrossRef]