Some Aspects of the Control of Root Growth and Georeaction: The Involvement of Indoleacetic Acid and Abscisic Acid

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ABSTRACT

Apical segments of roots of *Zea mays* L. cv. Orla and cv. Anjou show a strong georeaction during 7 hours geostimulation. This is abolished by detipping the segments and restored by replacing the tips upon the apical cut surfaces. After exodiffusion of endogenous indoleacetic acid (IAA) the retipped segments showed a significantly lower geocurvature. Application of low concentrations of IAA to the basal cut surface of root segments from which endogenous IAA had not been allowed to exodiffuse increased the geocurvature of retipped Orla segments but decreased geocurvature of Anjou segments. At appropriate concentration basally applied IAA restored the georeaction capacity of root segments from which the endogenous auxin had exodiffused. The implications of the interaction between exogenous and endogenous IAA in the control of root georeaction are discussed with special reference to the normal role of endogenous IAA in the regulation of root georeaction and the variation in endogenous IAA content of roots of different cultivars of maize. The probability that the normal control of root growth and georeaction involves concomitant actions in the elongation zone of IAA moving preferentially in the acropetal direction and basipetally transported growth inhibitors (such as abscisic acid) produced in the cap cells is stressed.

Since Juniper *et al.* (12) completed their original microsurgical experiments, workers in several laboratories (24, 33, 37) have carried out intensive investigations of the hormonal regulation of maize root growth and geotropism. The role originally ascribed to IAA in the classical Cholodny-Went hypothesis (3, 31, 32, 35) and in refinements of it (5) has been filled by AbA (and possibly other inhibitory compounds) (1, 6, 7, 20–23, 34, 36, 37) and the function of IAA reconsidered. Typically exogenous IAA (7) and AbA (20–22, 26) inhibit growth of intact maize roots but under certain conditions IAA at very low concentrations may stimulate elongation (7, 18). Several experiments now indicate that root growth and geotropism are controlled by concomitant actions, in the cells of the elongation zone, of basipetally moving AbA (20–23) and acropetally moving IAA (1, 6, 7, 15, 19, 20, 23). In support of our contention that endogenous IAA plays an essential role in the regulation of root elongation, we have reported (27) that elongation of maize (cv. Orla) root apical segments which had had their endogenous IAA concentration reduced by an exodiffusion technique, was stimulated by IAA applied to their basal cut ends, whereas growth of untreated segments was inhibited by all tested concentrations of IAA. In the former experiments the endogenous IAA content of detipped apical root segments was reduced dramatically by allowing the segments to transport IAA acropetally

into blocks of buffered agar. The changes in endogenous IAA were monitored by a highly specific and sensitive spectrophotofluorimetric method (27).

There have been several reports of effects of exogenous auxin on root georeaction (1) but the results have tended to be confusing. High concentrations of IAA have been shown to inhibit geocurvature in parallel with elongation (13) while low concentrations enhanced curvature (11, 14). Other workers have claimed that IAA abolished (10) or reversed (4, 29, 30) root geotropic responses. This confusing situation might be explicable on the basis of differences in endogenous auxin content before treatment (9, 18, 28). It is known that the auxin content of roots changes with age (18) and our unpublished data suggest that it also varies between varieties.

The present paper reports the results of experiments designed to elucidate the role of endogenous IAA in regulation of georeaction of apical segments of seedling roots of maize cultivars Orla and Anjou.

MATERIALS AND METHODS

The technique for preparing the primary roots has been described previously (20, 23). *Zea mays* L. cv. Orla 264 (Assoc. Suisse des Sélectionneurs, Lausanne) and cv. Anjou 210 (Hodée, La Ménitrée, France) were used. When the primary roots—which were maintained in a vertical position—reached 15 ± 3 mm in length, they were selected for uniformity in green light (530 ± 20 nm; 1.2 w cm^{-2} at the manipulation point) (25) and only straight apical segments (10 ± 0.2 mm long) were cut for use. For the straight growth experiments segments were mounted vertically, with their apices down, in plastic frames for 8 ± 0.5 h with moist buffered (pH 6.1; 10 mM phosphate-citrate) (8) filter paper on their basal cut ends. IAA or AbA was applied on the base (in the buffered filter paper) or on the tip (in a 5-μl droplet of buffered solution) (26). For the IAA treatments experiments were repeated five times with 30 ± 5 segments for each assay. For the AbA treatments experiments were repeated three times with 50 ± 8 segments for each assay. The mean results are reported with their standard deviations. For the geocurvature experiments all segments were mounted vertically, with their apices down, for 4 h in plastic frames. Where appropriate the tips (0.5 mm) were removed and replaced before the commencement of this period. For the exodiffusion treatments the detipped segments were kept with their apical cut surfaces in contact with 1.5% Difco purified agar buffered at pH 6.1 with 10 mM phosphate-citrate (8) buffer. For some treatments the root tips were reattached after exodiffusion of IAA.

For the geocurvature period the segments were mounted horizontally in plastic frames with their basal cut surfaces in contact with filter paper moistened with 7 mM phosphate-citrate buffer,

pH 6.1 (8). Where appropriate the buffer solution contained IAA at 10^{-4}, 10^{-6}, or 10^{-8} M. The frames were placed in temperature-controlled boxes (22 ± 1 C) in which a humid atmosphere (90 ± 5%) was maintained. The segments were kept in the light (25) from a white fluorescent lamp (0.9˙ ± 0.06 J m^{-2} s^{-1}: Philips, Eindhoven). Downward curvature (in degrees) after 7 ± 0.5 h was recorded by means of shadow photographs. Experiments were repeated three times with 40 ± 5 segments for each assay. The mean results are given with their standard deviations; significant differences were determined by the t test.

RESULTS AND DISCUSSION

Figure 1A shows that after 7 h of geostimulation intact root apical segments of both Orla and Anjou cultivars showed a strong geocurvature. It is now evident that the root cap is the site of geoperception (1, 23, 33) and of production and release of growth inhibitors (24, 28, 37) which mediate the differential growth response. As expected, therefore, detipped segments (Fig. 1B) showed no significant georeaction unless the tip was replaced (Fig. 1C) on the apical cut end at the beginning of the period of geostimulation. In the latter case, however, the geocurvature was reduced in comparison with the control (Fig. 1A). After the endogenous IAA level in the segments had been reduced (27) by exodiffusion into buffered agar, the detipped segments (Fig. 1D) still showed no georeaction, while the geocurvature of the retipped segments (Fig. 1E) was significantly lower than that of the segments (Fig. 1C) which had been retipped without reduction of the IAA level by exodiffusion.

The results of this series of experiments were compatible with the view that the reduction of endogenous IAA by exodiffusion (27) caused a decrease in georeactivity. If this were the case the supply of an appropriate concentration of IAA at the basal cut surface would have been expected to restore (at least partially) the georesponse—just as it was possible to enhance the elongation growth of endogenous IAA-depleted segments of roots of cv. Orla by basally applied IAA (27). When IAA was supplied to the basal cut surface of retipped root segments (as Fig. 1C) from which IAA had not been allowed to exodiffuse; 10^{-8} M IAA caused a small but significant enhancement of geocurvature in apical segments of roots of cv. Orla but higher concentrations inhibited geocurvature (Fig. 2A). In contrast, for apical segments of roots of cv. Anjou no concentration of IAA enhanced geocurvature and both 10^{-6} and 10^{-4} M IAA strongly inhibited curvature (Fig. 2A). If root segments were retipped after 4 h exodiffusion of IAA into buffered agar (as Fig. 1E), there was a dramatic enhancement of geocurvature of segments of cv. Orla by basally applied 10^{-6} IAA (Fig. 2B). Again, the results with cv. Anjou were in sharp contrast in that 10^{-6} M applied IAA strongly inhibited geocurvature.

The different responses of the root segments of the two varieties are explicable in terms of our proposals (7, 23), when we recognize that the IAA dose/root growth response curve passes from suboptimal through optimal to supraoptimal concentrations (31, 32). The data imply that for cv. Orla the endogenous IAA concentration at the time of excision of the apical segments was slightly
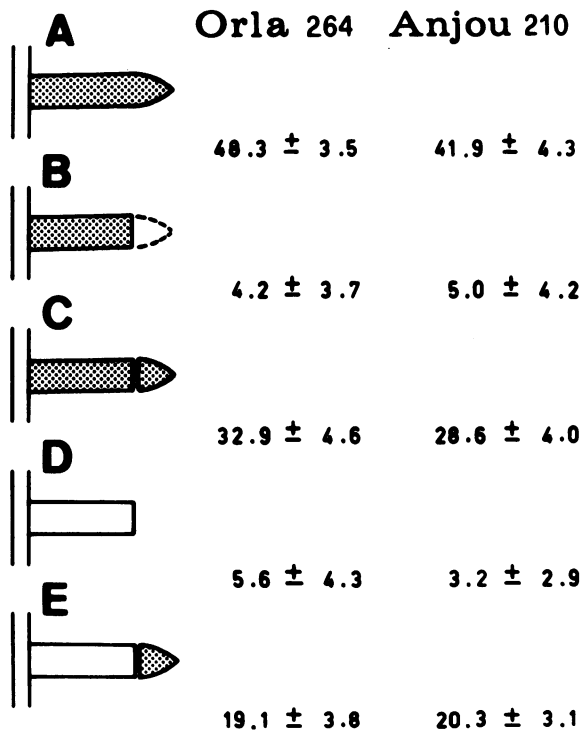

	Orla 264	Anjou 210
A		
	48.3 ± 3.5	41.9 ± 4.3
B		
	4.2 ± 3.7	5.0 ± 4.2
C		
	32.9 ± 4.6	28.6 ± 4.0
D		
	5.6 ± 4.3	3.2 ± 2.9
E		
	19.1 ± 3.8	20.3 ± 3.1

FIG. 1. Downward curvature (in degrees), after 7 ± 0.5 h of horizontally placed maize (cvs. Orla 264 and Anjou 210) apical root segments, with moist buffered (pH 6.1) filter paper on their basal cut ends. *Exodiffusion pretreatment.* Before the curvature assays, segments were kept vertical in darkness, either intact (segments type I: shaded) or decapitated (segments type II: not shaded) with their apical cut surfaces placed for 4 h on buffered (pH 6.1) agar. *Georeaction experiments.* (A), Intact segments (I). (B), Decapitated segments (I). (C), Decapitated segments (I) with their tips replaced on the apical cut ends. (D), Decapitated segments (II). (E), Stumps from segments (II) with the tips from segments (I) applied on the apical cut surfaces. Experiments repeated three times with 40 ± 5 segments for each assay and the mean results reported with their standard deviations. Segments not to scale.

	Orla 264	Anjou 210
A		
CONTROL	32.9 ± 4.6	28.6 ± 4.0
+ IAA		
10^{-8} M	44.8 ± 5.1	24.1 ± 4.7
10^{-6} M	29.0 ± 2.4	16.4 ± 3.5
10^{-4} M	22.4 ± 3.0	11.7 ± 2.3
B		
CONTROL	19.1 ± 3.8	20.3 ± 3.1
+ IAA		
10^{-8} M	28.4 ± 5.7	25.2 ± 4.2
10^{-6} M	39.0 ± 4.9	11.3 ± 2.8
10^{-4} M	17.2 ± 5.1	14.8 ± 3.3

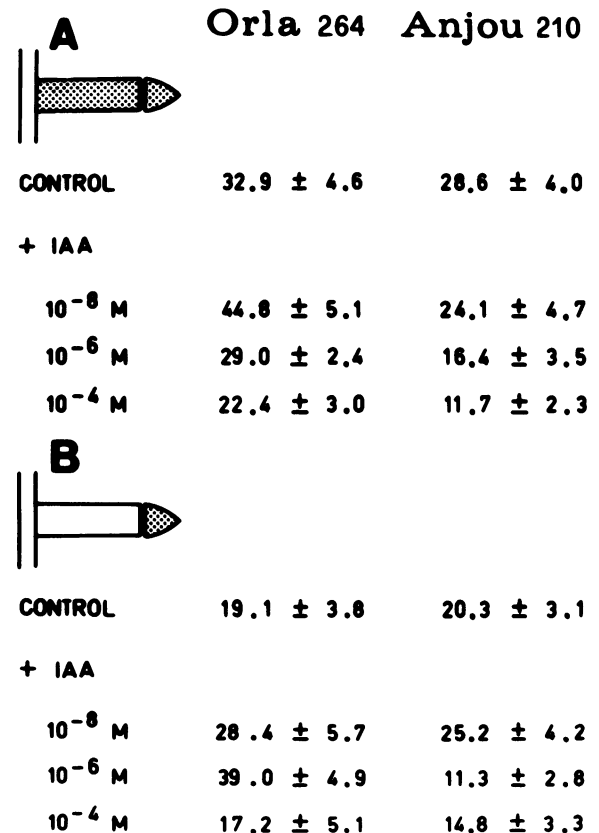FIG. 2. Downward curvature (in degrees) after 7 ± 0.5 h of horizontally placed maize (cvs. Orla 264 and Anjou 210) apical root segments with moist buffered (pH 6.1) filter paper containing or not containing IAA, on their basal cut ends. Experiments repeated three times with 40 ± 5 segments for each assay and the mean results reported with their standard deviations. Segments not to scale.

suboptimal, hence (Fig. 2A) basal application of 10^{-8} M IAA slightly enhanced geocurvature but higher concentrations reduced it. Table I shows that straight growth of freshly excised apical segments of roots of cv. Orla was stimulated by basally applied 10^{-8} M IAA but inhibited by higher concentrations. Exodiffusion of endogenous IAA reduced the IAA concentration in cv. Orla to a markedly suboptimal value (27) so that 10^{-8} M IAA enhanced geocurvature (Fig. 2) but 10^{-6} M IAA was required to produce the maximal enhancement and only the highest applied concentration (10^{-4} M) raised the endogenous IAA concentration to a supraoptimal value with a consequent decrease in geocurvature. For cv. Anjou on the other hand, the endogenous IAA concentration at the time of excision must have been supraoptimal (31, 32), so that all concentrations of basally applied IAA reduced geocurvature (Fig. 2A). Table I shows that all the concentrations of IAA tested (10^{-8}, 10^{-6}, and 10^{-4} M) inhibited straight growth of freshly excised apical segments of cv. Anjou when applied to the basal cut surface.

Table I. *Elongation of Vertically Placed Apical Root Segments of Maize cvs. Orla 264 and Anjou 210 in Response to Basally or Apically Applied IAA*

Length of segments were 10 ± 0.2 mm. IAA was applied in moist, buffered (pH 6) filter paper to cut basal ends of segments or in 5 μl droplets of buffer solution to the tips. Data are mean values with standard deviations for five experiments, each with 30 ± 5 segments for each treatment.

Treatments (8 ± 0.5 h)	Mean Increases in Length	
	cv. Orla 264	cv. Anjou 210
	mm	
Basal IAA application		
Control[a]	1.36 ± 0.14	1.21 ± 0.13
10^{-8} M	1.80 ± 0.16	0.88 ± 0.07
10^{-6} M	0.83 ± 0.06	0.60 ± 0.05
10^{-4} M	0.44 ± 0.05	0.23 ± 0.04
Apical IAA application		
Control[b]	1.25 ± 0.18	1.16 ± 0.14
10^{-8} M	1.34 ± 0.19	1.09 ± 0.15
10^{-6} M	1.12 ± 0.13	1.20 ± 0.15
10^{-4} M	0.87 ± 0.09	0.72 ± 0.07

[a] No IAA in the buffered filter paper applied on the basal cut end.
[b] No IAA in the buffered droplet applied on the tip.

Table II. *Elongation of Vertically Placed Apical Root Segments of Maize cvs. Orla 264 and Anjou 210 in Response to Basally and Apically Applied AbA*

Treatments with AbA are as with IAA in Table I. Data are mean values with standard deviations for three experiments with 50 ± 8 segments in each treatment.

Treatment (8 ± 0.5 h)	Mean Increases in Length	
	cv. Orla 264	cv. Anjou 210
	mm	
Basal AbA application		
Control[a]	1.28 ± 0.18	1.22 ± 0.11
10^{-7} M	1.07 ± 0.13	1.31 ± 0.16
10^{-5} M	1.29 ± 0.15	1.13 ± 0.12
Apical AbA application		
Control[b]	1.19 ± 0.13	1.10 ± 0.12
10^{-7} M	0.92 ± 0.10	0.86 ± 0.09
10^{-5} M	0.70 ± 0.08	0.56 ± 0.06

[a] No AbA in the buffered filter paper applied on the basal cut end.
[b] No AbA in the buffered droplet applied on the tip.

Four hours' exodiffusion of IAA reduced the concentration to a suboptimal value, so that basally applied 10^{-8} M IAA no longer inhibited geocurvature; but both 10^{-6} and 10^{-4} M IAA restored supraoptimal levels of IAA in the segments, and thus geocurvature was reduced. These conclusions are supported by our published data (27) about the stimulation of extension growth of segments of roots of cv. Orla and by the fact that, at all concentrations tested, IAA applied to the basal cut surfaces of apical root segments of cv. Anjou inhibited growth even after 4 h exodiffusion of the endogenous IAA. It has been shown (18) that the auxin content of *Lens culinaris* roots increased with age from an initially suboptimal level to a markedly supraoptimal value. Clearly, in addition to such changes in auxin content with age we must also note that the auxin content of roots of different cultivars of the same species at the same developmental stage may vary dramatically. It seems likely that apparently contradictory data of different workers using different cultivars of the same species can be explained on this basis.

The present paper provides further support for the contention that the regulation of root elongation and georeaction requires acropetally moving IAA (2, 7, 19, 20, 23). The possibility that there are two streams of endogenous auxin in the root (5)—one towards the tip in the stele (2, 15) and the other away from the tip in the cortex (16, 17)—must be considered. But it can be seen (Table I) that when IAA is applied to the tips of apical segments of roots of cvs. Orla and Anjou neither 10^{-8} nor 10^{-6} M IAA has an effect. This result one might anticipate because IAA transport through such segments is predominantly acropetal (1, 6, 7, 15, 19, 20, 22). Although apically applied 10^{-4} M IAA inhibits the growth of segments of roots of both cultivars, indicating that there is some movement of the compound back from the tip as previously observed (17, 17), it is noteworthy that basally applied 10^{-4} M IAA (Table I) causes far greater inhibition of growth. These observations are compatible with our view that IAA is not one of the inhibitors which move back to the elongation zone from the root cap (20, 21) and, by their asymmetrical distribution, cause the georeaction (22). Several other bioregulators are likely to be involved (6, 9, 28) including endogenous inhibitors such as AbA (20-22, 24, 26). In fact, Pilet and Rivier observed (unpublished) that the level of endogenous AbA in the lower halves of horizontally placed maize (cv. LG 11) roots increases significantly during geostimulation. In line with these results we note (Table II) that AbA applied to the tips of apical segments of both cvs. Orla and Anjou roots inhibited their elongation as a function of the concentration, while AbA applied to the cut basal ends of the segments had no effect, as expected, since it is transported basipetally (20, 24, 26). When AbA was applied asymmetrically to the tips of maize root segments the resulting curvatures were significantly enhanced by IAA supplied concomitantly to the bases (20, 21, 24).

The results reported here support the view that the control of root growth and georeaction in the elongation zone normally involves concomitant actions of acropetally moving IAA and basipetally moving growth inhibiting substances (such as AbA) derived from the root cap.

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