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EFFECT OF GROWTH SUBSTANCES ON PHENOTYPIC EXPRESSIONS OF THE KNOTTED (Kn) GENE OF **ZEA MAYS**

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INTRODUCTION

The mutant gent Knotted **(Kn)** in **Zea mays** is so named because it is responsible for bulges and outgrowths, or so-called "knots", associated with the lateral veins of the leaf blade. These knots may be clustered near the base of the blade or somewhat diffused to more distal portions of the blade. Knots may occur on the abaxial or adaxial surface of the leaf, but are predominant on the abaxial surface. The vascular bundles that pass through the knots are not disrupted nor distorted (1). The appearance of these abnormal growths, or knots, is only one of several phenotypic effects of the gene. Other expressions include dwarfing, decrease in length and increase in width of leaves, and decreased pollen production.

Bryan and Sass (1) reported this gene to be a simple dominant located on the first chromosome between floury$_1$ and brown-midrib$_2$. Nelson and. Postlethwait[1] hypothesized that knotting was due to a dispersion of the cells of the intercalary meristem. According to their hypothesis, meristematic cells are pushed outward as they divide, as dictated by the restraints of surrounding, differentiated cells. Other effects, such as dwarfing, they termed "secondary" to the knotting effect.

Rhodes and Myers (4) found that dwarfing regularly preceeded the appearance of knots. They also found significant differences in the time and amount of dwarfing, length and width of leaves, and the time and amount of knotting between homozygous **(KnKn)** plants and heterozygous **(Kn+)** plants. On the basis of these findings they suggest a definite dosage effect of the **Kn** gene for these phenotypic expressions.

Phinney (3) found that the gibberellin, GA_3, induced normal growth in four **Zea mays** dwarf mutants, d_1, d_2, d_3, and d_5, while other growth substances failed to elicit such a response. He suggested that these GA_3-responding mutants might be controlling different steps in a biochemical pathway leading to the production of naturally occurring gibberellin similar to GA_3, and necessary for normal growth.

Nickerson (2) applied growth hormones to **Kn** mutants and succeeded in modifying the effects of the gene with naphthalenacetic acid and

[1]Nelson, O. E., and S. H. Postlethwait. 1963. Characterization of development in maize through the use of mutants. II. The abnormal growth conditioned by the knotted mutant. (unpublished).

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gibberellic acid. Development of knots in four strains of maize carrying the gene was completely inhibited by daily treatment of 500 μg of naphthalenacetic acid. Other effects produced by these treatments included suppression of flowering, reduction of the abnormal thickness of knotted leaves, and the formation of flanges by the brace roots, with a single stele for the entire group. The dwarfing associated with the gene, however, was not altered by naphthlenacetic acid. Plants having the **Kn** gene were only one-half to two-thirds as tall as normal plants. The gibberellic acid, GA_3, produced similar, but less pronounced, results.

This study was undertaken in an attempt to clarify Nickerson's work and to elucidate the role of certain growth hormones in modifying the dwarfing and knotting expressions of the knotted gene.

MATERIALS AND METHODS

The material used in this study was derived from seed obtained from the Maize Genetics Cooperative in 1964, and is in a genetic background related to the single cross M14/W23.

The growth hormone experiment diagramed in Figure 1 was con-

Figure 1. Design of growth hormone experiment.

	4T					8T					12T				
	IAA	NAA	GA_3	K	C	IAA	NAA	GA_3	K	C	IAA	NAA	GA_3	K	C
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
I	abc	abc	abc	abc	abc	abc	abc	abc	abc	abc	abc	abc	abc	abc	abc
II	abc	abc	abc	abc	abc	abc	abc	abc	abc	abc	abc	abc	abc	abc	abc
III	abc	abc	abc	abc	abc	abc	abc	abc	abc	abc	abc	abc	abc	abc	abc
IV	abc	abc	abc	abc	abc	abc	abc	abc	abc	abc	abc	abc	abc	abc	abc
V	abc	abc	abc	abc	abc	abc	abc	abc	abc	abc	abc	abc	abc	abc	abc

Explanation of Symbols

4T — four treatments
8T — eight treatments
12T — twelve treatments
I to V — replications
1 to 15 — groups
IAA (indoleacetic acid)
NAA (naphthalenacetic acid)
GA_3 (gibberellic acid)
K (kinetin)
C (control)
a — normal plants(++)
b — heterozygous plants (Kn+)
c — homozygous plants (KnKn)

ducted in the fall of 1965. Two hundred and twenty-five, five inch pots were prepared and placed in rows of five in the greenhouse. The entire arrangement consisted of 15 groups, each group consisting of 15 pots. On September 27, normal (++), heterozygous (**Kn**+), and homozygous **(KnKn)** seed were planted. Plants began to emerge on October 3, and were later thinned to one plant per pot. Five treat-

ments, namely, indoleacetic acid (IAA), kinetin (K), gibberellic acid (GA_3), naphthalenacetic acid (NAA), and the control (C), were applied, beginning on October 6. Each treatment consisted of 10 µg. of one of the above substances dissolved in 1.0 ml. distilled water. The control consisted of 1.0 ml. distilled water. Sodium carbonate was used in small amounts to dissolve kinetin. Each treatment was applied by means of a pipette to the hollow formed by the first unfolding leaf. The first five groups were given twelve treatments, one every two days, beginning on October 6 and ending on October 28. The second five groups were given eight treatments, beginning on October 6 and ending on October 20. The third five groups were given four treatments, beginning on October 6 and ending on October 12. Groups 1, 6, and 11 received indoleacetic acid; groups 2, 7, and 12 received kinetin; groups 3, 8, and 13 received gibberellic acid; groups 4, 9, and 14 received naphthalenacetic acid; and groups 5, 10, and 15 received control treatments.

Solutions were freshly prepared each week and stored in the refrigerator.

Plant height and the number of knots were recorded every two days after emergence for a four week period. Height was designated as that distance from the base of the plant at soil level to the uppermost part of the plant. A knot was counted as a single visible protuberance on either side of the leaf blade.

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value found	F value required
Total	224	47,653.0			
Replication	4	2,222.8	555.7	8.3	
Time Period	2	495.5	249.7	3.7	8.65
Error A	8	532.3	66.5		
Treatment	4	16,739.5	4,184.8	49.9**	3.74
Treatment x Time	8	934.4	116.8	1.3	2.90
Error B	48	4,027.0	83.8		
Genotype	2	16,891.6	8,445.8	318.7**	4.78
Genotype x Time	4	192.8	48.2	1.8	3.47
Genotype x Treatment	8	862.6	107.8	4.0**	2.65
Gen. X Treat. X Time	16	1,568.3	98.0	3.6**	2.15
Error C	120	3,182.3	26.5		

** Highly significant.

Table 1. Analysis of variance of height of plants treated with growth hormones.

RESULTS AND DISCUSSION

Dwarfing. An analysis of variance for height is shown in Table 1. Highly significant F values were obtained for treatments, genotypes, genotype x treatment interaction, and genotype x treatment x time interaction. Time periods, with the exception of the genotype x treatment x time interaction, were not significant. This would seem to indicate that the duration of the treatments had little effect on the results obtained.

A ranking of means from all significant sources of variation is shown in Table 2. Duncan's new multiple range test was used to

Genotype		Genotype x Treatment x Time Period					
		Gibberellin			Kinetin (con't)		
++	46.0				Kn+	- 8t - K	28.4
Kn+	35.2				Kn+	- 12t - K	22.0
KnKn	24.8	++	- 8t - GA	75.5	KnKn	- 4t - K	21.2
		++	- 4t - GA	63.8	KnKn	- 12t - K	17.0
Treatment		++	- 12t - GA	54.7	KnKn	- 8t - K	15.9
		Kn+	- 8t - GA	51.1			
		Kn+	- 4t - GA	49.4			
GA	49.9	Kn+	- 12t - GA	45.0			
Control	40.6	KnKn	- 4t - GA	40.0	Naphthalenacetic Acid		
NAA	28.9	KnKn	- 8t - GA	34.7			
Kinetin	28.6	KnKn	- 12t - GA	34.7	++	- 12t - NAA	43.4
IAA	28.6				++	- 4t - NAA	39.7
		Control			++	- 8t - NAA	37.7
					Kn+	- 12t - NAA	29.7
Genotype x Treatment		++	- 4t - C	54.9	Kn+	- 8t - NAA	28.5
		++	- 12t - C	45.4	Kn+	- 4t - NAA	25.8
++ - GA	64.7	Kn+	- 4t - C	45.2	KnKn	- 12t - NAA	19.6
++ - C	49.0	++	- 8t - C	44.6	KnKn	- 8t - NAA	18.5
Kn+ - GA	48.4	Kn+	- 8t - C	43.6	KnKn	- 4t - NAA	18.4
Kn+ - C	42.3	Kn+	- 12t - C	38.1			
++ - NAA	40.3	KnKn	- 8t - C	38.0			
++ - K	40.2	KnKn	- 4t - C	27.6	Indoleacetic Acid		
KnKn - GA	36.4	KnKn	- 12t - C	26.2			
++ - IAA	35.8				++	- 4t - IAA	39.0
KnKn - C	30.6				++	- 12t - IAA	34.9
Kn+ - IAA	30.1				Kn+	- 8t - IAA	34.1
Kn+ - NAA	27.6	Kinetin			++	- 8t - IAA	33.6
Kn+ - K	27.4				Kn+	- 4t - IAA	31.6
KnKn - IAA	20.0	++	- 4t - K	44.4	Kn+	- 12t - IAA	24.7
KnKn - NAA	18.8	++	- 12t - K	41.9	KnKn	- 8t - IAA	20.4
KnKn - K	18.0	++	- 8t - K	34.3	KnKn	- 12t - IAA	19.9
		Kn+	- 4t - K	31.7	KnKn	- 4t - IAA	19.2

Vertical lines denote means not significantly different at 5% level of probability using Duncan's multiple range test. Means are in centimeters.

Table 2. Ranking of height means.

determine significant differences between means. Genotypes were significantly different at the 5% level of probability. In respect to treatments, height differences between NAA, K, and IAA were not significant, but all three treatments produced plants significantly shorter than control plants. Genotype x treatment means indicate that relative differences in height due to genotype are not eliminated by treatments. NAA, IAA, and K did not eliminate dwarfing, but GA significantly increased height of **KnKn** plants above the **KnKn** controls, **Kn**+ plants to the height of untreated ++ plants, and ++ plants to approximately one and one-fourth their normal height. Genotype x treatment x time means are ranked according to treatments. The relationship of genotype to height regardless of treatment is evident. There seems to be no relationship between genotype or treatment, and time period.

Knotting. An analysis of variance for knots is shown in Table 3. Since knots occur only on **KnKn** and **Kn**+ plants, the number of plants in the analysis is 150 rather than the 225 considered for height.

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value obtained	F value required
Total	149	1976.3			
Replication	4	107.2	26.8		
Time Period	2	35.2	17.6	0.70	8.65
Error A	8	20.9	26.1		
Treatment	4	276.6	69.2	4.87**	3.74
Treatment x Time	8	135.1	16.9	1.19	2.90
Error B	48	679.6	14.2		
Genotype	1	174.9	174.9	48.58**	7.08
Genotype x Time	2	12.1	6.1	1.69	4.98
Genotype x Treatment	4	136.7	34.2	9.50**	3.65
Gen. x Treat. x Time	8	179.9	22.5	6.25**	2.82
Error C	60	218.1	3.6		

** Highly significant.

Table 3. Analysis of variance of number of knots on mutant plants treated with growth hormones.

Highly significant F values were obtained for the same means as those for height. The duration of treatment had little effect on the amount of knotting on the plants of this experiment.

A ranking of the means for all significant sources of variation is shown in Table 4. Since the final count of knots was made four weeks after emergence when knotting was slight, differences in some instances, were not discernable. Differences that are valid statistically using Duncan's new multiple range test are shown in Table 4. All treatments

Treatments	
Control	4.3
Kinetin	2.4
IAA	2.2
GA	1.0
NAA	0.3

Genotype	
KnKn	3.0
Kn+	1.0

Genotype x Treatment	
KnKn - C	7.0
KnKn - IAA	3.8
KnKn - K	3.0
Kn+ - K	1.9
KnKn - GA	1.6
Kn+ - C	1.6
Kn+ - IAA	0.7
Kn+ - GA	0.5
KnKn - NAA	0.3
Kn+ - NAA	0.3

Genotype x Treatment x Time

Control			
KnKn - 8t - C	9.6		
KnKn - 4t - C	6.4		
KnKn - 12t - C	5.0		
Kn+ - 8t - C	2.0		
Kn+ - 12t - C	1.8		
Kn+ - 4t - C	1.0		

Kinetin			
KnKn - 4t - K	7.6		
Kn+ - 8t - K	3.2		
Kn+ - 12t - K	1.6		
KnKn - 12t - K	1.4		
Kn+ - 4t - K	0.8		
KnKn - 8t - K	0.0		

Indoleacetic Acid			
KnKn - 8t - IAA	7.4		
KnKn - 12t - IAA	3.2		
Kn+ - 8t - IAA	1.4		

Indoleacetic Acid (con't)			
KnKn - 4t - IAA	0.8		
Kn+ - 4t - IAA	0.4		
Kn+ - 12t - IAA	0.2		

Gibberellic Acid			
KnKn - 8t - GA	2.6		
KnKn - 12t - GA	1.2		
KnKn - 4t - GA	1.0		
Kn+ - 12t - GA	0.8		
Kn+ - 4t - GA	0.4		
Kn+ - 8t - GA	0.2		

Naphthalenacetic Acid			
Kn+ - 4t - NAA	0.6		
Kn+ - 12t - NAA	0.4		
KnKn - 8t - NAA	0.4		
KnKn - 12t - NAA	0.4		
KnKn - 4t - NAA	0.2		
Kn+ - 8t - NAA	0.0		

Vertical lines denote means not significantly different at 5% level of probability using Duncan's multiple range test.

Table 4. Ranking of knot means.

reduced the total number of knots when compared to the control population. **KnKn** plants had more knots when averaged over all experimental situations than **Kn**+ plants. NAA virtually eliminated knotting on both **KnKn** and**Kn**+ plants. IAA inhibited knotting to a lesser extent than NAA, but inhibited both genotypes to about the same degree.

The effects of NAA and IAA seemed to be primarily on knotting, with leaves returning to normal in respect to length and width. Plants treated with GA_3 and K had fewer knots in most instances than control plants, but other drastic effects produced suggest that the reduction of knotting was not due to the same mechanism involved in knot reduction by NAA and IAA. K-treated plants had necrotic leaves bearing a white compound, presumably the sodium carbonate used to keep K in solution. GA_3-treated plants were extremely spindly, with very long, narrow leaves.

In view of the fact that NAA treatments virtually eliminate knotting while not eliminating dwarfing, it seems that knotting is not responsible for dwarfing, but that these are separate and wholly independent expressions of the same gene or closely linked genes.

SUMMARY

The growth hormones gibberellic acid, indoleacetic acid, kinetin, and naphthalenacetic acid were tested for their effect on the knotting and dwarfing expressions of the Knotted (**Kn**) gene in **Zea mays.** Naphthalenacetic acid almost completely suppressed the production of knots. Gibberellic acid, indoleacetic acid, and kinetin reduced knotting to a lesser degree. Naphthalenacetic acid, indoleacetic acid, and kinetin did not eliminate dwarfing, but gibberellic acid significantly increased the height of both heterozygous and homozygous plants.

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