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Sensitized Bean First Internode Bioassay for Auxins and Brassinosteroids

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Abstract. The bean first internode bioassay of MEUDT and BENNETT (Physiol. Plant. 44: 422—428, 1978) is based on the curvature of bean internode sections after unilateral application of test material near to their base. The test was also used for estimation of biological activity of brassinosteroids which enhance the effect of auxin. Sensitivity of the assay to both IAA and 24-epibrassinolide was increased 1000 and 100 times, respectively, when internode sections were incubated in morphologically inversed position. Under these conditions test material was applied to the young auxin-sensitive morphologically apical internode tissues and auxin was basipetally translocated to the zone of curvature. Using this bioassay as little as 10 fmol of IAA and 1 pmol of 24-epibrassinolide can be estimated.

Bioassays for auxin are based either on the curvature of plant organs or enhancement of straight growth. The curvature tests are more specific, but require skilled labour and in most cases carefully standardized conditions. The exception to this rule is the curvature test of MEUDT and BENNET (1978) using partially etiolated first internode sections of beans. Sections are inserted with their morphological base into scintillation vials containing buffer and a piece of sponge as a support. Test compounds are applied in ethanol solution on small filter paper discs. Dry filter discs are sandwiched between the lower part of the internode and sponge support. Growth response is expressed as horizontal displacement of the apical end of the internode section measured 1 to 3 h after the application of test compound. The test has been also used for testing biological activities of brassinosteroids (THOMPSON *et al.* 1981, 1982), *i.e.* plant growth promoting substances which act synergistically with auxins (YOPP *et al.* 1981). In this case paper discs containing brassinosteroide are applied to internode sections for 1 h prior to application of IAA. The control sections are treated with auxin alone. The effect of brassinosteroide is expressed as a difference between the brassinosteroide + IAA treated sections and controls (THOMPSON *et al.* 1981, 1982).

The test is specific for auxin and its sensitivity is comparable to the oat coleoptile curvature bioassay of WENT (1928). Using this assay as little as 10 pmol of IAA can be estimated (MEUDT and BENNET 1978).

In the original procedure of MEUDT and BENNETT (1978) the bioassay is

carried out with internode sections incubated with their apical end up, *i.e.* in normal morphological orientation. In this arrangement the paper discs containing test material are applied to the morphological base of internode sections, *i.e.* to relatively old and matured tissues. Further, auxin applied is transported basipetally from the site of its application to the nearby morphological base of section where its effect on curvature cannot be realized.

Improvement of the bioassay presented here is based on incubation of sections in inversed position. Under these conditions substances under the test are applied to young auxin-sensitive apical tissues and are translocated basipetally to the zone of curvature.

MATERIAL AND METHODS

Chemicals: IAA purchased from Calbiochem was purified according to Goot *et al.* (1956), [2—^{14}C]-IAA (2.04 GBq nmol^{-1}) was from Amersham, 24-epibrassinolide was a gift from Dr. V. Černý, Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Praha.

Bean seeds (*Phaseolus vulgaris* L. cv. HS 1906) were germinated for 2 days on paper wool saturated with distilled water at 28 °C under weak fluorescent light. Selected germinating seeds were then transferred into vermiculite saturated with Knop's solution and placed into a light-controlled cultivation room (23 °C, irradiance 6—8 W m^{-2}, light/dark period 10/14 h). Seedlings 10 days old were harvested 3.5 h after the beginning of the light period. Four cm long sections of the first internode were cut off just under the second node. Sections were inserted either with their morphologically apical or basal part (5 mm) into 20 ml glass scintillation vials containing a piece of sponge for support (15 × 15 × 60 mm) and 20 ml of sodium phosphate buffer (1 mmol l^{-1}, pH 6.4) as previously described (Meudt and Bennett 1978). Two sections were placed inside the neck of each vial. Each treatment contained 10 internode sections. Substances under the test were applied in ethanol solution on squares (5 × 5 mm) of Whatman No. 1 paper. Dry squares were sandwiched between the internode and sponge 5 mm from the section base. When activity of 24-epibrassinolide (24-BR) was tested, paper squares containing 24-BR were applied to sections and after 1 h were replaced by other ones containing standard amount of IAA. Vials were then incubated at 26 °C in darkness. The horizontal displacement of the upper end of sections was measured at 1 h intervals after application of IAA.

Elongation of different parts of the first internode section after application of IAA was expressed as a change of length of 5 mm long internode subsections after their submersion in IAA solutions in water. Ten subsections were incubated in 5 ml of IAA solution for 3.5 h at 26 °C in darkness.

Translocation of [2—^{14}C]-IAA from paper squares into and in internode sections was estimated under the standard assay conditions. Sections were incubated either with their apical or basal end up. Paper squares containing [2—^{14}C]-IAA supplemented with a carrier (2.56 kBq 10^{-8} mol l^{-1} IAA paper disc^{-1}) were placed in contact with the lower part of sections as described above. After 3.5 h of incubation at 26 °C in darkness internodes were washed in cold water and cut transversally into 25 mm long pieces. These subsections were three times separately extracted with boiling methanol (5 ml per 10 subsections) for 3 min. The volume of combined extracts was

reduced to 1 ml under vacuo and its radioactivity was measured after addition of a scintillant using a Packard 300 CD scintillation counter. An external standard was used for correction to d.p.m.

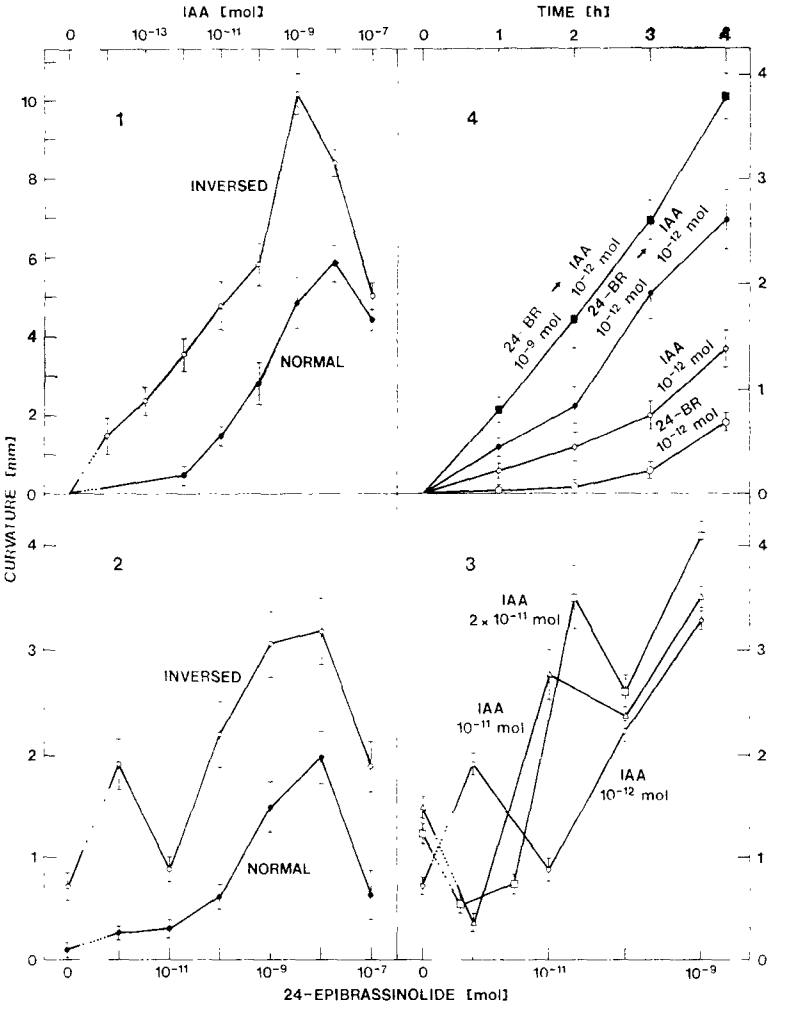

Fig. 1. Effect of IAA on curvature of the bean first internode sections (mol per section) incubated for 3 h with their morphological end up (normal) or in inversed position. Vertical bars denote standard errors.

Fig. 2. Effect of 24-epibrassinolide on curvature of the bean first internode sections (mol per section) incubated with their morphologically apical end up (normal) and in inversed position. 24-Epibrassinolide was applied for 1 h and then replaced by IAA (1 pmol per section). Curvature of sections was measured 3 h after application of IAA. Vertical bars denote standard errors.

Fig. 3. Effect of different ratios of IAA and 24-epibrassinolide on curvature of the bean first internode sections incubated in inversed position. 24-Epibrassinolide was applied for 1 h and then replaced by IAA. Curvature of sections was measured 3 h after application of IAA. Vertical bars denote standard errors.

Fig. 4. Rate of curvature of the bean first internode sections incubated in inversed position after application of different quantities of IAA and 24-epibrassinolide (mol per section). 24-Epibrassinolide was applied for 1 h and then replaced by IAA. Vertical bars denote standard errors.

TABLE 1

Relative activities of different indole derivatives in sensitized bean first internode bioassay

Indole derivative	Relative activity (% of IAA)	
	1 nmol	0.1 nmol
Indol-3-ylacetic acid	100.0	100.0
3-indolylacetonitril	18.3	13.0
Indol-3-ylpyruvic acid	21.2	8.7
Indol-3-ylpropionic acid	7.7	5.8
Indol-3-ylbutyric acid	18.3	3.8

RESULTS

Effect of IAA

Inversion of internode sections increased the sensitivity of the bioassay to IAA 1000 times (Fig. 1). As little as 10 fmoles (1.7×10^{-12} g) of IAA can be estimated. Growth response of sections is linearly dependent on the logarithm of IAA concentration over four orders of magnitude. Activity of different indole derivatives in sensitized bioassay is compared in Table 1. The assay is very specific to IAA. Other indole derivatives are much less active.

Effect of 24-BR

The dependence of the curvature of internode sections on the amount of applied 24-BR is expressed by a two-peaks curve (Fig. 2). When evaluated on the first peak basis inversion of sections increases the sensitivity of the bioassay by the factor of 100. For getting a significant growth-response 1 pmol (4.8×10^{-10} g) of 24-BR is required. The unusual two-peaks response is probably a result of interaction of two growth regulators applied success-

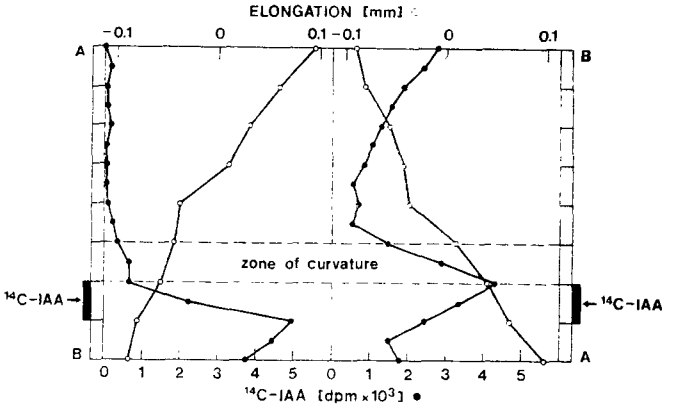

Fig. 5. Comparison of sensitivities of different subsections of the bean first internode sections to IAA and translocation of IAA. Sensitivity to IAA is expressed by elongation of individual subsections after their incubation in IAA solution (10^{-8} mol l^{-1}) for 3.5 h. Translocation of [2—^{14}C]-IAA was estimated after incubation of 40 mm long internode sections either with their morphologically apical (A/B) or basal (B/A) end up for 3.5 h. Dark bars indicate the site of [2—^{14}C]-IAA application.

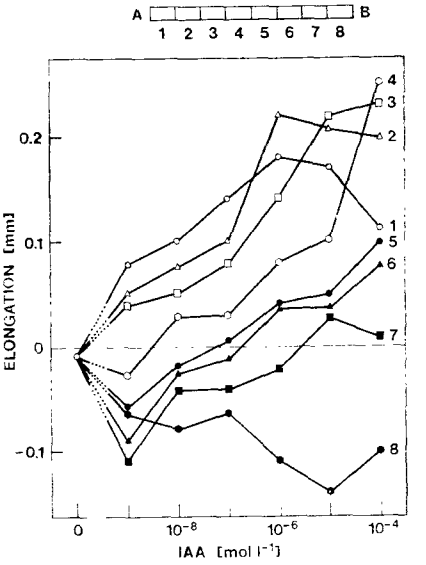

Fig. 6. Elongation of different subsections of bean first internode after their submersion for 3.5 h in IAA solutions of different concentrations. Numbers indicate position of individual 5 mm long subsections of the internode section starting from its apical (A) end.

ively to internode sections, *i.e.* 24-BR and IAA. The first peak was always recorded when equimolar quantities of the two regulators were applied (Fig. 3). The effect of 24-BR on enhancement of IAA-induced curvature of inversed sections is evident already 1 h after application of IAA and is gradually progressing for another 3 h (Fig. 4).

Factors Affecting Sensitivity of the Bioassay

It has been found that the following factors are responsible for the increased sensitivity of the sensitized bioassay: (1) High uptake of applied IAA by the young apical internode tissues; (2) polar transport of IAA from the site of its application to the zone of curvature; (3) accumulation of applied IAA in this zone, and (4) high sensitivity of the young apical tissue to auxin.

Inversion of internode sections significantly influenced the uptake and distribution of [2—^{14}C]-IAA applied to the lower part of the internode (Fig. 5). In the case of inversed sections 37.2 % of the label passed from the paper square into internode as compared with 18.1 % in controls. Different amounts of the uptaken label were found in the elongation zone: 30.7 % in inversed sections and only 3.3 % in controls, *i.e.* about 20 times more of applied label was accumulated in the curvature zone in inversed sections.

Sensitivity of subsections cut off the different parts of the internode sections to IAA was expressed by their alongation after submersion in IAA solutions of different concentrations (Fig. 6). Elongation of subsections derived from the morphologically apical part of the internode (0—15 mm, subsections 1, 2, 3) was stimulated by 10 to 1000 times lower IAA concentrations as compared with the middle part of the internode (subsections 4, 5, 6). Surprisingly the basal 10 mm part of the internode sections did not elongate after application of IAA concentrations tested.

Growth response of internode sections is significantly dependent on the

cultivar. Testing 8 different cultivars of bean plants we found that garden cultivars are in general more sensitive than the bush ones (results not shown). Selection of a sensitive cultivar is prerequisite for a sensitive bioassay.

DISCUSSION

Comparison of sensitivities of different bioassays for auxin (see NITSCH and NITSCH 1956, YOPP *et al.* 1981) with the sensitized bean first internode shows that this is nearly 1000 times more sensitive than the classical oat curvature bioassay of WENT (1928), considering the minimum amount of IAA required for positive growth response. It represents the most sensitive curvature test. Of the bioassays based on organ elongation only pea root section assay of AUDUS and TRESH (1953) is slightly more sensitive. Its specificity, however, has been questioned (NITSCH and NITSCH 1956). Broad linear dependence of the growth response on auxin concentration is an important advantage of the present bioassay. Comparison of the sensitivity of bean sensitized bioassay and the original test of MEUDT and BENNETT (1978) to various indole derivatives indicates that inversed sections are more sensitive to IAA and less sensitive to other auxins.

Inversion of bean first internode sections also increases sensitivity of the bioassay to brassinosteroids. For isolation of a new plant growth regulator, brassinolide, GROVE *et al.* (1979) used the bioassay based on elongation, swelling and splitting of bean second internode (MITCHELL and LIVINGSTONE 1968). Using this bioassay less than 2×10^{-11} mol of brassinolide can be detected (THOMPSON *et al.* 1981). Brassinolide, however, is 2—3 times more active than 24-BR which was used in our experiments (THOMPSON *et al.* 1982). Comparison of these data indicates that the bean second internode bioassay is about 10 times less sensitive to brassinosteroids than the sensitized one. This corresponds to the finding of KRIZEK and MANDAVA (1983) that the swelling and splitting responses require much higher concentrations of active component than to those of curvature. Rice lamina inclination bioassay seems to be the most sensitive requiring only 10^{-13} mol of brassinolide for positive growth response (WADA *et al.* 1984). When calculated on 24-BR basis it is about 2—3 times more sensitive than sensitized bean first internode bioassay. It requires, however, to handle the plant material under dim red light.

Increased sensitivity of the sensitized bean first internode bioassay is caused by the described factors which act simultaneously in the system. Their final effect is a result of their cooperative action on internode curvature. The experimental system involves conditions which are favourable and specific to auxin action (polar transport, sensitivity of young tissues) and which make the bioassay both sensitive and specific to IAA. Sensitivity of the assay to IAA is a condition for its sensitivity to brassinosteroids.

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