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Effect of abscisic acid on the cell cycle in the growing maize root

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Abstract. The mechanism by which the rate of cell proliferation is regulated in different regions of the root apical meristem is unknown. The cell populations comprising the root cap and meristem cycle at different rates, proliferation being particularly slow in the quiescent centre. In an attempt to detect the control points in the cell cycle of the root apical meristem of *Zea mays* L. (cv. LG 11), quiescent-centre cells were stimulated to synthesise DNA and to enter mitosis either by decapping or by immersing intact roots in an aqueous 3,3-dimethyl-glutaric acid buffer solution. From microdensitometric and flow-cytometric data, we conclude that, upon immersion, the G_2 phase of the cell cycle of intact roots was shortened. However, when 50 µM abscisic acid (ABA) was added to the immersion buffer, parameters of the cell cycle were restored to those characteristic of intact roots held in a moist atmosphere. On the other hand, decapping of primary roots preferentially shortened the G_1 phase of the cell cycle in the quiescent centre. When supplied to decapped roots, ABA reversed this effect. Therefore, in our model, applied ABA retarded the completion of the cell cycle and acted upon the exit from either the G_1 or the G_2 phase. Immersion of roots in buffer alone seems to trigger cells to more rapid cycling and may do so by depleting the root of some ABA-like factor.

Key words: Abscisic acid – Cell cycle regulation – Flow cytometry – Quiescent centre – *Zea*

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Abbreviations: *ABA* = *cis*-abscisic acid; DGA = 3,3-dimethyl-glutaric acid; DAPI = 4',6-diamidino-2-phenylindole; LI = labelling index

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Introduction

Abscisic acid (ABA) is generally associated in plants with stomatal control (Outlaw et al. 1992), cell growth (Pilet 1975; Pilet and Chanson 1981; Audus 1983; Zeevaart and Creelman 1988) and root gravireaction (Audus 1975; Wilkins 1984; Feldman 1985; Pilet and Barlow 1987; Pilet 1991). Some authors have postulated that ABA also has a cell-cycle-regulating function because exogenously supplied ABA affects the synthesis of nucleic acids and proteins (Pilet and Nocera-Przybecka 1978; Barlow and Pilet 1984; Owen and Napier 1988). In *Lemna*, for example, it has been shown that ABA reduced incorporation of tritiated thymidine into nuclear DNA (Stewart and Smith 1972). In radish, cell growth and DNA synthesis were both affected following ABA treatment (Sarrouy-Balat et al. 1973) and similar effects were found in the maize root meristem (Barlow and Pilet 1984). In sunflower roots, drought stress, which elevates endogenous ABA, as well as exogenously supplied ABA, inhibited both DNA synthesis and mitosis (Robertson et al. 1990b). Considering that ABA could be one of the growth inhibitors synthesized in the cap of maize roots and basipetally transported through the meristem (Pilet 1975; Rivier et al. 1977; Pilet and Rivier 1980), evidence for direct or indirect effects of the hormone on the cell cycle would be of interest. However, our preliminary findings have indicated that roots continuously immersed in a 3,3-dimethyl-glutaric acid (DGA) buffer solution grew faster than roots maintained in moist atmosphere (data not shown). Because the enhanced elongation of the immersed roots could be due to the exodiffusion of some inhibiting factor such as ABA, and in order to determine if cell cycling was disturbed by the immersion treatment, we compared the distribution of nuclear DNA contents of roots held in moist atmosphere with those of roots immersed in DGA buffer with or without added ABA. In addition, ABA was supplied to roots held in moist atmosphere by means of donor cubes of fibre-glass saturated with the buffered ABA solution. Fibre-glass donors provide a contrast with immersion treatment and were

chosen because, unlike the immersion treatment, root elongation was not altered by the buffer solution when supplied by the donor cubes.

The aim of our study can be summarized as being an analysis of the indirect effect of ABA on the cell cycle in apical cells of primary maize roots submitted to conditions that simulate flooding (i.e. buffer immersion) and that involve decapping of the roots, the latter treatment providing a stimulation to the cell cycle in the quiescent centre (Barlow 1974). Microfluorimetry and flow cytometry were used to estimate the proportions of cells belonging to different nuclear DNA content classes and of cells in replication (S) phase. The quiescent-centre model which has been used to trace the rate of DNA synthesis under several conditions is described elsewhere (Müller et al. 1993). The present experiments are also aimed at elucidating the controls over cell proliferation that operate in this unique zone of the root.

Materials and methods

Caryopses of *Zea mays* L. (cv. LG 11, Association Suisse des Sélectionneurs, Lausanne, Switzerland) were soaked 16 h and germinated 48 h (19.5°C, darkness) according to Pilet (1977). Roots 15 ± 1 mm long were selected for subsequent treatments.

Treatments. Roots of intact seedlings were treated with 1 μM, 50 μM and 1 mM ABA made up in DGA buffer (3 mM, pH 6.2). In some experiments, tritiated thymidine ([methyl-^{3}H]thymidine, 925 GBq·mmol^{-1}, 2.4·10^4 Bq·mL^{-1}) was also supplied with the ABA. Treatments were performed with vertical roots either by directly immersing them into DGA buffer solution (± ABA, aerated by continuous air bubbling), or by means of cubes of fibre-glass (about 1 mm^3 in volume) imbibed with the buffered solution and applied to the root apex (Müller et al. 1993). The roots (between 50 and 200, depending on the experiments), treated with their respective solutions, were incubated for 8, 16 or 24 h (19.5°C, darkness, 99 ± 1% relative humidity). Then, they were carefully wiped dry and cut into segments 0–1, 1–5 and 5–10 mm from the tip. These segments represent, respectively, cap and meristem, elongating zone, and differentiating zone (Pilet et al. 1983). Their nuclei were isolated, fixed and used in static fluorimetry measurements. The meristem and elongation zone were analyzed together by flow cytometry. Quiescent centres were excised and squashed in order to follow DNA replication by autoradiography.

Isolation of nuclei. Isolated nuclei were obtained according to a method described by Galbraith (1984). All operations were carried out at 4°C. Root segments were transferred to 5-cm-diameter Petri dishes and sterile lysis buffer (45 mM $MgCl_2$, 30 mM sodium citrate, 20 mM Mops (3-(N-morpholino)propanesulfonic acid), 0.1% Triton X-100, pH 7.0 adjusted with NaOH) was added (1.7 mL per 0.1 g fresh weight). The segments were thoroughly chopped with a razor blade and the homogenate so obtained was filtered through a 75-μm nylon mesh. The nuclei were then gently pelleted at 35·g for 20 min and stained for 20 min with either 10 μg·mL^{-1} ethidium bromide or 1 μg·mL^{-1} 4′,6-diamidino-2-phenylindole (DAPI). Stained nuclei were filtered (20 μm nylon mesh) and analyzed for DNA content.

Decapping and excision of the quiescent centre. Roots were decapped and the quiescent centre of each was excised and prepared as described elsewhere (Müller et al. 1993). On decapped roots, the acroscopic face of the quiescent centre appeared as a dome surrounded by a collar of remaining cap tissue (Barlow and Hines 1982). Inserting a scalpel at the distal edge of this collar allowed the lens-shaped quiescent centre to be separated from the proximal meristem. Quiescent centres that had incorporated [methyl-^{3}H]thymidine for 8 or 16 h (925 GBq·mmol^{-1}, 2.4·10^4 Bq·ml^{-1}, made up in DGA buffer) were fixed in FPA (formalin:propionic acid:50% ethanol, 1:1:18, by vol.) for 2 h at room temperature. They were washed, hydrolyzed, stained with Schiff's reagent and squashed on subbed microscope slides (slides dipped into 0.5% gelatin and 0.05% chromic potassium sulfate in water and dried). After drying, the squashed quiescent centres were covered with autoradiographic emulsion (Ilford L4; Ilford, Sussex, UK). The films were exposed for 8 d at 4°C in darkness, then developed in Kodak D-19 (Kodak, Rochester, N.Y., USA), washed and fixed in 30% $Na_2S_2O_3$. The preparations were finally mounted in DPX (BDH, Poole, Dorset, UK).

Analyses. Analyses of the DNA content of DAPI-stained nuclei were performed by means of a Leitz MPV 3 episcopic microdensitometer equipped with a stabilized 100-W mercury lamp, a Ploempack with A_2 filter system (340–380/430 nm) and a 40 × immersion objective (Leitz GmbH, Wetzlar, Germany). For each measured nucleus, readings from an empty background of similar area was subtracted. Nuclei stained by the Feulgen reaction were analyzed with a Vickers M85 (Haxby, York, UK) scanning microdensitometer using monochromatic light at 565 ± 1 nm and a measuring spot of 0.65 μm. Prophases and telophases were used as a reference to establish the 4C and 2C DNA values respectively. Ethidium-bromide-stained nuclei were scanned with a FACScan flow cytometer (Becton Dickinson, Mountain View, Cal., USA).

The proportion of quiescent centre nuclei that incorporated [methyl-^{3}H]thymidine was expressed as a labelling index (LI = number of radioactive nuclei/total number of nuclei).

Results and discussion

Immersion. The modulation of the cell cycle was first studied in experiments involving quiescent-centre nuclei. These came from either intact or decapped roots maintained in a moist atmosphere or similarly treated roots which had been immersed in solutions either with or without ABA. A radioactive DNA precursor ([methyl-

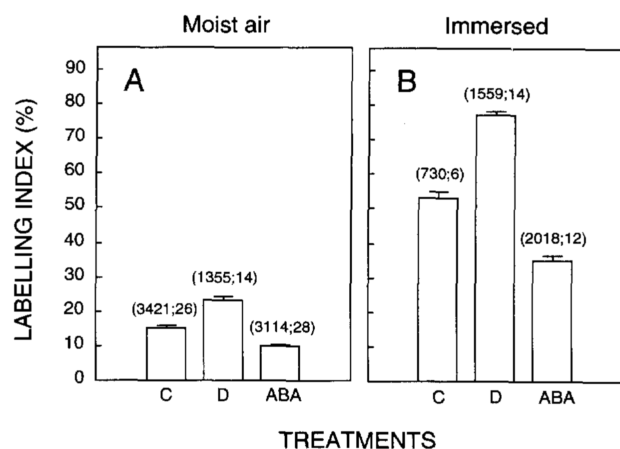

Fig. 1. Mean labelling indices (± SE) in quiescent centres of maize roots maintained for 16 h either in moist atmosphere (**A**) or immersed in buffer (**B**). *C*, control roots; *D*, decapped roots; *ABA*, decapped roots treated with ABA at 50 μM. [Methyl-^{3}H]thymidine and ABA were supplied either in the immersion buffer (**B**), or in fibre-glass cubes (**A**). The number of nuclei (x) and the number of samples (y) scored are given in parentheses (x;y). All pooled samples proved to be comparable as inferred by statistical analysis (χ^2 test, P = 0.05)

[^{3}H]thymidine, 925 GBq·mmol^{-1}, 2.4·10^4 Bq·mL^{-1}) was supplied to all roots for 16 h, and the nuclei were stained by the Feulgen reaction. (Feulgen staining was here more convenient than fluorochromes because the nuclei became visible for subsequent squashing.) Figure 1 shows the DNA replication activity in the quiescent centre of roots treated either by immersion in DGA buffer, or by means of fibre-glass cubes imbibed with the buffered treatment solution. Although it has been shown previously that [^{3}H]thymidine might disturb the cell cycle in some cases (activate it in quiescent-centre cells; De la Torre and Clowes 1974; Clowes 1975), this radioactive DNA precursor is commonly used by most authors (Cionini et al. 1985; Colombo 1987; Baíza et al. 1989). The amount applied in our experiments did not disturb root growth, and cells continued to cycle (Müller et al. 1993). Thus, [^{3}H]thymidine at 2.4·10^4 Bq·mL^{-1} can be used for comparative studies, provided a control is run under similar conditions. The LI values (continuous labelling) clearly show that upon immersion more quiescent-centre cells entered S phase during the 16 h of treatment than when roots were maintained in a moist atmosphere (Fig. 1). This may be interpreted as a shortening of the cell cycle. Our microdensitometry results (Fig. 2) show that, upon immersion, quiescent centres had significantly fewer nuclei in G_2 phase than when roots were incubated in a moist atmosphere (data subjected to χ^2 test, $P = 0.05$). Because the overall duration of the cell cycle is assumed to be shortened after immersion, the decreased proportion of G_2 nuclei suggests that the G_2 phase is shortened.

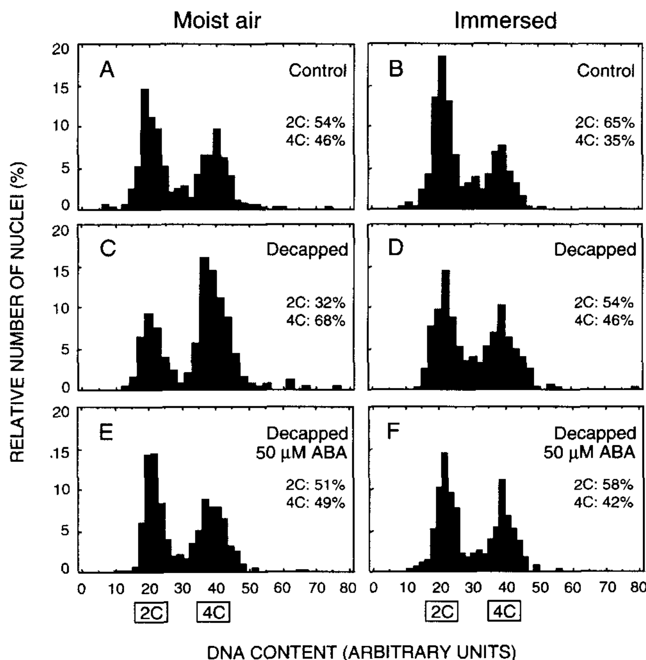

Fig. 2A–F. Frequency of Feulgen-stained quiescent-centre nuclei from maize roots as a function of their DNA content. Control roots (**A, B**), decapped roots (**C, D**) and roots treated with 50 µM ABA (**E, F**) were maintained for 16 h either immersed in buffer (**B, D, F**), or in moist atmosphere (**A, C, E**) (19.5°C, darkness, 99 ± 1% relative humidity). *cis*-Abscisic acid was supplied in the buffer solution or by means of fibre-glass cubes. The proportion of nuclei belonging to each DNA content class (2C and 4C) is reported for each treatment. Number of nuclei scored: 351 from three roots (**A**); 261 from three roots (**B**); 251 from three roots (**C**); 353 from four roots (**D**); 438 from three roots (**E**); and 354 from three roots (**F**). The root-to-root variation was statistically insignificant (χ^2 test, $P = 0.05$)

Decapping and ABA treatment. Some roots which provided quiescent centres for analysis were decapped prior to their incubation; others were decapped and treated with 50 µM ABA. Whatever the test conditions (roots held either immersed in solution or in moist atmosphere), the decapped roots showed a higher LI than the intact controls (Fig. 1). Thus, in decapped roots, more quiescent-centre nuclei entered the replication phase during the subsequent 16-h growth period than in intact ones, and hence, after decapping, as also during immersion treatment of intact roots, there was a general shortening of the cell cycle. This supports previous observations by Barlow and Pilet (1983, 1984) who reported that decapping accelerated the entry of nuclei into the DNA synthetic phase.

When decapped roots were treated with 50 µM ABA, the LI was maintained at, or below, its value in control roots, suggesting that ABA may have an inhibitory effect on cell-cycle progression (Fig. 1). When the DNA distributions for quiescent centres of intact (control) and decapped roots were compared, the proportion of G_2 nuclei was statistically significantly greater in the latter than in the former (Fig. 2A–D). Thus, decapping induces a shortening of G_1. This contrasts with immersion of the roots into buffer, which resulted in a shortening of G_2. If decapped roots were treated with ABA, their nuclear DNA distributions were similar to those of the controls (χ^2 test, $P = 0.05$). The comparison of the proportion of nuclei belonging to each class actually indicates that mainly the G_1 phase was lengthened in ABA-treated, decapped roots as compared with decapped roots without ABA, and this was so irrespective of whether ABA was supplied in the immersion buffer or by means of fibre-glass cubes (Fig. 2, compare the proportions of 2C nuclei in E and in C, and the proportion of 2C nuclei in F and in D), although the G_1 phase may be slightly shortened as compared to intact roots (compare Fig. 2A (54% 2C nuclei) with Fig. 2E (51% 2C nuclei) and Fig. 2B (65% 2C nuclei) with Fig. 2F (58% 2C nuclei)). It may therefore be concluded that ABA is able to restore the initial duration of the G_1 phase in roots where G_1 was shortened as a result of decapping and, hence, that ABA is able to retard the entry of nuclei into S phase as it may do when the cap is present.

Comparison of LI after 8 h and 16 h showed that DNA replication continued during the whole incubation period when roots are held in a moist atmosphere. Control roots had an LI of 10.0 ± 0.8% after 8 h and of 15.0 ± 0.7% after 16 h (decapped roots: $LI_{8h} = 13.9 \pm 0.8\%$, $LI_{16h} = 23.1 \pm 1.2\%$; ABA-treated, decapped roots: $LI_{8h} = 6.4 \pm 0.7\%$, $LI_{16h} = 10.0 \pm 0.5\%$). This was also the case in intact and decapped roots maintained in immersion culture. On the other hand, DNA replication was stopped in roots immersed in buffer containing 50 µM ABA ($LI_{8h} = 36.2 \pm 2.1\%$, $LI_{16h} = 35.1 \pm 1.2\%$), although the DNA-content histogram indi-

cates that after 16 h a few cells were still in S phase (cells between 2C and 4C; Fig. 2F). It is possible that in roots immersed in ABA at high concentration (50 μM), there was an exodiffusion of some inhibitor from the quiescent centre which resulted in an activation of the cell cycle (probably by shortening of G_2 phase). In contrast, applied ABA reached the quiescent centre and depressed or even blocked its cell cycle activity, the cells being arrested in the phases in which they were already engaged. Another possible explanation is that ABA regulated some step during G_1, all the cells beyond this step then complete S phase and arrest, as usual, in G_2. Finally, inhibition by endogenous ABA should not be neglected: after a certain incubation time, the cumulative effects of both endogenous and applied ABA may be limiting.

Concerning the somewhat puzzling high proportion of G_2 nuclei in all histograms presented (Fig. 2), a partial synchrony of the slow-cycling quiescent-centre cells may be suggested, since the synchrony observed in the quiescent centre after decapping (Barlow 1974) is also present as a general feature in intact newly germinated primary roots (data not shown).

Fluorimetry. In these experiments, the effects of ABA on the cell cycle were studied by comparing nuclei that came from several parts of whole roots immersed in solution. One micromolar ABA, added to the immersion buffer, clearly modified the distribution in the main ploidy classes of nuclei in the root apex (Fig. 3). After hormone treatment, a systematic enrichment of the higher ploidy class was observed in the meristematic zone (4C DNA; Fig. 3A, B) as well as in the growth zone (Fig. 3C, D) and differentiation zone (Fig. 3E, F) where 8C values accumulated. These observations suggested that a general feature of ABA could be to regulate the cell cycle by lengthening or arresting the G_2 phase. The tedious analysis by microfluorimetry allowed only small samples to be processed, and did not readily permit the replication phase to be studied. Subsequent analyses of larger samples were therefore performed by flow cytometry. Because we were interested primarily in the cells that were affected in their growth, we restricted our analysis to the most apical part of the root.

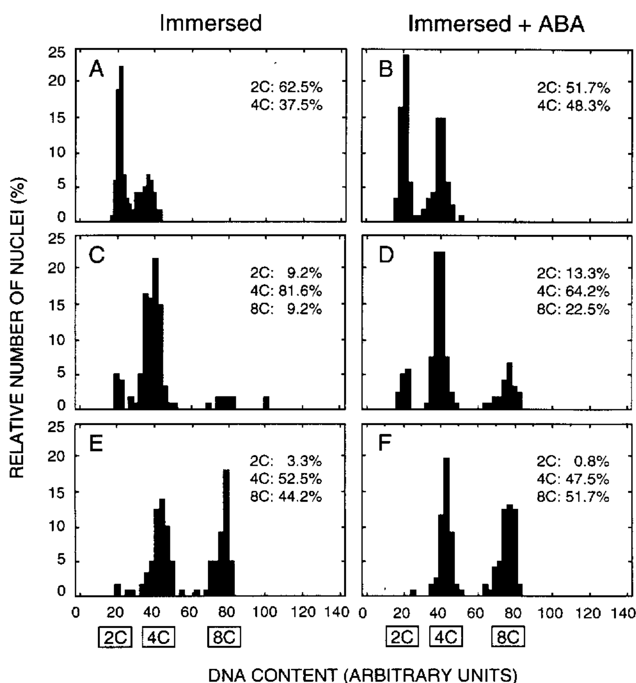

Fig. 3. Typical plot of the frequency of nuclei extracted from several maize root zones as a function of their DNA content (**A**, **B**: cap and meristem; **C**, **D**: elongating zone; **E**, **F**: differentiating zone). Fifty roots were immersed (**A**, **C**, **E**) or immersed and treated with 1 μM ABA (**B**, **D**, **F**) for 24 h (19.5°C, darkness). The DNA was stained with DAPI. After isolation, 120 nuclei were scored for each histogram. The proportion of nuclei belonging to the DNA classes 2C, 4C and 8C is also reported

Flow cytometry. Preliminary observations had indicated that continuously immersed roots were characterized by an increased growth rate in comparison with control roots grown in a moist atmosphere. Whereas control roots, during a 16-h period, elongated at a mean rate of 0.71 ± 0.14 mm·h^{-1}, which is in agreement with previous results (see Beffa and Pilet 1982; Versel and Mayor 1985; Pilet and Saugy 1985, 1987; Saugy et al. 1989), immersed roots reached 1.39 ± 0.14 mm·h^{-1}. One micromolar ABA in the buffered solution did not have any significant effect on the elongation rate (1.47 ± 0.14 mm·h^{-1}), but in 50 μM ABA the roots grew at 0.84 ± 0.38 mm·h^{-1}, a rate comparable to that of control roots grown in a moist atmosphere. When 1 mM ABA (a concentration assumed to be rather toxic) was supplied to the roots in immersion, they still grew at a mean rate of 0.50 ± 0.13 mm·h^{-1} during the 16-h test period.

Flow-cytometric analyses, performed on the cells that were principally affected in their growth (0–5 mm from the tip) confirmed the results obtained by static microfluorimetry (Fig. 4). The larger number of nuclei in the samples measured (10000 or even 40000 nuclei in each assay) allowed the proportion of cells in S phase to be estimated (arrows in Fig. 4). The hypodiploid nuclei that appeared at about 10 arbitrary units of DNA content are considered as artifactual and are therefore excluded from the percentage calculations. They represent nuclei broken during chopping of the roots, as can be deduced from analysis of their shape and size (data not shown). Ethidium bromide, rather than DAPI, had to be chosen as a stain for the flow-cytometry experiments (although DAPI is more specific for DNA) because the laser used allowed only ethidium bromide to be detected. Both stains however give comparable results.

A 16-h immersion in buffer without ABA resulted in a clear increase of the proportion of nuclei in replication phase, as compared with roots grown in a moist atmosphere (Fig. 4:2–4C and 4–8C nuclei), whereas the proportion of nuclei in the 4C and 8C classes were both reduced. One micromolar ABA in the immersion buffer had only a small effect on the proportion of nuclei present in several C classes in comparison with roots immersed in buffer alone. This might be due to the simultaneous analysis of meristematic and elongating cells. After ABA treatment, the proportion of nuclei in the 4C class increased in meristematic cells, whereas it decreased

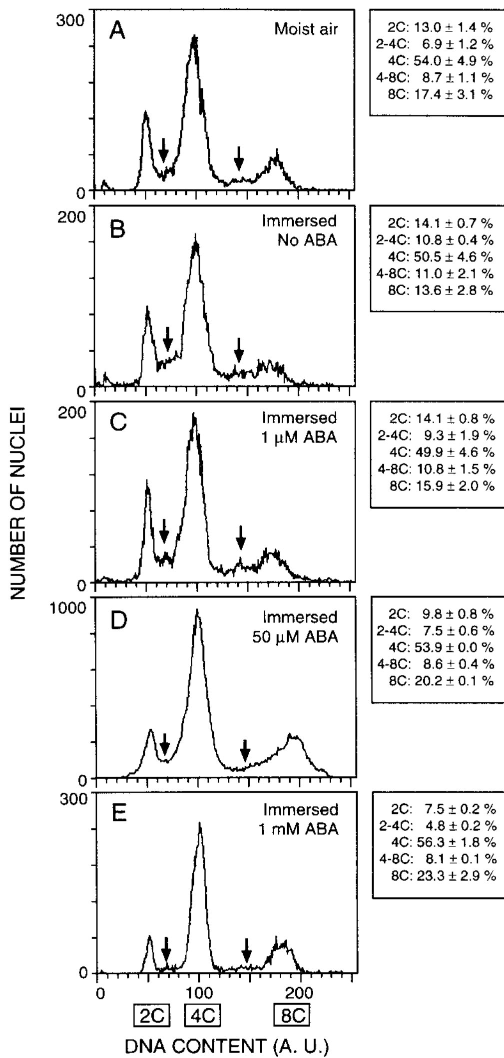

Fig. 4A–E. Flow-cytometric analysis of nuclei extracted from the apical 5-mm segment (cap, meristem and elongating zone) of primary roots of maize. Before being analysed, the roots were incubated for 16 h (19.5°C, darkness) in moist atmosphere (**A**), or in buffer containing 0 μM (**B**), 1 μM (**C**), 50 μM (**D**) or 1 mM (**E**) ABA. Ten thousand ethidium-bromide-stained nuclei were scored in each assay ($4 \cdot 10^4$ in **D**). *Arrows* emphasize nuclei in replication phases: 2–4C; 4–8C. The proportion of nuclei in each class was determined in between the apparent boundaries which separate G_1, G_2 and replicative nuclei, taken at the edge of the peaks of two independent traces. The experiment was performed twice

in elongating cells (Fig. 3). If both types were analyzed together, as in the flow-cytometry experiments, the loss of some classes in one zone made up for their gain in another zone and the proportion of nuclei in the 4C class therefore appeared constant in the combined populations. The same argument may apply to the 2C-class nuclei. Nevertheless, after treatment with 1 μM ABA, the flow-cytometry measurements showed that nuclear distributions tended towards the values observed in roots maintained in a moist atmosphere, especially if compared with roots immersed in buffer alone (Fig. 4B, C).

After treatment with 50 μM ABA, the proportion of nuclei with an 8C DNA content rose sharply to attain 20.2%. This occurred principally at the expense of the diploid G_1 nuclei, this 2C class being reduced to its lowest level overall. The proportion of S-phase nuclei, principally those of diploid cells (2–4C), was strongly decreased. The frequency of the 4C class, partly composed of diploid nuclei in G_2, the rest being tetraploid nuclei in G_1, was also modified by the treatment. At higher ABA concentration (1 mM) the hormone's effect on the cell cycle was amplified, resulting in very few cells in S phase (Fig. 4E) even though growth was not completely stopped as already mentioned.

The results obtained can be explained as follows. Consistent with the results obtained for quiescent centres, and assuming that a shortening of the cell cycle occurs as a general feature in all cells when roots are immersed, the large decrease of 8C nuclei after an immersion for 16 h suggests that the G_2 phase of the tetraploid (endoreduplication) cell cycle was preferentially reduced in these peculiar conditions. On the other hand, ABA treatment led to a sharp increase of the 8C-nuclei population, concomitant with a drastic reduction of the 2C population. This, together with the assumption that the cell cycle is lengthened by ABA treatment, accounts for the preferential lengthening of G_2, or for the arrest of some cells in G_2 phase. Both kinds of treatment (immersion and ABA supply) are therefore thought to act in opposite ways at this level of the cell cycle, namely by modulating G_2 phase. Moreover, intermediate 2–4C and 4–8C classes were also modified by the treatments in the expected way: if G_1 and/or G_2 was shortened, then the proportions of nuclei in the respective 2–4C and 4–8C classes should represent a larger part in relation to the whole. On the other hand, their proportions should be decreased if G_1 and/or G_2 was lengthened. This was effectively the case. One should however also consider that the spatial organization of meristem, elongating, and differentiating zones could have been altered in response to immersion and/or ABA treatment (as shown in sunflower roots in response to ABA or drought stress; Robertson et al. 1990a). If ABA treatment reduced the length of the meristematic zone, a shift towards the higher degree of polyploidy would be expected in the 0- to 1-mm root segment, although this would not account for the modified proportion of S-phase cells.

In quiescent-centre cells, as well as in meristematic and elongating cells, immersion in buffer seems to cause the shortening of phase G_2, most probably as a result of the exodiffusion of some inhibiting factor(s). Whether or not these cell-cycle inhibitors are of the growth-regulating type remains to be established, although there is some evidence from decapped roots for an involvement of growth regulators in the cell divisions preceding regeneration of a new cap (Barlow and Sargent 1978; Barlow and Pilet 1983). Whereas the shortening of G_2 as a consequence of immersion treatment seems to be a general feature in juvenile maize root cells, our results show that treatment with ABA reverses this effect in quiescent centres and most probably in all apical cells. Both kinds of response to ABA treatment (after decapping and during immersion) suggest that, in our system, ABA lengthens the cell cycle by acting preferentially on G_2, unlike the situation in sunflower roots where ABA treatment de-

pressed cell division, the cells coming to rest in G_1 (Robertson et al. 1990b).

Considering our findings, it might be asked if endogenous ABA is involved in the cell-cycle regulation in root apices. If the sharp stimulation of LI in quiescent-centre cells after the immersion of roots in buffer (shortening of the cell cycle), as well as the modified cell cycle in meristematic and elongating cells in similar conditions are due to the exodiffusion of some endogenous inhibiting factors, ABA could be a good candidate. According to our own observations and to GC-MS analyses by J.-M. Ribaut (Institute of Plant Biology and Physiology, University of Lausanne; personal communication), there is clear GC-MS evidence to show that under our conditions, the amount of endogenous ABA present in the apical 5 mm of the root declined after 1 h of immersion to 50% of its level in roots maintained in a moist atmosphere. When immersed roots were treated with ABA, a cell-cycle status similar to that of roots maintained in a moist atmosphere was restored, indicating that applied ABA may be active on the G_2 phase of the cell cycle.

The maize root cap, known as being a putative site of biosynthesis for one or several growth inhibitors (Pilet 1977, 1986; Feldman 1981; Wilkins 1984), could also be involved indirectly in the regulation of the cell cycle in apical tissues. Our results show that decapping induced an activation of the cell cycle in quiescent centre cells by shortening of the G_1 phase. Treatment of decapped roots by ABA restored DNA-content distributions close to those of intact roots, suggesting indirectly (i) that ABA could be one of the inhibitors coming from the cap (Pilet 1977), and (ii) that ABA could have a cell-cycle-inhibiting action also on the G_1 phase in quiescent-centre cells (see also Müller et al. 1993).

It was shown recently in tomato, that some genes are regulated by elevated levels of endogenous ABA during drought stress (Bray 1992). One of these genes (*le*20) is similar to the gene coding for histone H1. Because H1 histones are involved in the control of chromatin structure, an effect of ABA at the chromatin level, and as a regulator of the cell cycle, may be postulated.

In fact, even if the cell cycle is not directly regulated by ABA, the observed effects on both G_1 and G_2 phases (depending on whether the context is immersion or decapping) recall a type of regulation similar to that observed in "stationary phase" meristems by Van't Hof and Kovacs (1972). These authors postulated that the cell cycle was governed by two "principal control points", one in G_1 regulating the G_1/S transition, and another in G_2 controlling the G_2/M transition. Today, the existence of a universal control mechanism common to all eukaryotic cells and regulating entry into M and S phases is generally accepted (Nurse 1990). Central to this mechanism is a protein kinase, $p34^{cdc2}$, which forms a complex with cyclin in order to be active. Several components of such complexes have been isolated in yeast and in animals (Fosburg and Nurse 1991; Hartwell 1991) as well as in higher plants (Feiler and Jacobs 1990, 1991; Hemerly et al. 1992). A $p34^{cdc2}$-like protein has been shown to be a link in auxin-stimulated cell division (Gorst et al. 1991). It is not unlikely that an effect of ABA on the cell cycle could also be mediated more or less directly in the same way.

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References

Audus, L.J. (1975) Geotropism in roots. In: The development and function of roots, pp. 327–363, Torrey, J.G., Clarkson, D.T., eds. Academic Press, London

Audus, L.J. (1983) Abscisic acid in root growth and geotropism. In: Abscisic acid, pp. 421–477, Addicott, F.T., ed. Praeger, New York

Baíza, A.M., Vázquez-Ramos, J.M., Sánchez de Jiménez, E. (1989) DNA synthesis and cell division in embryonic maize tissues during germination. J. Plant Physiol. **135**, 416–421

Barlow, P.W. (1974) Regeneration of the cap of primary roots of *Zea mays*. New Phytol. **73**, 937–954

Barlow, P.W., Hines, E.R. (1982) Regeneration of the root cap of *Zea mays* L. and *Pisum sativum* L.: a study with the scanning electron microscope. Ann. Bot. **49**, 521–539

Barlow, P.W., Pilet, P.E. (1983) Mitotic activity in the maize root apex after freeze-decapping. Planta **157**, 286–288

Barlow, P.W., Pilet, P.E. (1984) The effect of abscisic acid on cell growth, cell division and DNA synthesis in the maize root meristem. Physiol. Plant. **62**, 125–132

Barlow, P.W., Sargent, J.A. (1978) The ultrastructure of the regenerating root cap of *Zea mays* L. Ann. Bot. **42**, 791–799

Beffa, R., Pilet, P.E. (1982) Elongation and gravireaction of intact and segmented roots: light effects. Physiol. Plant. **54**, 1–6

Bray, E.A. (1992) Regulation of gene expression by abscisic acid during drought stress. (Abstr.) Plant Physiol. **99** Suppl., 1

Cionini, P.G., Zolfino, C., Cavallini, A. (1985) Extra DNA synthesis in the differentiating cells of *Vicia faba* roots. Protoplasma **124**, 213–218

Clowes, F.A.L. (1975) The quiescent centre. In: The development and function of roots, pp. 3–19, Torrey, J.G., Clarkson, D.T., eds. Academic Press, London

Colombo, D. (1987) Root cell separation for a rapid and reliable determination of the labelling index. Protoplasma **137**, 63–64

De la Torre, C., Clowes, F.A.L. (1974) Thymidine and the measurement of rates of mitosis in meristems. New Phytol. **73**, 919–925

Feiler, H.S., Jacobs, T.W. (1990) Cell division in higher plants: a cdc2 gene, its 34-kDa product, and histone H1 kinase activity in pea. Proc. Natl. Acad. Sci. USA **89**, 5397–5401

Feiler, H.S., Jacobs, T.W. (1991) Cloning of the pea cdc2 homologue by efficient immunological screening of PCR products. Plant Mol. Biol. **7**, 321–333

Feldman, L.J. (1981) Root cap inhibitor formation in isolated root caps of *Zea mays*. J. Exp. Bot. **32**, 779–788

Feldman, L.J. (1985) Root gravitropism. Physiol. Plant. **65**, 341–344

Fosburg, S.L., Nurse, P. (1991) Cell cycle regulation in the yeast *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. Annu. Rev. Cell Biol. **7**, 227–256

Galbraith, D.W. (1984) Flow cytometric analysis of the cell cycle. In: Cell culture and somatic cell genetics of plants, vol. 1: Laboratory procedures and their applications, pp. 765–777, Vasil, I.K., ed. Academic Press, Orlando

Gorst, J.R., John, P.C.L., Sek, F.J. (1991) Levels of $p34^{cdc2}$-like protein in dividing, differentiating and dedifferentiating cells of carrot. Planta **185**, 304–310

Hartwell, L.H. (1991) Twenty-five years of cell cycle genetics. Genetics **129**, 975–980

Hemerly, A., Bergounioux, C., Van Montagu, M., Inzé, D., Ferreira, P. (1992) Genes regulating the plant cell cycle: Isolation of a mitotic-like cyclin from *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. USA **89**, 3295–3299

Müller, M.L., Pilet, P.E., Barlow, P.W. (1993) An excision and squash technique for analysis of the cell cycle in the root quiescent centre of maize. Physiol. Plant. **87**, 305–312
Nurse, P. (1990) Universal control mechanism regulating onset of M-phase. Nature **344**, 503–508
Outlaw, W.H. Jr., Hite, D.R.C., Zhang, S.Q. (1992) Molecular, cellular, and plant mechanisms of ABA control of stomatal aperture size. In: Progress in plant growth regulation, pp. 474–485, Karssen, C.M., Van Loon, L.C., Vreugdenhil, D., eds. Kluwer Academic Publishers, Dortrecht
Owen, J.H., Napier, J.A. (1988) Abscisic acid: new ideas on its role and mode of action. Plants Today **1**, 55–59
Pilet, P.E. (1975) Abscisic acid as a root growth inhibitor: physiological analyses. Planta **122**, 299–302
Pilet, P.E. (1977) Growth inhibitors in growing and geostimulated maize roots. In: Plant growth regulation, pp. 115–128, Pilet, P.E., ed. Springer, Berlin
Pilet, P.E. (1986) Importance of the cap in maize root growth. Planta **169**, 600–602
Pilet, P.E. (1991) Root growth and gravireaction. Implications of hormones and other regulators. In: Plant roots. The hidden half, pp. 179–204, Waisel, Y., Eshel, A., Kafkafi, U., eds. Marcel Dekker Inc., New York
Pilet, P.E., Barlow, P.W. (1987) The role of abscisic acid in root growth and gravireaction: a critical review. Plant Growth Regul. **6**, 217–265
Pilet, P.E., Chanson, A. (1981) Effect of abscisic acid on maize root growth. A critical examination. Plant Sci. Lett. **21**, 99–106
Pilet, P.E., Nocera-Przybecka, D. (1978) Abscisic acid effect on the DNA microgradients of decapped maize roots. Plant Cell Physiol. **19**, 1475–1481
Pilet, P.E., Rivier, L. (1980) Light and dark georeaction of maize roots: effect and endogenous level of abscisic acid. Plant Sci. Lett. **18**, 201–206
Pilet, P.E., Saugy, M. (1985) Effect of applied and endogenous IAA on maize root growth. Planta **164**, 254–258
Pilet, P.E., Saugy, M. (1987) Effect on root growth of endogenous and applied IAA and ABA. Plant Physiol. **83**, 33–38
Pilet, P.E., Versel, J.M., Mayor, G. (1983) Growth distribution and surface pH patterns along maize roots. Planta **158**, 398–402
Rivier, L., Milon, H., Pilet, P.E. (1977) Gas chromatography-mass spectrometric determinations of abscisic acid levels in the cap and the apex of maize roots. Planta **134**, 23–27
Robertson, J.M., Hubick, K.T., Yeung, E.C., Reid, D.M. (1990a) Developmental responses to drought and abscisic acid in sunflower roots. 1. Root growth, apical anatomy, and osmotic adjustment. J. Exp. Bot. **41**, 325–337
Robertson, J.M., Yeung, E.C., Reid, D.M., Hubick, K.T. (1990b) Developmental responses to drought and abscisic acid in sunflower roots. 2. Mitotic activity. J. Exp. Bot. **41**, 339–350
Sarrouy-Balat, H., Delseny, M., Julien, R. (1973) Plant hormones and DNA synthesis: evidence for a bacterial origin of rapidly labelled heavy satellite DNA. Plant Sci. Lett. **1**, 287–292
Saugy, M., Mayor, G., Pilet, P.E. (1989) Endogenous ABA in growing maize roots: light effects. Plant Physiol. **89**, 622–627
Stewart, G.R., Smith, H. (1972) Effects of abscisic acid on nucleic acid synthesis and the induction of nitrate reductase in *Lemna polyrhiza*. J. Exp. Bot. **23**, 875–885
Van't Hof, J., Kovacs, C.J. (1972) Mitotic cycle regulation in the meristem of cultured roots: the principal control point hypothesis. Adv. Exp. Med. Biol. **18**, 15–31
Versel, J.M., Mayor, G. (1985) Gradients in maize roots: local elongation and pH. Planta **164**, 96–100
Wilkins, M.B. (1984) Gravitropism. In: Advanced plant physiology, pp. 163–185, Wilkins, M.B., ed. Pitman, London
Zeevaart, J.A.D., Creelman, R.A. (1988) Metabolism and physiology of abscisic acid. Annu. Rev. Plant Physiol. Plant Mol. Biol. **39**, 439–473