

## **Abscisic acid effect on the DNA microgradients of decapped maize roots**

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Longitudinal and transversal total DNA gradients were analysed in 42- $\mu$ m thin sections of maize root tips. The optimum DNA concentration was found in the apex (quiescent centre and meristem). It significantly increased after removal of the root cap, which can be considered an essential source of abscisic acid (ABA). When the caps were taken off and agar blocks containing ABA were immediately placed on the apical cut sections, the total DNA level in the root apex decreased greatly. ABA regulation of cellular and nuclear behaviour is discussed.

**Key words:** ABA — Apex — Cap — DNA — Maize — Meristem — Nuclear behaviour — Nucleic acid — Root.

Geosensitivity in roots is lost after removal of the cap (9, 17, 18, 27, 28), but is regained after a few hours, which is considered the georecovery time (17, 27, 31). It has been assumed that the georecovery time is directly related to the formation of a new root cap (2, 12, 17). Decapping does indeed stimulate the cells of the quiescent centre to divide to form a new cap (3, 13). However, it has been clearly demonstrated that georecovery precedes cap reformation by many hours (3, 4). Consequently, georecovery is not related to regeneration of the cap. During the ageotropic period of the decapped roots, some ultrastructural changes in the cells of the apex have been reported (5-7) and the formation of some amyloplasts has been observed (7). It is quite clear now that the cap imposes quiescence on cells at the pole of the root. As expected, removing the cap stimulates the cells of the quiescent centre and the meristem to grow and divide (3, 5). This was also clearly demonstrated by Clowes (11): he cut off the distal half of the cap and, 5 hr later, observed that the average rate of cell division had declined in the cap initials but increased in the quiescent centre and the apex cells.

On the other hand, the cap is the source of growth inhibitors (15, 29) which move basipetally (30, 41). For horizontally placed roots, downward transport of these inhibiting substances occurs inside the apex (33, 36) and their level is greater in the lower than the upper half of the cap (36, 37, 41, 47). These findings indicate clearly that the cap inhibitors control root geotropism (37, 45, 48, 49). One of these growth regulators could be abscisic acid (ABA), which strongly inhibits root elongation (23, 24, 26) and seems to have the same effects on root growth as the cap-inhibiting substances (32-34, 46). Kundu and Audus (19, 20) detected the presence of a cap inhibitor, the chromatographic properties of which were similar to those of

ABA. According to GC/MS data (40), the cap and the apex of maize roots (cv Kelvedon 33) contain 36.1 and 66.5  $\mu\text{g}$  ABA  $\text{kg}^{-1}$  fresh weight, respectively.

Although the mechanisms controlled by ABA are still uncertain (23), physiological and biochemical analyses have led to a general proposal. Its primary effect could be the inhibition of DNA biosynthesis (43). Using *Lemna* in sterile cultures, Stewart and Smith (42) reported that the incorporation of  $^3\text{H}$  thymidine into DNA was inhibited by 80 to 90% within 1 to 3 hr of ABA application. It is well established now (23) that ABA reduces the content of DNA, inhibiting its biodegradation. But the decrease in the DNA level has been suggested to be only a reflection of earlier events (1). Time scale and the complexity of the response make it difficult to believe that DNA synthesis is the primary target of ABA (23).

The present study first analysed the longitudinal and transversal distribution of total DNA in the tip of intact maize roots. Second, the effect of the cap removal on the DNA content was analysed in the same tissues after a given time. Third, as the cap cells may be the source of ABA, the effect of this regulator on the DNA level of decapped roots was examined.

### Materials and methods

The conditions for preparing maize roots and root segments have been previously described in detail (32, 35, 37). Selected caryopses of *Zea mays* L. cv Kelvedon 33 (Hurst Gunsons, London, UK) were soaked and germinated in darkness (22°C) and the roots were allowed to elongate vertically on moist paper towels. When the primary roots reached  $15 \pm 3$  mm, root segments of  $10 \pm 0.2$  mm were prepared and mounted vertically in plastic frames which were then placed in closed, humidified ( $90 \pm 5\%$ ) boxes ( $22 \pm 1^\circ\text{C}$ ) under white light ( $0.9 \pm 6 \times 10^{-2}$   $\text{J m}^{-2} \text{s}^{-1}$ ) from a fluorescent lamp (Osram L 13 W/25). At zero time, some of the segments were decapped: their apical ends were removed at  $450 \pm 50$   $\mu\text{m}$  from the tip (25). For some assays, the apical cut sections were covered with an agar block. The cylindrical agar block (diameter, 2.5 mm; thickness, 1 mm) contained ABA at  $10^{-6}$  M. Buffered (pH 6.1) agar (Difco purified) at 1.8% was used (33, 35). For each experiment, intact or decapped segments, with or without agar blocks containing ABA, were kept 6 hr in a vertical position.

The technique for preparing root inclusions and thin sections was previously described (21, 22). Segments were quickly embedded and frozen in liquid  $\text{N}_2$ . The inclusion medium employed was "Tissue Teck" (Ames Prod., Miles Seravac). Roots were then unmoulded and cut while frozen into thin sections (42  $\mu\text{m}$ ) by using a Bright Cryostat ( $-25^\circ\text{C}$ ).

Of the many techniques for estimating nucleic acids the most suitable is that in which DNA and RNA are separated by hydrolysis with dilute alkali (14). The microgradient of total DNA was tested according to the method based on the measurement of the desoxyribose formed (39) and quantitatively estimated using a diphenylamine reagent (8) at 600 nm (SP-1800 Unicam Spectrophotometer). For standard determination, suitable amounts of hydrolysed calf thymus DNA were used. In some experiments using larger root sections, total DNA was analysed by a similar technique adapted to that previously described for lentil root extracts (38).

## Results and discussion

In the first set of assays, the longitudinal gradient of total DNA was analysed in each 42- $\mu\text{m}$  transversal thin cut section. Fig. 1 gives the data for the tips of apical maize root segments kept 6 hr in a vertical position. The DNA distribution is related to the histological structure of the maize root end (16, 25). In the intact root segments (Fig. 1A), there was practically no DNA in the cap cells, while the apex (quiescent centre and meristem) contained the most DNA. The total DNA concentration was lower in the differentiated part of the tip, but increased with distance from the end of the root. Such a distribution, closely related to the division pattern

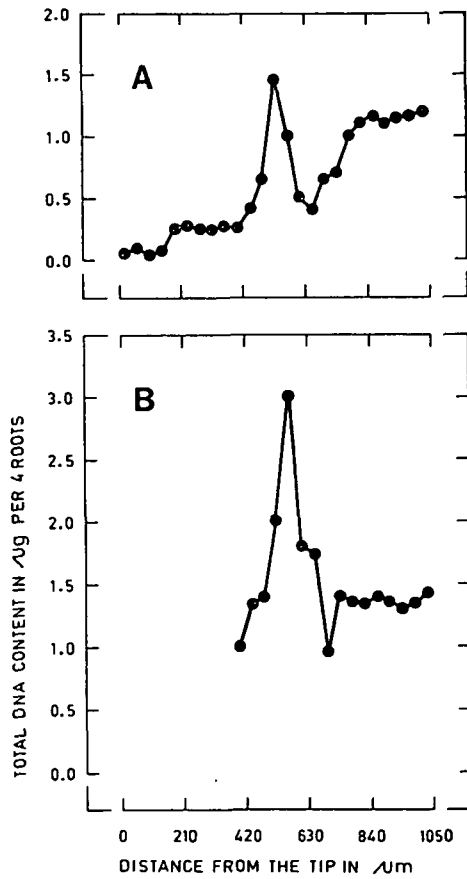


Fig. 1.

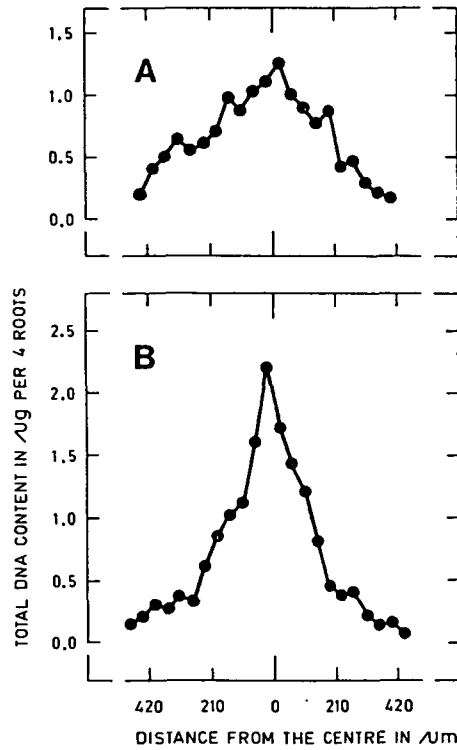


Fig. 2.

Fig. 1. Longitudinal gradient of total DNA for intact (A) and decapped (B) apical root segments of maize cv Kelvedon 33, kept 6 hr in a vertical position. Transversal sections of 42  $\mu\text{m}$  were analysed for DNA in  $\mu\text{g}$  per 4 roots from the tip (0  $\mu\text{m}$ ). For decapped segments, caps were removed at zero time.

Fig. 2. Transversal gradient of total DNA for intact (A) and decapped (B) apical root segments of maize cv Kelvedon 33, kept 6 hr in a vertical position. Longitudinal sections of 42  $\mu\text{m}$  were analyzed for DNA in  $\mu\text{g}$  per 4 roots (1050  $\mu\text{m}$  from the tip) from the centre. For decapped segments, caps were removed at zero time.

Table 1 *Total DNA content in intact and decapped root segments of maize cv Kelvedon 33*

Zone (in $\mu\text{m}$ )	Intact (I) segments	Decapped (D) segments	Stimulation (in %) <sup>a</sup>
0- 504	4.8	—	—
504- 672	23.0	52.5	128
672-1008	23.5	31.7	35

DNA (in  $\mu\text{g}$  per 100 thin sections of  $42 \mu\text{m}$  each) was analysed in three zones (counted from the tip) of apical segments kept 6 hr in a vertical position. For decapped segments, caps were removed (at  $450 \pm 50 \mu\text{m}$ ) at zero time.

<sup>a</sup> % =  $10^2 (D-I)/I$

of the cells, is very similar to that previously described (10, 11). When the root segments were decapped at zero time and their total DNA analysed 6 hr later (Fig. 1B), a similar distribution of DNA was found, together with a strongly enhanced nucleic acid level.

Using these histochemical data, the total DNA obtained for the thin sections was calculated for the three essential zones of the root tips. As can be seen in Table 1, when the cap was removed, the DNA content greatly increased. The relative values were significantly higher in the apex (the  $150\text{-}\mu\text{m}$  zone below the cap) than in the differentiated zones, as seen in Fig. 1.

In a second series of experiments, the transversal gradient of DNA was examined in  $42 \mu\text{m}$  longitudinal thin cut sections of the apical  $1050 \mu\text{m}$  segments. Fig. 2 gives the results for root segments kept 6 hr in a vertical position. In the intact segments (Fig. 2A), the highest level of DNA was found at the centre of the root. A similar distribution was obtained for decapped root segments (Fig. 2B), with the essential difference that the DNA concentration was much higher, as previously observed for the longitudinal gradient (see Table 1). Therefore, removal of the cap causes a significant increase in the DNA content of the apex cells.

From these data, we conclude that the cap forms some "regulators" which reduce the concentration of the total DNA and regulate aspects of cellular and nuclear behaviour. Barlow (5, 6) supposed the presence, in the root apex, of a gradient of auxins and cytokinins, the concentration of which maintains nuclear DNA synthesis in cells of the meristem and elsewhere in the apex. But, as discussed above, ABA could surely be considered as one of the growth inhibitors produced or released by the cap. And it seems clear now that ABA is transported basipetally from the cap to the apex and the elongating zone of the growing root. We also stated in the introduction that ABA may control the DNA biosynthesis.

Analyses of the DNA accumulation after decapping (from 0 to 6 hr) are now in progress. A possible correlation seems to exist to between the rate of formation of nucleic acids in the root apex and the reformation (not easy to test) of the new cap cells from the quiescent centre.

To analyse the possible role of ABA—as an endogenous regulator—on the DNA level of the apex cells, another experiment was done. Root caps were first removed and agar blocks, containing ABA or not, were immediately fixed on the apical cut sections. Decapped root segments were maintained vertically for 6 hr. The total

Table 2 Total DNA content in decapped root segments of maize cv Kelvedon 33 treated with ABA

ABA (in M)	DNA (in $\mu\text{g}/100$ mg FW)	Standard deviation	Inhibition (in %) <sup>a</sup>
$10^{-\infty}$ (C)	167	$\pm 25$	0
$10^{-8}$	140	$\pm 19$	16
$10^{-6}$	98	$\pm 20$	40
$10^{-4}$	63	$\pm 13$	62

DNA (in  $\mu\text{g}$  per 100 mg of fresh weight) was analysed in the  $1.5 \pm 0.1$  mm part below the apical end. For decapped segments, caps were removed (at  $450 \pm 50 \mu\text{m}$ ) at zero time. Agar blocks (with or without ABA) were immediately fixed on the apical cut sections. Measurement was made after the segments kept were for 6 hr in a vertical position.

<sup>a</sup> % =  $-10^2 (T-C)/C$  C: control T: treated

DNA was then analysed for the  $1.5 \pm 0.1$  mm region located just before the apical cut end. The results, reported in Table 2 indicate a significant decrease in the total DNA content: the lowest level of DNA was found with the highest ABA concentrations tested. The decapping causes major changes in the structure of the root apex (7, 11), and ABA also significantly changes the ultrastructure of the quiescent centre and the meristem of *Lepidium* roots (44).

Consequently, we can conclude that ABA, formed in the cap and moving in the basipetal direction from the root tip to the apex and then to the extending part of the elongating root, does decrease the total DNA. When the cap was removed, the concentration of endogenous ABA immediately decreased and that of total DNA greatly increased. This increasing accumulation of DNA must be related to the division of the quiescent centre and the meristematic cells. Consequently, these successive processes may induce the regeneration of the root cap.

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