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DATA ON THE SHOOT GROWTH-INHIBITING EFFECT OF 2, 3, 5-TRIIODIBENZOIC ACID

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Abstract

Treatment of bean seedlings with 2, 3, 5-triiodobenzoic acid (TIBA) below the cotyledon prevents the completion of the elongation of the hypocotyl and significantly inhibits the growth of the stem part above the treatment. The inhibitor decreases the fresh weight increase in parallel with the growth. The dry weight increases a little in the hypocotyl, and decreases considerably in the epicotyl.

TIBA applied to the uppermost internode inhibits the elongation of the more downward stem parts to only a small extent, but prevents the appearance of the parts above the treatment completely. Thus, not only the total length, but also the number of internodes decreases.

TIBA treatment significantly reduces the IAA content of the shoot, in the stem parts both below and above the site of treatment. The hypocotyl/epicotyl distribution ratio of the IAA content does not vary in the control as a result of the TIBA treatment. The TIBA therefore inhibits not only the transport but also the synthesis of IAA, in stem parts both below and above the treatment site.

Introduction

2, 3, 5-Triiodobenzoic acid (TIBA) is a known inhibitor of plant growth; the view is generally widespread in the literature that it specifically blocks the basipetally polar auxin transport (KUSE, 1953; NIEDERGANG—KAMIEN and SKOOG, 1956; HAY, 1956; ZWAR and RIJVEN, 1956; etc.). The majority of the authors explain the growth-inhibiting effect of TIBA exclusively in the blocking of the auxin transport from the apex towards the base, and only very few of them have dealt with its effects on the auxin content and its distribution (NIEDERGANG—KAMIEN and SKOOG, 1956; GOLDSMITH, 1968).

In the course of growth physiological experiments with bean seedlings, involving the use of TIBA, we have observed a number of phenomena which do not fit in too well with the classical conceptions of its inhibitory effect; it appeared desirable, therefore, to carry out a certain re-examination of the growth inhibition brought about by TIBA. With this aim, a study has been made of the effects of applying TIBA to various parts of the shoot on the growth of the parts of the stem above and below the site of treatment; a further investigation has been made of the influence of the inhibitor on the total auxin content of the shoot and on the apex/base distribution of the endogenous auxin (IAA).

Materials and Methods

The experiments were carried out with bean plants (*Phaseolus vulgaris* L., "White pearl"). The seeds were planted in perlite containing 70% water, and were watered with Prjanisnikov solution. The plants were grown in a green-house at 24 °C, with 16 hours of illumination daily.

TIBA in lanoline paste was used as the growth-inhibiting agent, in a concentration of 1.5%. The growth of the untreated shoots and those treated with TIBA was measured every 3 days until the 25th day after the sprouting, by determining the lengths of the internodes and also the fresh and dry weights of the shoots.

The extraction and chromatographic separation of indoleacetic acid (IAA) were carried out as described earlier (VARGA and BITÓ, 1968). In brief: a methanolic extract prepared from the tissue homogenizate was purified by shaking with petroleum ether, evaporated under vacuum, and chromatographed on a silica gel G layer with chloroform — ethyl acetate — formic acid 5:4:1 and isopropanol — 7% ammonia — water 8:1:1 as solvents. The IAA was identified by comparison of the Rf value with that of the authentic compound, by means of the colour given with the Ehrlich reagent, and from the UV fluorescence and UV absorption spectra. For quantitative determination the IAA spots were scraped off the plates, and eluted with methanol, and the concentration of IAA in the eluate was measured with a Spektromom 202 photometer at 280 nm (FLETCHER and ZALIK, 1964).

Every examination was carried out in two parallel series, repeated three times.

Results

1. Effect on the stem elongation of treating the hypocotyl with TIBA

Lanoline paste containing TIBA was smeared in a ring 2—3 mm wide onto the hypocotyl of 5-day seedlings, immediately below the cotyledon. The same number of untreated plants were left as controls. After 3, 6 or 9 days (i.e. at the age of 8, 11 or 15 days) the growths of the stem parts below and above the cotyledon were measured on the basis of three factors: the length, and the fresh and dry weights.

The effect of TIBA treatment on the stem growth is shown in Fig. 1. The untreated shoots became significantly elongated during the period of the experiment. The increase in the total length of the shoot is a result of the continuous growth

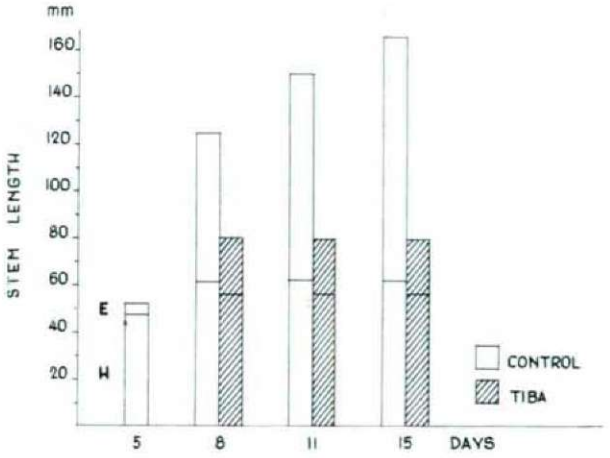

Fig. 1. Effect of TIBA treatment of the hypocotyl on the stem growth of bean seedlings. H=hypocotyl, E=epicotyl.

of the epicotyl, since the hypocotyl no longer grows after the 8th day. In the TIBA-treated shoots the total length was much lower, the growth of the hypocotyl being inhibited to a smaller extent (5—11%) and that of the epicotyl to a much larger extent (62—75%).

The TIBA inhibited the increase of the fresh weight of the hypocotyl only slightly up to the completion of the elongation, but the inhibition of the increase of the fresh weight of the epicotyl was much more significant (Table 1). The dry weight of the

Table 1. Effect of TIBA treatment on the increase of the fresh weight of bean shoots

Day	Hypocotyl			Epicotyl		
	Control g	TIBA-treated g	Inhibition %	Control g	TIBA-treated g	Inhibition %
5	0.314	0.314	—	0.121	0.121	—
8	0.566	0.510	9	0.512	0.374	27
11	0.633	0.641	—	0.879	0.536	39
14	0.583	0.590	—	1.244	0.809	35

Table 2. Effect of TIBA treatment on the increase of the dry weight of the shoots

Day	Hypocotyl			Epicotyl		
	Control mg	TIBA-treated mg	Inhibition %	Control mg	TIBA-treated mg	Inhibition %
5	26.6	26.6	—	21.6	21.6	—
8	31.6	33.3	—	75.0	48.0	36
11	37.5	39.7	—	96.6	68.6	29
14	46.0	46.8	—	164.0	111.5	32

hypocotyl of the treated seedlings did not fall behind that of the control, and in fact was even a little higher; that of the epicotyl, however, was considerably less than that of the control (Table 2).

2. Effect of TIBA applied to various internodes of the epicotyl on the elongation of the stem parts

Bean seedlings grown as described were divided into 4 groups. In the first group the lanoline ring containing the TIBA was applied to the first internode at the age of 7 days, in the second group to the second internode at the age of 10 days, in the third group to the third internode at the age of 13 days, and in the fourth group to the fourth internode at the age of 16 days, i.e. always to the uppermost internode then appearing. At 3-day intervals for 9 days after the treatment the growths of the stem parts below and above the ring were measured.

In the case of the treatment of the first internode (Fig. 2,1) it was not possible to observe the inhibiting effect of TIBA on the hypocotyl, and to only a slight extent on the first internode, for at this time these stem parts had already completely or

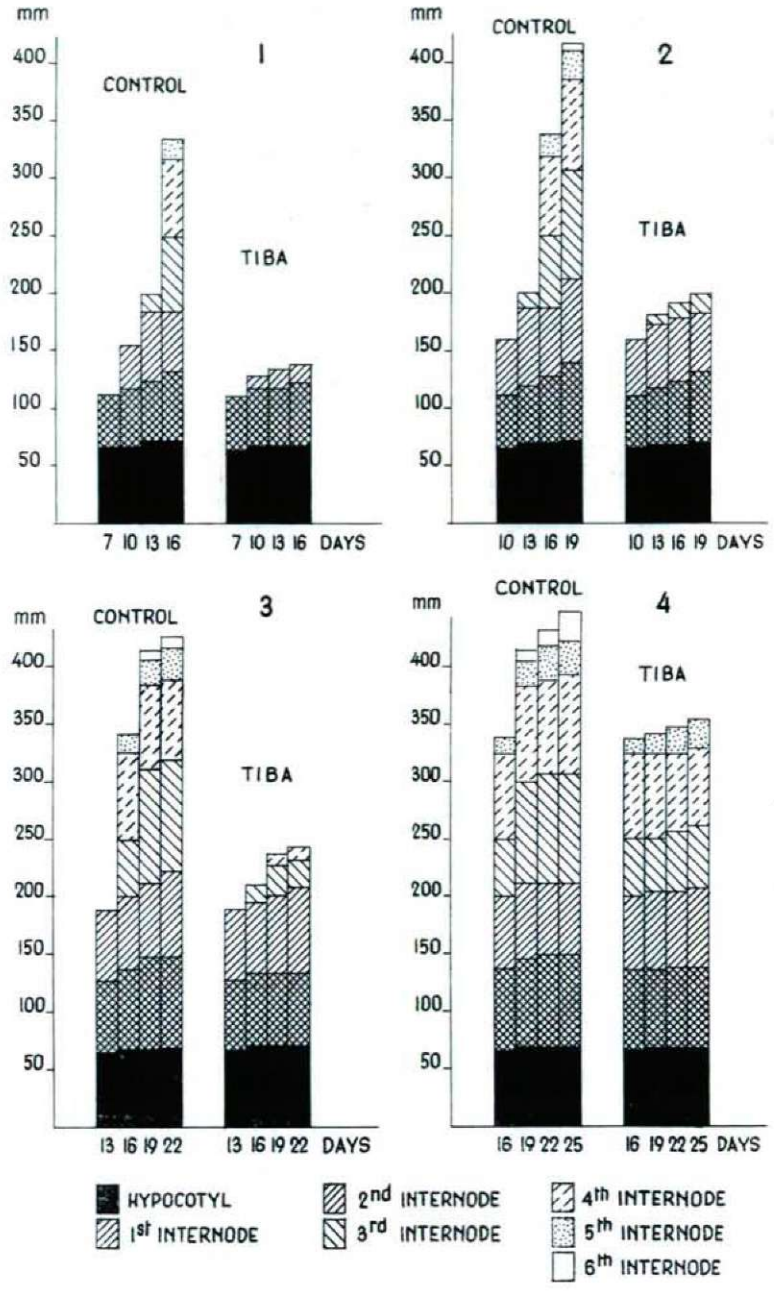

Fig. 2. Effect of application of TIBA to different internodes of the bean shoot on the elongation of the stem parts.

partially finished their elongation. On the other hand, the TIBA applied to the first internode caused a very large inhibitory effect on the development of the second internode, and completely blocked the occurrence of the following internodes. This could also be observed after treatment of the second, third and fourth internodes too: the TIBA had little effect on the elongation of the stem parts below the ring, whereas above the ring in every case only the following internode appeared to a very reduced extent, while the remaining internodes failed completely to develop (Fig. 2,2—4). It follows from this that the difference between the total lengths of the controls and the treated shoots is the smaller, the later the time of treatment of the appearing internode.

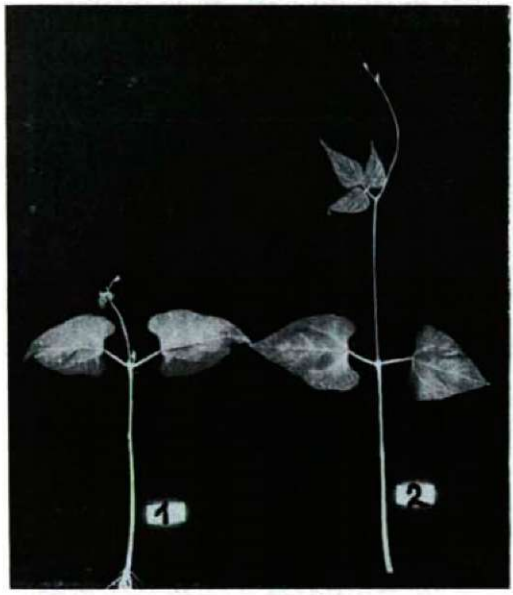

Fig. 3. Effect of application of TIBA to the first internode of the epicotyl on the shoot growth. 1 = treated, 2 = control.

Figure 3 shows the results of treatment for the first internode.

3. Effect of TIBA treatment on IAA content

The hypocotyl of 5-day bean seedlings was treated with a TIBA ring below the cotyledon, and three days later the amounts of IAA were determined in the entire shoot and in the hypocotyl and epicotyl parts separately. The results were calculated referred to one organ, and the data thus express the actual auxin contents of the shoots and the shoot parts.

The results indicate (Fig. 4) that TIBA treatment led to a marked reduction of the IAA contents of the whole shoot and the individual shoot parts (hypocotyl and epicotyl) compared to the control. This effect was somewhat more pronounced tn the hypocotyl (58%) than in the epicotyl (50%). As regards the distribution of ihe IAA content within the shoot, in the case of the control shoots less of the overall IAA content is provided to the hypocotyl (40%) than the amount remaining at the

sites of synthesis, in the apical part (60%). With TIBA treatment, although the total amount of IAA was significantly less, these ratios of the auxin distribution did not change (Fig. 4).

Discussion

When the TIBA ring was applied below the cotyledon to the stem of the 5-day seedlings, in the period following the treatment the TIBA completely inhibited the conclusion of the elongation of the hypocotyl, and kept it at the original level. At the same time the hypocotyls of the control shoots continued to grow until the eighth day, and then remained constant in length. This explains why, although the effect of the TIBA in restricting the total length of the hypocotyl is apparently slight, the inhibition calculated as a △ value attains 45—50%.

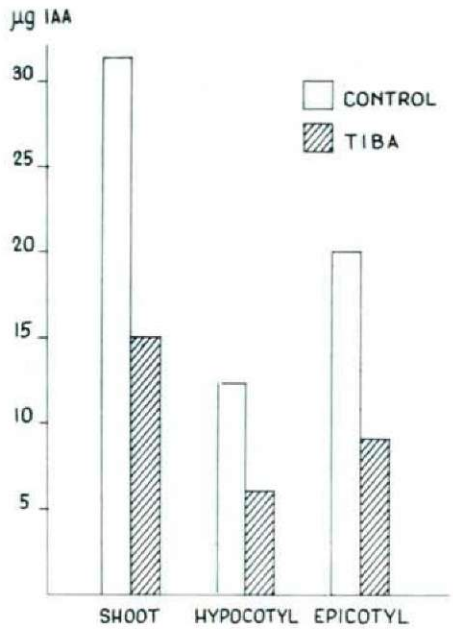

Fig. 4. Effect of TIBA treatment on the IAA content of the shoots.

The TIBA treatment exerted a striking and considerable inhibitory effect on the elongation of the stem part above the cotyledon (first internode), the analogous elongation in the control being very intensive in the experimental period. The TIBA induced inhibition of the elongation of the epicotyl stem part above the site of treatment proved to be 62, 73 and 90% in the individual measurements. This strong decrease of the elongation is in agreement with the inhibition of the fresh weight and dry matter content increase of the epicotyl.

When the TIBA ring was applied to newly appearing internodes of the epicotyl, the inhibition of the elongation of the internodes lying below the site of application was only slight, whereas the growth of those above the treated internode was com-

pletely inhibited. As a consequence of this, the TIBA treatment caused a significant decrease not only in the total length of the shoot, but also in the number of internodes, compared to the control.

As regards the effect of TIBA, the view is extremely widespread in the literature that, in accordance with the blocking of the basipetal IAA transport, inhibition of the stem growth occurs only below the site of treatment. For example, BOUCK and GALSTON (1967) report that application of TIBA to the third internode of pea stimulated the elongation of the stem above the ring, but significantly inhibited the growth of the stem parts below the ring. All this is explained by the accumulation above the ring of the auxin amounts migrating downwards under polar effects. TANIMOTO et al. (1967) similarly treated the middle of the third internode of pea shoots with TIBA; the upper half of the internode elongated strongly, whereas the lower half hardly grew. According to the authors, the cause of the phenomenon is almost certainly the uneven distribution of the IAA originating from the apex in the two halves of the internode, as a consequence of the blocking of the IAA transport by the TIBA. In contrast with these publications, our results show that the inhibitory effect of the TIBA on the stem elongation is definitely exerted upwards from the site of treatment. Thus, the blocking of the basipetal auxin transport can not be the only reason for the effect of TIBA.

A TIBA ring applied beneath the cotyledon to the young shoots of the seedlings significantly inhibited the increase of the IAA contents of both the hypocotyl and the epicotyl, i.e. the stem parts not only below, but also above the ring. These data are likewise in contrast with the results of certain publications. A number of authors have reported that the application of a TIBA ring to the stem or a stem-segment retains the bulk of the auxin in the apical part of the stem, and inhibits its accumulation in the basal part below the treatment (HEJNOVICZ and TOMASZEWSKI, 1967; LEOPOLD and FUENTE, 1967; HERTEL and FLORY, 1968).

In our experiments the TIBA treatment did not change the normal apex/base ratio of the total amount of auxin in the shoot, i.e. it inhibited the increase of IAA in the parts below and above the ring to roughly the same extent. In their investigations on tobacco stem segments, NIEDERGANG—KAMIEN and SKOOG (1956) similarly observed an inhibitory effect of TIBA on the total auxin content; study of the distribution of the amount of endogenous auxin within the stem segment, however, revealed a shift of the apex/base ratio in favour of the base.

Our data lead to the overall finding that the TIBA inhibits not only the transport of IAA in the treated shoots, but also the auxin content (synthesis). This dual effect is manifested both in the stem parts below the ring and in those above it. In the event of the application of TIBA in other concentrations (0.5, 1.0 and 2.0% inhibitor), its growth-inhibitory effect was exerted in a similar way.

The details of the mode of the TIBA effect observed by us require and are worthy of further investigations.

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