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## **Effects of 2, 3, 5-Triiodobenzoic Acid (TIBA) on Stem Growth Induced by Indole-3-acetic acid (IAA) and Naphthylacetic Acid (NAA) in Precooled Rooted Tulip Bulbs**

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Effects of 2, 3, 5-triiodobenzoic acid (TIBA) on stem growth induced by indole-3-acetic acid (IAA) and naphthylacetic acid (NAA) in precooled rooted tulip bulbs were studied. TIBA is effective in inhibiting either IAA- or NAA-induced elongation either below or above the point of treatment. Time lag of the treatments between IAA and TIBA did not give significant differences in the length of any internode. When IAA was applied additionally on the middle of the 3rd internode, it restored the growth inhibition of the 1st to 3rd internodes caused by TIBA applied on the 4th internode, but the restored growth did not reach to the length promoted by IAA only. The difference in the total internode length between the treatments with 0.1% IAA and 0.1% IAA+TIBA on the middle of the 4th internode was much smaller than that between the treatments with 2.0% IAA and 2.0% IAA+TIBA. Presented results using TIBA, an inhibitor of basipetal transport of auxin, confirmed crucial role of auxin in tulip stem growth.

### **INTRODUCTION**

It is well known for many years that 2, 3, 5-triiodobenzoic acid (TIBA) inhibits the basipetal polar auxin transport in plants (Niedergang-Kamien and Leopold, 1957; Morris *et al.*, 1973). There have been reports showing that TIBA, the non-phytotropin auxin efflux inhibitor, competes for the same binding sites as indole-3-acetic acid (IAA) (Thomson *et al.*, 1973; Jablanovic and Nooden, 1974). Okubo and Uemoto (1985) showed that TIBA treatment at the first internode of sprouting tulip shoot inhibited the dark-induced elongation of the first internode and decreased the amount of diffusible auxin from the upper organs into the first internode but did not affect the gibberellin amount. Recently, Saniewski and Okubo (1997) found that IAA applied in the place of removed flower bud and after excision of leaves promoted flower stalk elongation in the nonprecooled and precooled, rooted and derooted tulip bulbs, and TIBA applied in the middle of the 4th internode (below IAA application) greatly inhibited the growth of lower internodes. Thus, the important role of auxin in stem elongation of nonprecooled as well as of precooled tulip bulbs was confirmed in that study, but the role of auxin in stem elongation is still not clear.

In this study, to clarify the role of auxin in tulip stem growth, more detail experiments by changing the application methods of TIBA with two auxins, IAA and naphthylacetic

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acid (NAA) in precooled rooted bulbs were carried out.

## MATERIALS AND METHODS

### **Plant materials, temperature treatment and culture conditions**

Bulbs of tulip (*Tulipa gesneriana* L.) cv. Gudoshnik, with a circumference of 10–11 cm, were used throughout the experiments. The bulbs, after lifting, were stored at 18–20 °C until 15 October, and then kept at 4 °C for dry cooling. After cooling period, a minimum 12 weeks, the bulbs were planted individually in plastic pots and grown in a greenhouse at 17–20 °C in natural light conditions.

### **Effects of TIBA on stem growth induced by IAA or NAA**

All leaves and flower bud were removed when the length of the stem was about 12 cm and plain lanolin (control) or lanolin containing IAA or NAA at different concentrations was applied on the cut surface of the flower bud on 8 April. Besides auxin treatment, 0.2% TIBA in lanolin was applied on the middle of the 4th internode. Intact plants and the plants with flower bud but without leaves were also as control. Seven plants were used for each treatment. Growth measurements were made every two days during experiment period, but only the results on 19 April are presented.

### **Effects of site of TIBA application on stem growth induced by IAA**

When the length of the stem was about 5 cm, lanolin or 0.2% IAA in lanolin was applied around the base of flower bud of the plants of which the leaves were removed but the flower bud was intact or applied in the place of flower bud of the plants of which the leaves and flower bud were excised on 27 February. Then, 0.2% TIBA in lanolin paste was applied on the middle of different internodes. Also, TIBA alone was applied under the base of flower bud, and the mixture of TIBA and IAA was applied on the top of the 4th internode after removal of flower bud. Twelve plants in each treatment were used. The growth was recorded every two days but the results on 15 March are presented.

### **Effects of order, time lag and site of TIBA and IAA application on stem growth**

TIBA and IAA were applied in different order with different time lag on the middle of different internodes of the plants without leaves and flower bud on 24 January. Length of the stem at treatment was about 4 cm. Ten plants were used for each treatment. The growth was measured every two days but that of 10 February is only presented.

### **Effects of re-application of IAA on TIBA-induced reduction of stem growth induced by IAA**

IAA and TIBA were applied in the same manner as above to the plants of which the leaves and flower bud were removed on 11 February, when the length of the stem was about 6 cm. Subsequently or one day after the treatment, 0.1% IAA was applied on the middle of the 3rd internode. Seven plants were used and the length of each internode was measured every two days and the results on 22 February are presented.

### Effects of site of TIBA application and concentration of IAA on stem growth-1

IAA at the concentration of 0.1 and 2.0% was applied on the top of the last internode after decapitation of flower bud and leaf excision as described above and 0.2% TIBA was applied on the middle of different internode on 31 January. The stem length at treatment was 5 cm. Seven plants were used per treatment. Measurements of stem growth were made every two days but those of on 15 February are only presented.

### Effects of site of TIBA application and concentration of IAA on stem growth-2

IAA at different concentrations and TIBA were applied in the same manner as above on 6 March. Length of the stem at treatment time was about 5 cm. The results were recorded every two days but those of 17 March are presented. Seven plants were used per treatment.

## RESULTS AND DISCUSSION

### Effects of TIBA on stem growth induced by IAA or NAA

It is well known that auxin, IAA and NAA, applied in the place of removed flower bud of sprouted precooled tulip bulbs and after excision of all leaves, induced growth of all internodes and that flower bud, after excision of all leaves, induced growth of tulip stem (Saniewski and De Munk, 1981; Banasik and Saniewski, 1985). The results were fully confirmed in this study.

IAA alone was more promoting than NAA alone on any internode elongation, consequently on total internode growth (Table 1 and Fig. 1). NAA had no effect on the

**Table 1.** Effects of TIBA on stem growth induced by IAA or NAA.

Treatment	Length of internode (cm)						Total
	1st	2nd	3rd	4th			
				Total	Below <sup>w</sup>	Above <sup>w</sup>	
1. Intact control	8.1 b <sup>a</sup>	7.7e	10.0dc	21.1f			46.9g
<i>All leaves removed, flower bud intact</i>							
2. Control (lanolin)	8.0 b	8.1ef	11.2cf	24.3h	11.0d	13.3e	51.7h
<i>All leaves and flower bud removed</i>							
3. Control (lanolin)	5.4a	3.5a	2.5a	2.4a			13.8a
4. IAA 0.2%	9.3c	8.7fg	10.2dc	19.8cf	9.1c	10.7d	48.0g
5. IAA 0.2% + TIBA <sup>w</sup>	7.3b	6.5d	9.9d	19.4e	10.5d	8.9c	43.1f
6. IAA 0.1%	10.0c	9.2g	12.3f	22.6g	10.5d	12.1c	54.1h
7. IAA 0.1% + TIBA	6.4a	5.5bc	9.1cd	19.0c	10.0cd	9.0c	39.9e
8. NAA 0.1%	5.7a	4.8b	6.6b	13.5c	6.1b	7.4b	30.6c
9. NAA 0.1% + TIBA	5.7a	3.7a	3.1a	7.0b	2.2a	4.8a	19.5b
10. NAA 0.05%	5.7a	5.6c	7.9c	16.0d	7.1b	8.9c	35.3d
11. NAA 0.05% - TIBA	5.7a	3.5a	2.6a	6.6b	2.1a	4.5a	18.4b

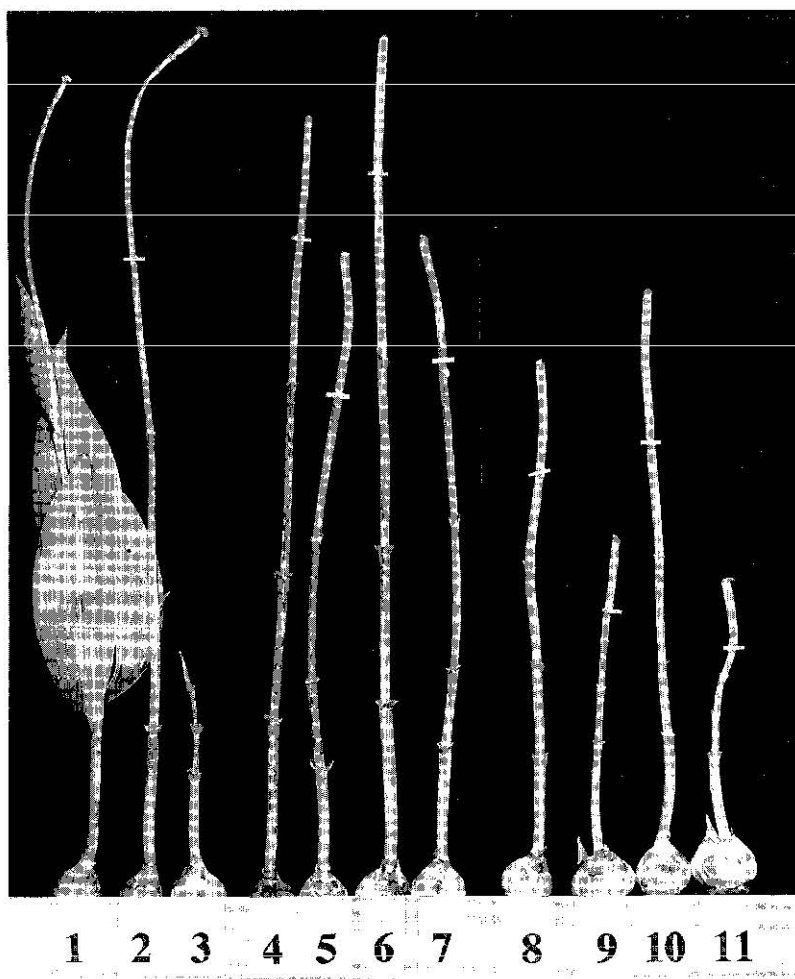
<sup>a</sup> Below; Length of the 4th internode below the point of lanolin or TIBA treatment.

<sup>a</sup> Above; Length of the 4th internode above the point of lanolin or TIBA treatment.

<sup>a</sup> Mean separation within columns by Duncan's multiple range test at 5% level.

<sup>w</sup> TIBA=0.2%

Initial length at treatment; 1st=4.6, 2nd=3.3, 3rd=2.2, 4th=2.2 cm.



**Fig. 1.** Effects of TIBA on stem growth induced by IAA or NAA. 1–11; see Table 1.

1st internode elongation. The elongation of the 3rd and 4th internodes was promoted more with 0.1% IAA than with 0.2% IAA, but the growth of the 1st and 2nd internodes was not significantly different between the treatments. The growth of the 2nd to 4th internodes was promoted more with 0.05% NAA than with 0.1% NAA.

TIBA is effective in inhibiting either IAA- or NAA-induced elongation either below or above the point of treatment. It is different from our previous results with derooted bulbs (Saniewski and Okubo, 1997); length of the top internode above the position of TIBA treatment was longer.

### Effects of site of TIBA application on stem growth induced by IAA

IAA at a concentration of 0.2% applied under flower bud after excision of all leaves, induced flower bud blasting and promoted stem growth (Table 2 and Fig. 2). It was found previously that auxins applied under the flower bud cause flower bud blasting in tulips (De Munk and Gijzenberg, 1977; Saniewski, 1986) probably through the stimulation of the ethylene production in the flower bud (Saniewski, 1986). It is well known that flower bud blasting is caused by ethylene in tulips (Kamerbeek and De Munk, 1976). TIBA applied under flower bud in the absence of leaves inhibited the growth of all internodes and stems were twisted (Fig. 2). Application of TIBA on the middle of the 4th, 3rd and 2nd internodes greatly inhibited internode elongation induced by IAA applied under flower bud. TIBA applied on the 1st internode only slightly inhibited the growth of stem induced by IAA.

The growth of stem induced by IAA 0.2% applied in the place of removed flower bud was strongly inhibited by TIBA applied to the 3rd and 4th internodes (Table 2). Much

**Table 2.** Effects of site of TIBA application on stem growth induced by IAA.

Treatment	Length of internode (cm)				
	1st	2nd	3rd	4th	Total
1. Intact control	11.0f <sup>*</sup>	8.9c	9.7cde	14.5b	44.1d
<i>All leaves removed, flower bud intact</i>					
2. Control (lanolin) <sup>†</sup>	11.4f	8.3de	9.4cd	21.1f	50.2e
3. IAA <sup>‡</sup>	13.4g	9.3c	10.5de	20.9f	54.1f
4. IAA <sup>‡</sup> +TIBA <sup>§</sup> (IV) <sup>‡</sup>	5.4bc	5.4b	7.0b	17.3cd	35.1bc
5. IAA <sup>‡</sup> +TIBA (III)	5.1b	4.5b	7.3b	16.0bc	32.9bc
6. IAA <sup>‡</sup> +TIBA (II)	5.8bc	6.7c	10.0de	19.0c	41.5d
7. IAA <sup>‡</sup> +TIBA (I)	9.6e	8.9e	10.7e	20.7f	49.9e
8. TIBA (U)	6.8cd	5.5b	6.5b	17.7de	36.5c
<i>All leaves and flower bud removed</i>					
9. Control (lanolin)	3.1a	1.5a	1.1a	1.3a	7.0a
10. IAA	12.0f	9.0e	10.5de	17.7de	49.2c
11. IAA + TIBA (IV)	5.6bc	4.8b	7.3b	15.7b	33.4bc
12. IAA + TIBA (III)	5.2b	4.7b	7.1b	14.8b	31.8b
13. IAA + TIBA (II)	5.7bc	6.9c	10.0de	19.2e	41.8d
14. IAA + TIBA (I)	7.8d	7.5cd	10.3de	18.3de	43.9d
15. IAA + TIBA (T)	9.6e	7.1c	8.7c	18.7de	44.1d

<sup>\*</sup> Mean separation within columns by Duncan's multiple range test at 5% level.

<sup>†</sup> Lanolin or IAA was applied under flower bud.

<sup>‡</sup> IAA=0.2%. <sup>§</sup>TIBA=0.2%.

<sup>¶</sup> IV, III, II, and I; TIBA was applied on the middle of the 4th, 3rd, 2nd and 1st internode, respectively, U; TIBA was applied on the 4th internode just below the flower bud and T; TIBA was mixed with IAA and applied on the top cut surface of the 4th internode.

Initial length at treatment; 1st=2.4, 2nd=1.3, 3rd=0.9, 4th=0.9 cm.



**Fig. 2.** Effects of site of TIBA application on stem growth induced by IAA. 1-15; see Table 2.

smaller inhibitory effect was observed after application of TIBA on the 2nd and 1st internodes. Application of IAA together with TIBA on top cut surface of the 4th internode slightly affected the growth of the 1st to 3rd internodes in comparison to IAA treatment alone.

#### **Effects of order, time lag and site of TIBA and IAA application on stem growth**

Two or 4hrs time lag of the treatments between IAA and TIBA did not give significant differences in the length of any internode (Table 3 and Fig. 3).



**Fig. 3.** Effects of order, time lag and site of TIBA and IAA application on stem growth. 1-12; see Table 3.



**Table 3.** Effects of order, time lag and site of TIBA and IAA application on stem growth.

Treatment	Length of internode (cm)						
	1st	2nd		3rd	4th		Total
		Total	Below <sup>a</sup>	Above <sup>a</sup>	Total	Below	Above
1. Intact control	10.8e <sup>x</sup>	7.4d		8.8ef	20.3c		47.3e
<i>All leaves removed, flower bud intact</i>							
2. Control (lanolin)	8.3d	5.9c		7.1cde	19.6c		40.9d
<i>All leaves and flower bud removed</i>							
3. Control (lanolin)	2.5a	1.2a		1.0a	1.1a		5.8a
4. IAA <sup>w</sup>	10.8e	7.5d		8.9f	19.6c		46.8c
5. TIBA <sup>x</sup> (IV) <sup>y</sup> was applied first, then IAA was applied	3.9b	2.7b		4.1b	12.4b	6.8a	5.6a
6. IAA first, then TIBA (IV)	3.4ab	3.0b		5.8c	12.7b	5.3a	7.4b
7. IAA first, TIBA (IV) 2 hrs later	4.0b	3.6b		6.1c	14.2b	5.7a	8.5b
8. IAA first, TIBA (IV) 4 hrs later	4.4b	3.7b		6.8cd	14.6b	7.2a	7.4b
9. TIBA (II) first, then IAA	5.8c	5.0c	2.7a	2.3a	8.4def	18.6c	
10. IAA first, then TIBA (II)	5.9c	5.2c	2.6a	2.6a	8.9f	19.1c	
11. IAA first, TIBA (II) 2 hrs later	6.0c	5.2c	2.6a	2.6a	8.3def	17.9c	
12. IAA first, TIBA (II) 4 hrs later	6.0c	5.6c	2.4a	3.3b	8.8f	18.6c	

<sup>a</sup> Below; Length of the 2nd or 4th internode below the point of lanolin or TIBA treatment.<sup>a</sup> Above; Length of the 2nd or 4th internode above the point of lanolin or TIBA treatment.<sup>x</sup> Mean separation within columns by Duncan's multiple range test at 5% level.<sup>w</sup> IAA=IAA 0.1%, <sup>x</sup>TIBA=0.2%.<sup>y</sup> IV and II, TIBA was applied on the middle of the 4th and 2nd internode, respectively.

Initial length at treatment; 1st=1.9, 2nd=0.8, 3rd=0.6, 4th=0.8 cm.

**Table 4.** Effects of re-application of IAA on TIBA-induced reduction of stem growth induced by IAA.

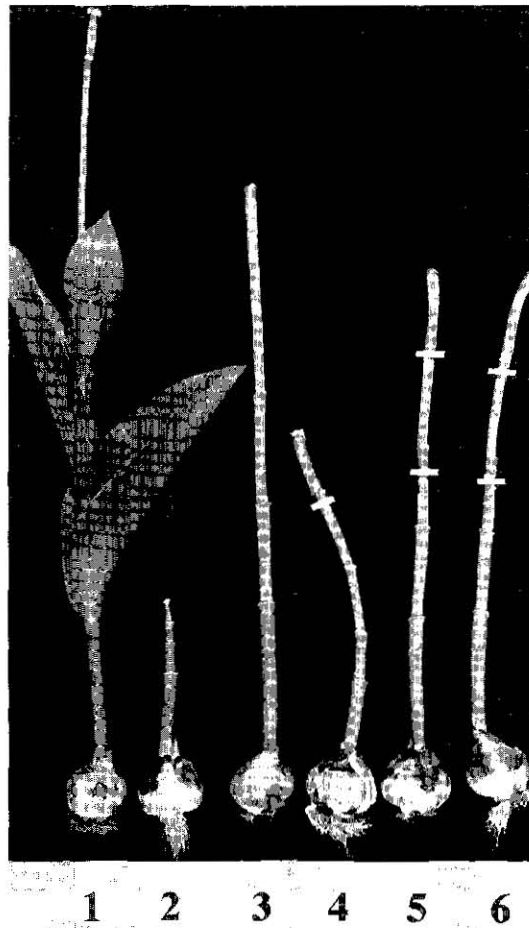
Treatment	Length of internode (cm)				
	1st	2nd	3rd	4th	Total
1. Intact control	7.5b <sup>z</sup>	6.0c	6.4c	14.8c	34.7d
<i>All leaves and flower bud removed</i>					
2. Control (lanolin)	3.6a	1.6a	1.1a	1.4a	7.7a
3. IAA <sup>z</sup>	8.7c	6.2c	6.5c	13.8c	35.2d
4. IAA + TIBA <sup>x</sup> (IV) <sup>w</sup>	3.9a	2.3b	3.6b	9.9b	19.7b
5. IAA + TIBA (IV) + IAA (III)	7.2b	5.5c	4.2b	11.5b	28.4c
6. IAA + TIBA (IV) first, IAA(III) one day later	7.6bc	5.9c	4.1b	10.8b	28.4c

<sup>z</sup> Mean separation within columns by Duncan's multiple range test at 5% level.<sup>z</sup> IAA=IAA 0.1%, <sup>x</sup>TIBA=0.2%.<sup>w</sup> IV and III, TIBA and IAA were applied on the middle of the 4th and 3rd internode, respectively.

Initial length at treatment; 1st=2.8, 2nd=1.3, 3rd=0.9, 4th=1.2 cm.

**Effects of re-application of IAA on TIBA-induced reduction of stem growth induced by IAA**

Similarly to the results in Tables 1 to 3, TIBA applied on the middle of the 4th internode inhibited the IAA-induced elongation of all the internodes (Table 4 and Fig. 4). When IAA was applied additionally on the middle of the 3rd internode, it recovered the growth inhibition of the 1st to 3rd internodes caused by TIBA, but it did not reach to the length promoted by IAA only. The elongation of the 4th internode was not affected. There was no significant differences in the lengths of all the internodes between the re-treatments of IAA on the middle of the 3rd internode on the same day of TIBA treatment and of IAA on the middle of the 3rd internode one day after the TIBA treatment.



**Fig. 4.** Effects of re-application of IAA on TIBA-induced reduction of stem growth induced by IAA. 1-6; see Table 4.

### Effects of site of TIBA application and concentration of IAA on stem growth-1

IAA 2.0% was less effective than IAA 0.1% on elongation of all the internodes (Table 5 and Fig. 5). TIBA application on the lower internodes with IAA 0.1% on the top of the 4th internode was less effective in inhibition of all internodes induced by the auxin in comparison to TIBA treatment on higher internodes.

When the difference in the total internode length between the treatments with 0.1% IAA and 0.1% IAA+TIBA on the middle of the 4th internode was compared with that between the treatments with 2.0% IAA and 2.0% IAA+TIBA, the latter was much smaller than the former.

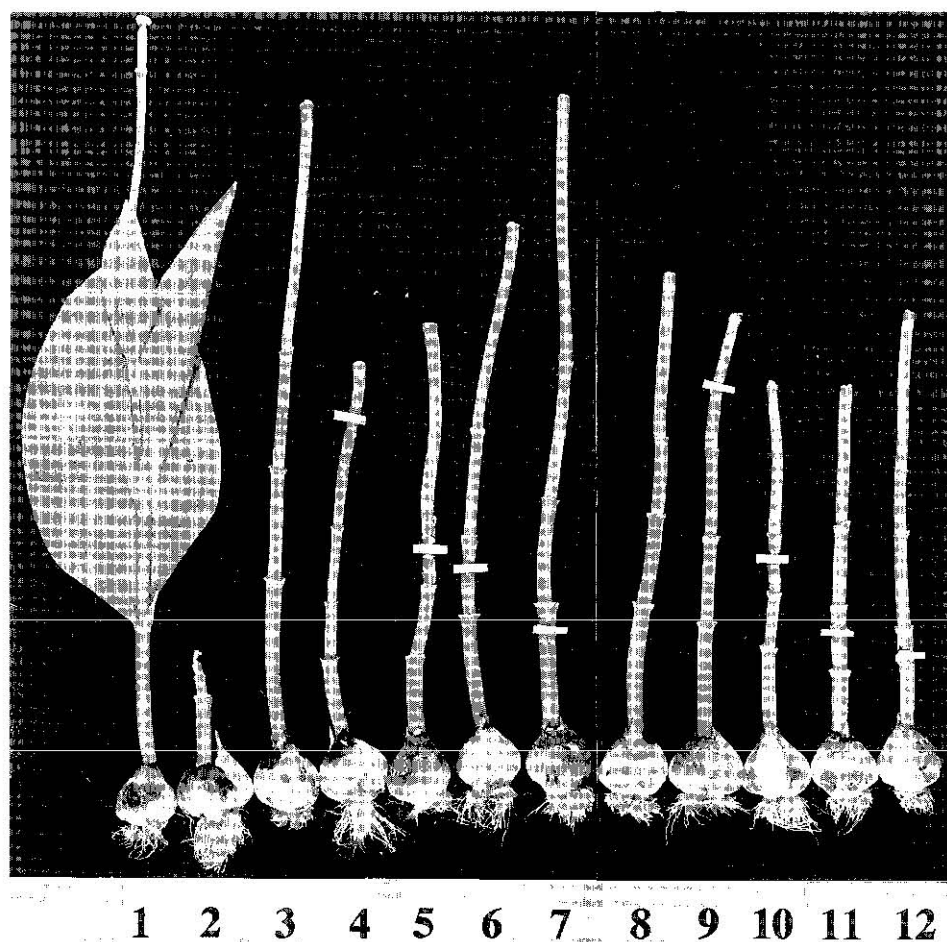


Fig. 5. Effects of site of TIBA application and concentration of IAA on stem growth-1. 1-12; see Table 5.

**Table 5.** Effects of site of TIBA application and concentration of IAA on stem growth-1.

Treatment	Length of internode (cm)				
	1st	2nd	3rd	4th	Total
1. Intact control	8.1gh <sup>z</sup>	6.7f	6.4e	18.8g	40.0g
<i>All leaves and flower bud removed</i>					
2. Control (lanolin)	3.2a	1.6a	1.0a	1.2a	7.0a
3. IAA 0.1%	8.9h	6.7f	7.1c	13.3e	36.0f
4. IAA 0.1% + TIBA <sup>y</sup> (IV) <sup>x</sup>	4.2b	3.0b	4.7bc	10.2cd	22.1bc
5. IAA 0.1% + TIBA (III)	4.2b	4.0cd	4.3b	10.9d	23.4bcd
6. IAA 0.1% + TIBA (II)	5.8de	4.7de	5.6d	12.6e	28.7e
7. IAA 0.1% + TIBA (I)	7.2f	6.1f	6.9e	14.9f	35.1f
8. IAA 2.0%	7.3fg	5.3e	4.9bcd	10.1cd	27.6e
9. IAA 2.0% + TIBA (IV)	5.2cd	4.2cd	5.3cd	9.3bc	24.0cd
10. IAA 2.0% + TIBA (III)	4.5bc	3.6bc	4.1b	9.8cd	22.0bc
11. IAA 2.0% + TIBA (II)	4.0ab	3.5bc	4.7bc	8.6b	20.8b
12. IAA 2.0% + TIBA (I)	6.0e	5.3e	4.9bcd	10.6cd	26.2de

<sup>z</sup> Mean separation within columns by Duncan's multiple range test at 5% level.

<sup>y</sup> TIBA=TIBA 0.2%.

<sup>x</sup> IV, III, II and I; TIBA was applied on the middle of the 4th, 3rd, 2nd and 1st internode, respectively.

Initial length at treatment; 1st=1.9, 2nd=1.2, 3rd=0.7, 4th=1.0 cm.

### Effects of site of TIBA application and concentration of IAA on stem growth-2

Similarly to the results shown in Table 5, TIBA applied on the middle of the 3rd internode with 0.1% IAA on the top cut surface of the 4th internode reduced the length of

**Table 6.** Effects of site of TIBA application and concentration of IAA on stem growth-2.

Treatment	Length of internode (cm)				
	1st	2nd	3rd	4th	Total
<i>Intact plants</i>					
1. Control	8.0d <sup>z</sup>	6.1bc	8.0bc	14.4bc	36.5b
2. TIBA <sup>y</sup> (I) <sup>x</sup>	5.3b	6.9c	9.1c	16.3bcd	37.6b
<i>All leaves removed, flower bud intact</i>					
3. Control	6.1c	5.6b	8.3bc	16.7cd	36.7b
4. TIBA (III)	4.4a	3.3a	7.2b	18.6d	33.5b
<i>All leaves and flower bud removed</i>					
5. Control (lanolin)	1.8	1.1	1.0	1.2	5.1
6. IAA 0.1%	10.5f	8.0d	10.4d	17.4d	46.3c
7. IAA 0.1% + TIBA (III)	4.4a	2.7a	5.4a	13.7ab	26.2a
8. IAA 2.0%	8.9e	6.4bc	8.1bc	13.6ab	37.0b
9. IAA 2.0% + TIBA (III)	4.1a	2.5a	4.6a	11.3a	22.5a

<sup>z</sup> Mean separation within columns by Duncan's multiple range test at 5% level.

<sup>y</sup> TIBA=TIBA 0.2%.

<sup>x</sup> I and III; TIBA was applied on the middle of the 1st and 3rd internode, respectively.

Initial length at treatment; 1st=1.8, 2nd=1.1, 3rd=1.0, 4th=1.2 cm.



**Fig. 6.** Effects of site of TIBA application and concentration of IAA on stem growth-2. 1-9; see Table 6.

all the internodes (Table 6 and Fig. 6). The length of all the internodes was less in 2.0% IAA than in 0.1% IAA treatments, whereas it was not significant when TIBA was applied with 0.1% or 2.0% IAA. It is well known that ethephon greatly inhibited tulip stem growth induced by flower bud or by IAA application after removal of flower bud and all leaves (Saniewski and Kawa, 1988). IAA 2.0% promoted 26 times higher ethylene production than IAA 0.1% in the 4th internode of tulip in the same experiment system (Saniewski *et al.*, 1990), and it is possible that ethylene itself inhibits polar transport of auxins via feedback control. It is well known that ethylene inhibits basipetal transport of auxin in many plants and their organs (Kang, 1987; Suttle, 1988). It is, therefore, considerable

that TIBA inhibits the auxin-induced ethylene production in tulip. TIBA inhibited ethylene production induced by IAA in etiolated mung bean hypocotyl segments (Tsai and Arteca, 1984). Presented results using TIBA confirmed crucial role of auxin in tulip stem growth.

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