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Over-expression of the oat arginine decarboxylase cDNA in transgenic rice (*Oryza sativa* L.) affects normal development patterns in vitro and results in putrescine accumulation in transgenic plants

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Abstract Transgenic rice (*Oryza sativa* L.) cell lines and plants expressing an oat arginine decarboxylase (*Adc*) cDNA under the control of the CaMV 35 S promoter were recovered using particle bombardment. Molecular analyses confirmed stable integration of the transgene and active transcription (mRNA). A four- to sevenfold increase in arginine decarboxylase (ADC) activity was observed in transformed plants compared to wild-type controls. Biochemical analysis of cellular polyamines (PAs) indicated up to fourfold increase in putrescine (Put) levels in transgenic callus and regenerated plants. This is the first report which demonstrates an increase in PA levels in plants engineered with the *Adc* gene. Implications of this increase are discussed in terms of development, physiology and nutrition. We observed a correlation between high levels of *Adc* gene expression and inability of callus tissue to develop normally into differentiated plants. This correlates well with reports in other species, in which perturbation of the PA pathway using genes involved in PA biosynthesis results in aberrant phenotypes. We have shown for the first time that PA biosynthesis can be manipulated in cereal species using genetic engineering.

Key words Transgenic plants · Development · *Oryza sativa* · Arginine decarboxylase · Polyamines

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Introduction

The polyamines (PAs) spermidine (Spd) and spermine (Spm) and their diamine precursor putrescine (Put) are found ubiquitously in the plant kingdom (Smith 1985). They occur either in their free (positively charged) form or bound as conjugates with hydroxycinnamic acids. The positively charged PAs have been implicated in a number of different biological processes and also in many physiological functions (Evans and Malmberg 1989). Examples include: regulation of light-induced growth response, somatic embryogenesis, organogenesis, fruit development, pollen formation, flower development, senescence and response to physical and biological stresses (Cohen et al. 1983; Evans and Malmberg 1989; Galston 1989; Minocha 1988; Pegg 1986; Rastogi and Kaur-Sawhney 1990; Slocum and Flores 1991; Slocum et al. 1984).

The key enzymes in PA biosynthesis are arginine decarboxylase (ADC; EC 4.1.1.19) and ornithine decarboxylase (ODC; EC 4.1.1.17). ADC catalyses the decarboxylation of L-arginine (arg) to the diamine Put, via agmatine (agm) and *N*-carbamoylputrescine (NCPut). This is one of two main pathways of Put synthesis in plants and some bacteria, in contrast to the pathway in animals and most fungi in which the sole route to Put biosynthesis is the direct decarboxylation of L-ornithine (orn) in a reaction catalysed by ODC. The biosynthesis of Spd and Spm is carried out by addition of an aminopropyl moiety from decarboxylated *S*-adenosylmethionine (dSAM) to one or both primary amino groups of Put by spermidine and spermine synthases (EC 2.5.1.16; EC 2.5.1.22), respectively (Smith 1985). The biosynthetic pathway has been well-documented, and a number of inhibitors of these enzymes are available and have been used to study their physiological functions in plants (Evans and Malmberg 1989).

Until very recently, most experimental procedures in PA biosynthesis relied on correlations between inhibitor action and observed effects *in vitro* (Evans and Malmberg 1989). Results from these experiments suggest a strong involvement of PAs in various physiological and biochemical processes. A major uncertainty remains, however, as it is not possible to ascertain conclusively that exogenously fed PAs would be taken up and transported to the appropriate cells or cell compartments within organized tissue. Costa and Bagni (1983) suggested that [^{3}H]-Put absorbed by leaves and translocated to the fruit was metabolized to Spd and Spm, and these results could explain an increasing fruit growth, fruit set, and yield per tree of apple trees.

The use of molecular biological techniques, in conjunction with genetic transformation to modulate metabolic pathways in plants, will facilitate a better understanding of the role of PAs in different plant developmental processes and remove uncertainties inherent to feeding experiments.

Availability of cloned genes for PA biosynthetic enzymes provides a unique opportunity to manipulate cellular PA metabolism through genetic engineering. In early experiments, Bastola and Minocha (1995) and DeScenzo and Minocha (1993) expressed a mammalian cDNA for ODC in transgenic tobacco and carrot under the control of plant-specific promoters. This caused a significant increase in cellular Put levels. Put levels were also increased in tobacco roots (Hamill et al. 1990) as a result of expressing a yeast *Odc* cDNA. An increase in cellular levels of Spd has been achieved by introduction of a human *Samdc* cDNA into tobacco plants (Noh and Minocha 1994) and by transformation of potato with potato *Samdc* cDNA under the control of a tetracycline-inducible promoter (Kumar et al. 1996). Over-expression of the oat *Adc* cDNA in tobacco caused a 65-fold increase in agm accumulation but no concomitant change in Put levels (Burtin and Michael 1997).

We are currently modifying the PA pathway in cereals using rice as a model. In this report data are presented on the over-expression of oat *Adc* cDNA in transgenic rice callus and plants and its effect on cellular PA levels. We found that: (1) cellular Put concentration in rice callus can be increased following stable transformation with an oat *Adc* cDNA; (2) variation in PA levels in transgenic callus lines affects normal development patterns and differentiation; and (3) phenotypically normal transgenic rice plants with increased Put levels were recovered.

Materials and methods

Plasmids

The 2124-bp oat *Adc* cDNA (Bell and Malberg 1990) was excised as an *Eco*RI fragment and subcloned into the *Eco*RI site of pJIT60 (Gurineau et al. 1992) which contains a CaMV 35 S promoter with duplicated enhancer sequences and a CaMV transcriptional termination region (Burtin and Michael 1997). This plasmid is subsequently referred to as pAMC2.

Transformation and plant regeneration

Rice immature embryos 12–15 days old were harvested from expanded panicles and sterilized with 2% sodium hypochlorite for 5 min. They were subsequently rinsed repeatedly with sterile distilled water, and glumes were removed under a dissecting microscope. The embryos were then aseptically removed and plated on a water-agar plate with the adaxial side in contact with the medium. Transformation was performed as described previously (Christou et al. 1991). Following transformation, embryos were plated on selection medium (Vain et al. 1993) supplemented with hygromycin (50 mg/l) and 2,4-dichlorophenoxyacetic acid (2, 4D, 2 mg/l) in the dark. Embryogenic callus and plantlets were recovered on regeneration medium, in the light, as described (Christou et al. 1991).

Southern and northern blot hybridization

DNA and RNA were isolated from callus and plant material according to the procedure of Creissen and Mullineaux (1995). Following digestion with *Eco*RI and electrophoresis (Sambrook et al. 1989) DNA was transferred to Hybond membranes (Amersham, Aylesburg, UK). Filters were prehybridized at 65°C for 2 h in 6 × SSC (900 m*M* NaCl, 90 m*M* sodium citrate), 5 × Denhart's solution, 50 m*M* sodium phosphate, 0.1% SDS and 100 μg/ml salmon sperm DNA. The 2124-bp *Eco*RI *Adc* fragment from pAMC2 was labelled with [^{32}P]-dCTP using the Random Primer Labelling Kit from Boehringher-Mannheim (Lewes, UK), and the probe was purified on Sephadex G50 columns prior to use. Hybridization was performed at 65°C overnight at high-stringency conditions (0.5% SDS, 0.1% SSC). Blots were exposed to X-ray film and/or visualized on BAS 1000 phosphoimager analyzer (Fuji Photofilm, Kanawa, Japan). RNA analysis was carried out following similar procedures.

Polymerase chain reaction (PCR) and reverse transcription (RT)-PCR analyses

PCR was carried out in 40-μl total reaction volume containing 300 ng of genomic DNA, 1 × PCR buffer (50 m*M* KCl, 10 m*M* TRIS-HCl pH 9.0, 0.1% Triton X-100); 400 μ*M* deoxynucleoside triphosphates, 100 n*M* of both forward and reverse sequence primers (Genosys, Cambridge, UK) and 2.5 U of *Taq* polymerase (Boehringher-Mannheim, Lewes, UK). The thermocycler was programmed for denaturation at 96°C for 40 s, annealing at 60°C for 30 s, and extension at 72° C for 40 s. The reaction was carried out for 35 cycles. The forward sequence primer started from position 1 in the oat *Adc* cDNA open reading frame and consisted of 5′-CGGCGATGTGTACCATGTCGAGGG-3′; the reverse sequence consisted of 5′-GCGGTGTGGAGCGAGTTGATGCGG-3′, resulting in a fragment of 267 bp. The final product was checked on a 2% agarose gel.

Aliquots of 2 μg of total RNA were used in each RT-PCR reaction. Reverse transcription was performed according to standard procedures (Sambrook et al. 1989). cDNAs were amplified for 35 cycles in 50-μl volumes. Conditions and primer sequences used were the same as those in the PCR reaction.

Determination of ADC activity

Callus and plant material were used for ADC activity measurements. Tissue was extracted in buffer (100 m*M* MOPS, 2 m*M* DTT and

1 m*M* EDTA) at a ratio of 300 mg/ml buffer. PVP (100 mg) was added during grinding. Following centrifugation at 12 000 *g* for 10 min, the supernatant was used directly in enzyme activity assays. Tissue was always processed fresh, and all assays were performed using fresh extracts. Enzyme assays were carried out in 1.5-ml Eppendorf tubes. A 6-mm-diameter filter paper disc impregnated with 50 µl of 2 *N* KOH and transfixed with a 1.5-inch-long needle was used to trap the $^{14}CO_2$ liberated during the reaction. The reaction mixture contained 20 µl of extraction buffer, 160 µl of crude enzyme and 20 µl of the substrate [20 µl of L-(U-^{14}C) arg (740.7 Bq/mmol, Amersham, UK) diluted with 20 µl cold arg (500 m*M*) and 60 µl of distilled water to give a final concentration of 10 m*M* arg]. Assays were run at 30° C for 45-min in a waterbath. Two hundred microliters of 10% (v/v) perchloric acid (PCA) was added to stop the reaction. After a further 45 min incubation, the filter paper was placed in scintillation minivials with 2 ml scintillation liquid (OptiPhase hisafe II, Fisons Chemicals, UK), and radioactivity was measured in a Wallac 1219 Rackbeta liquid scintillation counter. Enzyme activity was expressed as µmol $^{14}CO_2$ released/min per gram fresh weight.

Polyamine analysis

A crude extract from callus and leaf samples was obtained after homogenizing in 5% (v/v) cold PCA (300 mg fresh weight/ml). The homogenates were centrifuged at 27 000 *g* for 20 min, and the supernatant fraction was collected. Dansyl-PAs were separated on high-resolution silica gel TLC plates (Whatman LK6DF) and developed using chloroform:triethylamine (4:1, v/v) (Tiburcio et al. 1985). Standard Put, Spd and Spm were included each time that PA levels were analysed. After development of the plates, bands were scraped into 6 ml of ethyl acetate and quantified using a Kontron SFM 25 spectrofluorophotometer at an excitation wavelength of 350 nm and an emission wavelength of 495 nm.

Results

Generation of transformed callus and plants

Immature rice embryos were co-transformed with a plasmid pAMC2 (Burtin and Michael 1997) and pWRG1515 (Christou et al. 1991) containing the hygromycin phosphotransferase (*hpt*) gene as the selectable marker. Twenty-four transgenic clones were analysed.

Transgenic rice plants transformed with pAMC2 which accumulated increased levels of Put exhibited a normal phenotype.

We observed a general trend whereby clones which were able to regenerate plants did not express the oat *Adc* cDNA at high levels (Fig. 1g, h), whereas clones expressing high levels of *Adc* mRNA and high ADC activity were severely inhibited in their capacity to regenerate plants. This behaviour was compounded by exposure to light. Figure 1 illustrates representative examples of phenotypes we recovered. These transgenic lines grew normally on selection and regeneration medium in the absence of light (Fig. 1a), however, upon exposure to light they could only regenerate small shoots which failed to develop further (Fig. 1b). In extreme cases transgenic lines were necrotic (Fig. 1c) and unable to differentiate (Fig. 1d) compared with wild-type callus (Fig. 1e, f).

PCR analyses and Southern blot hybridization

All callus lines that were resistant to hygromycin were screened by PCR and Southern blot hybridization. In PCR experiments, we used oligonucleotides (see experimental procedures) to amplify a 267-bp fragment of the oat *Adc* cDNA (data not shown).

To confirm stable integration of the gene of interest (*Adc*) in callus lines and regenerated plants, we digested genomic DNA with *Eco*RI and the hybridized it with the labelled oat *Adc* cDNA. Southern blot hybridization of rice genomic DNA digested with *Eco*RI yielded a 2.1-kb fragment which confirmed the presence of the intact gene in most of the callus lines and plants we regenerated (data not shown).

Northern hybridization and RT-PCR analyses

Northern hybridization analysis permitted us to classify independently derived plants and callus into different categories in terms of levels of expression (mRNA). Representative examples are shown in Fig. 2. In extreme cases in which high steady-state transcript levels were observed (Figs. 3a, b) tissues became necrotic or produced small shoots which aborted when transferred to light (Fig. 1a–d). Such callus phenotypes failed to regenerate plants. Normal plantlet development was negatively correlated with increasing levels of oat *Adc* mRNA. *Adc* transcripts could not be detected in Northern blots (Fig. 2a) of callus lines which followed the normal regeneration pattern when transferred to light (Fig. 1g, h). However, when a more sensitive detection method (RT-PCR) was performed, transcripts could be detected easily (Fig. 3). The low level of expression of the oat *Adc* mRNA detected by RT-PCR appears to be near the threshold for normal plant development.

Arginine decarboxylase activity

ADC activity was measured in callus and regenerated plants that had been co-transformed with pAMC2 and pWRG1515. Different amounts of *Adc* mRNA and enzyme activity were detected in various transgenic lines (callus and plants). An increase in ADC activity, ranging from 2.7 to 8-fold over wild-type controls, was measured in lines with detectable levels of mRNA (Northern blots and RT-PCR). Representative examples are shown in Fig. 4. After exposure to light, ADC enzyme activity in shoots and plants were, in general, comparable to levels measured in the parental callus lines. Some lines had increased Put levels, and upon

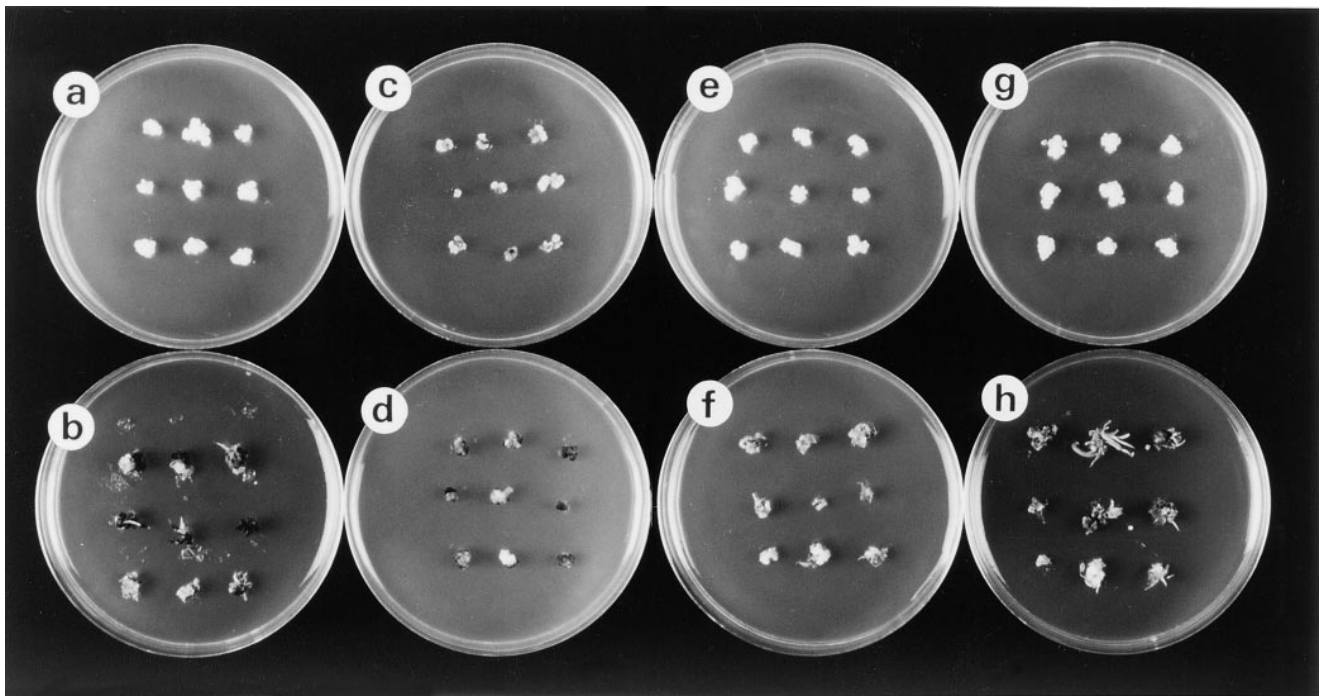

Fig. 1a–h Callus lines engineered with pAMC2 and the *hpt* gene. **a** Transgenic clone 66; callus growing on selection medium (dark), **b** Transgenic clone 66; callus growing on regeneration medium (light), **c** Transgenic clone 39; callus growing on selection medium (dark), **d** Transgenic clone 39; callus growing on regeneration medium (light), **e** Control callus growing on selection medium (dark), **f** Control callus growing on regeneration medium (light), **g** Transgenic clone 5; callus growing on selection medium (dark), **h** Transgenic clone 5; callus growing on regeneration medium (light)

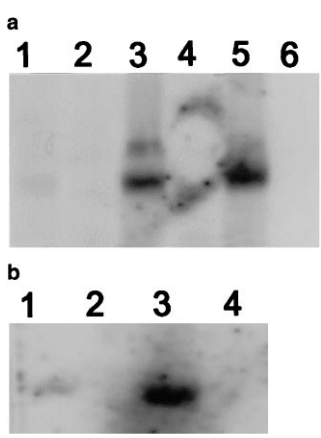

Fig. 2a, b Northern blot analyses of total RNA extracted from callus transformed with pAMC2. Each lane was loaded with 50 μg RNA. Hybridization probe, [^{32}P]-labelled *Eco*RI fragment of pAMC2, exposure time, 2 h. **a** *Lane 1* control, *lane 2* clone 5 grown on selection medium (dark), *lane 3* clone 39 grown on selection medium (dark), *lane 4* clone 5 grown on regeneration medium (light), *lane 5* clone 39 grown on regeneration medium (light), *lane 6* plant regenerated from clone 5. **b** Lane *1* clone 24 grown on selection medium (dark), *lane 2* clone 69 grown on selection medium (dark), *lane 3* clone 66 grown on selection medium (dark), *lane 4* clone 33 grown on selection medium (dark)

exposure to light failed to differentiate shoots. A number of callus lines which did not show measurable mRNA levels in Northern blots but showed expression by RT-PCR exhibited a decrease in ADC activity ranging from 31% to 66% (Fig. 5). These lines regenerated plants which also showed low ADC activity (Fig. 5). In these lines gene-silencing mechanisms may have been in operation. Such lines were indistinguishable from controls (cell lines as well as plants) in all respects other than the presence of the oat *Adc* cDNA.

Polyamine levels

The PA content of transformed callus was determined following subculture on selection medium. This was standardized for all PA measurements. Most transformed cell lines exhibited increased amounts (up to twofold) of cellular Put (Fig. 6). Transformed callus lines with measurable levels of mRNA in Northern blots and with increased ADC activity showed an increase in Put levels of twofold, in the dark. A further increase in Put levels (three–fourfold, Fig. 7) was observed upon exposure of such transgenic cell lines to light. These increases were consistent with a loss of regeneration ability in all cases, suggesting an inhibitory role for Put in developmental processes *in vitro* (Fig. 1b, d).

Transformed callus lines with moderate mRNA expression (only detectable by RT-PCR; Fig. 3) which showed an increase in ADC activity resulting in up to twofold increases in Put levels were competent for regeneration. The twofold Put increased levels in regenerated plants were comparable to levels measured in

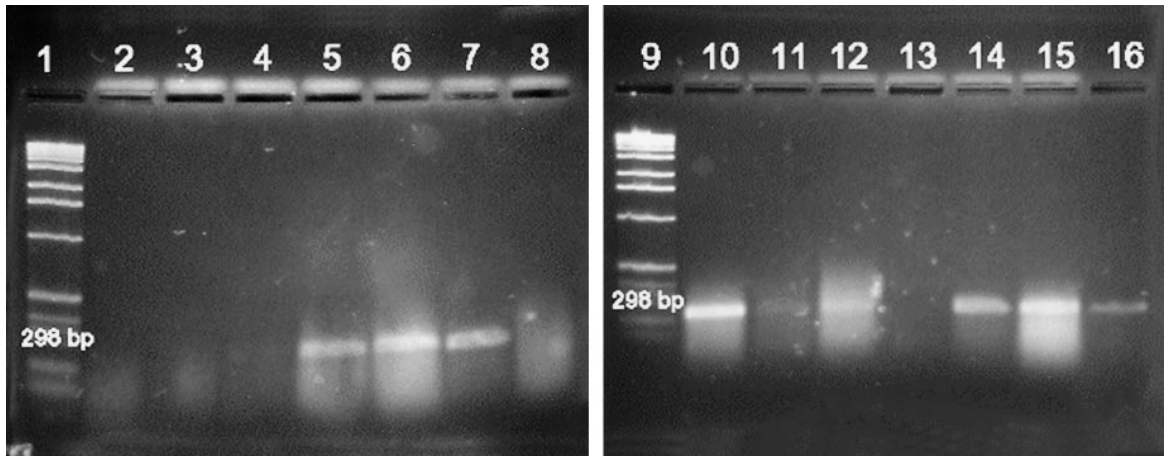

Fig. 3 RT-PCR analysis of cDNA (267 bp) from total RNA extracted from callus and plants transformed with pAMC2; 298-bp maker size is shown in the figure. *Lanes 1* Molecular-size marker, *2* control callus grown on selection medium (dark), *3* control callus grown on regeneration medium (light), *4* control plant, *5* clone 5 callus grown on selection medium (dark), *6* clone 5 callus grown on regeneration medium (light), *7* plant regenerated from clone 5, *8* clone 36 callus grown on selection medium (dark), *9* molecular size marker, *10* clone 24 callus grown on regeneration medium (light), *11* clone 43 callus grown on selection medium (dark), *12* clone 45 callus grown on selection medium (dark), *13* clone 46 callus grown on selection medium (dark), *14* clone 69 callus grown on selection medium (dark), *15* clone 69 callus grown on regeneration medium (light), *16* plant regenerated from clone 69

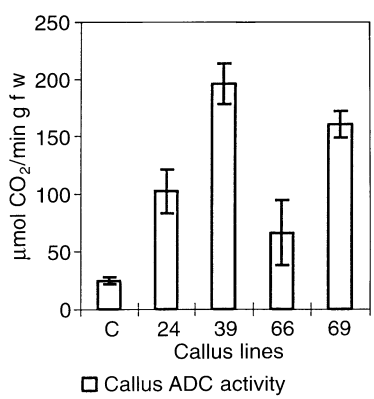

Fig. 4 Enzyme ADC activity in different transgenic callus lines compared to control (*C*) callus. Values are mean ± SE for three replicates. Significantly different from control at $P < 0.05$

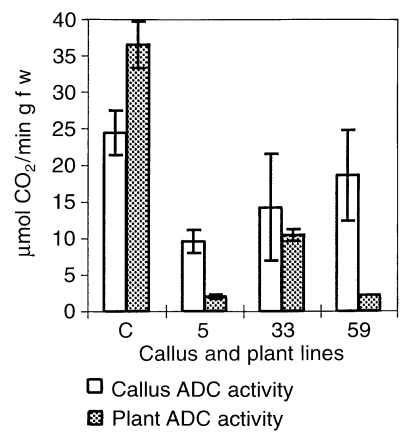

Fig. 5 Enzyme ADC activity in different transgenic callus lines and plants compared to control (*C*) callus and plants. Values are mean ± SE for three replicates. Significantly different from control at $P < 0.05$

the parental callus lines in all cases (Fig. 7). Some of these transformed callus lines with moderate levels of mRNA expression (only detectable by RT-PCR; Fig. 3) exhibited lower ADC enzyme activity, resulting in various levels of Put (ranging from a 50% decrease to a twofold increase compared to non-transformed controls). Normal plants could also be regenerated from all these lines. Cell lines in which silencing mechanisms appeared to be in operation did not exhibit any changes in Put levels at any stage of development (callus, differentiation, regenerated plants; Fig. 7). Spd levels were not affected, and Spm levels appeared to be lower than those of the nontransformed callus controls (Fig. 6). Total PAs were significantly higher in all of the transgenic callus lines (Fig. 6), mostly as a result of Put fluctuation.

Discussion

Changes in the normal development patterns of cell lines with altered PA metabolism have previously been reported in tobacco and potato (DeScenzo and Minocha 1993; Kumar et al. 1996) but not in any monocotyledonous species. We investigated the consequences of constitutively over-expressing an oat *Adc* cDNA in transgenic rice.

We report here dramatic changes in the development of transgenic rice engineered with *Adc*. Our results

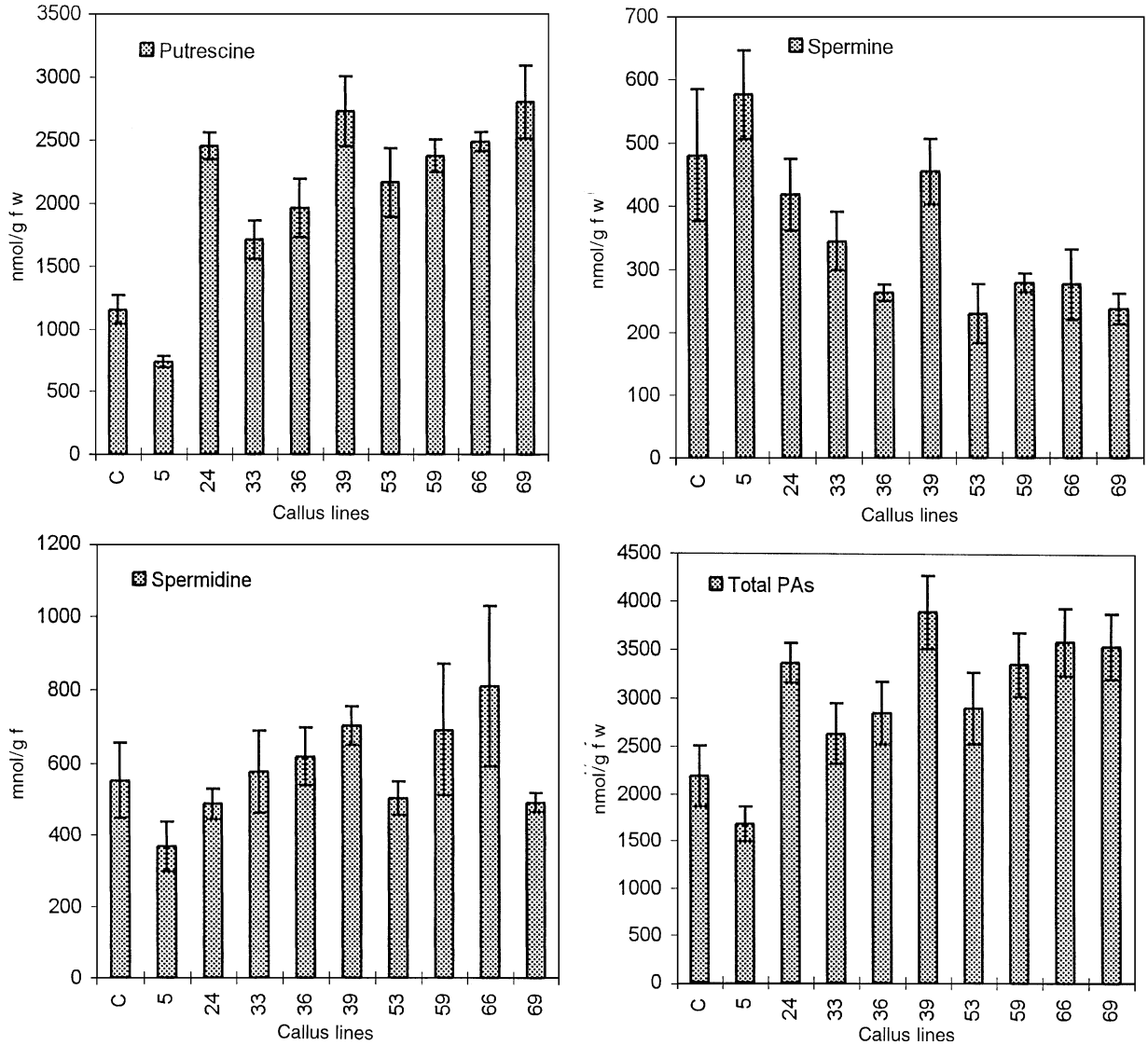

Fig. 6 Cellular PA levels in control (*C*) callus and different transgenic callus lines grown on selection medium in darkness. Values are mean ± SE for three replicates. Put levels significantly different from control at $P < 0.05$

provide new evidence for the existence of a direct correlation between PA levels, plant differentiation and plant morphogenic responses *in vitro*, particularly in cereals. Such responses could be classified in different behaviour patterns resulting from differences at the *Adc* transcript level, ADC enzyme activity and Put accumulation. In order to confirm that the phenotype of callus and shoots was due to changes in ADC expression and accumulation of end product (Put), we performed a detailed molecular and biochemical characterization of all transgenic tissues. We found that increased transcript levels (detectable by Northern blot), elevated enzyme (ADC) activity and changes in PA levels strongly influence the phenotype and morphogenic response of engineered tissue. The relationship between *Adc* transcript level and ADC activity was not directly proportional. However, in general, higher activity was observed in callus lines which expressed the transcript at a high level (Figs. 2–4). Results of PA analysis showed a twofold increase in Put levels in the transformed plants compared to wild-type (Fig. 7). Normal morphogenic development and differentiation of callus lines which expressed mRNA at the highest levels was blocked by exposure to light. In such cases, a significant increase in Put levels (up to fourfold) was observed (Fig. 7). We concluded that there is a maximum threshold of product accumulation which, if exceeded, will result in an inability of the tissue to differentiate into plants, suggesting an inhibitory role for Put in developmental processes in vitro. It has been reported that higher levels of PAs in plant tissues are negatively correlated with normal development (Tiburcio et al.

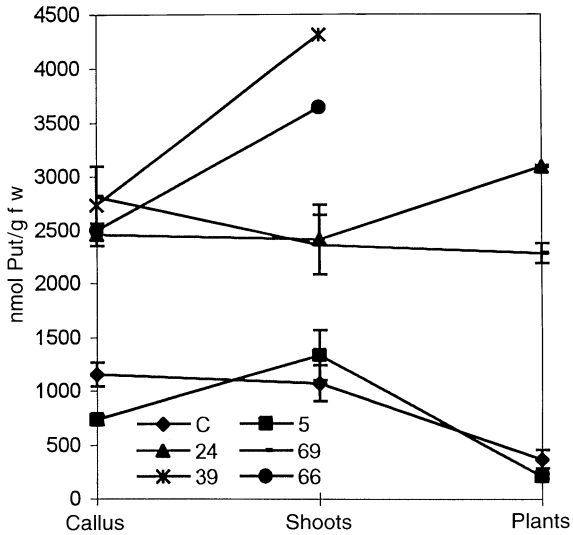

Fig. 7 Cellular Put levels in control (*C*) callus and different transgenic callus lines grown on selection medium in the dark, on regeneration medium in the light and plants regenerated from these tissues. Values are mean ± SE for three replicates. Significantly different from control at $P < 0.05$

1986; Kumar et al. 1996). Bajaj and Rajam (1995) showed that a decrease in plant regeneration in long-term cultures of rice is due to both an accumulation of Put and a high Put/Spd ratio. These cultures reverted to normal developmental patterns following exogenous application of Spd. We concluded that an increase in the ratio of Put to Spd + Spm was not due to limited substrate (arg) availability in the tissue because when exogenous arg was supplied to the callus tissues this did not cause any further increase in cellular Put levels. DeScenzo and Minocha (1993) reported comparable results in engineered tobacco following the introduction of a mouse *Odc* followed by an exogenous supply of orn. In cases in which low mRNA levels were detected, a near two-fold increase in Put was measured in callus and shoots. These cell lines could regenerate normally and other than this twofold Put increase, they were indistinguishable from the parental lines (Fig. 7). Rabiti et al. (1989) showed that when [^{3}H]-labelled Put was fed to maize seedlings, only 14% of the labelled Put was taken up by the plants and only 50% of the labelled Put (7%) was further metabolized to higher PAs (Spd and Spm). Consequently, feeding experiments cannot be relied on to provide conclusive information on the effect of specific PAs in physiological or developmental processes. Transgenic plants expressing PA biosynthetic genes provide a reliable tool for correlating specific phenotypes to enzyme activity and accumulation of specific PAs.

As ADC is directly involved in the biosynthesis of Put, an increase in enzyme activity is expected under normal conditions following the introduction of the oat *Adc* cDNA, which in turn should result in increased Put levels. A number of transgenic plants we recovered expressed ADC at low or negligible levels. Accumulation of the transcript in these plants could be detected by RT-PCR but not by Northern blots. This appears to be in agreement with the biochemical switch model of co-suppression suggested by Meins and Kunz (1994) and Matzke and Matzke (1995) as the introduced oat *Adc* gene shows significant homology to the endogenous rice homologue. In plants in which ADC activity decreaseed significantly (Fig. 5), Put concentration remained at the same level as in the callus. Occasionally a significant decrease in Put levels was observed (Fig. 7).

Our results show clearly that increased Put in transgenic callus lines does not result in increased Spd or Spm levels. This is consistent with experiments in which transgenic tobacco callus overexpressing mouse *Odc* cDNA showed a 4- to 12-fold increase in Put accumulation, with no concomitant changes in Spd or Spm concentrations (DeScenzo and Minocha 1993). Burtin and Michael (1997) reported similar findings in transgenic tobacco plants engineered with an oat *Adc* cDNA. In those experiments a 10- to 20-fold increase in agm *in vivo* did not result in elevated Put accumulation. The levels of Spd and Spm were unchanged in transgenic plants. These results suggest a very strong regulation of the PA pathway.

Conventional biochemical studies in the PA field have up to now focused on the use of metabolic inhibitors to block the action of particular enzymes involved in the pathway. In general, these experiments resulted in a reduction of inhibition of enzyme activities, resulting in a concomitant reduction in PA levels (Bagni et al. 1983; Burtin et al. 1989; Evans and Malmberg 1989; McCann et al. 1987; Minocha 1988; Slocum and Flores 1991). By using transgenic approaches we are now in a position to alter levels of enzyme activity and perhaps PA levels, and we are now beginning to manipulate the PA pathway in rice and other cereals using genetic engineering. It is reasonable to expect that the oat *Adc* gene would be expressed differently in a cereal such as rice than in dicots. By having access to transgenic cell lines and plants with altered PA levels we can begin to investigate in detail the involvement of these compounds in morphogenesis and development. We were able to recover fertile rice plants which accumulated increased levels of Put. Such plants will be of considerable interest to nutritionists as PAs play an important role in growth and development processes in mammals, including humans (Bardocz et al. 1993). In addition, PAs have been implicated in stress responses (Bay et al. 1992; Roy and Ghos 1996; Willadino et al. 1996). We now have in hand plants which can be tested for tolerance against a number of biotic and abiotic stresses. Such experiments have been initiated and no chlorophyll loss has been detected in a number of these transgenic rice plants after 8 days of drought stress (manuscript in preparation).

We have shown that (1) the oat *Adc* cDNA is stably integrated into the rice genome; (2) it is expressed and produces an active oat ADC enzyme; (3) increased ADC activity results in increased cellular Put; (4) transgenic tissues show a range of developmental patterns in vitro, depending on levels of mRNA, ADC enzyme activity and Put levels.

Our results expand the field of PA biosynthesis to cereal crops, which constitute the most important component of the diet, particularly in developing countries. We have shown conclusively that not only can we recover transgenic rice plants accumulating increased Put but also that there is a direct involvement of Put in morphogenesis and development.

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