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Review of Plant Biotechnology and Applied Genetics

Involvement of polyamines in root development

Ivan Couée*, Irène Hummel, Cécile Sulmon, Gwenola Gouesbet & Abdelhak El Amrani

*Université de Rennes 1, Centre National de la Recherche Scientifique, UMR 6553 ECOBIO, Campus de Beaulieu, bâtiment 14A, F-35042 Rennes Cedex, France (*requests for offprints; Fax: 33-223235026; E-mail: Ivan.Couee@univ-rennes1.fr)*

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Abstract

Root development is under the control of hormonal, metabolic, and environmental cues that can act on genetically-controlled developmental programmes and thus affect the plasticity of root architecture. These processes involve not only the five 'classical' plant hormones, but also other growth regulators, such as polyamines. The present review emphasises the importance of polyamines in the different aspects of root development: primary root growth and lateral and adventitious root formation. Free (agmatine, putrescine, spermidine, spermine), conjugated (such as hydroxycinnamate conjugates) and macromolecule-bound polyamines are reported to be present in root systems. Modifications of their endogenous levels by inhibitor treatment, by mutation, by gene manipulation, or by exogenous treatment can have drastic effects on root development and subsequent architecture. These effects may be related to the involvement of polyamines in the control of cell division and differentiation, which plays an important role in the root apex and during lateral and adventitious root formation. The exact mechanisms of action remain to be elucidated, but accumulating evidence in plant and animal cells supports the idea that, besides biophysical effects on membranes and nucleic acids, polyamines interact with protein kinases and transcription factors and are thus involved in signal transduction pathways. The high flexibility of polyamine metabolism in response to environmental stress and the metabolic link between polyamine and ethylene synthesis strongly suggest that polyamines may play a role in environmentally-induced plasticity of root development. Moreover, polyamines may be implicated in the establishment of biotic interactions between roots and rhizospheric micro-organisms.

Abbreviations: ADC – arginine decarboxylase; DFMA – difluoromethylarginine; DFMO – difluoromethylornithine; DW – dry weight; ODC – ornithine decarboxylase

Introduction

Root systems show considerable variation of architecture among plant species, among genotypes within the same species, and among the different regions of the same individual (Lynch, 1995). This plasticity of root development probably represents an important adaptation of terrestrial plants in order to optimise utilisation of heterogeneous soil resources (Bell and Sultan, 1999). Development of root architecture mainly depends on

– the growth of root individual axes,

- the emergence of lateral roots from these root axes, and
- the direction of elongation (Lynch, 1995). Moreover, plants develop new roots from stems, petioles or leaves through adventitious processes.

The developmental stages of root growth and lateral and adventitious root formation result from the activation of various genetic programmes (Rost and Bryant, 1996). A number of developmental genes involved in root apical meristem activity and in lateral and adventitious root formation have thus been characterised (Smith and Fedoroff, 1995; Scheres et al.,

1996). It is also well established that root development is controlled by hormonal signals, especially auxins (Celenza et al., 1995), and that environmental cues can act on the genetic programmes of root development and on their hormonal and metabolic control (Zhang et al., 1999). In this complex network of interactions resulting in root developmental plasticity, other growth regulators than the five 'classical' plant hormones (Kende and Zeevart, 1997) may be involved (Mathesius et al., 1998). Thus, a large corpus of literature gives ample evidence that polyamines play an important role in primary, lateral and adventitious root development.

Polyamines are low molecular mass polycations that are found in all living organisms (Martin-Tanguy, 2001). They are generally considered to be growth regulators, and, in higher plants, they have been implicated in a range of developmental processes (Smith, 1985; Evans and Malmberg, 1989; Galston and Flores, 1991; Martin-Tanguy, 2001). Like in animals and in bacteria, polyamines could play a major role in proliferation and growth of plant cells, since application of exogenous polyamines stimulates development in several higher plants, suggesting that endogenous concentrations of these amines could be growth-limiting (Galston and Flores, 1991). As polyamine metabolism is responsive to external conditions, polyamines may play an important role in interactions between environment and development (Galston et al., 1997; Bouchereau et al., 1999).

In plants, the diamine putrescine is generally synthesized by two pathways (Figure 1). One pathway starts with ornithine to give putrescine via ornithine decarboxylase (ODC), the other produces agmatine via arginine decarboxylase (ADC) followed by additional steps to produce putrescine (Smith, 1980). A number of plant species have been shown to possess both enzyme systems. In other cases, such as that of *Arabidopsis thaliana*, ADC genes and enzymes have been clearly identified (Galloway et al., 1998), whereas evidence for ODC is lacking (Hanfrey et al., 2001). Putrescine conversion to spermidine and then to spermine (Figure 1) occurs by successive transfers of aminopropyl groups from decarboxylated S-adenosylmethionine (Martin-Tanguy and Carré, 1993). However, the dynamics of polyamine metabolism is complexified by the existence of degradation (Figure 1) and conjugation pathways and of transport and uptake mechanisms (Martin-Tanguy, 2001). Besides biophysical effects through their positive charge at physiological pH (Smith, 1985), polyam-

ines may be involved in signal transduction pathways, through effects on calcium fluxes (Thomas et al., 1993), and interactions with transcription factors (Wang et al., 1999), protein kinases (Datta et al., 1987), and 14-3-3 proteins (Athwal and Huber, 2002). Finally, polyamines have been shown to interact with phytohormones (Altman, 1989; Alabadi et al., 1996; Tonon et al., 2001).

The aim of this review is to assess the physiological importance of these correlations between polyamines and root development, and to present possible mechanisms of action of polyamines at the biochemical and molecular levels.

Identification and characterisation of polyamines in roots

Classical procedures for extracting and analysing polyamines (Flores and Galston, 1982; Smith and Davies, 1985) have identified in root tissues the presence of

- free polyamines, such as agmatine, putrescine, spermidine, and spermine,
- soluble conjugated polyamines, such as caffeineylputrescine and feruloylputrescine, and
- insoluble bound polyamines (Shen and Galston, 1985; Martin-Tanguy et al., 1990; Ben-Hayyim et al., 1994, 1996; Aribaud et al., 1995; Watson et al., 1998; Hennion and Martin-Tanguy, 2000; Tassoni et al., 2000; Hummel et al., 2002).

For instance, perchloric acid extraction from roots of *Arabidopsis thaliana* yields free polyamines and also conjugated or bound polyamines, in the perchloric-acid-soluble and perchloric-acid-insoluble fractions (Tassoni et al., 2000). Acetyl conjugates of polyamines have been reported in a very small number of higher plants. Thus, in the case of the subantarctic cruciferous species *Pringlea antiscorbutica*, Kerguelen cabbage, N1-acetylputrescine has been shown to exist in the roots (Hennion and Martin-Tanguy, 2000). Besides, polyamines can be the precursors of several hundred alkaloids (Martin-Tanguy, 2001), some of which can be associated with root development. For instance, in species of the Asteraceae producing pyrrolizidine alkaloids, the synthesis is exclusively restricted to roots, and seems to be correlated with root growth, as it is reduced or ceases when root growth stops (Ober and Hartmann, 1999). Some plant cells seem to have the ability to synthesize uncommon long-chain polyamines previously repor-

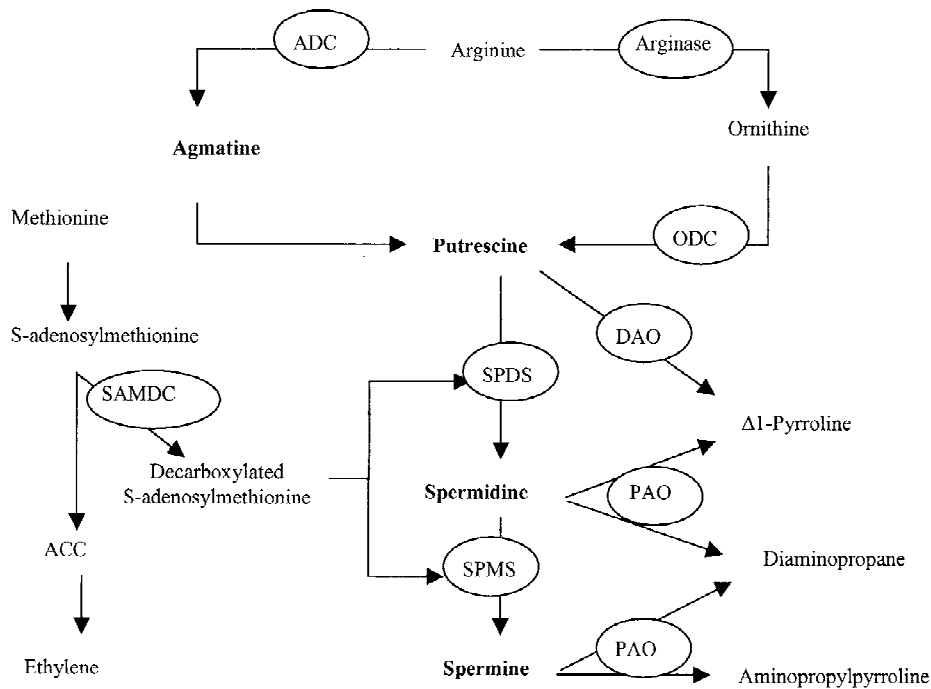


Figure 1. Pathways of polyamine metabolism and ethylene synthesis. Abbreviations: 1-aminocyclopropane-1-carboxylic acid (ACC), arginine decarboxylase (ADC), diamine oxidase (DAO), ornithine decarboxylase (ODC), polyamine oxidase (PAO), S-adenosylmethionine decarboxylase (SAMDC), spermidine synthase (SPDS), spermine synthase (SPMS).

ted only in thermophilic bacteria (Bouchereau et al., 1999). Thus, Roy and Ghosh (1996) reported the production of long-chained polyamines in rice callus under heat stress. Such long-chained polyamines do not appear to have been reported yet in root tissues. However, other unusual polyamines, such as 4-aminobutylcadaverine, have been described in root nodules of bean plants (Fujihara et al., 1995). Finally, polyamine-binding transglutaminase-like activity and macromolecule-bound polyamines have been identified in roots initiated from leaf explants (Aribaud et al., 1995).

Modifications of polyamine levels and root development

Studies in a number of plant species have shown that overall depletion of polyamine pools was linked with root growth inhibition. Indeed, decrease of polyamine level is often associated with ageing and senescence whereas accumulation, more precisely spermidine and spermine accumulation, is associated with growing tissue activity and organogenesis (Perez-Amador et al., 1995). For example, in *Phaseolus vulgaris*, deple-

tion of putrescine, spermidine, and spermine levels, resulting from decreased ADC activity, leads to decrease of root length (Palavan-Unsal, 1987). Polyamine accumulation has also been correlated to adventitious root formation (Friedman et al., 1982, 1985; Jarvis et al., 1983; Altamura et al., 1991, 1993).

Difluoromethylarginine (DFMA), a specific inhibitor of ADC (Burtin et al., 1989), has been shown to decrease root elongation in lateral roots in hairy root cultures (Biondi et al., 1993). Martin-Tanguy and Carré (1993) also showed that DFMA inhibited adventitious root growth from grapevine microcuttings. This probable involvement of the ADC pathway in root development was confirmed by the characterization of *spe* mutants of *Arabidopsis*, affected in ADC activity levels (Watson et al., 1998). Thus, *spe* double mutants exhibit highly kinked roots forming a tight cluster (Watson et al., 1998). High ADC activity has been found in roots of *Arabidopsis* (Watson et al., 1998; Hanfrey et al., 2001) and high ADC mRNA levels are present in roots of soybean seedlings (Nam et al., 1997). In *Arabidopsis*, one of the two ADC genes is strongly expressed in roots (Piotrowski et al., 2003), and one of the two ADC promoters, which show distinct patterns of activity (El Amrani

et al., 2002), is highly active in roots (Hummel I, Bourdais G, Gouesbet G, Couée I, Malmberg RL, El Amrani A, submitted article). In Kerguelen cabbage, modifications with inhibitors revealed a positive correlation between increase of endogenous agmatine and stimulation of root growth (Hummel et al., 2002). Thus, it could be hypothesized that the size of the agmatine pool might be a direct link between ADC activity and root development. Such a link would imply the direct involvement of agmatine in mechanisms of plant development, which requires further investigation. At present, only studies on animal cells point out to the existence of protein targets of agmatine, such as receptors or enzymes (Reis and Regunathan, 2000).

A positive correlation between spermidine or spermine contents and primary root growth is also generally found. Moreover, decrease of spermidine and spermine levels by cyclohexylammonium treatment, which inhibits spermidine synthase (Martin-Tanguy and Carré, 1993), has been shown to result in a decrease of lateral root development (Hummel et al., 2002). Since it is well established that primary root growth and lateral root formation involve intense mitotic activity and are dependent on an array of endogenous physiological factors (Burtin et al., 1990), the positive connection that is observed between spermidine and spermine contents and root development could be related to spermidine and spermine involvement in cell cycle activity, growth and differentiation (Fuller et al., 1977; Rupniak and Paul, 1978). Endogenous spermine content of less than $0.1 \mu\text{mol g}^{-1}$ DW in roots of untreated plantlets of Kerguelen cabbage was in the range of concentrations that gave large variations of root growth rate (Hummel et al., 2002). Finally, genes encoding spermine synthase and putative spermidine synthases have been shown to be strongly expressed in roots of *Arabidopsis* (Hanzawa et al., 2002). Spermidine and spermine may thus be good candidates for regulation of root development.

In some plant species, treatments leading to putrescine accumulation in seedling root suggest a negative connection, within a certain concentration range, between putrescine level in root and growth rate of primary root. Thus, in roots excised from plants of *Nicotiana tabacum*, difluoromethylornithine (DFMO), the specific inhibitor of ODC (Burtin et al., 1989), was found to cause an increase in root length in parallel with putrescine depletion, agmatine accumulation and no variation of spermidine pool (Ben-Hayyim et al., 1996). In these excised roots, DFMO caused a fundamental change in root architecture with increase

of primary root length. These changes were reversed when 1 mM putrescine was included in the DFMO treatment, thus indicating that effects of DFMO on development were indeed due to putrescine limitation. In this study, DFMO decreased free putrescine level in excised roots by a factor of 3 and exogenous putrescine prevented this effect whereas the spermidine titer remained unchanged (Ben-Hayyim et al., 1994). In other plant species, such as Kerguelen cabbage, large variations of the endogenous putrescine pool, from 2 to $12 \mu\text{mol.g}^{-1}$ DW, did not have any effect on root growth, but at concentrations lower than $2 \mu\text{mol g}^{-1}$ DW, decrease of the putrescine pool seemed to enhance primary root growth (Hummel et al., 2002). However, Lee (1997) had shown that exogenously-added putrescine in concentrations varying from 0.01 to 1 mM enhanced elongation in excised rice roots under *in vitro* conditions at 25 °C. In the same study, DFMO was found to inhibit root elongation and polyamine levels and these effects were reversed by putrescine. In contrast, spermidine inhibited root elongation at concentrations greater than 0.1 mM and spermine, at all concentrations used, inhibited elongation of roots grown at 25 °C (Lee, 1997). Similarly, Tarengi et al. (1995) showed that, in strawberry microcuttings, 1 mM putrescine treatment led to increase of putrescine levels, greater number of adventitious roots and increase of root length. Taken together, all of these studies emphasize important inter-species differences, which might also depend on culture conditions. In spite of these discrepancies, a number of unrelated dicot species, such as *Nicotiana tabacum*, *Phaseolus vulgaris*, *Vitis vinifera*, *Arabidopsis thaliana* and *Pringlea antiscorbutica* seem to share common features of polyamine involvement in root development.

In contrast to the effects of endogenous increase (Hummel et al., 2002), addition of 1 mM spermidine to culture media has been found to result in significant inhibition of root growth in *Arabidopsis* (Tassoni et al., 2000). Similarly, we have found that exogenous spermine significantly inhibited root growth of *Arabidopsis* (our unpublished data). Exogenous treatments with spermidine and spermine result in major increases respectively of spermidine and putrescine (Tassoni et al., 2000), and of spermine (our unpublished data), in root tissues. Thus, exogenous treatments would seem to give significantly different results from those of inhibitor-based modifications of endogenous levels. This difference may be ascribed to the range of concentrations that were used. Locke et al. (2000)

reported enhanced root growth of barley seedlings treated with 1 μ M putrescine, spermidine, or spermine. However, other mechanisms may be involved. Thus, uptake of exogenous polyamines and endogenous control of polyamine biosynthesis may not have the same developmental impact. Verification of such hypothesis would require further characterization of polyamine uptake systems in plant cells. Finally, the overall balance of the different free polyamines may be more important than a specific role of each polyamine. Thus, correlated changes of putrescine, spermine, spermidine are observed during seedling root development (Shen and Galston, 1985), and regulations induced by inhibitor treatments or mutations result in modifications of more than one free polyamine (Burtin et al., 1989; Ben-Hayyim et al., 1996; Watson et al., 1998; Hummel et al., 2002).

Besides direct effects, the relationship between polyamine metabolism, ethylene synthesis, and aminoacid metabolism may have an impact on root development. For instance, genetic modification of tryptophan decarboxylase and tyrosine decarboxylase pathways, which yield monoamines, can result in root-curling phenotypes, which have been ascribed to depletion of tryptophan pool (Guillet et al., 2000). Ethylene is known to be involved in root development (Ross and O'Neill, 2001), and treatment with methylglyoxal-*bis*-guanyldiazide, which inhibits (Bagni et al., 1981) the production of decarboxylated S-adenosylmethionine (Figure 1) and may thus provide more S-adenosylmethionine for ethylene production (Locke et al., 2000), has a strongly negative impact on root development in Kerguelen cabbage (Hummel et al., 2002) and *Arabidopsis* (Tassoni et al., 2000). In a similar way, Locke et al. (2000) examined the effects of inhibitors of the ethylene pathway on germination in terms of modifications of polyamine levels, and came to the conclusion that during barley germination polyamines played a complementary, growth-promotive role to ethylene. The relationship between polyamine and gibberellin actions, which has been described in more detail for floral development (Alabadi et al., 1996; Hanzawa et al., 2000), may also potentially be responsible for modifications of root development. Thus, Ben-Hayyim et al. (1996) showed that treatment with gibberellic acid could reverse the effects of DFMO on tobacco roots. In the *acaulis5* mutant of *Arabidopsis*, which is mutated in a gene encoding a spermine synthase, transcript levels of gibberellin-related genes are affected (Hanzawa et al., 2000). However, the *acaulis5*

mutant plants apparently show little morphological defects in other organs than stems and flowers (Hanzawa et al., 2000), although the *ACAULIS5* gene is strongly expressed in roots (Hanzawa et al., 2002).

The machinery for polyamine metabolism is generally present in the cells or tissues where their action takes place (Martin-Tanguy, 2001). However, despite scarcity of relevant studies, the possibility of long-distance transport, for instance in xylem, of polyamines must be considered (Bagni and Pistocchi, 1991). Furthermore, numerous polyamine-derived alkaloids are synthesised in roots and transported to shoots by the xylem (Martin-Tanguy, 2001). Further studies on such long-distance transport may open the possibility that polyamines or their derivatives play a role in the exchange of information between root and shoot development.

Control of cell division and differentiation in the root apex and during lateral and adventitious root formation

Longitudinal growth of root individual axes largely relies on the activity of root apical meristems, which involves active division and differentiation in meristem initials and active expansion and differentiation in the elongation zone (Scheres et al., 1996). Lateral root formation requires a succession of steps with induction of primordia showing high meristematic activity and then organisation of lateral root meristems (Malamy and Benfey, 1997). Adventitious root formation follows similar steps and share common mechanisms with lateral root formation (Lund et al., 1996; Ermel et al., 2000). Such importance of meristematic activity for root growth has been shown to depend on gene expression control. For instance, control of cyclin gene expression and therefore abundance of cyclin may function to allow flexible root growth control and induction of lateral roots (Doerner et al., 1996). Lateral root formation is initiated by auxin-mediated mitogenic activation, which lifts the G2 blockage of pericycle cells and launches a G2/M transition (Clark et al., 1999). The induction of lateral root formation is thus related to a specific step of the cell cycle.

Polyamines have been associated with cell proliferation in animal and plant cells (Theiss et al., 2002). Increased levels of spermidine and spermine have often been found to occur during the transition from G1 to the S phase of cell cycle in animals and plants (Fuller et al., 1977), and conversely, inhibition of

polyamine synthesis has been found to block the cell cycle (Rupniak and Paul, 1978). One can relate this involvement in G1/S transition with effects of polyamines on DNA configuration (Bouchereau et al., 1999). Other studies also point out to the involvement of polyamines in the control of G2/M transition (Fowler et al., 1996).

It is also generally accepted that major changes of polyamine levels are associated with the control of the balance between division and differentiation (Shen and Galston, 1985), and it has been suggested that the ODC pathway is active in dividing cells, whereas the ADC pathway predominates in mature tissue (Flores, 1991). At any rate, there is clear evidence from *in situ* studies that increased polyamine synthesis is associated with lateral root formation and meristematic activity (Schwartz et al., 1986). However, complete experimental characterisation of this differential role for ADC- and ODC-mediated pathways remains to be carried out (Granell and Carbonell, 2000), and plants that appear to lack ODC, such as *Arabidopsis*, necessarily rely on considerably distinct mechanisms or on different roles for ADC (Hanfrey et al., 2001). It must be pointed out however that DFMO treatment of *Arabidopsis* seedlings results in severe stunting of roots and of aerial organs (Hanfrey et al., 2001; our unpublished data).

Polyamine metabolism therefore shows plasticity in direct relationship with cellular processes of division and differentiation that are essential in root development. Indeed, in the roots of different species such as pea, tomato and maize, putrescine content increases as elongation progresses and is higher in differentiating zones, whereas spermine and spermidine are most abundant near apices (Shen and Galston, 1985). However, this would imply that other steps of polyamine metabolism than ADC and ODC must be differentially regulated during root growth and development. This remains to be verified experimentally. Moreover, involvement of polyamines in other essential aspects of root development such as columella or root hair development does not appear to have been studied yet.

Polyamines and environmental regulation of root development

Roots are often a primary target of environmental stresses, such as soil flooding or drought, which in turn have considerable impact on root development

(Vartanian et al., 1994). A lot of these responses to stress involve changes in the balance of phytohormones which are known to have effects on root development (Ross and O'Neill, 2001). The relationship between ethylene and polyamines (Locke et al., 2000) has been discussed above, and Lee et al. (1997) have reported the effect of abscisic acid on modulating the levels of polyamines.

On the other hand, independently of hormone-mediated effects, environmental stress has been shown to induce considerable increase or decrease of polyamine levels, depending on type of stress, plant species and time of stress application (Flores, 1991; Galston et al., 1997; Ali, 2000; Hennion and Martin-Tanguy, 2000). Moreover, in some cases, artificial modulations of polyamine contents have been shown to mimic external stimuli (Hennion and Martin-Tanguy, 2000). In a number of stresses or of stress-related situations, such as salinity (Chattopadhyay et al., 1997; Soyka and Heyer, 1999) or acid stress (Nam et al., 1997), which are known to affect root development, the response to stress involves elevated levels of ADC or ODC activity or mRNA. This may have a direct impact on root development for the reasons presented above. However, the relationship between polyamines and response to stress is more complex than mere induction of polyamine biosynthetic pathways, with involvement of all the different classes of polyamines and of biosynthetic and degradation pathways (Bouchereau et al., 1999). Thus, increase of the activities of biosynthetic enzymes may finally result in increase of free polyamines or conjugated polyamines, which are likely to have different impacts on root growth.

Situations of acute stress are generally associated with reduction of root growth, or changes of root developmental programmes (Vartanian et al., 1994). However, root development under sub-optimal conditions, short of situations of acute stress, has received little attention. For instance, little is known about root development under chilling temperatures, although they may have a strong impact on root growth rate. When cultivated at 10 °C, soybean (*Glycine max* cv Aldana) primary root growth is slowed down to 1 mm.d⁻¹, which corresponds to a 10-fold decrease relative to growth at 25 °C (Janas et al., 2000). In contrast, rate of root growth in Kerguelen cabbage showed a 2-fold decrease, relative to growth at 5–10 °C, when plants were cultivated at 25 °C (Hennion and Martin-Tanguy, 2000). This species is characterised by high levels of polyamines, especially agmatine (Hennion and Martin-Tanguy, 2000), and agmatine

levels show a drastic decrease upon heat treatment in parallel with termination of root growth (Hennion and Martin-Tanguy, 2000). This result and the fact that, as discussed above, root growth rate seems to be positively related to agmatine content (Hummel et al., 2002) strongly indicate that agmatine may be important not only in normal root growth, but also in sustaining root growth under chilling conditions.

Under such non-optimal or low-stress conditions where root growth is maintained, active changes of root architecture may participate in the response to environmental constraints. In heterogeneous soil, plasticity of root architecture could confer an advantage depending on resource distribution, including water, and edaphic constraints (Lynch, 1995). Ben-Hayyim et al. (1994) showed that, in *Nicotiana tabacum*, modifying polyamine contents by DFMO treatment could convert a root system consisting of short roots of similar length to one having a dominant tap root from which numerous lateral roots were formed. This increase of overall root length and of lateral roots, with potentially deeper foraging of water resource, can be considered as a potentially adaptive response to drought stress (Ben-Hayyim et al., 1994; Tepfer et al., 1994).

In the soil, root development and acquisition of root architecture as well as root functions greatly rely on interactions of root tissues with the surrounding biotic environment (O'Connell et al., 1996), especially micro-organisms. El Ghachtouli et al. (1996) showed that DFMO treatment, with reversion by exogenous putrescine, strongly inhibited root growth and arbuscular mycorrhizal infection of *Pisum sativum*. A possible role of polyamines in arbuscular mycorrhizal infection was therefore postulated. Similarly, ectomycorrhizal fungi were recently shown to promote root growth of scots pine through the potential involvement of the diamine cadaverine (Niemi et al., 2002).

Free polyamines, mainly putrescine, have been detected in root exudates of tomato (Kuiper et al., 2001) and *Arabidopsis* (our unpublished data). On the other hand, bacteria, including species that are known to be present in the soil, are known to possess a complex array of polyamine transport systems in the plasma membrane (Kuiper et al., 2001). It is therefore reasonable to assume that polyamines may be part of the exchanges between root tissues and surrounding bacteria in the rhizosphere (Fogel, 1985). Thus, increased uptake of putrescine has been shown to have a bacteriostatic effect on *Pseudomonas* sp. (Kuiper et al., 2001). At another level of involvement, inter-

action between plants and soil-borne *Agrobacterium rhizogenes* results in possible introduction of a root-inducing, left-hand transferred DNA in plant tissues (Tepfer, 1984), which carries genes that may profoundly modify root and shoot development. Polyamine metabolism in root cultures is altered by expression of *rol* genes from this left-hand transferred DNA (Altabella et al., 1995). For instance, over-expression of the *rolA* gene under the control of the 35S promoter increases growth and ramification of excised roots, and results in depletion of putrescine and putrescine conjugates (Ben-Hayyim et al., 1996).

Conclusion and perspectives

All of these relationships between polyamines and the different aspects of root development, i.e. meristem activity, elongation, differentiation, lateral and adventitious root formation, may be extremely useful for biotechnological purposes. Various protocols for modifying polyamine levels thus appear to promote rooting in thin layer explants, leaf explants, microcuttings, and shoots (Friedman et al., 1982, 1985; Jarvis et al., 1983; Burtin et al., 1990; Altamura et al., 1991; Hausman et al., 1994; Tonon et al., 2001). Such protocols may obviously be of great interest for applications to *in vitro* culture and micropropagation.

Roots are vital to plant growth and therefore crop productivity (Lynch, 1995). Studies of the impact of polyamines on root architecture may suggest novel improvement strategies, through marker-assisted genetic breeding or genetic transformation, in order to obtain cultivars with larger root development. Polyamine metabolism and regulation are complex, and involve a great number of genes. However, it has been shown that single-gene genetic transformation was sufficient to obtain important modifications of polyamine balance (Lepri et al., 2001; Thu-Hang et al., 2002). Cultivars with wider or deeper root systems may be useful for cultivation on land with poor water or mineral resources (Klepper, 1990). It may also be envisaged to utilise such cultivars for bioremediation of polluted land, if ever they are able not only to develop a wide root system under adverse conditions, but also to take up pollutants from surrounding soil. Polyamines have for instance been reported to enhance tolerance to xenobiotics such as herbicides (Zheleva et al., 1994). However, in the case of xenobiotics that use polyamine transport systems, as reported for paraquat in maize

(Hart et al., 1992), elevated polyamine levels may not be compatible with xenobiotic uptake.

The recent identification and characterisation of *Arabidopsis* mutants of polyamine biosynthesis pathways (Watson et al., 1998; Hanzawa et al., 2000) has confirmed the relationship between polyamine metabolism and developmental processes. However, further mutant screening and reverse genetics approaches will certainly yield in the near future more information on the involvement of genes related to polyamine synthesis and the roles of polyamines in root development. At present, only the two complementation groups of *spe* mutants (Watson et al., 1998) provide a link between polyamines and root development. The existence of families of polyamine-related genes with tissue-specific expression and potentially different functions (Galloway et al., 1998; Watson et al., 1998; El Amrani et al., 2002; Hanzawa et al., 2002) raises the possibility that specific genes in these families are involved in root development. However, other aspects of polyamine metabolism, such as degradation and conjugation pathways, mechanisms of polyamine action, such as signal transduction pathways, and mechanisms of polyamine uptake remain poorly characterised in plants. Characterisation of these processes will be essential to explain the classical view of polyamine involvement in cell division and differentiation, or to elucidate the links that may exist between polyamines and regulation of genes controlling root development.

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