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Inhibitory Effect of Naphthylphthalamic Acid (NPA) on Stem Growth Induced by Auxin in Precooled Tulip Bulbs

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The effect of phytotropin naphthylphthalamic acid (NPA), a well known auxin transport inhibitor, on tulip stem growth induced by indole-3-acetic acid (IAA) in low (0.1%) and high (2.0%) concentration in lanolin paste was studied. IAA 0.1 and 2.0% applied on the place of removed flower bud of sprouted precooled tulip bulbs and after excision of all leaves induced growth of all internodes; IAA 0.1% induced stem growth in higher degree than IAA 2.0%. NPA 0.2% applied on the 4th, 3rd and 2nd internodes strongly inhibited the growth of internodes induced by IAA 0.1 and 2.0%; the inhibition was stronger in case of IAA 0.1 than IAA 2.0%. The inhibitory effect of NPA on stem growth induced by IAA was restored by additional application of IAA below NPA treatment. Tulip stem growth induced by flower bud after excision of all leaves was also inhibited by NPA below its application. The inhibitory effect of NPA on stem growth promoted by IAA and that additional application of IAA below NPA treatment restored the growth indicate on crucial role of auxin in tulip stem growth.

INTRODUCTION

Tulip bulbs with terminal buds containing a complete flower require a period of 12–14 weeks of low temperature treatment for shoot elongation (De Hertogh, 1974). It is well known that elongation of the stem and leaves of tulips is due almost entirely to the elongation of cells produced during earlier developmental stages of flower bud formation (Gilford and Rees, 1973). The leaves and gynoecium provide auxins which control the elongation growth of the stem (Op den Kelder *et al.*, 1971; Hanks and Rees, 1977; Saniewski and De Munk, 1981; Banasik and Saniewski, 1985). Saniewski and De Munk (1981) and Banasik and Saniewski (1985) showed that elongation of all internodes in precooled tulip bulbs is promoted by application of auxins in the place of removed flower buds in the absence of leaves. Recently, it has been suggested that the elongation of all the internodes in tulips is controlled by the interaction of endogenous auxins and gibberellins (Okubo and Uemoto, 1985, 1986; Okubo *et al.*, 1986; Saniewski, 1989). Okubo and Uemoto (1985) showed that 2,3,5-triiodobenzoic acid (TIBA) treatment at the first internode of sprouting tulip shoot inhibited the dark-induced elongation of 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 stalk elongation in the nonprecooled and precooled, rooted and derooted

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tulip bulbs, and TIBA applied in the middle of the 4th internode (below IAA application) greatly inhibited the growth of lower internodes. TIBA also inhibited tulip stem growth induced by IAA and NAA in precooled rooted tulip bulbs after removal of flower bud and all leaves (Saniewski and Okubo, 1998).

The phytotropin naphthylphthalamic acid (NPA) is known to inhibit polar auxin transport in dicotyledons and monocotyledons by inhibiting active auxin secretion (Depta *et al.*, 1983). NPA inhibits auxin transport by specifically binding to the auxin efflux carrier (Katekar and Giessler, 1980). The endogenous auxin IAA does not compete with NPA for binding site (Lomax *et al.*, 1995) and NPA–binding site is important for auxin transport (Ruegger *et al.*, 1997).

In this report the effect of NPA on tulip stem growth induced by auxins, IAA and NAA, in precooled rooted tulip bulbs is presented.

MATERIALS AND METHODS

Plant materials, temperature treatment and culture conditions

Bulbs of tulip (*Tulipa gesneriana* L.) cv. Gudoshnik, with 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 NPA applied on the middle of the 4th internode on stem growth induced by IAA 0.1%

All leaves and flower bud were removed when the length of the stem was about 8 cm and plain lanolin (control) or lanolin containing IAA at a concentration of 0.1% was applied on the cut surface of the flower bud on 11 February. Then, 0.2% NPA in lanolin paste was applied on the middle of the 4th internode and in other treatments additionally IAA 0.1% was applied on the middle of the 3rd internode directly after NPA treatment or one day later after NPA application. Seven plants were used for each treatment. The growth of all internodes was measured every two days but that of 22 February is only presented.

Effects of NPA application on different internodes on stem growth induced by IAA at a concentration of 0.1 or 2.0%

IAA at a concentration of 0.1 or 2.0% was applied on the top of the last internode after decapitation of flower bud and leaf excision as described above and then 0.2% NPA was applied in the middle of different internodes on 31 January. The stem length at treatment was about 7 cm. Seven plants were used per treatment. Measurements of different internode growth were made every two days but those of on 15 February are presented.

Effects of NPA on the growth of stem of intact plants, growth induced by flower bud after excision of all leaves and growth induced by IAA

When the length of the stem was about 5 cm 0.2% NPA was applied on the middle of

the 1st internode of intact plants or on the middle of the 3rd internode after removal of all leaves in the presence of flower bud. In the case when IAA 0.1 or 2.0% was applied on the top of the last internode after decapitation of flower bud and excision of all leaves 0.2% NPA was applied on the middle of the 3rd internode. Seven plants were used per treatment. Treatments were made on 6 March and measurements were done every two days during experiment period, but only the results on 17 March are presented.

RESULTS AND DISCUSSION

Effects of NPA applied on the middle of the 4th internode on stem growth induced by IAA 0.1%

IAA 0.1% applied in the place of removed flower bud of sprouted precooled tulip bulbs and after excision of all leaves induced growth of all internodes (Table 1, Fig. 1). The results fully confirmed previous studies (Saniewski and De Munk, 1981; Banasik and Saniewski, 1985; Saniewski, 1989). NPA 0.2% applied on the middle of the 4th internode inhibited the growth of all internodes induced by IAA 0.1% applied on the top of the last internode after decapitation of flower bud and removal of all leaves. The inhibitory effect of NPA on tulip stem growth induced by IAA was restored by additional application of IAA on the middle of the 3rd internode (below NPA) when applied directly or one day later after NPA treatment. It was found recently that inhibitory effect of TIBA on tulip stem growth induced by IAA was greatly restored by application of IAA below TIBA treatment place (Saniewski and Okubo, 1998). The growth of maize coleoptiles and pea internodes was greatly suppressed when the organ was ringed with NPA and the growth suppressed by NPA was restored by IAA applied directly to the zone below NPA treatment (Moritoshi Iino, personal communication).

Table 1. Effects of NPA (0.2%) applied on the middle of the 4th internode on stem growth induced by IAA (0.1%).

Treatment	Length of internode (cm)				
	1st	2nd	3rd	4th	Total
1. Intact control	7.5 b[z]	6.0 b	6.4 d	14.8 e	34.7 d
All leaves and flower bud removed					
2. Control (lanolin)	3.6 a	1.6 a	1.1 a	1.4 a	7.7 a
3. IAA	8.7 b	6.2 b	6.5 d	13.8 de	35.2 d
4. IAA+NPA (IV)[y]	3.8 a	2.0 a	1.9 b	9.9 b	17.6 b
5. IAA+NPA (IV)+IAA (III)	8.2 b	5.8 b	5.1 c	12.2 cd	31.3 cd
6. IAA+NPA (IV) first, then IAA (III) one day later	8.0 b	5.9 b	4.4 c	11.3 bc	29.6 c

[z]Mean separation within columns by Duncan's multiple range test at 5% level.

[y]IV and III; NPA 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.

Fig. 1. Effects of NPA (0.2%) applied on the middle of the 4th internode on stem growth induced by IAA 0.1%. 1–6; see Table 1.

Effects of NPA application on different internodes on stem growth induced by IAA at a concentration of 0.1 or 2.0%

IAA 0.1% induced stem growth of tulip in higher degree than IAA 2.0% (Table 2, Fig. 2). NPA applied on the middle of the 4th, 3rd and 2nd internodes greatly inhibited the growth of all internodes induced by 0.1 and 2.0% IAA, and the inhibitory effect of NPA was stronger in case of 0.1% than 2.0% IAA. NPA applied on the 1st internode did not inhibit or only slightly inhibited the growth of the stem induced by either concentration of IAA.

Table 2. Effects of NPA (0.2%) application on different internodes on stem growth induced by IAA at a concentration of 0.1 or 2.0%

Treatment	Length of internode (cm)				
	1st	2nd	3rd	4th	Total
1. Intact control	8.1 e[a]	6.7 f	6.4 g	18.8 g	40.0 g
All leaves and flower bud removed					
2. Control (lanolin)	3.2 a	1.6 a	1.0 a	1.2 a	7.0 a
3. IAA 0.1%	8.9 f	6.7 f	7.1 h	13.3 f	36.0 f
4. IAA 0.1% + NPA (IV)[b]	3.9 ab	1.9 ab	2.3 b	8.9 bc	17.0 b
5. IAA 0.1% + NPA (III)	4.1 b	2.4 b	4.3 cde	11.3 e	22.1 c
6. IAA 0.1% + NPA (II)	4.3 b	4.8 c	6.3 g	12.0 e	27.4 d
7. IAA 0.1% + NPA (I)	7.1 d	6.2 f	6.3 g	12.4 ef	32.0 e
8. IAA 2.0%	7.3 d	5.3 e	4.9 def	10.1 d	27.6 d
9. IAA 2.0% + NPA (IV)	4.2 b	2.9 c	4.1 c	8.6 b	19.8 c
10. IAA 2.0% + NPA (III)	3.6 ab	3.0 c	4.2 cd	9.0 bcd	19.8 c
11. IAA 2.0% + NPA (II)	3.8 ab	3.5 d	5.3 f	8.7 bc	21.3 c
12. IAA 2.0% + NPA (I)	6.3 c	5.0 e	5.0 ef	9.9 cd	26.2 d

[a]Mean separation within columns by Duncan's multiple range test at 5% level.
[b]IV, III, II and I; NPA 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.

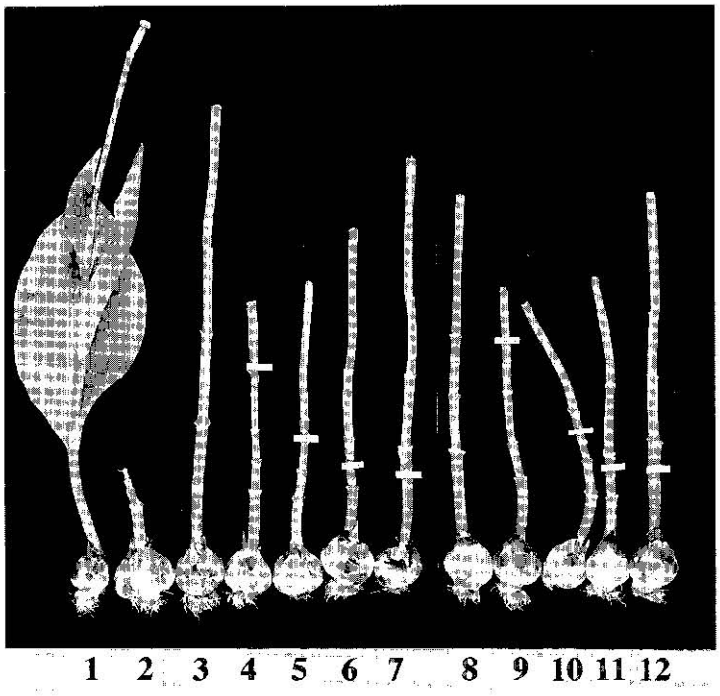

Fig. 2. Effects of NPA (0.2%) application on different internodes on stem growth induced by IAA at a concentration of 0.1 or 2.0%. 1–12; see Table 2.

Effects of NPA on the growth of stem of intact plants, growth induced by flower bud after excision of all leaves and growth induced by IAA

Tulip stem growth induced by flower bud after excision of all leaves was also inhibited by NPA below its application (Table 3, Fig. 3). NPA applied below the 1st node of intact plants only inhibited the growth of the 1st internode.

It is well known that high concentration of IAA or NAA stimulates ethylene production in tulip stem and promoted internode elongation less and thickening more than low concentrations of these auxins do (Saniewski *et al.*, 1990). Silver thiosulphate (STS), an inhibitor of ethylene action, applied simultaneously with high concentration of IAA or NAA greatly stimulated the stem growth of tulip in comparison with the application of these auxins alone in the same concentrations (Saniewski *et al.*, 1990). Ethephon greatly inhibited tulip stem growth induced by flower bud or by IAA after removal of flower bud and all leaves and STS completely reversed the inhibitory effect of ethylene on stem elongation (Saniewski and Kawa, 1988). It is possible that ethylene induced by high concentration of auxin itself inhibits polar transport of auxins via feedback control. In pea seedlings where polar auxin transport is inhibited by ethylene, NPA binding sites were found to be reduced in number by the gas (Kang, 1987; Suttle, 1988).

The inhibitory action of NPA on tulip stem growth induced by auxin and the fact that IAA applied below the place of NPA treatment restored the growth indicate on the crucial role of auxin in tulip stem growth.

Table 3. Effects of NPA (0.2%) on the growth of stem of intact plants, growth induced by flower bud after excision of all leaves and growth induced by IAA.

Treatment	Length of internode (cm)				
	1st	2nd	3rd	4th	Total
Intact plants					
1. Control	8.0 c[z]	6.1 bc	8.0 c	14.4 a	36.5 c
2. NPA (I)[y]	5.8 b	6.5 c	8.0 c	14.4 a	34.7 bc
All leaves removed, flower bud intact					
3. Control (lanolin)	6.1 b	5.6 b	8.3 c	16.7 b	36.7 c
4. NPA (III)	4.6 a	2.8 a	6.7 b	18.5 b	32.6 bc
All leaves and flower bud removed					
5. Control (lanolin)	1.8	1.1	1.0	1.2	5.1
6. IAA 0.1%	10.5 c	8.0 d	10.4 d	17.4 b	46.3 d
7. IAA 0.1% + NPA (III)	5.8 b	2.8 a	5.1 a	17.0 b	30.7 b
8. IAA 2.0%	8.9 d	6.4 c	8.1 c	13.6 a	37.0 c
9. IAA 2.0% + NPA (III)	4.4 a	2.5 a	5.3 a	12.4 a	24.6 a

[z]Mean separation within columns by Duncan's multiple range test at 5% level.
[y]I and III; NPA 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. 3. Effects of NPA (0.2%) on the growth of stem of intact plants, growth induced by flower bud after excision of all leaves and growth induced by IAA. 1–9; see Table 3.

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