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SEED PRIMING EFFECTS OF YUHUANGJIN ON SPRING MAIZE

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ABSTRACT

The chemical regulation of crops is becoming a leading agro-technical measure for combating corn lodging. Yuhuangjin (YHJ) is a plant growth regulator which mainly affects on stem lodging. This study was aimed to provide a theoretical reference for control and prevention technologies for the cultivation of high-density maize. A split pot experiment design was conducted in 2012 for the field condition whereas four YHJ treatment concentrations (0ml, 4ml, 8ml and 16ml) were conducted in Jilin province during 2011 under greenhouse conditions. Optimum application of YHJ (4mL/Kg) on maize seed effectively influenced stalk-lodging resistance, bending strength, ear height and yield. When the concentration was ≥8mL/kg, the chemical regulation-mediated inhibition of the underground portion of the plant was greater than its aboveground counterpart. Moreover, dramatic reduction was also observed in height of the treated plants (31.99% for 8mL/kg and 39.91% for 16mL/kg). In the field, YHJ treatment reduced the internode length between internodes I-III and increased the length between internodes IV-VII. These differences in the length between internodes I-III and internodes IV-VII were accompanied by a slight increase in ear height. Under high-density conditions, the seed dressing effect on lodging resistance was pronounced but lodging rate decreased by 10.41%.

Key words: Chemical regulation, seed dressing, maize lodging, growth and development.

INTRODUCTION

Maize (*Zea mays*) is one of the most significant and widely grown crops for animal, human nutrition and agro-industrial purposes worldwide (Huffaker *et al*. 2011). The plants are commonly grown on soils presenting high heavy metal contents particularly iron and Chromium (Mallick *et al.* 2010). Increased maize production is directly associated with the high plant density (Tokatlidis *et al.* 2011), but, high density plants produce thinner maize stems as well as contribute extensively towards lodging that ultimately results in yield losses (Tokatlidis *et al*. 2010). Lodging in maize is *one* of the major problems in maize production worldwide, which accounts for annual yield losses of 5–25% and the application of chemical growth regulators to shorten crops and reduce lodging is widespread in many countries (Berry *et al.* 2003). Resistance of maize to stalk lodging is important, especially when grown for grain and the development of high yielding cultivars and the increased use of fertilizers have resulted in plants with more weighty ears that may not stand heavy winds (Esechie *et al.* 2004). Lodging resistance in maize has been related to some of its morphological characters, such as plant height, diameter and length of basal internode, thickness of rind and weight of 5 cm basal section, stalk breaking strength, crushing strength, stalk diameter, weight and density as well as rind penetrometer resistance (Zuber, 1973; Abedon *et al.* 1999; Esechie *et al.* 2004).

The complexity of variable environment makes it difficult to interpret the reduction in yield under natural conditions of lodging Kenneth (1999). This is because when lodging occurs several other factors usually change apart from the bending of shoots. Lodging most likely occur during the second or third months preceding harvest and occurs through interactions between plant, wind, rain and soil (Carter and Hudelson, 1988; Setter *et al.* 1997) reported that lodging reduced canopy photosynthesis by 60–80%, and every 2% of lodging caused a decrease of 1% in grain yield.

To quantify the effect of lodging on grain yield, the yield potential of different high yielding genotypes requires a thorough study Richards (2000); (Tams *et al*. 2004). Although maize breeders have made great efforts to solve the stalk-lodging problem by developing varieties of maize with superior resistance to lodging, the amount of natural stalk lodging varies from field to field depending upon the environmental factors (Sheri *et al*. 2004). Inbreeding for high yield potential and lodging resistance is always a key target because lodging is one of the major factors limiting the yield potential (Zhu *et al.* 2008).

Plant growth regulators (PGRs) impart vital role in combating lodging resistance to increase maize production (Schluttenhofer *et al*. 2011). The application of PGRs was considered to be the most economic, simple and effective method to prevent lodging in crop production. These PGRs are now used worldwide on a diversity of crops, such as maize (Gaska and Oplinger, 1988; Khosravi and Anderson, 1991). Likewise other plant growth regulators, YHJ (30% Amine fresh grease, YHJ) is a Chinese agricultural product. Although a number of studies have focused on the responses of lodging to environmental factors such as N fertilization and light (Hiroyuki *et al.* 2007), but not a single study has evaluated the effects of chemical regulation on growth and development of spring corn. The aim of the present work was to observe the effect of chemical regulation on the yield, its component factors and plant morphology of spring corn in central region of Jilin Province, China. Therefore, this experiment was designed to test whether the application of YHJ will ameliorate lodging resistance in *Xianyu 335* populations, and to determine whether the application of YHJ can enhance the maize yield, its component factors and plant morphology of spring corn in central region of Jilin Province.

MATERIALS AND METHODS

Test materials, sources and experiment location: Corn breed: *Xianyu 335* (XY335) was provided by Pioneer seed company; Chemical modulation, while YHJ (amine fresh ethephon, fresh fat amine3% ethephon content of 27%) made available by Fujian Haolun Biotechnology Engineering Ltd., Co.

The test was conducted in Jilin Provincial Academy of Agricultural Sciences, Changchun network control room during 2011 in summer. However, during 2012, the test was propagated using different parameters at Dachengzi Township high-yield corn demonstration base, Lishu County, Siping, Jilin Province (43°20'N, 124°18'E). The region is predominantly under rain-fed agriculture that has typical black and fertile soil whose pH is 5.9 with rich Organic contents (27.1 g kg^{-1}). The annual precipitation is 500 to 900 mm with 60% of the total rainfall occur during the summer (May–September). Average monthly precipitation from May to September was 38.4mm, 136.8mm, 149.1mm, 122 mm and 71.6mm respectively in 2012. The experimental region was not Irrigated anymore because of the plenteous rainfall in that year.

Test design: Initially, 4 uniform dressings were treated separately with 4 different treatment concentrations of YHJ (0, 4, 8 and 16 mL/kg), and each concentration of YHJ was diluted with purified water in the ratio 20:1. In addition, each dressing treatment contained 200g maize and the mixture was placed on plastic film to let it dry under sunlight for 30 minutes. The first experiment test was conducted during May 2011. A total of 48 pots (12 pots per treatment) were sown with 3 seeds in each selected seed treatment. The pots were overseeded with hand planters and watered adequately during the whole experiment duration.

However, for the second experiment, the maize (*Xianyu 335* (XY335)) was planted on 5 May 2012 and subsequently harvested at October 1st 2012. Two planting densities, 70,000/ha and 90,000/ha were used. The experimental design was a split-plot with 3 replications by using plant densities as main plots and YHJ treatment as subplot. In all plots, 80 cm distance was kept between evenly spaced rows. Each plot is 10m long and 6m wide.

Survey on germination rate: Survey emergence rate during 5, 10, 15, 20 days was recorded for morphological index such as plant height, leaf length and width. At this moment, SPAD-502 was used to determine leaf chlorophyll's SPAD. At two or three leaves stages, roots were cut out and their parameters were analyzed using DT-SCAN (United Kingdom Delta-Devices Ltd Co.). Aboveground and underground biomass were separated by using clippers, fresh weighed and packed in brown paper bags. Subsequently, they were put in the oven for 30 minutes at 105^0C to remove water followed by drying for 24-48 hours at 80^0C . At last, samples were cooled in desiccators and reweighed (dry weight).

Determination of indicators and methods: Plant height and ear height were determined after teaseling with the help of a ruler. After jointing and silking stage, meter scale was used to determine the lengths between internode below ear. Stalk strength tester YY-2 (TOP Instrument Company) was used to determine the flexural strength during jointing and silking stage between different corn internode sections. Five plants per plot were selected at the silking stage to measure the stretch bending strength. Digital force gauge instrument was used to raise shoot about 1.5m high from the ground level for the determination of the 45° angle. Three plants were selected for experimental analysis in $10m^2$ of plot. In the middle of each plot, four rows of ripe *Xianyu 335* plant were selected to investigate the number of lodging plant and barren plant. In addition, panicles number, row number, kernels row, kernels ear, barren tip length and 100-kernel weight were also determined.

RESULTS

Corn seedling germination rate: Germination rate increased gradually until day nine when it attained maximum value. It should be noted that the germination rate of 4mL/kg treatment was very similar to that of the control and rates were higher than 8mL/kg and 16mL/kg treatments. Thus, the higher concentration of YHJ had little influence on the germination rate of maize.

Height of the seedling: Twenty days after sowing, dramatic reduction in height of the treated plants (20.84% for 4mL/kg; 31.99% for 8mL/kg and 39.91% for

16mL/kg) was noticed compared with the controls. This showed that plants treated with YHJ were shorter than the control and the height of the plant decreased according to the concentration of YHJ (4 mL/kg >8 mL/kg >16 mL/kg).

Leaf area of the corn seedling: Thirty days after sowing, the leaf area decreased as follows: 31.10% (4mL/kg), 53.07% (8mL/kg) and 59.40% (16mL/kg) compared with the control. Increase in YHJ concentration led to reduction in leaf area.YHJ treatment also influenced leaf blade's length and width. Different concentrations of YHJ showed different reductions in the length of the leaf blade and seedling leaf width compared with the control. This reduction was more evident on the second and third leaf.

Above ground and underground of corn seedlings: The accumulation of aboveground and underground dry material gradually decreased upon increasing the YHJ concentration. Right after 20 days of sowing, the above ground biomass of corn seedling reduction levels were 23.56%, 33.13% and 51.25% while the underground root system of corn seeding showed 5.50%, 12.92% and 21.76%, for the given YHJ concentrations of 4mL/kg, 8mL/kg and 16mL/kg respectively as compared with the control.

Root length of root layer: The length decreased with a consistent increase in concentration from 4mL/kg>8mL/kg> to 16mL/kg. In the first layer, root length increased slightly as compared to the second layer where the length increased greatly. At 20 days after emergence, data could not let us to conclude about the 3rd layer, but the results obtained enabled us to conclude that the increase in root length was inversely proportional to the concentration of YHJ.

The radicle length: It was observed that YHJ inhibited the radicle growth and this inhibitory effect kept on increasing with the concentration. After performing seed dressing, the radicle length was measured at 15, 20 and 25 days. However, the use of YHJ reduced these lengths by 28.40%, 38.13% and 55.20% for 4mL/kg, 8mL/kg and 16mL/kg respectively.

Root shoot: Variance analysis showed that there was an obvious difference in shoot ratio between treated plants and the control. Increased YHJ concentration initially resulted in decrease root shoot ratio, but this ratio later increased with time. Interestingly, 8mL/kg treatment gave better ratio on the 20th day. Furthermore, when the concentration of YHJ was less than 8mL/kg, the inhibition on above ground growth was greater than the inhibition on the root. However, when the concentration was greater than 8mL/kg the inhibitory effect on the root was greater than the shoot growth (Table 1).

Effect of YHJ on plant height and ear height: YHJ showed no significant effect on plant height, and this can be seen under 70,000/ha and 90,000 /ha high plant density, where plant seeds with dressing (BD7 and BD9) had slightly decreased height as compared to the plants without dressing (D7 and D9). Analysis of variance showed insignificant difference. Regarding ear height, significant difference was found between plants with seed dressing and those without seed dressing. Under 70,000/ha, the ear height reduced about 8.96cm for the plants with seed dressing as compared to the control and about 9.77cm under 90,000/ha high density planting (Fig. 1)

Effect of YHJ on flexural strength and stretch bending: There was gradual decrease in flexural strength of II, III and IV nodes. Under 70,000/ha or 90,000/ha plant density, treated plant stalks offered more resistance to bending as compared to the control, and this was shown by the average increase of 7.14% in flexural strength for II-IV internodes under 90,000/ha density. Furthermore, under 70,000/ha density, there was no significant difference in corn stalk stretch strength between treated plants and control. However, under 90,000/ha plant density, it was fairly significant with 25.40% in flexural tensile for treated plants (Fig. 2)

Effect of YHJ on internode length and lodging rate: Internodes lengths for seven internodes (I-VII) below ear were measured and labelled as 1, 2, 3, 4, 5, 6 and 7th internodes. Under 70,000/ha and 90,000/ha high density planting, the length of I-III internodes at jointing stage (Fig. 3-a, 3-b) and silking stage (Fig. 3-c, 3-d)–were shorter (1-2cm) compared with the controls. From IV to VII internodes, the length of internodes at silking stage was greater for YHJ treatment than the controls (Fig. 3-c, 3-d). The lodging resistance of treated plants was slightly higher than the control. Under 70,000/ha and 90,000/ha plant density, the lodging rate decreased by 10.41% for the treated plants (Fig. 3).

Effect of YHJ on yield and yield components: Variance analysis showed that yield under different densities was greatly different ($p<0.05$). Under 70,000/ha density, the difference between seed with dressing and seed without dressing was not significant, but under 90,000/ha density, the difference was 6.92% (Table. 2).

For the yield components, no difference was found for corn line numbers, hundred-grain weight and the number of productive ears between seed with dressing and seed without dressing under 70, 000/ha plant density. However, seed without dressing do have greater number of kernels per spike and barren tips as compared to the seeds with dressing. Under the 90,000/ha density, the number of productive ears per unit area increased, barren tips decreased along with line grain number as well as hundred grain weight and kernels per spike. Above analysis concluded that the influence of YHJ on yield was not significant under low density, but yield was significantly increased under high plant density mainly due to the increased effect of YHJ on the number of productive ears per unit area.

Table 1. Root shoot ratio change under different concentrations of chemical regulation seed dressing at different days after sowing.

Treatment	Days after sowing (d)			
	5d	10d	15d	20d
0mL	2.69d	1.09c	1.36c	1.09c
4mL	3.92c	2.71a	1.44b	1.30b
8mL	5.93a	2.82a	1.74a	1.76a
16mL	5.40b	2.31b	1.50b	1.38b

Note：The different letters mean difference is significant at 0.05 levels.

Table 2. Influence on corn yield under different chemical regulation and yield components.

Treatments	Yield（kg/hm^2）	Effective panicles number ($P/10m^2$）	Row number	Kernels/row	kernels/ Ear	Barren tip length（cm）	100-kernel weight（g）
D7	10803.01c	67.0c	15.84a	34.00a	538.34a	1.76a	32.18a
BD7	10438.34c	67.7c	15.89 a	32.29b	512.99b	1.54c	32.40a
D9	11405.51b	81.7b	16.00 a	29.94c	479.09 c	1.64b	32.43a
DB9	12253.06a	85.3a	16.11 a	26.89d	432.75 d	1.14d	31.83b

Note：The different letters mean difference is significant at 0.05 level.

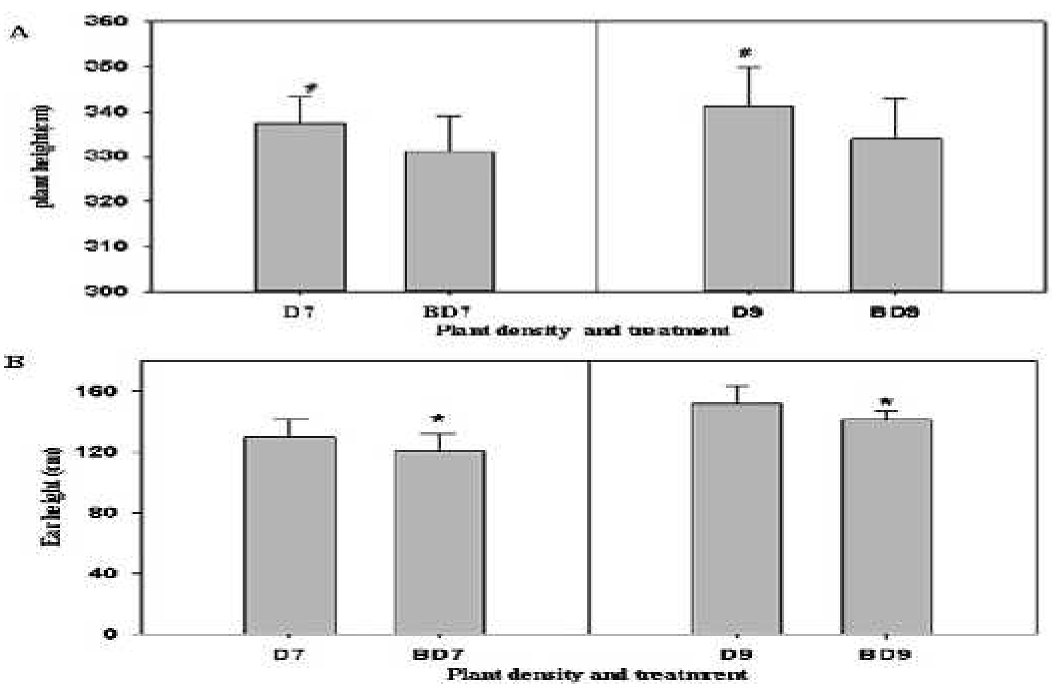

Figure. 1: (A) Comparison of plant height under different densities of seed dressing and without seed dressing: D7= 70,000/ha without YHJ; BD7= 70,000/ha with YHJ; D9= 90,000 without YHJ; BD9= 90,000 with YHJ: (B) Comparison of ear height under different density between seed with dressing and seed without dressing, indicating positive effect of YHJ.

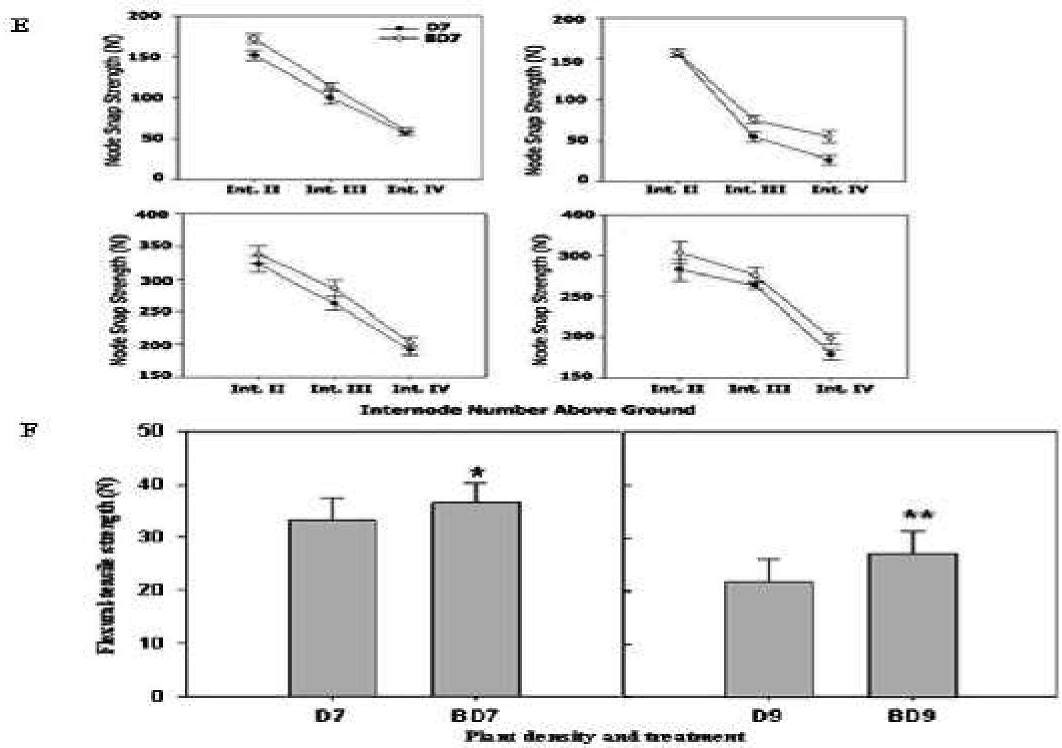

Figure 2: (E) Comparison of flexural strength between different internodes of seed with dressing and seed without dressing under different densities during jointing stage (upper) and silking stage (bottom): (F) Comparison of stretch strength under different densities between seed with dressing and seed without dressing.

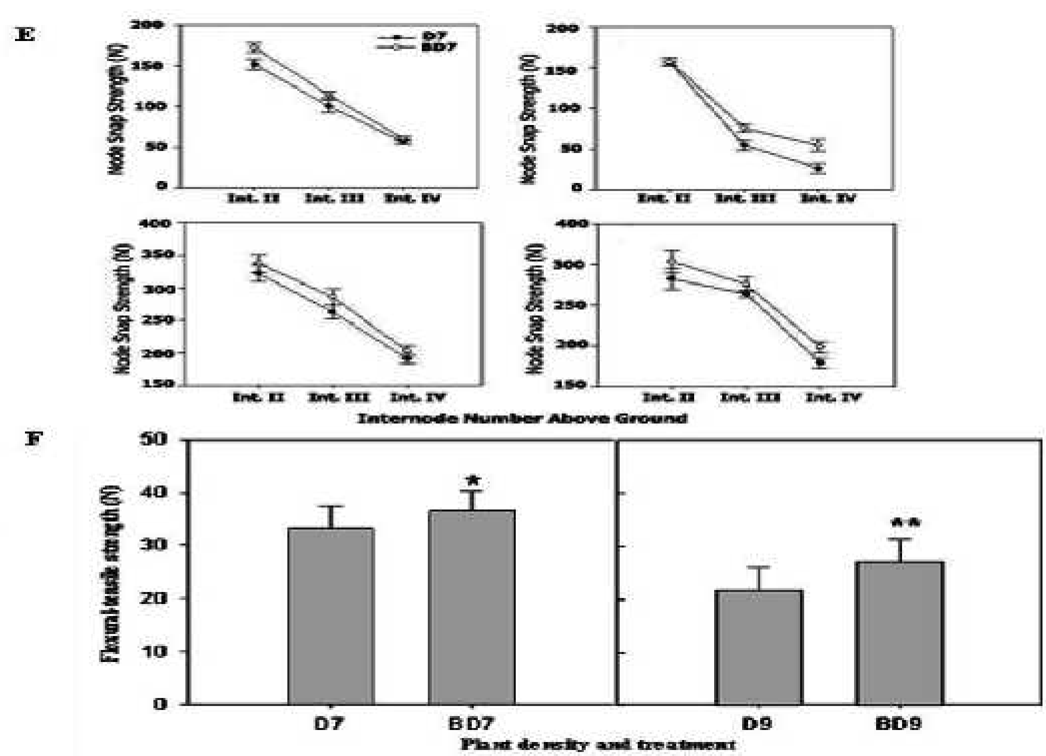

Figure 3: (G) Comparison of different internodes length of seed with dressing and seed without dressing under different densities at joining stage (a, b) and silking stage (c, d): (H) Comparison of lodging resistance under different densities between seed with dressing and seed without dressing.

Discussion: Lodging is a significant problem for farmers because it causes huge reductions in grain yield and quality (Mallick *et al.* 2010). It has been hypothesized that root lodging might become the most common form in wheat due to the advent of varieties with shorter and more rigid stems. This may also be the case for other cereal species, such as barley and oats. Therefore, new means must be developed to further increase maize yield, and reducing root loading.

Based on biochemical effects, plant growth regulations were divided up into three categories: plant growth promoter, plant growth inhibitor and plant growth retardant. (Guodong *et al.* 2012) reported that the germination percentage of aged corn seeds treated with H_2O_2 was significantly greater than those without H_2O_2 treatment. Corn seeds without H_2O_2 imbibed significantly more slowly than those with oxygen fortification by 0.15% H_2O_2. However, (Ramezani *et al.* 2013) mentioned that the percentage of any corn seeds to germinate was improved after treatment with different concentrations of polyethylene glycol (PEG). Among the three different concentrations, priming with 0.50% chitosan had the best enhancement effects for chilling-sensitive Mo17, while no significant different effects among the three concentration treatments were observed for chilling-tolerant. (Guan *et al.* 2009). In the present study, the data implied that YHJ might be an important regulator of maize lodging. These results are consistent with previous studies that demonstrated that chemical regulation is an important assistant measure for cultivation (Shannon *et al*. 2011; Carl and Paul, 2014).

Growth regulating chemicals that have positive influences on major agronomic crops can be of valuable importance. However, the final test states that harvested yields and crop quality must be enhanced for plant growth regulators to be profitable. Among many current uses of plant growth regulators, indirect yield effects were often applied. Some of these effects include: (1) preventing lodging in cereals, (2) preventing preharvest fruit drop, (3) synchronizing maturity to facilitate mechanical harvest, (4) hastening maturity to decrease turnover time and (5) reducing labour requirements. Studies conducted on major crops, such as corn, soybean, wheat and rice have identified materials capable of altering individual agronomic characteristics like lodging, plant height, seed number, and maturity (Harms *et al.* 1988).

Different concentrations of YHJ affected the germination rate of corn seed. High concentrations of YHJ (8mL/Kg or 16mL/Kg) had little influence on seed germination. Germination rates of the seeds treated with 8mL/Kg and 16 mL/Kg of YHJ were lower by 6.7% and 21.3% respectively than the control ones ($P< 0.05$). However, germination rate of the seeds treated with low concentration of YHJ (4 mL/Kg) was not significantly different with control group. The present results showed that the germination of corn seeds was not influenced by the presence of low concentration of YHJ. Therefore, the presence of small concentration of YHJ cannot account for inhibition of corn germination process, suggesting that the toxicity of YHJ at this concentration was not evident at seed germination.

The size of plant organs is tightly controlled by environmental and other factors that must spatially and temporally coordinate cell expansion and cell cycle activity. Since leaf development has a strong relationship with crop growth, knowing the change in leaf area may be useful for estimating crop growth. Different variables such as leaf length, leaf width or a combination of these variables were used to estimate leaf area. Considering that leaf area and crop growth are both affected by nutritional conditions, more reliable results may be obtained through the addition of nutritional factors to the models (Cho *et al.* 2007).

After maize seed treatment, there was obvious inhibition in length and width of the second leaf at trefoil stage resulting in reduced leaf surface area. Priming with different concentrations of YHJ reduced maize seedling height and leaf area. Extensive studies have measured the rooting pattern of maize and its relationship to nutrient uptake and yield because of the important root system for the acquisition of nutrients and water. Seed priming with 4mL/Kg YHJ slightly decreased both dry matter weight and dry root weight as compared with the control. However, when priming was greater or equal to 8mL/Kg YHJ, both dry matter weight and dry root weight were extremely lower than 4mL/Kg, suggesting that YHJ application at ≥8mL/Kg negatively affected corn development. Thus, it is inferred from this observation that the optimum concentration of YHJ was 4mL/Kg.

In maize, patterns of internode elongation were also studied previously (Bennouma *et al.* 2004). In the first phase, the distribution of cell lengths was unchanged over the entire internode and the extension was exponential. During the second phase, cell length gradient developed at the distal end of internode followed by an increased rate of internode elongation. The third phase involves the formation of internode length, which have adequately constant elongation rate of internode that in turn corresponds to a linear phase.

Chemical YHJ could make internodes below the spike coarser and enhance its lodging-resistance. This chemical was effective in reducing not only plant height but also internode length. The 3rd internode below ear was greatly influenced by YHJ treatment and its length was significantly shortened. These results are similar to those with treated chemical growth regulators (Guan *et al.* 2009).

In this study, the corn hybrid *Xianyu 335* cultivated in density of 90000 plants/ha, gave the highest yield while those in density of 70000 plants/ha, resulted in greatly reduced yield because of lodging barren plant, barren ear tip and smaller ear. In addition, the lodging rate was significantly decreased because plant height and ear height were reduced and the basal stem became tonic.

This work has provided the evidence that the application of YHJ (4mL/kg) on maize seed could effectively influence stalk resistance and other characteristics. YHJ has also been shown to affect yield components such as decrease in ear's line number, number of kernel per spike, and barren tips but the overall yield increased in high density planting due to increase in productive ears.

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