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Gene expression and characterization of a stress-induced tyrosine decarboxylase from *Arabidopsis thaliana*

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ABSTRACT

Full-length tyrosine decarboxylase cDNA (*TyrDC*) from *Arabidopsis thaliana* was identified by rapid amplification of cDNA ends-PCR and isolated by RT-PCR. The *TyrDC* mRNA was substantially induced by drought stress and wounding, and was considerably decreased by salt stress. By using TyrDC protein fusions with green fluorescent protein, an intracellular localization to the cytoplasm was shown. Recombinant $(His)_6$-TyrDC was expressed in *Escherichia coli* and enzymatically characterized: it exclusively catalyzed the conversion of L-tyrosine to tyramine, exhibited an optimum temperature of 50 °C, and an optimum pH at approximately 8.5–9. Recombinant TyrDC protein formed tetramers, as shown by blue native gel electrophoresis.

Structured summary:
MINT-7040408:*TyrDC* (uniprotkb:Q8RY79) and *TyrDC* (uniprotkb:Q8RY79) *bind* (MI:0407) by *blue native page* (MI:0276)

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1. Introduction

Aromatic L-amino acid decarboxylases (AADCs) are suggested to play crucial roles in the biosynthesis of a number of different secondary metabolites in plants. These compounds are not of general importance for the normal development of the plant, but often exhibit specialized functions in the interaction between plants and their abiotic and biotic environment.

Tyrosine decarboxylase (TyrDC) mediated decarboxylation of L-tyrosine is assumed to be a common feature distributed throughout the entire plant kingdom, and it seems to have a vital function in several different metabolic pathways. Besides the involvement in the biosynthesis of verbascoside in *Syringa vulgaris* [1] and of hordinine in *Hordeum vulgare* [2], TyrDC appears to be involved in plant defense, as both *TyrDC* transcript level and TyrDC activity transiently respond to fungal elicitor treatment in cell suspension cultures [3,4].

Here we report the identification of the full-length *TyrDC* cDNA from *Arabidopsis* and the functional characterization of the corresponding protein. Furthermore, we give an account of the stress-dependent regulation of *TyrDC* gene expression.

Abbreviations: AADC, aromatic L-amino acid decarboxylase; GFP, green fluorescent protein; MeJA, methyl jasmonic acid; OPDA, 12-oxo-phytodienoic acid; RACE, rapid amplification of cDNA ends; qRT-PCR, quantitative reverse transcription polymerase chain reaction; TyrDC, tyrosine decarboxylase

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2. Materials and methods

2.1. Plant material

All experiments were carried out using *Arabidopsis thaliana* ecotype Col-0 (NASC, stock N1092). Seedlings were sterilely raised on solidified ½ MS-medium supplemented with 1% (w/v) sucrose [5]; plantlets were kept under constant environmental conditions (8 h light at 24 °C, 16 h darkness at 20 °C, photosynthetically active radiation 105 μE m^{-2} s^{-1}) for 2–4 weeks.

2.2. Stress conditions

To assess the influence of various different stress conditions on *TyrDC* mRNA levels, 4 weeks old seedlings were incubated under different conditions for time periods indicated, before 100 mg plant material was harvested, frozen in liquid nitrogen, and total RNA was extracted. To induce cold stress, plants were incubated at 4 °C; for heat stress, plants were kept at 38 °C. To mimic drought conditions, plates were opened to allow the plants and the medium to be dehumidified in a drying chamber. To induce salt stress, medium was supplemented with 3 M NaCl; other plates were transferred to darkness. Wounding was performed by using sterile tweezers. Plants kept at constant conditions in a phytochamber were used as controls. In case of oxylipin treatment, 7 days old sterilely grown plants were used. Plates were either supplemented with 50 μM 12-oxo-phytodienoic acid (OPDA), 50 μM methyl

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jasmonic acid (MeJA) or 10 μM coronatine dissolved in ½ MS solution. As control, plants treated with ½ MS solution without any further supplementation were used. Pathogen studies made use of the avirulent bacteria strain *Pseudomonas syringae* DC3000(*avrRPM1*). Pathogen infiltration was performed with a 5×10^7 cfu ml^{-1} suspension [6].

2.3. RNA isolation and RT-PCR

Total RNA was prepared from 100 mg plant tissue by using TRIzol reagent (Invitrogen, Karlsruhe, Germany) according to the manufacturer's instructions. RNA to be used in subsequent rapid amplification of cDNA ends (RACE)-PCR applications or qPCR was further purified using an Oligotex mRNA kit (Qiagen, Hilden, Germany). First-strand synthesis was performed according to the supplier's instructions, using M-MLV-reverse transcriptase and oligo(dT)$_{15}$ primer (Promega, Madison, USA). All subsequently performed PCR applications made use of intron-spanning, gene-specific oligonucleotides (Table 1).

2.4. 5′-RACE-PCR

For RACE a 5′/3′ RACE kit (2nd generation, Roche Diagnostics, Mannheim, Germany) was used according to the manufacturer's instructions. Based on the *TyrDC* sequence, as is deposited in GenBank (NM_119010), two oligonucleotides were designed and used (see Table 1). Finally, the obtained extended cDNA fragment was commercially sequenced (GATC, Konstanz, Germany).

2.5. Expression analysis by qRT-PCR

To quantify the expression of *TyrDC* in response to multiple environmental cues, quantitative reverse transcription polymerase chain reaction (qRT-PCR) was performed in a total volume of 25 μl using the QuantiFast SYBR Green RT-PCR kit (Qiagen) on a DNA Engine Opticon 2 instrument (MJ Research, Waltham, USA) according to the manufacturer's instructions. Relative quantification of expression was calculated after data analysis using Opticon monitor software (ver. 2.02.24, MJ Research) by the comparative $2^{-\Delta\Delta C_T}$ method [7] with *EF-1α*, *APT1*, and *UBQ10* as reference genes [8]. For normalization and determination of amplification efficiencies the geNORM and LinRegPCR algorithms, respectively, were used [9,10].

2.6. Vector construction

Molecular techniques were carried out following standard protocols according to [11]. Full-length *TyrDC* cDNA was amplified by PCR using gene specific primers (TyrDC-RACE-for/TyrDC-PstI-rev, Table 1) and cloned into pGEM-T (Promega). The PCR parameters were 95 °C for 10 min followed by 35 cycles of 95 °C for 45 s, 58 °C for 45 s, and 72 °C for 90 s; for the amplification of the coding region a proofreading polymerase (iProof, Bio-Rad, Munich, Germany) was used. After sequence verification of the PCR product (GATC), the corresponding fragment was cloned into each of the vectors pQE-30 (Qiagen) and pTrcHis2 (Invitrogen) by using BamHI/PstI sites. For generation of green fluorescent protein (GFP) fusion constructs, the BamHI/SalI fragment from pTrcHis-TyrDC was introduced into the BamHI/SalI sites of pUC-GFPn and pUC-GFPc. These vectors facilitate 5′ and 3′ fusion, respectively, of target genes with the coding sequence of the *GFP* gene. Expression of these fusion constructs is driven by a *35S* promoter and terminated by a *NOS* terminator sequence.

2.7. Heterologous expression of TyrDC

N-terminally hexahistidine-tagged TyrDC was expressed in *Escherichia coli* strain M15 [pREP4] harboring the pQE-TyrDC construct. For this, 300 ml of 2YT medium were inoculated with 30 ml of an overnight culture and incubated at 30 °C under constant shaking. Once an optical density (OD$_{600}$) of 0.8–1.0 was reached, protein expression was induced by adding 1 mM IPTG. After an additional incubation time of 18 h, cells were harvested by centrifugation (4000×*g*, 15 min, 4 °C). Then pellets were resuspended in 30 ml of lysis buffer (50 mM sodium phosphate buffer pH 7.5, 300 mM NaCl, 25 mM imidazole, 1 mg ml^{-1} lysozyme). Following a 30 min incubation on ice, bacteria were disrupted by sonification (three burst of ultrasound, 30 s, 40 W), and the soluble protein fraction was isolated by centrifugation (10 000×*g*, 20 min, 4 °C). The resulting supernatant was filtered through 0.22 μm Millex-GP filter units (Milipore, Billerica, USA) and (His)$_6$-tagged proteins purified by affinity chromatography on a Ni^{2+} agarose matrix (Qiagen) following the supplier's instructions. Eluates were desalted using PD-10 columns (GE Healthcare, Freiburg, Germany), equilibrated with 50 mM sodium phosphate buffer at pH 7.5, and protein concentrations determined according to [12].

2.8. Confocal laser scanning microscopy

Transient transformation of pavement cells and subsequent microscopic analysis of the expressed GFP-tagged proteins was carried out as previously described [13]. *Arabidopsis* plantlets were transformed by particle bombardment. After incubation for 16–18 h, TyrDC-GFP expressing cells were analyzed by confocal laser scanning microscopy.

2.9. Enzymatic assays

Screening for appropriate substrates of recombinant TyrDC was performed by thin layer chromatography (TLC). Different

Table 1
Primers used for cloning and expression analysis of *Arabidopsis TyrDC*.

Name	Sequence (5′–3′)	Experiment
5′-RACE-TyrDC-02	CAGTAGCGGCAGGAGAAGTGAGCCAGG	5′-RACE-PCR
5′-RACE-TyrDC-03	CCCCAATGGGACCAAAGGATCAACCGC	5′-RACE-PCR
TyrDC-qPCR-for	GGCGATGAAGACCAATGTAACGAAC	qRT-PCR
TyrDC-qPCR-rev	GCACCAACAGCAAATCGTAAAACG	qRT-PCR
APT1-qPCR-for	TCGTGCTGTTCCTTGCAACCG	qRT-PCR
APT1-qPCR-rev	GCGGAGGAGAAGAGGCGGAGT	qRT-PCR
EF-1α-qPCR-for	CTTGCTTTCACCCTTGGTGT	qRT-PCR
EF-1α-qPCR-rev	TCCCTCGAATCCAGAGATTG	qRT-PCR
UBQ10-qPCR-for	TTGGAGGATGGCAGAACTCTTGCT	qRT-PCR
UBQ10-qPCR-rev	AGTTTTCCCAGTCAACGTCTTAACGAAA	qRT-PCR
TyrDC-datab-for	TATGGATCCATGTTTAAACCCCAACATATGTATG	RT-PCR
TyrDC-RACE-for	TAAGGATCCATGGAATTTGGTACCGG	RT-PCR, cloning
TyrDC-PstI-rev	TATCTGCAGGCATAATGGTCGTTGCGTGTAAATTTG	RT-PCR, cloning

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