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Effects of arbuscular mycorrhizal colonization and phosphorus application on nuclear ploidy in *Allium porrum* plants

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Abstract Arbuscular mycorrhizal (AM) colonization can strongly affect the plant cell nucleus, causing displacement from the periphery to the center of the cell, hypertrophy and polyploidization. The hypertrophy response has been shown in a variety of AM plants whilst polyploidization has been reported only in *Lycopersicon esculentum*, a multiploid species with a small genome. In order to determine whether polyploidization is a general plant response to AM colonization, analyses were performed on *Allium porrum*, a plant with a large genome, which is much less subject to polyploidization than *L. esculentum*. The ploidy status of leaves, complete root systems and four zones of the adventitious roots was investigated in relation to phosphorus content, AM colonization and root differentiation in *A. porrum* plants grown under two different regimes of phosphate nutrition in order to distinguish direct effects of the fungus from those of improved nutrition. Results showed the presence of two nuclear populations (2C and 4C) in all treatments and samples. Linear regression analyses suggested a general negative correlation between phosphorus content and the proportion of 2C nuclei. The percentage of 2C nuclei (and consequently that of 4C nuclei), was also influenced by AM colonization, differentiation and ageing of the root cells, which resulted in earlier occurrence, in time and space, of polyploid nuclei.

Keywords *Allium porrum* · Endopolyploidy · Arbuscular mycorrhizae · Roots · Phosphorus

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Introduction

Polyploidization is a common phenomenon in plants, especially in angiosperms (D'Amato 1998), where it may occur in any cell type with the exception of the gametes, the meristems and the guard cells. Only in some families, namely Compositae and Umbelliferae, does tissue differentiation occur in a diploid state. In most cases polyploidy results from DNA duplication without intervening chromosome condensation, segregation and cytokinesis (D'Amato 1998; Larkins et al. 2001). During differentiation, most cells expand and undergo a chromosome endoreduplication cycle. In onion roots, for example, only the cells of the meristems, procambium and pericycle are diploid, whilst in the cortex and central cylinder, cells are mainly tetraploid (D'Amato 1998). Endoreduplication is activated in response to developmental and environmental signals that control a large variety of processes, including cell differentiation, expansion and metabolism (Larkins et al. 2001; Gutierrez et al. 2002). Their constant tissue-specific patterns in different organs suggest that endoreduplication cycles constitute an essential part of the plant developmental programs that lead to differentiation and specialization of cells and tissues (Cebolla et al. 1999).

To date, the physiological significance of polyploidy is poorly understood (Kondorosi and Kondorosi 2004). According to some authors, increased ploidy levels may be a prerequisite for increase in cell size in certain tissues (Kondorosi et al. 2000; Mizukami 2001), but the precise correlation between these two parameters is not clear (Gendreau et al. 1999; Martin et al. 2001). Additionally, endoreduplication has been related to the metabolic activity of cells, to increased availability of nuclear templates, and to the amount and rate of RNA synthesis (D'Amato 1998). In fact, transcription and translation increase with each doubling of the genome, and endoreduplication increases the number of copies of "useful" genes more simply than by selective gene amplification (Galbraith et al. 1991). Moreover, there is evidence for the involvement of endoreduplication in the regulation of gene expression; in the rearrangement of the entire nuclear organization after

each replication event, genes can be either activated or repressed, as the structure of both active and repressed chromatin is disrupted (Wolffe 1991; Baluska et al. 1995). In addition, ploidy may control gene expression, inducing or repressing genes in relation to the number of chromosome sets, as has been shown in yeast and in transgenic maize (Mittelsten Scheid et al. 1996; Galitski et al. 1999).

In arbuscular mycorrhiza (AM), arbuscule formation is associated with extensive reorganization of the host cortical cells; the nucleus moves from the periphery of the cell to a central position, often among the arbuscule branches (Balestrini et al. 1992), and, in various AM systems, shows significant hypertrophy caused by chromatin decondensation related to increased transcription (Berta et al. 1996; Berta and Fusconi 1998). To date, an increase in the proportion of polyploid nuclei following AM colonization has been demonstrated only in *Lycopersicon esculentum* (a multiploid plant with small genome—about 2 pg DNA/diploid nucleus) colonized by *Glomus mosseae*, where a close correlation was identified between the presence of arbuscules and nuclei of the highest ploidy class, 8C (Berta et al. 2000).

In contrast, flow and static cytometry failed to demonstrate polyploidization in whole root systems of *Allium porrum* colonized by the AM fungus *Glomus* sp. strain E3 (from Rothamsted Experimental Station, Herts, UK). In this case, in both mycorrhizal and uncolonized plants, only a relatively low proportion of nuclei had doubled (4C) DNA content and there was no correlation with colonization (Berta et al.1990a). However, *A. porrum* is an auto-tetraploid species (Brewster 1994) and has a genome with a DNA content about 25 times that of *L. esculentum* (Bennett and Leitch 1995). Due to this large DNA content, *A. porrum* is therefore much less subject to polyploidization than *L. esculentum*, because polyploidization is especially frequent in cells of plants with small genomes (Galbraith et al. 1991). Nevertheless, the more branched pattern of mycorrhizal root systems of *A. porrum*, in comparison to uncolonized ones (Berta et al. 1990b), likely influences the mean age and the metabolic activity of root cells and, consequently, may also affect the ploidy status of the root systems. For this reason, we have more accurately analyzed ploidy levels in *A. porrum* by flow cytometry, in complete root systems and adventitious root segments taken at increasing distance from the apex. In order to discriminate mycorrhizal from nutritional effects on polyploidization, the ploidy level of nuclear populations was determined in non-mycorrhizal and mycorrhizal plants fed with differing rates of phosphate. The ploidy state and P content of the leaves were considered as reference values of an organ not invaded by fungal structures but showing increased growth at higher P concentrations (Torelli et al. 2000).

Materials and methods

Plant material

Seeds of leek, *A. porrum* L. cv. Mostruoso di Carentan, were surface-sterilized, rinsed with sterile water and sown in 0.5 l pots containing quartz sand (0.3–0.7 mm coarse grade). Some were inoculated at sowing with the AM fungus *G. mosseae* (Nicol and Gerd) Gerdemann and Trappe (BEG12). The inoculum, from Biorize (Dijon, France), consisting of AM fungal spores and colonized root fragments suspended in a quartz sand carrier, was given at the rate of 10% (v/v). Controls received an equal amount of the quartz sand carrier. A nutrient solution was applied to the sand to saturation, on alternate days ($MgSO_4$ 0.75 mM; $NaNO_3$ 1 mM; K_2SO_4 1 mM; $CaCl_2$ 2 mM + micronutrients). Phosphorus was added as NaH_2PO_4 at one of two concentrations: 3.2 µM (P I) and 96 µM (P II). The seedlings were maintained in a growth chamber with a 16/8 h photoperiod, 24°/20°C thermoperiod, and 150 µE m^{-2} s^{-1} irradiance at pot height.

Plants were harvested 8 weeks after sowing. Four treatments were thus established: control plants grown with the P I nutrient solution (C I), AM plants grown with the P I solution (M I), control plants grown with the P II nutrient solution (C II) and AM plants grown with the P II solution (M II).

Analysis of complete root systems and leaves

Biomass and mycorrhizal colonization

Biomass of leaves and roots was assessed for 15 plants per treatment, randomly chosen from each batch of the final harvest, and the percentage of colonized root length was evaluated microscopically after clarification in 10% KOH and staining with 1% methyl blue (Sigma, St. Louis, Mo.) in lactic acid, according to Trouvelot et al. (1986).

Phosphorus analysis

Entire root systems and leaves of five plants per treatment were analyzed for total P content according to Torelli et al. (2000). Briefly, milled samples of 20–40 mg were wet-digested in 50 ml Kjeldhal flasks in 1 ml 10 *N* H_2SO_4, and 10–20 drops 30% H_2O_2. After neutralization and addition of an ammonium molybdate-sulfuric acid solution, samples were boiled for 1 min with 100 mg ascorbic acid, and the optical density determined with a Pharmacia 3000 Ultrospec spectrophotometer (λ 660 nm; Pharmacia, Uppsala, Sweden); P content values were obtained from a 10–350 µg P calibration curve.

Flow cytometry of root and leaf nuclei

Nuclei were extracted from fresh root systems, without apices, and from leaves of five plants per treatment, chopped with a razor blade in extraction buffer (0.1 M citric acid, 0.5% Tween 20). Nuclei were extracted after 30 min incubation in this buffer by filtering through a 30 μm nylon mesh. The samples were diluted with 0.4 M dibasic phosphate to adjust suspensions to 2.5×10^5 nuclei ml^{-1} and nuclei were then stained with 5.6 μM DAPI (4′, 6-diamidino-2-phenil-indole; Sigma). Polyscience beads (4.2 μm; Polyscience, Warrington, Pa.) were used as a standard. A mercury arc lamp coupled with BG1, UG1 filters and a TK420 dichroic mirror was employed to select the exciting wavelength (UV) of a Partec PAS III flow cytometer. DAPI fluorescence was detected using a GG415 barrier filter.

Analysis of selected segments of adventitious roots

Segments of adventitious roots of complete root systems were cut from four zones measured from the apex: Z1, 0–1.5 cm; Z2, 1.5–4.5 cm; Z3, 4.5–7.5 cm; Z4, 7.5–10.5 cm.

Mycorrhizal colonization and alkaline phosphatase activity

The percentage of colonized root was determined for each adventitious root zone in five M I and M II plants, according to Trouvelot et al. (1986). Activity of the intraradical fungal structures was evaluated by cytochemical localization of alkaline phosphatase (ALP) activity, a fungal enzyme that is considered a useful marker for the symbiotic efficiency of AM in terms of phosphate nutrition (Tisserant et al. 1993; Fries et al. 1998). For this purpose, Fast Blue RR salt/methyl blue double staining was performed, to simultaneously observe ALP activity and the total intraradical fungus. About 30 root pieces, representing each zone of mycorrhizal roots, were digested in 1% cellulose plus 1% macerozyme R-10 Onozuka for 2 h and stained in 0.05 M Tris/citric acid buffer (pH 9.2), containing 1 mg ml^{-1} Fast Blue RRsalt, 1 mg ml^{-1} alpha-naphthyl acid phosphate, 0.5 mg ml^{-1}, $MgCl_2$ and 0.8 mg ml^{-1} $MnCl_2{\cdot}4H_2O$ (Tisserant et al. 1993). The same root pieces were then fixed for 3 h in 3.5% aqueous formaldehyde, rinsed, cleared with 10% KOH, and stained with lactic methyl blue, as previously reported. This double staining technique allowed discrimination of the active (stained black) and inactive (stained blue) mycelium.

Phosphorus analysis and flow cytometry

The P content and the percentages of the nuclear populations were analyzed in the four zones of adventitious roots: three groups of five plants were used for each experiment.

Statistical analysis

Mean values and standard errors were calculated and the data compared by analysis of variance (ANOVA), with $P<0.05$ as the significance cut-off. The data concerning fresh weights, percentage of 2C nuclei and root P content were plotted and linear regression was applied. To verify the relationship between ploidy and P content, linear regression analyses were calculated on plots of the percentage of 2C nuclei versus the P content. The coefficient of regression (r) was then calculated and its level of significance evaluated.

Results

Analysis of complete root systems and leaves

Both AM colonization and P nutrition caused an increase in leaf and root fresh weights. This increase was evident at the lower P level of nutrition (C I versus M I) in AM-colonized plants, in which leaf and root weights increased about 7.5 and almost 3.5 times, respectively, compared to controls. At the higher P level, differences between AM treatments were not significant (Table 1).

The degree of mycorrhizal colonization was 77.14±1.88 and 42.79±3.83 in M I and M II, respectively; hence it decreased by about 45% in plants grown at the higher P level.

The P content of complete root systems (formed by adventitious and lateral roots of each order), and of leaves, also increased with P application and/or AM colonization. Roots and leaves had similar P concentrations, except for M I plants, which showed significantly higher values in roots. Treatment C I showed a significantly lower percentage of P with respect to the other treatments (Fig. 1a). A significant correlation was found between P content and fresh weight by linear regression analysis (Table 2).

Two ploidy levels were found in all roots and leaves of *A. porrum* plants: we considered as 2C the nuclei with the lower ploidy level (with four sets of chromosomes) and 4C those with doubled genome. Rarely, a very small number of 8C nuclei was observed but, as their proportion was hardly detectable (lower than 0.5%), and not in all samples, they

Table 1 Mean leaf and root weights of non-mycorrhizal (*C*) and mycorrhizal (*M*) plants grown at low (*I*, 3.2 μM NaH_2PO_4) and high (*II*, 96 μM NaH_2PO_4) phosphorus (P) concentration. Values followed by different letters are significantly different in each column

Treatment	Leaf weight (g)	Root weight (g)
C I	0.147±0.011 a	0.203±0.023 a
M I	1.109±0.140 b	0.705±0.073 b
C II	1.792±0.101 c	1.333±0.110 c
M II	2.036±0.104 c	1.444±0.078 c

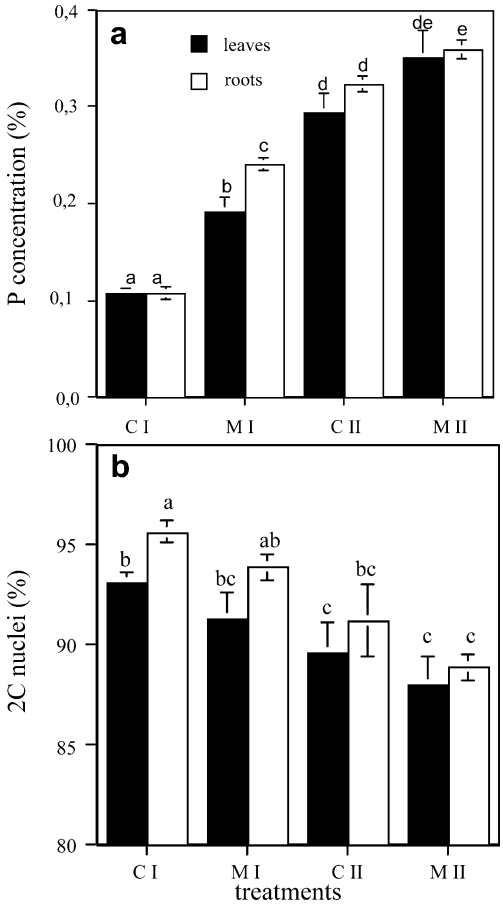

Fig. 1a, b Analysis of complete root systems and shoots of non-mycorrhizal and mycorrhizal *Allium porrum* plants. *C I*, *M I* Non-mycorrhizal and mycorrhizal plants, respectively, grown with 3.2 µM NaH_2PO_4; *C II*, *M II* non-mycorrhizal and mycorrhizal plants, respectively, grown with 96 µM NaH_2PO_4. Different letters indicate significantly different values in each figure. **a** Percentage of phosphorus (P) concentration. **b** Percentage of 2C nuclei; two main classes of ploidy were found, 2C and 4C, hence the percentage of 4C nuclei is complementary to that of 2C

were not considered in data analyses. The percentage of 2C nuclei of complete root systems (deprived of apices), and leaves decreased (consequently, the percentage of 4C nuclei must have increased correspondingly) in the following order: C I>M I>C II>M II; significant differences were detected between C I and C II or M II, and between M I and M II in the roots (Fig. 1b).

Linear regression analysis showed a significant correlation between the percentage of 2C nuclei and both fresh weight and P content in leaves and roots, with a high level of significance for the leaves (Table 2).

Analysis of selected segments of adventitious roots

Percentage mycorrhizal colonization was significantly higher in M I than in M II in all zones. It was lower in Z1 and increased significantly in Z2; thereafter it remained almost constant in M I, and increased more slowly in M II, where it was constant only from the third zone (Table 3). ALP activity—indicated by a black staining of the mycelium—was localized in the vacuoles of appressoria (Fig. 2a), intercellular hyphae (Fig. 2b), arbuscule branches (Fig. 2b) and some extraradical hyphae (Fig. 2c). The mean proportion of fungal tissue showing ALP activity was always high, with values ranging between 70 and 95% of the total intraradical mycelium in all zones and for all treatments (Table 3). This suggests that the extent of colonization within the different zones is likely to be closely related to efficient phosphate metabolism in the fungus. In young infection units, i.e., during the earliest phases of infection, a large proportion of hyphae were unstained and arbuscules were among the first structures showing ALP activity (Fig. 2d, e).

The P concentration in the selected root zones increased in mycorrhizal plants and, following P nutrition, in a manner similar to that of complete root systems (Fig. 3a). In each M I zone, P content was about twice that of the corresponding C I root zone. M I had a lower P content than that of the corresponding C II and M II zones, with significant differences being evident, except in the third zone. C II plants had a lower P content than M II plants in the different root zones, although these differences were significant only in the second zone. The percentage of P decreased from the apical to the basal zone of the root, with significant differences between the first and the second root zone in all treatments (Fig. 3a).

The percentage of 2C nuclei in the selected zones decreased in the same order as observed in complete root systems (C I>M I>C II>M II). In C I, the percentage of 2C nuclei decreased only in Z4, although no significant differences were found among the four zones, and in C II it decreased from Z3 onwards. In mycorrhizal plants, the percentage of 2C nuclei followed a rather different pattern: a significant decrease occurred between the first and the

Table 2 Linear regression analysis of root systems and leaves

x	y	Plant organ	Equation of the line	*r*	*P*
% 2C	Fresh weight	Leaves	y=−0.37x+34.99	0.975	0.0249
		Roots	y=−0.19x+18.28	0.965	0.0349
P content	Fresh weight	Leaves	y=7.71x−0.55	0.983	0.0165
		Roots	y=5.13x−0.40	0.987	0.0131
P content	% 2C	Leaves	y=−20.74x+95.42	0.984	0.0165
		Roots	y=−25.42x+99.00	0.952	0.0485

Table 3 Degrees of colonization and percentage of alkaline phosphatase (ALP) activity of adventitious roots at different distances from the root tip. Values followed by different letters in each group of data are significantly different

Root zone[f]	Degree of colonization (%)		Percentage of ALP activity	
	M I	M II	M I	M II
Z1	39.26±4.12 a	15.90±3.07 c	35.11±3.65 a	12.72±1.46 d
Z2	65.23±5.88 b	28.46±2.72 d	50.83±5.04 b	20.33±1.94 e
Z3	71.72±7.09 b	49.23±5.02 a	63.99±6.32 bc	38.77±3.95 a
Z4	71.98±7.51 b	51.37±5.21 a	68.38±7.13 c	46.96±4.76 ab

[f]*Z1* Apex–1.5 cm of root length, *Z2* 1.5–4.5 cm, *Z3* 4.5–7.5 cm, *Z4* 7.5–10.5 cm

second zone, with the percentage then remaining almost constant between the second and the third zone, and reducing further only in M II (Fig. 3b). The result of this different pattern is that the major differences between control and mycorrhizal plants grown at the same P level were found in Z2. Significant differences were not found between corresponding zones of C II and M I (Fig. 3b).

A significant correlation (>95%) existed between P content and percentage of 2C nuclei in all four zones, except in Z2, where the percentage of 2C nuclei in mycorrhizal roots was proportionally lower than that observed in controls (Fig. 4).

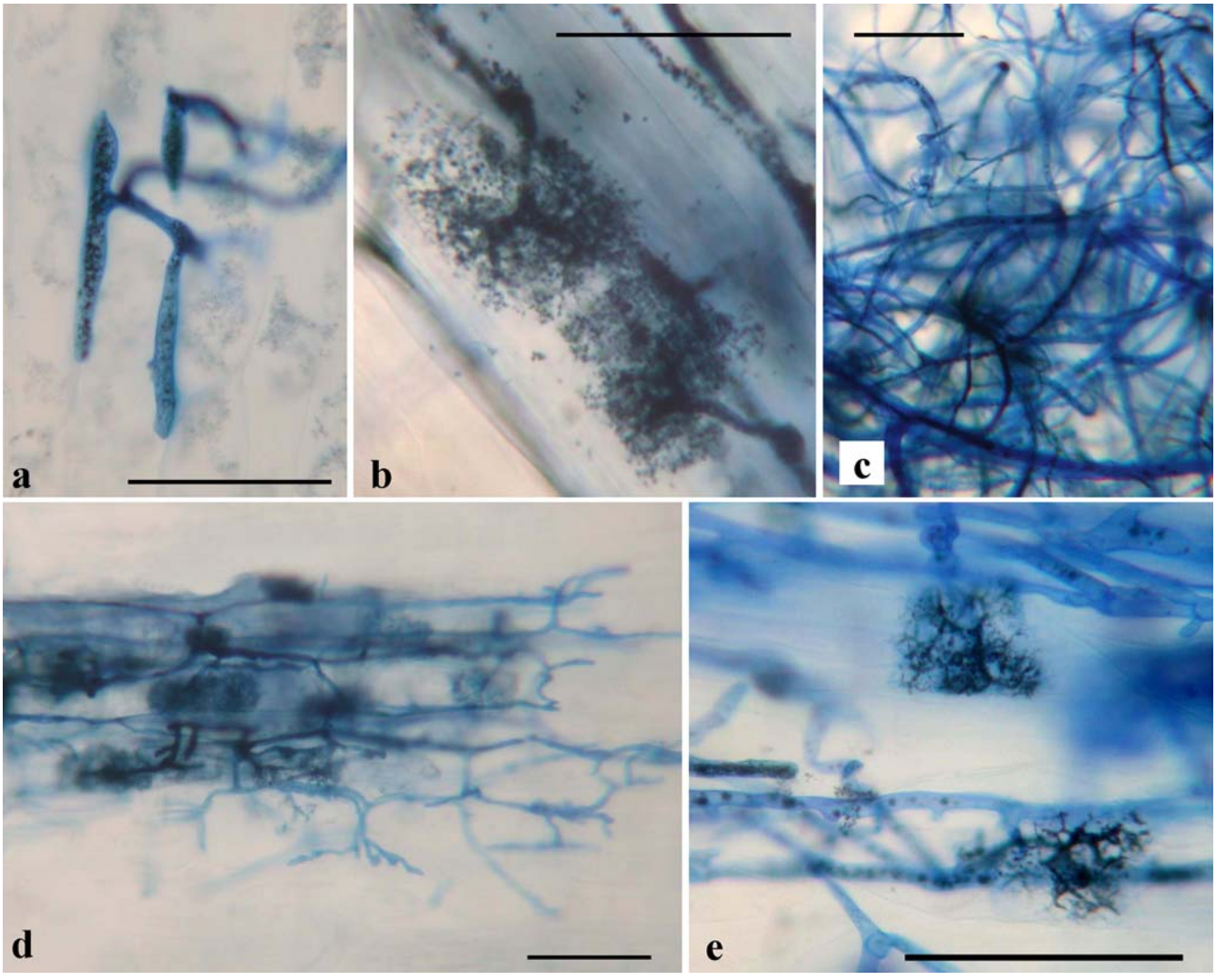

Fig. 2a–e Double staining to visualize total mycelium (*blue*) and fungal alkaline phosphatase (ALP) activity (*black*) in adventitious roots of *A. porrum*. **a** appressoria; **b** intercellular hyphae and arbuscule branches; **c** extraradical hyphae; **d**, **e** young infection unit —only some intercellular hyphae and the arbuscules are stained. *Bars* **a**, **c**, **d** 100 µm; **b**, **e** 50 µm

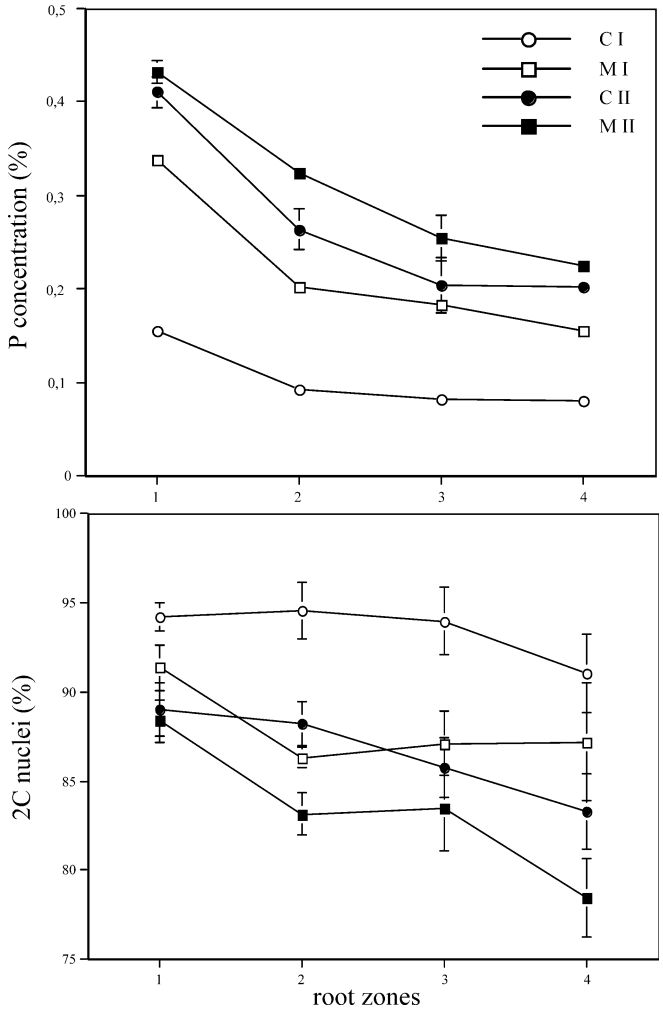

Fig. 3a, b Analysis of selected areas of adventitious roots. *A. porrum* plants, grown at low and high P concentration. *C I*, *M I*, *C II* and *M II* defined in the legend to Fig. 1. Root zones: *1* Apex–1.5 cm; *2* 1.5–4.5 cm; *3* 4.5–7.5 cm; *4* 7.5–10.5 cm. **a** Percentage of P concentration (error bars not visible when masked by symbols). **b** Percentage of 2C nuclei

Discussion

Our results concerning the effects of AM and P nutrition on fresh weights and P percentages of complete root systems and leaves are in accord with literature data (Amijee et al. 1989, Trotta et al. 1991, Torelli et al. 2000). In addition, AM colonization and P nutrition decreased the percentage of 2C nuclei, and consequently increased the percentage of 4C nuclei, in complete root systems and leaves of all treatments. However, 4C nuclei were always present in low numbers, in agreement with previous results from *A. porrum* + *Glomus* strain E3 (Berta et al. 1990a). This further supports the statement that polyploidy is less frequent in plants with large genomes (Galbraith et al. 1991). Variations in ploidy class frequency may be tentatively ascribed, as in other experimental systems, to variations in hormonal balance (Müller et al. 1994; Valente et al. 1998; Gendreau et al. 1999), which affect expression of key regulatory cell-cycle genes (Larkins et al. 2001). Both AM symbiosis and improved P nutrition have been shown to increase the concentration of cytokinins in roots (Barker and Tagu 2000; Torelli et al. 2000). In contrast, data on indole acetic acid are less consistent (Torelli et al. 2000; Shaul-Keinan et al. 2002) and those concerning gibberellins (Shaul-Keinan et al. 2002) and abscisic acid (Danneberg et al. 1992) are very scanty. So far, therefore, we cannot hypothesize a mechanism of hormonal regulation that could fully explain the observed decrease in the population of 2C nuclei.

In this work, the plant fresh weight and P content were negatively correlated with the percentage of 2C nuclei. However, the correlation between P content and ploidy distribution is stronger in leaves than in roots. In order to explain this result, it is necessary to consider that the root apparatus is a more complex and heterogeneous system than a leaf system. In *A. porrum*, the proportion of lateral roots increases with P nutrition and mycorrhization (Berta et al. 1990b; Trotta et al. 1991), and this could affect the mean age of the root cells, the P distribution, and the ploidy levels in the root apparatus.

In this context, the analysis of successive areas from the apex to the base of adventitious roots has proven helpful in elucidating the relationship between mycorrhization, P content and polyploidization, alongside the age of the cells, even if some limitations occur due to the influence of AM colonization and P nutrition on morphology, and rates of growth and root differentiation (Berta et al. 1990b; Trotta et al. 1991; Fusconi et al. 2000). In this study, P content increased in the order C I<M I<C II<M II, whilst it decreased in all treatments from the apical to the basal zone of the root, with significant differences between the first and the second root zone. These results are in agreement with the fact that plant roots, while extending in the soil, primarily take up P directly through apices, root hairs and epidermal cells, whilst older regions do not normally take up P, following the creation of a depletion zone around the root (Smith at al. 2003). Mycorrhizal plants, however, have an additional pathway by which P is obtained: P uptake via extraradical fungal hyphae. This latter pathway can provide the dominant route for plant P supply whilst direct uptake by the roots may be lost (Smith et al. 2003). One might thus expect a localized increase in P content in heavily AM-colonized root zones, compared to the corresponding zones in controls, and consequently a different distribution of P content along the root in the two treatments. This does not occur, and P analysis of consecutive zones from the apex to the base of the adventitious roots showed a similar pattern in both control and mycorrhizal roots. A possible explanation could exist in studies focused on the expression of root phosphate transporter genes, *LePT1* (Liu et al. 1998a; Rosewarne et al. 1999) and *MtPT1* (Liu et al 1998b; Chiou et al. 2001). The transcripts of these genes, which are detected in the epidermis and root hairs of phosphate-starved roots, are down-regulated in response to AM colonization (Liu et al 1998b; Rosewarne et al. 1999; Smith et al. 2003), resulting in a reduced function of the direct uptake pathway in AM roots and activation of the fungal-mediated pathway (Smith

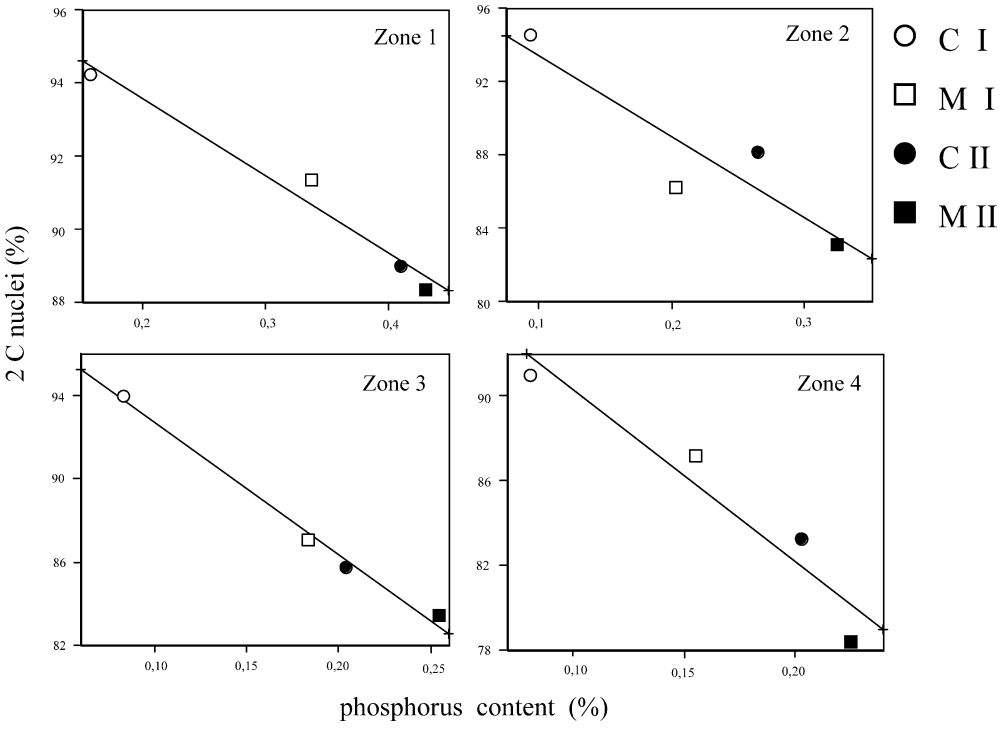

Fig. 4a–d Regression lines obtained from the percentage of 2C nuclei and of P content of all treatments (C I, M I, C II, M II) in each adventitious root zone (Z1, Z2, Z3, Z4, from the apex to the root base). **a** Z1, y=−20.98x +97.75, r=0.985, P=0.015; **b** Z2, y=−44.59x + 97.92, r=0.911, P=0.089; **c** Z3, y=−62.94x +98.97; r=0.996, P=0.004; **d** Z4, y=−80.19x + 98.28; r=0.956, P=0.043

et al. 2003). This switch in the pattern of P absorption could result in similar rates of P uptake by the plants, and hence in similar percentage of P in the root zones.

Analysis of ploidy levels in selected root zones revealed a general decrease in the percentage of 2C nuclei in corresponding zones of all treatments, with the same sequence observed in complete root systems (C I>M I>C II>M II). In addition, the percentage of 2C nuclei decreased from the apex to the base, both in mycorrhizal and control plants. This decrease along the adventitious roots, corresponding to an increase in the percentage of 4C nuclei, suggests a dependence of polyploidy on differentiation and ageing, in agreement with the observation that older tissues generally exhibit higher levels of multiploidy than younger tissues within the same plant (Galbraith et al. 1991; Kudo and Kimura 2001).

In the experimental system examined in this paper, the overlapping effects of P content and age of the cells, the gradients of which are oriented in opposite directions, promoted polyploidization—P content is probably involved in overall ploidy status, while differentiation and age mostly affect ploidy variations along the adventitious roots. The zone-by-zone comparison of all treatments by regression analysis of P and 2C percentages revealed a significant correlation between the two parameters, and hence a dependency of ploidy level on P status, except for the second zone, where the proportion of 2C is lower in mycorrhizal plants than in controls. In fact, our results showed a different pattern of distribution of the two ploidy classes along the adventitious roots: in controls, the percentage of 2C showed an almost linear pattern, whilst in mycorrhizal plants an abrupt decrease occurred between the first and the second zone both in M I and M II. This decrease is probably related to intensity of colonization, which showed lower values in the first root zone, and a strong increase in the second zone in both mycorrhizal treatments.

These findings suggest a direct involvement of AM colonization in the polyploidization phenomenon, which occurs closer to the apex (in younger tissues) in mycorrhizal roots. Moreover, this direct effect probably contributes to the poor correlation between P and percentage 2C nuclei observed in complete root systems. It is possible to propose hypotheses to explain this earlier occurrence of the endoreduplication process. Increases in ploidy levels have often been related to increases in cell size (see for example Melaragno et al. 1993; Cebolla et al. 1999; Kudo and Kimura 2002). In the study reported here, the cortical root cells of *A. porrum* varied in size according to the treatment. C I presented the narrowest and longest cells, with the minimum volumes, M I had intermediate size, C II and M II had the thickest and shortest cells, with the largest volumes, but there were no significant differences between the two (not shown). However, measures of cell size do not reflect the real size of arbuscule-containing cells. An uninfected cortical cell has a more or less cylindrical shape, with the cytoplasm forming a thin layer adjacent to the cell wall. Arbuscule formation induces extensive cytoplasm modifications: the vacuole decreases in size as the relative volume of the cytosol increases, cell organelles proliferate (Gianinazzi-Pearson 1996), and an extensive biogenesis of the host membrane occurs (Cox and Tinker 1976). It follows that an arbuscule-hosting cell will possess a higher "effective" volume than an uninfected cortical cell of the same size calculated simply on the basis of its cell wall dimensions. In addition, extensive rearrangement of the microtubular (MT) cytoskeleton occurs during arbuscule formation (Genre and Bonfante 1998; Blancaflor et al.

2001). MT cytoskeleton and nucleus are structurally and functionally interrelated (Baluska et al. 1997), and endoplasmic MTs are probably involved in the regulation of chromatin structure (Baluska et al. 1997) and in the activation of nuclear replication (Baluska et al. 1995).

We thus conclude that the increase in cytoplasmic volume and the rearrangement of the MT cytoskeleton, both of which occurring in cortical cells during arbuscule formation, are likely to be related to the precocious increase in ploidy observed in mycorrhizal roots in the second zone. However, other factors may be involved, among them, hormonal regulators produced by the fungus (see Barea and Azcon-Aguilar 1982; Esch et al. 1994) or other fungal signals involved in the symbiosis (Kosuta et al. 2003).

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