

Effects of Ethephon and Kinetin on Lodging Resistance and Yield of Maize Stalks: Postprint

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Abstract

Lodging is one of the primary factors affecting maize yield. To investigate the effects of spraying different growth regulators at different stages on maize lodging resistance and yield, the maize varieties ‘Demeya 1’ and ‘Demeya 2’ were used as experimental materials. Ethephon (ETH) and kinetin (KT) were sprayed at the 5-, 7-, and 9-leaf stages, with water spray as the control. Basal stalk morphology, mechanical properties, chemical components at the grain filling stage, and lodging rate and yield after maturity were measured. The results showed that, compared with the control, for ‘Demeya 1’ sprayed with KT at the 5-leaf stage, internode diameter increased significantly by 21.11%, and internode density increased by 13.23%. Compared with the control, for ‘Demeya 2’ sprayed with ETH at the 7-leaf stage, basal internode total length decreased significantly by 14.41%, internode diameter increased significantly by 10.70%, and internode density increased significantly by 15.46%. For ‘Demeya 1’ and ‘Demeya 2’ sprayed with ETH at the 7-leaf stage, breaking force increased significantly by 26.04% and 16.77%, and puncture strength increased significantly by 22.77% and 14.62%, respectively, compared with the control. For ‘Demeya 2’ sprayed with ETH at the 9-leaf stage, the total content of chemical components composed of internode hemicellulose, cellulose, and lignin increased significantly by 25.49% compared with the control. KT showed different effects on stalk mechanics between the two varieties, and ETH demonstrated better regulatory effects on stalk mechanics than KT treatment for both varieties. Both regulator treatments reduced the lodging rate of both maize varieties at harvest. Internode diameter was highly significantly positively correlated with breaking force ($r=0.905^{**}$), and internode total chemical component content and internode diameter were significantly or highly significantly negatively correlated with lodging rate. For both varieties sprayed with KT at the 9-leaf stage, yield increased highly significantly by 22.24% and 19.98% compared with the control, while ETH spraying had no significant effect on yield. In conclusion, spraying

KT at the 9-leaf stage for both varieties can significantly increase yield, while spraying ETH at the 7-leaf stage for both varieties provides better regulatory effects on lodging resistance.

Full Text

Effects of Ethephon and Kinetin on Lodging-Resistance and Yield of Maize

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Abstract

Lodging is one of the major factors affecting maize yield. To determine the effects of plant growth regulator application timing on lodging resistance and yield, two maize varieties ('Demeiya-1' and 'Demeiya-2') were sprayed with $400 \text{ mg} \cdot \text{L}^{-1}$ ethephon (ETH) and $10 \text{ mg} \cdot \text{L}^{-1}$ kinetin (KT) at the 5-, 7-, and 9-leaf stages, with water as control. Basal stem morphology, mechanical properties, chemical composition at grain-filling stage, and lodging rate and yield at maturity were measured. Compared with the control, KT application at the 5-leaf stage increased internode diameter by 21.11% and internode density by 13.23% in 'Demeiya-1'. ETH application at the 7-leaf stage reduced total basal internode length by 14.41% while increasing internode diameter by 10.70% and internode density by 15.46% in 'Demeiya-2'. ETH treatment at the 7-leaf stage increased internode break strength by 26.04% and 16.77%, and internode penetration strength by 22.77% and 14.62% in 'Demeiya-1' and 'Demeiya-2', respectively. ETH treatment at the 9-leaf stage increased the total content of hemicellulose, cellulose, and lignin in 'Demeiya-2' by 25.49%. ETH demonstrated superior regulatory effects on stem mechanics compared with KT, though both regulators reduced lodging rates in both varieties. Internode diameter showed a highly significant positive correlation with break strength ($r = 0.905^{**}$), while chemical composition content and internode diameter showed significant or highly significant negative correlations with lodging rate. KT application at the 9-leaf stage significantly increased grain yield by 22.24% and 19.98% in the two varieties, while ETH had no significant effect on yield. In conclusion, KT application at the 9-leaf stage significantly increased maize yield, while ETH application at the 7-leaf stage provided better lodging-resistance regulation.

Keywords: Ethephon; Kinetin; Maize; Stem morphology; Mechanics of crop stalk; Lodging-resistance; Yield

Introduction

Heilongjiang Province is China's largest maize-producing region. However, soil conditions, tillage practices, cultivation management, and planting density often result in excessively tall plants with slender stems and poor lodging resistance, directly affecting harvest yield and economic value [1-5]. Maize lodging includes root lodging, stem lodging, and combined root-stem lodging [6-7], with stem lodging causing greater damage than root lodging [8]. When stem nodes break, transport of substances between above- and below-ground parts and translocation of photosynthates to sinks are disrupted, while the original canopy structure is destroyed, reducing leaf photosynthesis, leaf area, grain-filling efficiency, ear length, kernel number, 100-kernel weight, and biological yield [4-5]. Plant height and ear position show significant positive correlations with lodging—taller plants with higher ear placement are more prone to lodging [9]. Martin et al. [10] reported that maize lodging resistance is related to basal internode diameter, with the diameter of the third basal node showing a significant negative correlation with lodging; thicker third nodes provide stronger lodging resistance. Yao et al. [11] demonstrated that in density experiments, basal internode cortex structure (mechanical tissue thickness, number of mechanical cell layers, cortex thickness/radius) showed significant positive correlation with internode diameter and negative correlation with internode length and field lodging rate. Liu et al. [12] found that lodging-resistant varieties had higher stalk crushing strength, penetration strength, and break strength in the third, fourth, and fifth basal nodes compared with lodging-susceptible varieties, with basal stem mechanical characteristics showing significant or highly significant negative correlations with lodging rate. Gou et al. [13] reported that stalk mechanical traits are closely related to agronomic characteristics, with varieties having higher dry matter accumulation in internodes, particularly upper nodes, showing stronger lodging resistance, and internode density (dry matter per unit stem length) having the greatest positive effect on stalk crushing strength and rind penetration strength. Wang et al. [14] conducted regression analysis on ten maize varieties and concluded that the main factors affecting break strength were rind thickness/radius, proportion of mechanical tissue, vascular bundles, and cellulose and lignin content, while the main factors affecting penetration strength were cellulose and lignin content and vascular bundle number per unit area.

Plant growth regulators are widely used for maize lodging resistance, but application conditions (including concentration, timing, and environmental factors) are critical. Currently, the major maize varieties planted in Heilongjiang are 'Demeiya-1' and 'Demeiya-2', but few studies have examined lodging resistance regulators for these varieties. Ethephon, an ethylene-releasing agent, reduces transverse microtubule arrangement while increasing longitudinal arrangement, which correspondingly increases longitudinal deposition of microfibrils and limits cell longitudinal expansion, favoring cell expansion in the transverse direction driven by turgor pressure [15], but improper use can cause yield reduction [16-

17]. Kinetin, a synthetic cytokinin, increases plant height, thickens ear nodes, and increases chlorophyll content [18], with effects on delaying crop senescence, but few studies have reported its effects on lodging resistance. This study was conducted at the Heilongjiang Nongken Jiusan Farm during August, when violent storms frequently occur during the maize grain-filling period. Two common plant growth regulators (ethephon and kinetin) were applied at the 5-, 7-, and 9-leaf stages to investigate their effects on maize stem agronomic traits, mechanical properties, and chemical composition, aiming to provide a theoretical basis for developing and applying lodging resistance regulators for Heilongjiang maize production.

Materials and Methods

1.1 Materials and Experimental Design The maize varieties ‘Demeiya-1’ and ‘Demeiya-2’, locally dominant cultivars with ear nodes generally at the sixth and seventh basal positions, were used as test materials. The experiment was conducted in 2015 at the Heilongjiang Nongken Jiusan Administration Bureau on chernozem soil with 0-20 cm tillage layer containing $266 \text{ mg} \cdot \text{kg}^{-1}$ alkali-hydrolyzable nitrogen, $38.1 \text{ mg} \cdot \text{kg}^{-1}$ available phosphorus, $183 \text{ mg} \cdot \text{kg}^{-1}$ available potassium, $55.1 \text{ g} \cdot \text{kg}^{-1}$ organic matter, and pH 6.25. Sowing occurred on May 20 at a density of $90,000 \text{ plants} \cdot \text{ha}^{-1}$ in double rows on wide ridges (1.1 m ridge width). Each plot was 4.4 m wide and 10 m long, with four replicates per treatment and control, totaling 72 plots arranged randomly. Field management followed local practices. Ethephon (ETH, $400 \text{ mg} \cdot \text{L}^{-1}$) and kinetin (KT, $10 \text{ mg} \cdot \text{L}^{-1}$) were sprayed at the 5-, 7-, and 9-leaf stages, with water as control. Spray volume was $225 \text{ L} \cdot \text{ha}^{-1}$. Concentrations were selected based on screening by the Heilongjiang Bayi Agricultural University Chemical Control Laboratory. Plastic sheets were placed between plots during spraying to prevent drift.

1.2 Measurement Items and Methods At grain-filling stage (20 days after regulator application at the 9-leaf stage), four representative plants per plot were selected. After removing leaf sheaths and blades, internode lengths of the basal five nodes were measured with a tape measure and diameters with a vernier caliper. Internode break strength (with 15 cm distance between two support points) and penetration strength (using a 1 mm^2 probe) of the third, fourth, and fifth basal nodes were measured using a SY-S03 plant stem strength tester (Shijiazhuang Shiya Technology). After measurement, stem nodes were segmented, killed at 105°C for 30 minutes, dried at 80°C to constant weight, and weighed. The third basal node was selected, ground, and sieved (0.25 mm) for analysis. Hemicellulose, cellulose, and lignin contents were determined using the Van Soest detergent method [19]. At harvest, yield was measured and lodging was assessed in 50 consecutive plants. Yield was adjusted to 14% moisture content.

1.3 Data Analysis Microsoft Excel 2013 was used for data calculation and graphing. SPSS 21 software was used for significance analysis of differences.

Results

2.1 Effects of Plant Growth Regulators on Basal Internode Length

Basal internode length is an important agronomic indicator in maize. As shown in [Figure 1: see original paper], compared with the control, kinetin application at the 5-, 7-, and 9-leaf stages did not significantly change total basal internode length in either variety, though the fourth internode at the 9-leaf stage in ‘Demeiya-2’ showed a significant increase. ETH application at the 7-leaf stage significantly reduced total basal internode length in both varieties; compared with the control, internodes 1-5 were shortened by 12.50%, 10.81%, 6.12%, 15.90%, and 37.93% in ‘Demeiya-1’, and by 21.88%, 18.25%, 6.25%, 11.36%, and 19.23% in ‘Demeiya-2’. ETH application at the 5- and 9-leaf stages showed no significant differences in total basal internode length compared with the control. For both varieties, ETH application at the 7-leaf stage significantly reduced the length of the fourth and fifth basal internodes. Reduced basal internode length lowers the center of gravity and plant height, thereby increasing lodging resistance.

2.2 Effects of Plant Growth Regulators on Basal Internode Diameter

Internode diameter shows a significant negative correlation with lodging rate—larger diameters confer stronger lodging resistance. As shown in [Figure 2: see original paper], both regulators increased internode diameter at all basal positions in both varieties. In ‘Demeiya-1’, kinetin at the 5-leaf stage significantly increased internode diameters of nodes 1-5 by 21.45%, 24.79%, 25.86%, 19.65%, and 13.78%, respectively, while 7- and 9-leaf applications showed no significant differences. In ‘Demeiya-2’, kinetin increased mean internode diameter by 6.83%, 6.20%, and 8.61% at the 5-, 7-, and 9-leaf stages, respectively. In ‘Demeiya-1’, ETH increased mean internode diameter by 6.68% and 5.54% at the 5- and 7-leaf stages, respectively, with the 9-leaf stage significantly increasing nodes 1-5 by 9.74%, 9.46%, 12.17%, 10.09%, and 9.29%. In ‘Demeiya-2’, ETH at the 7-leaf stage significantly increased nodes 1-5 by 10.96%, 11.41%, 11.71%, 9.71%, and 9.71%, while 5- and 9-leaf stages increased mean diameter by 4.74% and 5.52%, respectively. Kinetin at the 5-leaf stage in ‘Demeiya-1’ and ETH at the 7-leaf stage in ‘Demeiya-2’ showed superior regulation of internode diameter compared with other treatments within the same variety. Increased basal internode diameter reduces the probability of stem lodging, thereby enhancing lodging resistance.

2.3 Effects of Plant Growth Regulators on Basal Internode Density

Internode density is defined as internode dry weight per unit length. As shown in [Figure 3: see original paper], compared with the control, kinetin at the

3-leaf stage and ETH at the 7- and 9-leaf stages increased internode density in both varieties. In ‘Demeiya-1’, kinetin increased mean internode density by 13.23%, 8.26%, and 5.97% at the 5-, 7-, and 9-leaf stages, respectively. In ‘Demeiya-2’, kinetin significantly increased mean internode density by 22.66% and 23.36% at the 5- and 7-leaf stages, respectively, with the fifth internode at the 9-leaf stage increasing by 30.47%. In ‘Demeiya-1’, ETH at the 5- and 9-leaf stages showed no significant differences in mean internode density, while the 7-leaf stage significantly increased the third and fourth internodes by 25.48% and 68.67%, respectively. In ‘Demeiya-2’, ETH increased mean internode density by 15.46% and 16.70% at the 7- and 9-leaf stages, respectively, while the 5-leaf stage showed a non-significant decrease. ETH at the 7-leaf stage in ‘Demeiya-1’ and kinetin at the 5- and 7-leaf stages in ‘Demeiya-2’ showed superior regulation of basal internode density compared with other treatments. Greater internode density indicates greater stem mechanical strength.

2.4 Effects of Plant Growth Regulators on Basal Internode Break Strength

Internode break strength is the force required to break an internode at its midpoint when supported at two fixed points 15 cm apart. As shown in [Figure 4: see original paper], compared with the control, kinetin significantly increased mean break strength by 38.38% and 24.08% at the 5- and 7-leaf stages, respectively, with a non-significant 7.45% increase at the 9-leaf stage. In ‘Demeiya-2’, kinetin increased mean break strength by 5.96%, 5.54%, and 5.32% at the 5-, 7-, and 9-leaf stages, respectively, with no significant differences. ETH at the 7-leaf stage showed superior regulation of basal internode break strength compared with other ETH treatments in both varieties, increasing mean break strength by 26.04% in ‘Demeiya-1’ and 16.77% in ‘Demeiya-2’. Specifically, nodes 3-5 increased by 17.65%, 24.73%, and 35.74% in ‘Demeiya-1’, and by 20.36%, 8.21%, and 21.74% in ‘Demeiya-2’. Other ETH treatments showed no significant differences from the control. Greater internode break strength indicates better stem mechanical performance.

2.5 Effects of Plant Growth Regulators on Basal Internode Penetration Strength

Penetration strength shows a highly significant negative correlation with lodging rate [13]—greater penetration strength confers stronger lodging resistance. As shown in [Figure 5: see original paper], both regulators increased mean internode penetration strength in both varieties, except for kinetin at the 9-leaf stage in ‘Demeiya-2’. In ‘Demeiya-1’, kinetin significantly increased mean penetration strength by 17.68% and 16.82% at the 7- and 9-leaf stages, respectively, with an 8.26% increase at the 5-leaf stage. In ‘Demeiya-2’, kinetin at the 5-leaf stage significantly increased mean penetration strength by 14.97%, while 7- and 9-leaf stages showed no significant differences. ETH in ‘Demeiya-1’ significantly increased penetration strength by 13.12%, 22.77%, and 15.80% at the 5-, 7-, and 9-leaf stages, respectively. In ‘Demeiya-2’, ETH increased mean penetration strength by 9.00% and 9.02% at the 5- and 9-leaf stages, respectively, with the 7-leaf stage increasing nodes 3, 4, and 5 by 11.34%,

7.59%, and 24.91%, respectively. ETH at the 7-leaf stage showed superior regulation of internode penetration strength compared with other treatments.

2.6 Effects of Plant Growth Regulators on Stem Hemicellulose, Cellulose, and Lignin Content Hemicellulose and cellulose provide the cell wall with framework and elasticity, while lignin provides rigidity and mechanical strength. Cellulose is the main component of both primary and secondary cell walls. As shown in [Figure 6: see original paper], cellulose constituted the major portion of chemical components, with lignin representing a small proportion. All treatments increased chemical component content compared with the control, except for ETH at the 9-leaf stage in ‘Demeiya-1’. In ‘Demeiya-1’, kinetin increased chemical component content by 3.02%, 4.65%, and 5.29% at the 5-, 7-, and 9-leaf stages, respectively, with no significant differences from the control. In ‘Demeiya-2’, kinetin increased chemical component content by 2.04% and 11.10% at the 5- and 9-leaf stages, respectively, with the 7-leaf stage showing a 16.10% increase, including significant increases in hemicellulose and cellulose content of 15.69% and 20.22%, respectively. In ‘Demeiya-1’, ETH at the 5-leaf stage significantly increased chemical component content by 8.82%, including significant increases in hemicellulose and cellulose of 29.33% and 5.72%, respectively, while 7- and 9-leaf stages showed no significant differences. In ‘Demeiya-2’, ETH at the 9-leaf stage significantly increased chemical component content by 25.49%, including significant increases in cellulose and lignin of 37.85% and 27.33%, respectively.

2.7 Correlation Analysis Between Lodging-Related Traits and Lodging Rate All regulator treatments reduced lodging rates at grain-filling stage in both varieties (Table 1). Control lodging rates exceeded 11.25%, while treatment rates were below 10%. Kinetin at the 9-leaf stage in ‘Demeiya-1’ reduced lodging rate by 8.75%, showing superior regulation compared with other treatments in the same variety. ETH at the 7-leaf stage in ‘Demeiya-2’ reduced lodging rate by 10%.

Table 2 shows that internode diameter, penetration strength, and chemical component content showed significant or highly significant negative correlations with lodging rate. The correlation coefficient between internode density and both break strength and penetration strength was 0.156* and 0.540*, respectively, while internode break strength showed a highly significant positive correlation with internode diameter (0.905**). These results indicate that reducing internode length and increasing internode diameter, density, break strength, penetration strength, and chemical component content can all reduce maize lodging.

2.8 Effects of Plant Growth Regulators on Yield As shown in Table 3, kinetin at the 9-leaf stage produced the highest yield increases in both varieties, reaching 22.24% and 19.98% (highly significant). Compared with the control, kinetin at the 7-leaf stage increased yield by 19.90% in ‘Demeiya-1’ and 16.02% in ‘Demeiya-2’ (both highly significant). Kinetin at the 5-leaf stage

significantly increased yield by 17.29% and 11.25% in ‘Demeiya-1’ and ‘Demeiya-2’, respectively. In contrast, ETH at the 5-, 7-, and 9-leaf stages increased yield by 2.05%, 5.26%, and 4.67% in ‘Demeiya-1’, and by 0.88%, 4.64%, and 9.44% in ‘Demeiya-2’, with no significant differences from the control.

Discussion and Conclusion

Plant growth regulators are commonly used to regulate basal internode length, diameter, density, break strength, penetration strength, and chemical composition to improve maize lodging resistance. Ethepon application can reduce basal internode length. This study showed that ETH application at the 7-leaf stage significantly reduced total basal internode length and increased stem diameter in both varieties, consistent with previous research. Wei et al. [20] reported that ethepon reduced internode lengths 1-6 in multiple maize genotypes. Ye et al. [21] found that ethepon significantly shortened internodes 7-14 and increased internode diameter in maize treated with ethepon and nitrogen gradients. This study found that kinetin had no significant effect on internode length, neither shortening nor lengthening internodes.

The study found that kinetin at the 5-leaf stage in ‘Demeiya-1’ and ETH at the 7-leaf stage in ‘Demeiya-2’ showed superior regulation of internode diameter compared with other treatments within the same variety. This likely reflects that different application timings with the same regulator concentration produce different results, demonstrating the time-sensitive nature of plant growth regulators. The effects of kinetin and ethepon on internode diameter are generally consistent with previous studies. Li et al. [18] reported increased internode diameter after cytokinin application at the tasseling stage. Regarding regulator effects on internode density, this study found that kinetin at different leaf stages increased maize internode density, while ETH at the 5-leaf stage showed no significant difference from the control. Dourado et al. [22] reported that kinetin seed soaking increased total plant dry matter accumulation. This study found that ETH at the 7-leaf stage in ‘Demeiya-1’ and kinetin at the 5- and 7-leaf stages in ‘Demeiya-2’ showed superior regulation of internode density. Correlation analysis revealed that lodging rate was highly significantly negatively correlated with internode diameter, with internode length having a positive effect and internode density a negative effect on lodging rate. Comprehensive results and correlation analysis indicate that the optimal timing for plant growth regulator application is: kinetin at the 5-leaf stage for ‘Demeiya-1’ and ETH at the 7-leaf stage for ‘Demeiya-2’.

Regarding effects on internode break strength, this study found that kinetin at the 5- and 7-leaf stages significantly increased break strength in the third, fourth, and fifth basal nodes of ‘Demeiya-1’, but showed inconsistent effects in ‘Demeiya-2’, with weaker regulation. Previous studies indicate that although kinetin was discovered over 40 years ago, stable commercialization has not been

achieved mainly due to complex influencing factors [23]. This study showed that for internode break strength, kinetin at the 5-leaf stage in ‘Demeiya-1’ and ETH at the 7-leaf stage in ‘Demeiya-2’ showed superior regulation compared with other treatments within the same variety. Correlation analysis revealed a highly significant positive correlation between internode diameter and break strength, consistent with Gou et al. [13] regarding population density effects on maize lodging mechanics. For penetration strength, this study found that kinetin at the 5-leaf stage in ‘Demeiya-2’ and ETH at the 7-leaf stage in ‘Demeiya-1’ showed better regulation, with penetration strength showing a highly significant negative correlation with lodging. Comprehensive mechanical and correlation analysis indicates that kinetin application timing needs to be variety-specific, while the optimal timing for ethephon is at the 7-leaf stage for both varieties.

Hemicellulose, cellulose, and lignin are essential components of the cell wall skeleton. This study found that chemical composition showed a significant negative correlation with lodging. In ‘Demeiya-1’, the effect of kinetin at three leaf stages on chemical component content followed the order: 5-leaf stage < 7-leaf stage < 9-leaf stage, consistent with yield performance, where later application produced greater yield increases. In ‘Demeiya-2’, kinetin at the 7- and 9-leaf stages significantly increased chemical components, with yields at these stages reaching highly significant levels compared with the control. ETH at the 9-leaf stage in ‘Demeiya-2’ significantly increased chemical components, but this study also found that ETH had no significant effect on yield increase, due to reduced single-ear weight but increased plant number per unit area, consistent with previous research [24].

This study only analyzed basal internode morphology, mechanics, and chemical composition at the grain-filling stage. Due to the specificity and time-sensitivity of regulators, as well as potential “rebound” effects, systematic understanding of kinetin and ethephon effects on maize requires further research on various related enzymes and deeper investigation of hormonal regulation mechanisms.

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