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Effects of plant density and nitrogen rate on lodging-related stalk traits of summer maize

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ABSTRACT

Stalk lodging is a major constraint to limit grain yield under increased planting density in modern maize (*Zea mays* L.) production. A 3-year field experiment was imposed to study the effects of plant density and nitrogen (N) rate on stalk lodging and lodging-related stalk traits of two maize hybrids of contrasting susceptibility to lodging. The results indicated that the stem diameter, rind penetration strength (RPS), bending strength (BS), rind thickness, vascular bundle sheath's thickness (Vbs) and number of vascular bundle (Vb) of both hybrids all significantly decreased at the high density, while ear height and ear ratio clearly increased in Ludan981 (LD981; lodging-susceptible cultivar) with plant density increase. The lower plant height and ear height, stronger RPS and BS, and more Vb at high plant density might be reasons for Zhengdan958 (ZD958, lodging-resistant cultivar) had lower rate of stalk lodging. Meanwhile, N supply can significantly improve the stalk quality and decrease the risk of stalk lodging, however, little effects were observed in cv. LD981. Therefore, using lodging-resistant cultivar, planting at 82 500 plant/ha and supplying 180 kg N/ha can obtain high grain yield and low stalk lodging.

Keywords: maize lodging; lodging resistance; mechanical property; microstructure

Stalk lodging in maize has been a major problem in maize producing areas worldwide, which may result in loss of the ear at harvest (Zhang et al. 2014). When lodging occurs, the normal canopy structure is destroyed, resulting in the reduction of leaf photosynthetic activity and grain yield (Islam et al. 2007). Apart from decreases in yield, stalk lodging may result in a more difficult harvest and reduction in crop quality (Kang et al. 1999).

Many of the studies (Dudley 1994, Ma et al. 2014) on the relationship between stalk lodging and stalk traits in maize have demonstrated that plant height, ear height, stem diameter, length of basal internodes, rind thickness, rind penetration strength (RPS), and bending strength (BS) in maize are critical stem traits that can be used to estimate the lodging resistance of maize. Besides, stalk chemical constituents such as lignin, cellulose, potassium, nitrogen (N) and sodium were used to estimate lodging in maize (Esechie 1985).

Increasing plant density is a major measure for improving grain yield in maize production. At high density, however, plants may compete for light and soil resources available (e.g., water and nutrients; Tollenaar et al. 2006), which results in thinner stems that increase the risk of lodging. Nitrogen supply can enhance the lodging resistance by increasing the activity of the key enzymes regulating the lignin biosynthesis, the content of lignin and stalk diameter (Peng et al. 2014). Studies in the past decades focused primarily on the effects and reasons of stalk lodging and selection of stalk lodging indicators, but little attention was given to the stalk mechanical performance and microstructure of different density-tolerant cultivars at high plant density. Therefore, the objectives of this study were to (i) quantify the effects of plant density and N rate on the stalk morphology, mechanical properties and anatomical structure of summer maize; (ii) discuss the adaptive changes for stalk

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traits of lodging-resistant cultivar in response to plant density increase.

MATERIAL AND METHODS

Experimental site and design. A 3-year field experiment was conducted at the Corn Research Center (36°10'N, 117°09'E) of the Shan-dong Agricultural University, Tai'an, Shandong province, China in 2013–2015. The soil type was sandy loam (Typic Cambisols) composed of 6.0 g/kg organic carbon, 0.91 g/kg total N, 47.19 mg/kg available phosphate (Olsen method; Zandstra 1968) and 84.23 mg/kg available potassium (Dirks-Sheffer method; Melich 1953) in the top 0–20 cm arable soil layer. The seasonal average temperatures and precipitation were 24.55, 26.35, and 25.42°C and 247.7, 225.4, 236.1 mm in 2013, 2014 and 2015.

For this study, Zhengdan958 (ZD958, lodging-resistant cultivar) and Ludan981 (LD981, lodging-susceptible cultivar), were used for experimental materials. Two plant densities (D1 – 52 500, D2 – 82 500 plants/ha), and three N rates (N0 – 0, N120 – 120, N180 – 180 kg N/ha as urea) were conducted for the two cultivars. The experiments were established in a split-plot design of three replicates (36 subplots), with cultivar as the main plot, with density and nitrogen rate as the sub-plot. These subplots were arranged randomly and the size of each subplot was 15.0 m × 3.0 m (rows spaced 60 cm apart).

Basal fertilization of each subplot was applied before tillage during both years; it included phosphorus as calcium superphosphate and potassium as chloride at the rate of 53 kg P/ha and 119 kg K/ha, respectively. The N fertilizer source was half applied at the sixth leaf stage (V6) and half at the twelfth leaf stage (V12). The seeds were sown on 17 June and harvested on 5 October in each test year. Water, weeds, insects, and diseases were controlled as required to avoid yield loss.

Grain yield. In this experiment, thirty successive ears were hand harvested from the centre rows of each plot at physiological maturity (R6). Grains of all harvested ears were separated manually from cobs and weighed to determine the average individual yield. The grain yield per hectare was the product of ear density and average individual yield.

Stalk lodging estimates. All plants that bent and broke in the central three rows of each plot were counted to calculate stalk lodging at R6. Stalk lodging was the ratio of the number of lodged plants to the total number of plants in each subplot.

Stalk trait measurements. At the silking stage (R1), 15 successive plants in the centre three rows of each subplot were chosen to measure the plant height, ear height and stem diameter. Ear ratio was the ratio of ear height to plant height, expressed as a percentage of plant height.

The RPS and BS at the middle point of the basal third internodes were measured using a Stalk Strength Tester (YYD-1, Top Instrument Co., Ltd, Zhejiang, China) at R1. Measurements of RPS, BS were probed at 1×10^{-6} m^2 and 0.5×10^{-4} m^2, respectively (Ma et al. 2014).

Four stems in each treatment were selected at R1 in 2014 growing season. About 1.5 cm stem at the upper middle part of the third basal internodes was fixed in the Carnoy fixative. Thin slices were cut using the method of free hand section and stained with safranin. The slices were further microscopically examined using a fluorescence microscopy imaging system (OlympusBX51, Olympus Corporation, Tokyo, Japan) and photographed to calculate the number of big, small and total vascular bundle (Vb) per plant (Fu et al. 2013). At the same time, the thickness of the rind and vascular bundle sheath (Vbs) were measured with a micrometer (Olympus Corporation, Tokyo, Japan).

Statistical analysis. Treatments effects on stalk lodging, grain yield and stalk morphology and mechanical properties in the three years, and stem anatomical structure in 2014 were analysed according to the principles of analysis of variance, using the general linear model (GLM) in SPSS 17.0 (SPSS Inc., Chicago, USA). Significant differences among means were determined by the Duncan's multiple range tests at 5% level; correlations between lodging rate and stalk traits were estimated as the Pearson's correlation coefficient.

RESULTS AND DISCUSSION

Lodging rate and grain yield. Increasing plant density can increase grain yield per unit area significantly, but it also increase the possibility of lodging (Tokatlidis et al. 2010). In this study, averaged for N rates, the lodging rate of cvs. LD981 and ZD958 increased significantly by 119.5–145% and 11.3–19.1%, respectively, over the three years

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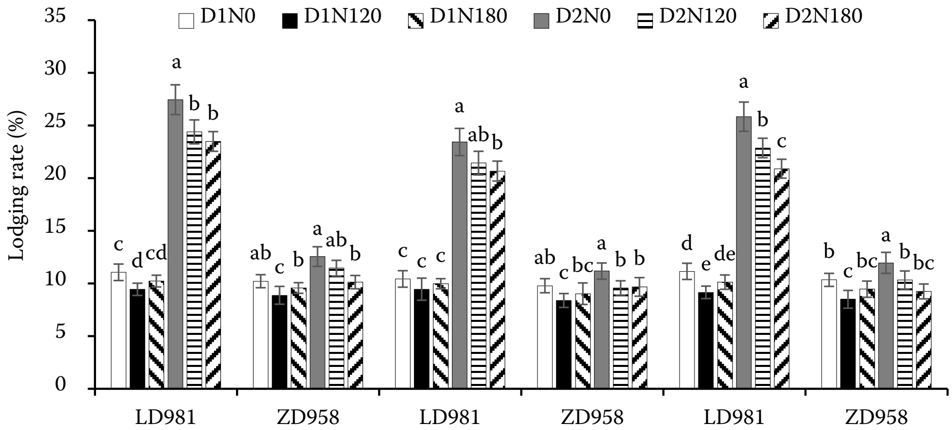

Figure 1. Effects of density increase and nitrogen (N) rate on the lodging rate of summer maize. Means (columns) and standard error (error bars) are presented (n = 3). Different small letters indicate differences between densities and N rates within each cultivar and year at P < 0.05. D1 – 52 500, D2 – 82 500 plants/ha; N0 – 0, N120 – 120, N180 – 180 kg N/ha

(Figure 1). The higher increase of the lodging rate in cv. LD981 resulted in only 3.2–4.9% increase in grain yield with the plant density increased; to the contrary, the grain yield of cv. ZD958 increased by 29.2–33.7% for the lower lodging rate (Figure 2). Thus, using the lodging-resistant cultivar can reduce the risk of lodging, effectively, and obtain high grain yield at high plant density.

N fertilizer is an important agronomic practice for maize production to obtain high grain yield, and previous studies (Li et al. 2010) indicated that N supply can significantly increase the quality of stalk. In this study, under D1, significantly lowest lodging rate was observed at N 120 for both cultivars, while a consistent decrease was clearly visible under increasing N rates at D2 (Figure 1). The grain yield of both cultivars showed the opposite trend with N rate increase (Figure 2). In addition, no significant differences were observed between N120 and N180 in grain yield of cv. LD981, while the grain yield of cv. ZD958 in N180 was significantly higher than in N120 at D2.

Stalk morphological traits. The plant height, ear height, ear ratio and stem diameter were significantly affected by plant density and genotype (Table 1). With plant density increased, the plant height, ear height and ear ratio of cv. LD981 increased by 1.1–2.1, 7.9–9.8 and 4.9–7.3%, respectively, and that of cv. ZD958 increased by 1.6–2.0, 0.8–1.7 and 0–2.3%, respectively, over the three years. At the same time, the stem diameter of cvs. LD981 and ZD958 decreased by 8.7–9.1% and 7.2–8.0%,

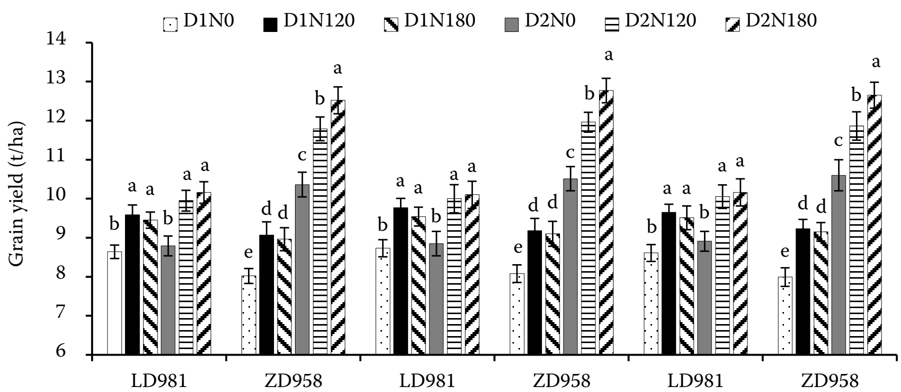

Figure 2. Effects of density increase and nitrogen (N) rate on the grain yield of summer maize. Means (columns) and standard error (error bars) are presented (n = 3). Different small letters indicate differences between densities and N rates within each cultivar and year at P < 0.05. D1 – 52 500, D2 – 82 500 plants/ha; N0 – 0, N120 – 120, N180 – 180 kg N/ha

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Table 1. Analysis of variance of lodging rate, grain yield; stalk traits as affected by year cultivar, plant density and nitrogen rate

Analysis of variance	Lodging rate	Grain yield	Plant height	Ear height	Ear ratio	Stem diameter	RPS	BS
Year (Y)	***	ns	**	***	**	*	***	***
Genotype (G)	***	***	***	***	***	ns	***	***
Density (D)	***	***	***	***	***	***	***	***
N rate (N)	***	***	*	ns	ns	***	***	***
Y × G	ns	ns	ns	ns	ns	ns	**	ns
Y × D	***	ns	ns	ns	ns	ns	*	ns
G × D	***	***	ns	***	ns	ns	**	ns
G × N	ns	**	ns	ns	***	ns	ns	ns
D × N	***	***	ns	ns	ns	ns	*	ns
Y × G × D	*	ns	ns	*	*	ns	ns	ns
G × D × N	ns	*	ns	ns	ns	ns	ns	ns

ns – not significant; *P < 0.05; **P < 0.01; ***P < 0.001; RPS – rind penetration strength; BS – bending strength

respectively (Table 2). Previous study (Esechie et al. 2004) showed that the plant morphology can significantly affect stalk lodging resistance. Ma et al. (2014) reported that ear height or ear ratio was closely related to stalk lodging resistance. In this study, the lodging rate was significantly related to the plant height, ear height, ear ratio and stem diameter in cv. LD981, while it only was significantly related to the stem diameter in cv. ZD958 (Table 3). In addition, due to the differences of

Table 2. Effects of plant density and nitrogen (N) rate on plant height, ear height and stem diameter of summer maize

Cultivar	Plant density	N rate	Plant height (cm)			Ear height (cm)			Ear ratio			Stem diameter (cm)		
			2013	2014	2015	2013	2014	2015	2013	2014	2015	2013	2014	2015
LD981	D1	N0	302.4^{b}	298.2^{c}	301.8^{b}	123.7^{b}	121.3^{b}	121.3^{b}	0.41^{b}	0.41^{b}	0.40^{b}	2.63^{a}	2.65^{a}	2.64^{a}
		N120	305.7^{ab}	304.1^{b}	304.0^{b}	125.3^{b}	123.7^{b}	124.8^{b}	0.41^{b}	0.41^{b}	0.41^{b}	2.65^{a}	2.68^{a}	2.65^{a}
		N180	306.6^{ab}	304.1^{b}	301.9^{b}	126.4^{b}	123.8^{b}	123.9^{b}	0.41^{b}	0.41^{b}	0.41^{b}	2.66^{a}	2.67^{a}	2.65^{a}
	D2	N0	307.0^{ab}	304.5^{b}	305.3^{b}	135.8^{a}	130.6^{a}	132.9^{a}	0.44^{a}	0.43^{a}	0.44^{a}	2.39^{c}	2.42^{c}	2.42^{c}
		N120	311.2^{a}	308.3^{a}	309.9^{a}	136.5^{a}	128.2^{a}	132.5^{a}	0.44^{a}	0.42^{a}	0.43^{a}	2.44^{b}	2.46^{bc}	2.45^{b}
		N180	312.3^{a}	308.2^{a}	310.1^{a}	134.9^{a}	129.6^{a}	131.8^{a}	0.43^{a}	0.42^{a}	0.43^{a}	2.46^{b}	2.49^{b}	2.46^{b}
ZD958	D1	N0	260.2^{b}	259.5^{b}	259.7^{b}	114.6^{a}	110.9^{a}	109.9^{a}	0.44^{a}	0.43^{a}	0.42^{a}	2.60^{b}	2.64^{b}	2.62^{b}
		N120	264.4^{ab}	262.2^{ab}	261.2^{ab}	115.2^{a}	110.3^{a}	113.8^{a}	0.44^{a}	0.42^{a}	0.44^{a}	2.66^{a}	2.70^{a}	2.68^{a}
		N180	264.5^{ab}	262.4^{ab}	263.3^{a}	116.5^{a}	111.7^{a}	113.5^{a}	0.44^{a}	0.43^{a}	0.43^{a}	2.67^{a}	2.71^{a}	2.69^{a}
	D2	N0	265.5^{a}	263.8^{a}	264.8^{a}	115.5^{a}	112.8^{a}	114.0^{a}	0.44^{a}	0.43^{a}	0.43^{a}	2.41^{d}	2.45^{d}	2.41^{d}
		N120	267.0^{a}	264.3^{a}	264.3^{a}	117.2^{a}	115.8^{a}	115.6^{a}	0.44^{a}	0.44^{a}	0.44^{a}	2.45^{c}	2.50^{c}	2.47^{c}
		N180	266.8^{a}	264.8^{a}	264.6^{a}	117.9^{a}	116.2^{a}	115.7^{a}	0.44^{a}	0.44^{a}	0.44^{a}	2.47^{c}	2.52^{c}	2.50^{c}

Different small letters within a column indicate significant differences across all treatments of the same hybrid at P < 0.05. D1 – 52 500, D2 – 82 500 plants/ha; N0 – 0, N120 – 120; N180 –180 kg N/ha

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Table 3. Correlation coefficient (*r*) between lodging rate and stalk traits of the two cultivars

Cultivar	Plant height	Ear height	Ear ratio	Stem diameter	RPS	BS	Rind thickness	Vbs thickness	Big Vb	Small Vb	Total Vb
LD981	0.552**	0.864**	0.765**	–0.923**	–0.794**	–0.944**	–0.872**	–0.919**	–0.799**	–0.737	–0.776**
ZD958	0.255	0.193	0.053	–0.606**	–0.687**	–0.670**	–0.719**	–0.742**	–0.651**	–0.727**	–0.700**

**$P < 0.01$; RPS – rind penetration strength; BS – bending strength; Vb – vascular bundle

genotype, the plant height and ear height of cv. LD981 were much higher than that of cv. ZD958, which results in higher lodging rate at D2.

N supply can increase the plant height, ear height and stem diameter of both cultivars, however, no significant differences were observed in cv. ZD958 with N rate increased (Table 2), indicating that N supply improves stalk lodging resistance in stalk morphological traits mainly by increasing the stem diameter. In addition, from the facts discussed above, it may be concluded that the non-significant increase in plant height and ear height might be the main reason for cv. ZD958 low rate of lodging at high plant density, and for the non-significant correlation between lodging rate and plant height or ear height.

Stalk mechanical resistance. The RPS and BS of cv. LD981 at N0 decreased significantly by 12.1–21.1% and 29.5–31.9%, respectively, and that of cv. ZD958 decreased by 10.4–13.7% and 22.9–23.6%, respectively, with plant density increased over the three years. In addition, influenced by genotype, the values of RPS and BS at any plant density of cv. ZD958 were higher than that of cv. LD981 (Figures 3 and 4). Correlation analysis showed that the RPS and BS were significantly and negatively correlated to stalk lodging in both cultivars (Table 3), which was consistent with the results of previous research (Hondroyianni et al. 2000, Esechie et al. 2004).

In this study, the RPS and BS of both cultivars significantly increased with the N rate increased, while no significant difference was observed between N120 and N180. However, Chen et al. (2012) reported that N supply had no significant effects on the RPS and BS. The differences in the results between this study and previous studies may be due to the amount of N fertilizer supplied. It may be concluded that N supply can increase the stalk lodging resistance by improving its mechanical strength.

Stalk microstructure. As plant density increased, the rind thickness, Vbs thickness and number of Vb of cv. LD981 at N0 decreased significantly by 18.0, 25.9 and 10.33%, respectively, and that of cv. ZD958 decreased significantly by 9.9, 13.4 and 5.2%, respectively (Table 4). Meanwhile, the number of big and small Vb of cv. LD981 decreased by 16.8% and 6.9%, while that of cv. ZD958 only decreased

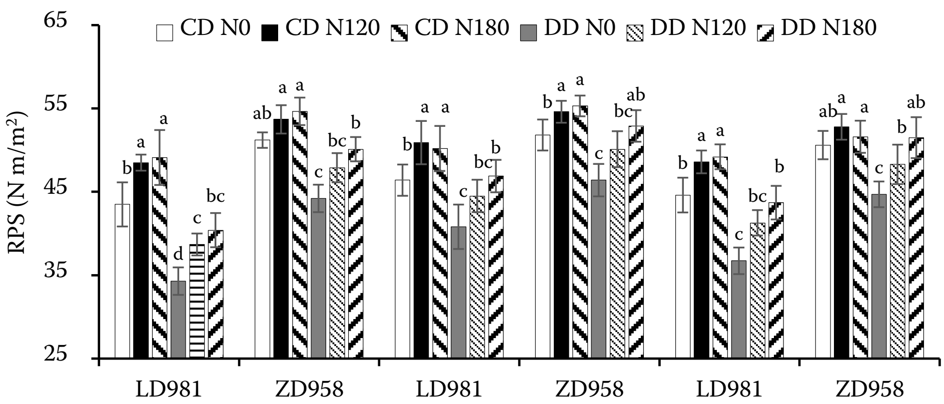

Figure 3. Effects of plant density and nitrogen (N) rate on the rind penetration strength (RPS) of the 3rd basal internodes of stem. Means (columns) and standard error (error bars) are presented ($n = 3$). Different small letters indicate differences between densities and N rates within each cultivar and year at $P < 0.05$. D1 – 52 500, D2 – 82 500 plants/ha; N0 – 0, N120 – 120, N180 – 180 kg N/ha

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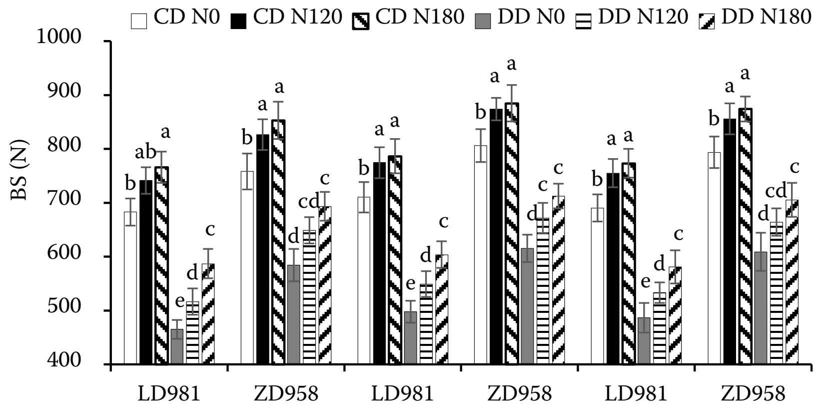

Figure 4. Effects of plant density and nitrogen (N) rate on the bending strength (BS) of the 3rd basal internodes of stem. Means (columns) and standard error (error bars) are presented (n = 3). Different small letters indicate differences between densities and N rates within each hybrid and year at P < 0.05. D1 – 52 500, D2 – 82 500 plants/ha; N0 – 0, N120 – 120, N180 – 180 kg N/ha

by 8.8% and 3.0%, indicating that plant density had a greater effect on the stalk microstructure traits of cv. LD981 and the effect of plant density on the number of big Vb was greater than that of small Vb. Correlation analysis showed that the rind thickness, Vbs thickness and number of Vb had

Table 4. Effects of plant density and nitrogen (N) rate on stalk microstructure of summer maize in 2014

Cultivar	Plant density	N rate	Rind thickness (mm)	Vbs thickness (µm)	Number of vascular bundle per plant		
					big Vb	small Vb	total Vb
LD981	D1	N0	1.28^b	49.27^b	208.27^b	363.00^c	571.27^c
		N120	1.41^a	54.60^a	228.27^a	398.83^a	627.10^a
		N180	1.40^a	53.80^a	229.50^a	400.47^a	629.97^a
	D2	N0	1.05^d	36.50^d	173.53^d	338.47^d	512.00^d
		N120	1.20^c	43.43^c	200.60^c	364.23^c	564.83^c
		N180	1.23^c	43.90^c	209.53^b	373.53^b	583.07^b
ZD958	D1	N0	1.31^b	50.20^b	222.57^d	367.40^d	589.97^d
		N120	1.43^a	56.30^a	254.27^a	417.93^a	672.20^a
		N180	1.43^a	56.50^a	260.03^a	419.20^a	679.23^a
	D2	N0	1.18^c	43.47^c	203.37^e	355.83^f	559.20^f
		N120	1.31^b	49.40^b	231.03^c	382.13^c	613.17^c
		N180	1.33^b	50.83^b	246.30^b	393.63^b	639.93^b
Analysis of variance							
Genotype (G)			***	***	***	***	***
Density (D)			***	***	***	***	***
N rate (N)			***	***	***	***	***
G × D			***	***	**	ns	**
G × N			ns	ns	**	*	***
D × N			ns	ns	**	***	*
G × D × N			ns	ns	ns	*	*

Different small letters within a column indicate significant differences across all treatments of the same hybrid at 0.05 level. ns – not significant; *P < 0.05; **P < 0.01; ***P < 0.001. D1 – 52 500, D2 – 82 500 plants/ha; N0 – 0, N120 – 120, N180 – 180 kg N/ha; Vb – vascular bundle

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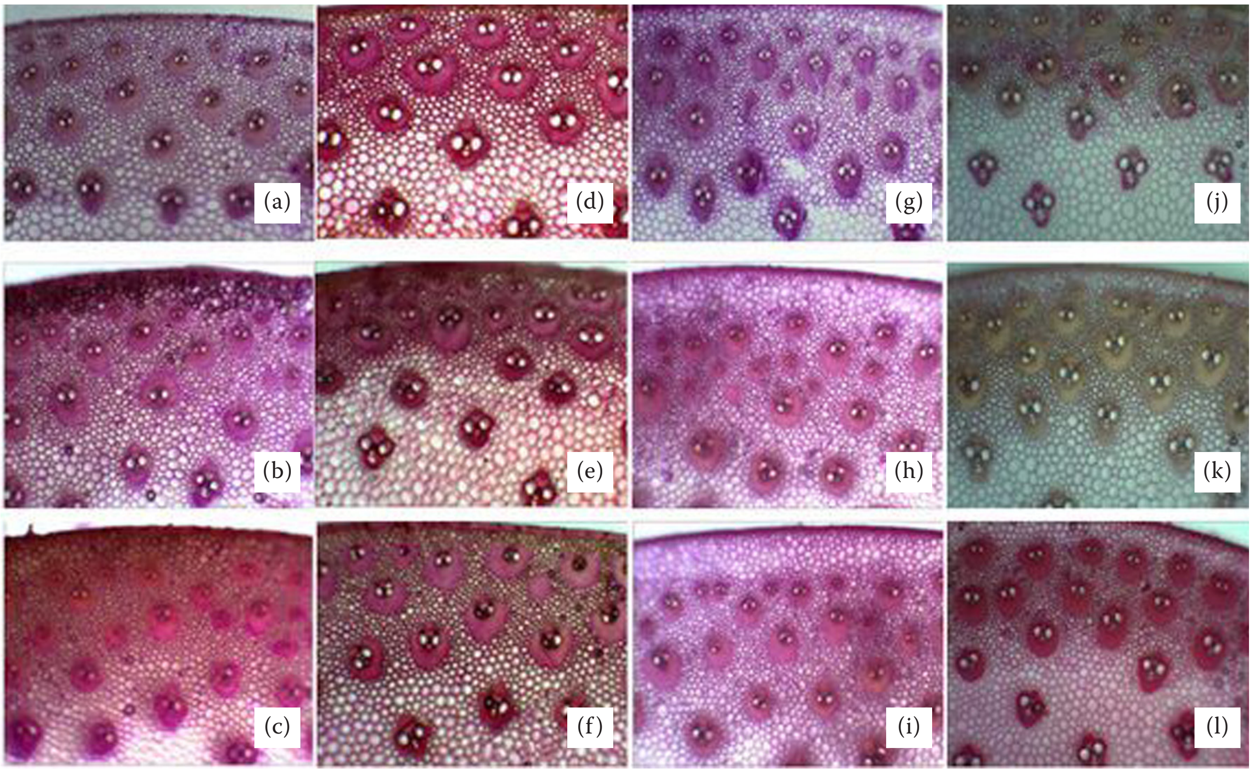

Figure 5. The structure of vascular bundle of the 3rd basal internodes of stem in 2014. (a, b, c) N0 – 0, N120 – 120, N180 – 180 kg N/ha; (d, e, f) D1 – 52 500, D2 – 82 500 plants/ha for cv. LD981 (× 40); (g, h, i) N0 – 0, N120 – 120, N180 – 180 kg N/ha under D1 and D2 for cv. ZD958; (j, k, l) D1 – 52 500, D2 – 82 500 plants/ha for cv. ZD958 (× 40)

a significant negative correlation with stalk lodging (Table 4), which was consistent with the results of Norberg et al. (1988), who pointed out that these traits can be used to measure the stalk strength. In addition, due to the differences of genotype, the number of Vb of cv. LD981 was less than cv. ZD958, which results in higher lodging rate at high plant density.

In this study, N supply can significantly increase the rind thickness, Vbs thickness and number of Vb of both cultivars, while no significant differences were observed between N120 and N180 at D1. Peng et al. (2014) pointed out that optimal N rate can significantly increase the content of lignin synthesis and the density of Vb, which can improve the stalk lodging resistance.

In conclusion, the stalk traits (morphology, mechanical properties and anatomical structure) were related or significantly related to stalk lodging (Table 4). With plant density increased, the plant height, ear height and ear ratio increased, while stem diameter, RPS, BS, rind thickness, Vbs thickness, and the number of big, small and total Vb decreased, which led to the increase of stalk lodging. Compared with cv. LD981, the lower plant height, ear height and ear ratio and higher RPS, BS and number of Vb of cv. ZD958 resulted in less stalk lodging at the high density. Meanwhile, N supply might improve the stalk quality and decrease the risk of stalk lodging, however, this cannot compensate a too high increase of lodging rate under the high plant density for the lodging susceptible cultivars. Therefore, using the lodging-resistant hybrid (cv. ZD958), planting at 82 500 plant/ha and supplying 180 kg N/ha was the optimized strategy to obtain a high grain yield and reduce the risk of stalk lodging.

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