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Structure and Molecular Characterization of Diadenosine Polyphosphate Hydrolase in *Brachypodium distachyon*

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Abstract

Putative diadenosine polyphosphate (Ap_nA) hydrolase gene, which encodes an amino acid sequence showing homology with that of Arabidopsis long-chain (Ap_nA) (n=5–6) hydrolase (AtNUDX13) and which conserves nudix motif and glycine tripeptide motif, was identified from *Brachypodium distachyon*. The mature form of Brachypodium (Ap_nA) hydrolase (BraNUDX15) catalyzed long-chain (Ap_nA) and Ap_4A, Ap_4G, Gp_4G, and dCTP, showing different substrate specificity from Arabidopsis (Ap_nA) hydrolases AtNUDX13, 25, 26, and 27. BraNUDX15 required Mg^{2+} and produced ATP from (Ap_nA), indicating asymmetrical (Ap_nA) hydrolase as the same as Arabidopsis (Ap_nA) hydrolases. Results show that BraNUDX15 gene was up-regulated by UV-A, UV-B, and UV-C irradiation and down-regulated by drought stress, but it was left unchanged by salt stress. Subcellular localization indicated that the BraNUDX15 protein was colocalized with the surface of the chloroplasts. These results suggest BraNUDX15 as a unique (Ap_nA) hydrolase with different substrate specificity from those of Arabidopsis (Ap_nA) hydrolases. It might play a role in regulating (Ap_nA) levels in chloroplasts under conditions of drought stress and UV irradiation.

Keywords: *Brachypodium distachyon*; Diadenosine polyphosphate; Drought stress; Nudix hydrolase; UV irradiation

Introduction

Diadenosine polyphosphate (Ap_nA) is a ubiquitous family of nucleotides in which two nucleoside moieties are linked 5-5' through a polyphosphate chain containing 3-7 phosphoryl groups [1,2]. Ap_4A is implicated in coupling DNA replication to cell division [3,4], initiation of DNA replication [5,6], recovery from stress by modulating protein refolding [2,7,8], and regulation of ATP-sensitive K^+ channels [9,10]. Because the Ap_4A level is increased in cells exposed to stress conditions such as oxidative, heat, nutritional, and DNA damage [7,8,11-13] and because Ap_4A increases the gene expression of phenylalanine ammonia-lyase and 4-coumarate: CoA ligase consisting of phenylpropanoid pathway by heavy metals in Arabidopsis [14], Ap_4A has been proposed as an 'alarmone'. The long-chain (Ap_nA) (n=5-6) produces cytotoxic effects, although it is also an intracellular and extracellular signaling molecule [2,15-18]. Distributed among humans, bacteria, fungi, and plants, Ap_4A hydrolase metabolizes and regulates (Ap_nA) levels. It is classified into two groups. One group cleaves Ap_4A symmetrically to produce two moles of ADP. Its structure is related to serine/threonine protein phosphatase [19-23]. The other group, which shows asymmetrical Ap_4A hydration to produce ATP and AMP, belongs to the nudix hydrolase (NUDX) family [24]. Some asymmetrical Ap_4A hydrolases catalyze not only Ap_4A but also long-chain (Ap_nA) and other nucleotide polyphosphates, for which specific activities and their products depend on enzyme characteristics [24-27].

In plants, AtNUDX13, AtNUDX25, AtNUDX26, and AtNUDX27 from Arabidopsis thaliana belong to (Ap_nA) hydrolase of the NUDX family [28-31]. Actually, AtNUDX13 is active toward Ap_6A and Ap_5A, but it has no activity to Ap_4A and other substrates for Ap4A hydrolases. AtNUDX25 hydrolyzes NADH, coenzyme A (CoA), and guanosine-3′, 5′-tetraphosphate (ppGpp), whereas AtNUDX26 hydrolyzes ppGpp, in addition to the activities of AtNUDX25 and 26 toward Ap_5A and Ap4A. AtNUDX27 hydrolyzes only Ap_5A. These NUDXs have a well-conserved nudix motif, $GX_5EX_7REUXEEXGU$, where U is usually Ile, Leu, or Val [24]. AtNUDX25, AtNUDX26, and AtNUDX27 had a tyrosine residue downstream of the nudix motif found in other Ap4A hydrolases and located in chloroplasts, whereas AtNUDX13 had a glycine tripeptide motif downstream of the nudix motif. The subcellular location was in mitochondria [28,29]. These results suggest that enzymatic properties and biological functions differ between (Ap_nA) hydrolases that have long-chain (Ap_nA)-specific activity and which have wide substrate specificity, but most studies of enzymatic properties and diversity of Ap_4A hydrolases and long-chain (Ap_nA) hydrolases have scope that is limited to Arabidopsis NUDXs in plants.

Brachypodium distachyon is a model plant of Pooideae subfamily including wheat and barley, which has tractable features such as small genome size with diploid, small plant size, and short life cycle [32]. It is expected to serve as a useful function model for identification of genes and biological functions related to agronomic interest from Triticeae crops. This study identified the putative gene from *Brachypodium* which encodes the homologue of AtNUDX13 that hydrolyzes Ap_6A and Ap_5A specifically. Furthermore, this study elucidated the structure, enzymatic properties, subcellular location, and expression profiles under stress conditions.

Materials and Methods

Plant cultivation and stress treatment

Seeds of Brachypodium, *Brachypodium distachyon* Bd21, were incubated on filter paper kept moist with water at 23°C for 5 days in the dark. Seedlings were selected randomly from the germinated seeds. Three seedlings were planted on one Wagner pot (1/5000 a) filled with soil under a metal halide light (350 μmol/m^2/s) with a light/dark cycle of 16 h/8 h in a growth chamber. After 7 days of cultivation, plants were irradiated with 186, 431, and 438 μW/cm^2 of 340, 312, and 260 nm of UV light for 6 h to induce UV stress. The plants were pulled up and dehydrated on a paper towel for 6 h to stimulate drought stress, were soaked in a pot with 100 mM NaCl solution for 24 h to stimulate salt stress or were cultivated under metal halide light as a control. After exposure to stress conditions, the shoots were harvested, frozen in liquid nitrogen, and stored at -80°C.

Quantitative RT-PCR analysis

Total RNA was isolated from shoot samples using the RNeasy Plant mini kit (Qiagen Inc., Tokyo, Japan) following the manufacturer's instructions. Poly(A)$^+$ RNA was purified from total RNA with the Poly (A) Purist MAG (Ambion Inc., Austin, Texas). Then the purified poly(A)$^+$ RNA was dissolved in the RNA storage solution. First-strand cDNA was synthesized from poly(A)$^+$ RNA using a PrimeScript RT Master Mix (Takara Bio Inc., Shiga, Japan). Quantitative RT-PCR was performed in a mixture of 20 μl containing first-strand cDNA, SYBR Premix Ex Taq (Takara Bio Inc.), and 0.2 μmol of each forward primer, 5′-TGCACTGCTGGAGCGGTTAT-3′, and reverse primer, 5′-ATCAGATGTCGTTTGGAGCA-3′ using LightCycler 2.0 (Roche Applied Science, Mannheim, Germany). The thermal cycle profile was 1 cycle of 95°C for 10 s, followed by 40 cycles of 95°C for 5 s and 60°C for 20 s. The cDNA quantities of each gene were calculated using software (LightCycler 4.0; Roche Applied Science) and were normalized with that of the S-adenosylmethionine decarboxylase gene [33]. The expression analysis was conducted three times.

Expression and purification of BraNUDX15

The active form of HvNUDX 15 genes was amplified with first-strand cDNA from control shoots and the primers, 5′-CCATATGAAGAAGGACGAGGGGAACCC-3′, which creates a Nde I site (denoted as underlined), and 5′-CCTCGAGGCACAATGCAACTGCGCC-3′, which creates a Xho I site (denoted as underlined). The PCR product of 525 bp length was cloned into the pGEM-T vector. Then the fragments of the plasmids digested by Nde I and Xho I were subcloned into a pET-20b (+) vector, in which a polyhistidine tag gene is fused upstream from the start codon. The resulting plasmid, pBraNUDX15-ACT, was transformed into *E. coli* BL21 cells. *E. coli* cells harboring pBraNUDX15-ACT were grown at 37°C in Luria-Bertani (LB) medium containing 50 μg/ml ampicillin. When the OD600 reached 0.5, isopropyl-μ-D-thiogalactopyranoside (IPTG) was added to the culture at a final concentration of 0.5 mM. After cultivation at 25°C for 18 h, the cells were harvested by centrifugation and were frozen at -80°C for at least 2 h. The frozen cell pellets were suspended in a protein extraction reagent (BugBuster™ HT; Merck, Darmstadt, Germany) according to the manufacturer's instructions. The resulting recombinant protein, which showed an insoluble form, was dissolved in 20 mM Tris-HCl buffer (pH 8.0) containing 0.5 M NaCl, 5 mM imidazole, and 6 M guanidine HCl (Buffer A) was purified using an Ni-NTA agarose column (Qiagen Inc.) initially equilibrated in Buffer A. The column was washed with Buffer A, followed by 60 mM imidazole in Buffer A, with the absorbed protein eluted with 200 mM imidazole in Buffer A. The protein solution was dialyzed against 20 mM Tris-HCl buffer (pH 8.0) containing 0.5 M NaCl, 5 mM imidazole, and 3 M guanidine HCl, followed by 20 mM Tris-HCl buffer (pH 8.0) containing 1 mM DTT, 100 mM NaCl, 0.5% n-dodecyl-μ-D-maltoside, and 10% ethylene glycol. The dialyzed solution was then concentrated (Vivaspin 4; Sartorius, Goettingen, Germany).

Enzyme and protein assays

The hydrolytic activity of BraNUDX15 was assayed according to a method described previously [34]. The reaction mixture (100 μl), which consists of 10 mM Tris-HCl (pH 8.0), 5 mM $MgCl_2$, 10 mM DTT, 100 μM substrate, and recombinant protein, was incubated at 37°C for 30 min. After the reaction was terminated by 17 μl of 100 mM EDTA, the reaction mixture was subjected to HPLC using a column (Cosmosil C18, 4.6 × 250 mm, Nacalai Tesque Inc., Kyoto, Japan) equilibrated with 100 mM phosphate buffer (pH 6.0) and 5% methanol at a flow rate of 0.6 ml/min. The reaction products were detected by absorption at 293 nm for 8-oxo-dGTP, 8-oxo-dGDP, at 260 nm for (Ap_nA) (n=4–6), ADP-ribose, NADH, UDP-Gal, dATP, ppGpp, and CoA, at 252 nm for dGTP, at 264 nm for dTTP, and at 271 nm for dCTP. Protein concentrations were quantified according to Bradford [35] with bovine serum albumin as the standard.

GFP transient assay

A DNA fragment encoding a putative transit peptide of BraNUDX15 predicted to the residue within the first 45 N-terminal amino acid residues was amplified by PCR with the first-strand cDNA from control shoot and primers, 5′-GGTCGACATGTCCAGCCTCGTTCTCGC-3′, which creates a Sal I site (denoted as underlined), and 5′-TCCATGGTGTACGGGACACACCCTGCA-3′, which creates a Nco I site (denoted as underlined). The PCR product of 144 bp length was cloned into the pGEM-T vector. Then the fragments of the plasmids digested by Sal I and Nco I were subcloned into a plasmid pTH-2, in which a GFP is fused downstream from the transit peptide [36]. The plasmid, pBraNUDX15-SIG+GFP, was transformed into Arabidopsis protoplasts according to the method explained by Miura et al. [37].

Results

Identification of Brachypodium (Ap_nA) hydrolase genes

The genes, which encode amino acid sequences showing homology with those of 28 Arabidopsis NUDX families (AtNUDX1–27 and AtDCP2) and nudix motif (e<0.0001), were searched using a BLAST program [38,39] and the RIKEN *Brachypodium distachyon* Full-Length cDNA Clone Database. The full-length cDNAs of 19 putative Brachypodium NUDX genes, BraNUDX1-19, were identified. The deduced amino acid sequences of BraNUDX1-19 showed 71-43% identities with those of AtNUDXs and nudix motif (Table 1).

Gene	Gene ID	Identity (%)		Subfamily
BraNUDX1	Bradi1g35490.1	AtNUDX2:	55	ADP-ribose/NADH
		AtNUDX10:	50	
		AtNUDX7:	45	
		AtNUDX6:	43	
BraNUDX2	Bradi3g53887.1	AtNUDX3:	66	n.d.
BraNUDX3	Bradi1g44170.1			n.d.
BraNUDX4	Bradi2g37517.1	AtNUDX9:	60	GDP-mannose
BraNUDX5	Bradi2g32550.1			n.d.
BraNUDX6	Bradi5g08460.1			n.d.
BraNUDX7	Bradi1g49810.1	AtNUDX14:	58	ADP-ribose/ADP-glucose
BraNUDX8	Bradi3g56830.1	AtNUDX17:	51	n.d.
		AtNUDX4:	49	
BraNUDX9	Bradi1g51060.1	AtNUDX19:	59	NADPH
BraNUDX10	Bradi4g28030.2	AtNUDX20:	61	Thiamin diphosphate
		AtNUDX24:	58	
BraNUDX11	Bradi4g37360.1	AtNUDX23:	53	FAD
BraNUDX12	Bradi5g26560.2	AtNUDX26:	58	ApnA/ppGpp
		AtNUDX25:	49	
		AtNUDX27:	47	
BraNUDX13	Bradi3g35160.1	AtNUDX22:	55	CoA
		AtNUDX11:	51	
BraNUDX14	Bradi5g17500.1	AtNUDX8:	50	n.d.
BraNUDX15	Bradi3g44460.1	AtNUDX13:	47	ApnA/ppGpp
		AtNUDX12:	46	
BraNUDX16	Bradi3g35150.1	AtNUDX15:	57	CoA
BraNUDX17	Bradi1g54020.1	AtNUDX16:	71	n.d.
BraNUDX18	Bradi3g56830.1	AtNUDX18:	52	n.d.
		AtNUDX21:	47	
BraNUDX19	Bradi3g54700.1	AtDCP2:	58	mRNA cap
n.d., not detected.				

Table 1: Identity of deduced amino acid sequences of BraNUDX genes with those of AtNUDX genes.

Alignment analysis of amino acid sequences of BraNUDX1-19 obtained using the Clustal W program showed that the nudix motif comprising 23 amino acid residues was conserved in the amino acid sequences of *Brachypodium* NUDXs, except for the insertion of 22 amino acid residues in BraNUDX4 (Figure 1).

According to the substrate specificities of Arabidopsis NUDXs, 15 *Brachypodium* NUDXs are classified into the following subfamilies: BraNUDX1 belongs to ADP-ribose/NADH hydrolase; BraNUDX4 belongs to GDP-mannose hydrolase; BraNUDX7 belongs to ADP-ribose/ADP-glucose hydrolase; BraNUDX9 belongs to NADPH hydrolase; BraNUD×10 belongs to thiamin diphosphate hydrolase; BraNUDX11 belongs to FAD hydrolase; BraNUDX12 and 15 belong to

(Ap_nA)/ppGpp hydrolase; BraNUDX13 and 16 belong to CoA hydrolase; BraNUDX19 belongs to mRNA cap; although BraNUDX2, 3, 5, 6, 8, 14, 17, and 18 were not assigned to any established subfamily (Table 1). These results suggest that two (Ap_nA) hydrolase genes, BraNUDX12 and 15, are present in *Brachypodium*. BraNUDX12 conserved Tyr downstream of nudix motif as did Arabidopsis (Ap_nA) hydrolases, AtNUDX25, 26, and 27, whereas BraNUDX15 conserved glycine tripeptide motif, GX_2GX_6G, as did AtNUDX13, which hydrolyzes Ap_6A and Ap_5A specifically (Figure 2).

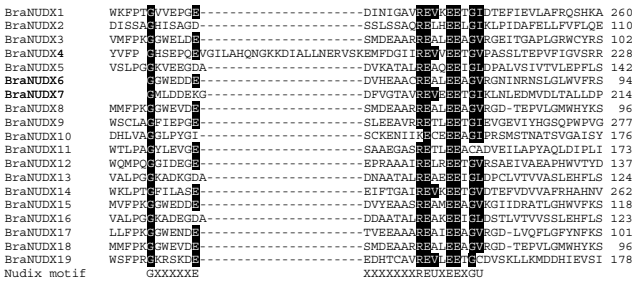

Figure 1: Alignment of the deduced amino acid sequences around the nudix motif in putative *Brachypodium* NUDXs. Gaps, denoted by a dash were introduced into the sequences to maximize the homology. The nudix motif is shown below the sequence. Identical amino acid residues to those of nudix motif are shown as reversed letters.

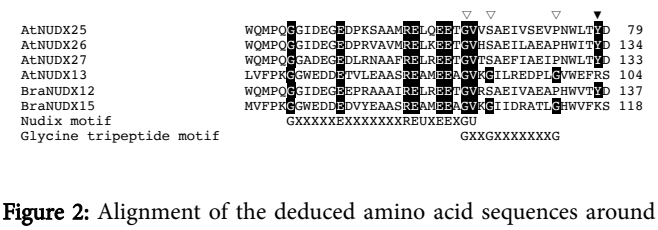

Figure 2: Alignment of the deduced amino acid sequences around the nudix motif in *Brachypodium* and Arabidopsis ApnA hydrolases. Glycine residues of glycine tripeptide motif and a tyrosine residue downstream from nudix motif are shown respectively by white and black triangles.

Purification and enzymatic characterization of recombinant BraNUDX15

BraNUDX15 protein, which was encoded by the open reading frame of cDNA, was produced in *E. coli* cells. However, the protein made an inclusion body and could not be made soluble by refolding (data not shown). Results of alignment analysis of the amino acid sequence of BraNUDX15 with that of AtNUDX13, which had the transit peptide [29], suggest that the N-terminal peptide of 46 amino acid residues of BraNUDX15 is the transit peptide (Figure 3).

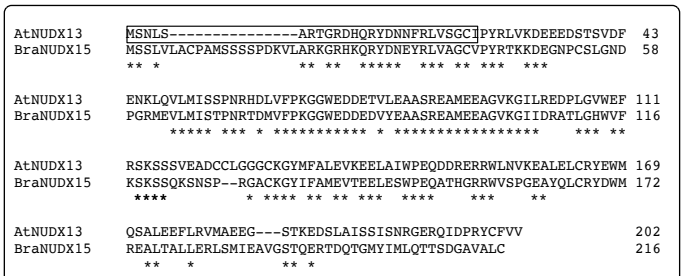

Figure 3: Alignment of the deduced amino acid sequences of full-length amino acid sequences of AtNUDX13 and BraNUDX15. The black frame and asterisks respectively denote the transit peptide of AtNUDX13 and identical amino acid residues between AtNUDX13 and BraNUDX15.

The mature form of BraNUDX15 protein, of which the transit peptide at the N-terminus was eliminated, tagged with a His-Tag at its C-terminus, was produced in *E. coli* cells. An extra protein with a molecular mass of 22 kDa, which is similar to that calculated from the amino acid sequence, was produced as an inclusion body in *E. coli* cells and was refolded to soluble form (Figure 4).

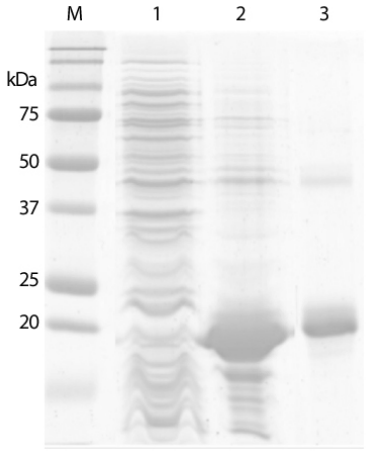

Figure 4: Analysis of the expression of BraNUDX15 in *E. coli* cells using SDS-polyacrylamide gel. E. coli cells harboring pBraNUDX-ACT were harvested after IPTG induction at 25°C for 18 h. The soluble protein (1), insoluble protein (2), and refolded BraNUDX15 purifying with Ni-NTA column (3) were subjected to 12% SDS-PAGE with molecular mass marker (M) followed by Coomassie Brilliant Blue R-250 staining.

The purified BraNUDX15 incubated with Ap_6A showed production of ATP, of which the specific activity was 1.23 μmol/min/mg. The enzyme activity was inhibited completely by EDTA and was recovered by $MgCl_2$. BraNUDX15 had maximum activity toward Ap_6A at pH 8.0 and relative activities of 95% for Ap_5A and 76% for Ap_4A to Ap_6A hydrolyzing activity, with barely any activity toward dCTP (Table 2).

	Specific activity (mmol/min/mg)				
Substrate	**BraNUDX15**	**AtNUX13 [28]**	**AtNUDX25 [30,31]**	**AtNUDX26 [30,31]**	**AtNUDX27 [30]**
Ap_3A	n.d.	n.d.	n.d.	n.d.	n.d.
Ap_4A	0.94 ± 0.02	n.d.	0.026 ± 0.02	13.3 ± 0.36	n.d.
Ap_5A	1.17 ± 0.11	4.2	0.017 ± 0.001	21.6 ± 0.58	0.22 ± 0.01
Ap_6A	1.23 ± 0.03	10.5	-	-	-
Ap_4G	0.72 ± 0.09	-	-	-	-
Gp_4G	0.74 ± 0.04	-	-	-	-
ADP-ribose	n.d.	-	n.d.	n.d.	n.d.
NADH	n.d.	-	0.016 ± 0.001	n.d.	n.d.
CoA	n.d.	-	0.012 ± 0.001	0.11 ± 0.01	n.d.
UDP-Gal	n.d.	-	n.d.	n.d.	n.d.
ppGpp	n.d.	-	0.06 ± 0.01	0.19 ± 0.05	-
8-oxo-dGTP	n.d.	-	n.d.	0.02 ± 0.01	n.d.
dGTP	n.d.	-	n.d.	0.05 ± 0.01	n.d.
dATP	n.d.	-	n.d.	0.07 ± 0.01	n.d.
dTTP	n.d.	-	n.d.	0.05 ± 0.01	n.d.
dCTP	0.04 ± 0.001	-	n.d.	0.07 ± 0.01	n.d.
n.d., not detected; -, not reported.					

Table 2: Substrate specificities of *Brachypodium* and Arabidopsis Ap_nA hydrolases.

Expression of BraNUDX15 gene under abiotic stress

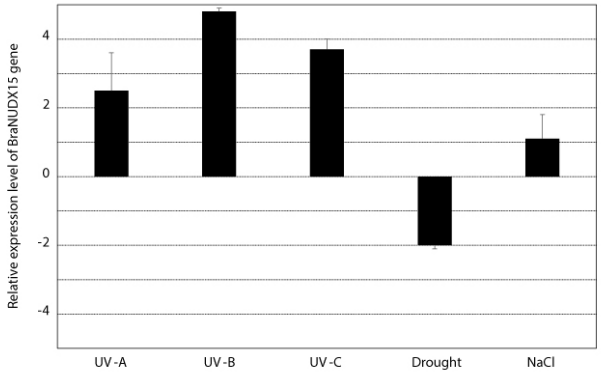

Figure 5: Expression profiles of BraNUDX15 gene in *Brachypodium* under abiotic stress. Total RNAs isolated from shoots of *Brachypodium* under UV-A, UV-B, UV-C, drought, and 150 mM NaCl conditions were subjected to quantitative RT-PCR. Expression levels were normalized with that of S-adenosylmethionine decarboxylase gene as an internal control. The error bar represents the standard error of the mean for three experiments.

Brachypodium was cultivated under UV irradiation, drought, and salt conditions to evaluate the response of BraNUDX15 gene to environmental stresses (Figure 5).

The expression level of BraNUDX15 gene was up-regulated considerably: 2.5, 4.8, and 3.7-fold, respectively, by UV-A, UV-B, and UV-C irradiation. Drought stress reduced the expression level to about half. The expression level was unchanged by salt stresses, which increased it about 10%.

Subcellular localization of BraNUDX15 protein

A DNA fragment corresponding to predicted transit peptide from BraNUDX15 cDNA sequence was fused in frame with GFP at the C-terminus and was expressed in protoplasts under the control of the CaMV 35S promoter. The GFP fusion protein fluorescence in the transgenic cells was colocalized with the surface of chloroplasts (Figure 6).

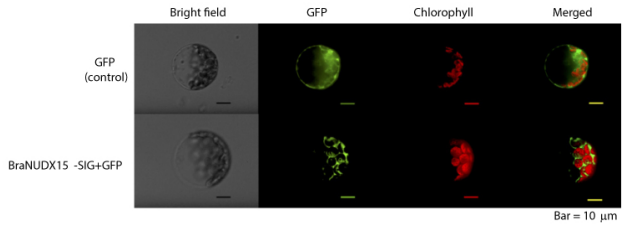

Figure 6: Subcellular localization of BraNUDX15. Arabidopsis cells were transformed with either pBraNUDX15-SIG+GFP or pTH-2 (GFP control). GFP fluorescence (GFP) and chlorophyll autofluorescence (Chlorophyll) signals were merged (Merged).

The subcellular localization of BraNUDX15 w as also predicted in chloroplasts using WoLF PSORT server.

Discussion

Genes encoding homology with (Ap_nA) hydrolase were searched from *Brachypodium.* Of 19 putative NUDX genes, BraNUDX12 and 15 genes showed homology with Arabidopsis (Ap_nA) hydrolases NUDXs. BraNUDX12 showed identity with AtNUDX25, 26, and 27, which conserved the tyrosine residue found in (Ap_nA) hydrolases and hydrolyzed Ap_4A and/or Ap_5A, whereas BraNUDX15 showed identity with AtNUDX13, which had the glycine tripeptide motif and hydrolyzed Ap_6A and Ap_5A but not Ap_4A [28,29]. These results suggest BraNUDX15 as the long-chain (Ap_nA) specific hydrolase.

The purified BraNUDX15, of which the predicted transit peptide was eliminated, required Mg^{2+} for hydrolyzing (Ap_nA), as did other (Ap_nA) hydrolases. The enzyme had the highest activity toward Ap_6A, with relative activities of 95% for Ap_5A and 76% for Ap_4A to Ap_6A hydrolyzing activity. It produced ATP from these substrates, whereas Arabidopsis long-chain (Ap_nA) hydrolase, AtNUDX13, showed activity toward Ap_6A, preferentially toward Ap_6A, and relative activity of 40% for Ap_5A to Ap6A hydrolyzing activity. However, it showed no activity toward Ap_4A. It produced ADP+p_4A from Ap_6A and AMP +p_4A from Ap_5A [28]. AtNUDX25 and 26 showed activity not only toward Ap_4A and Ap_5A but also toward NADH, CoA, 8-oxo-dGTP, ppGpp, or dNTPs [30,31], which were not hydrolyzed by BraNUDX15 except for slight activity toward CoA. These results indicate that BraNUDX15 is a unique (Ap_nA) hydrolase that has different substrate specificity from Arabidopsis (Ap_nA) hydrolases and indicate that glycine tripeptide motif is necessary for hydrolyzing long-chain (Ap_nA).

In plant cells, AtNUDX13 was localized in mitochondria; AtNUDX26 and 27 were localized in chloroplasts [28,30]. Reportedly, AtNUDX26 hydrolyzed ppGpp, of which the level in chloroplasts was increased under environmental stress. Moreover, the expression level of the gene increased under drought stress, suggesting that AtNUDX26 regulates the ppGpp level in chloroplasts [31]. Tomato Ap_4A hydrolase gene decreased by $CdCl_2$ [40]; Ap_4A increased the gene expression of phenylalanine ammonia-lyase and 4-coumarate: CoA ligase consisting of phenylpropanoid pathway by heavy metals in Arabidopsis [14], indicating that the Ap_4A level is regulated by Ap_4A hydrolase to induce stress tolerance genes as alarmone. An earlier study showed that Ap_6A inhibits ATP-sensitive K^+ channels [17] and that extracellular Ap_6A and Ap_5A influence cytosolic free Ca^{2+} concentrations [18]. The accumulation of long-chain (Ap_nA) can produce cytotoxic effects through the inhibition of various kinases [15,16]. Our result demonstrated that BraNUDX15 was localized around the chloroplast surface. The gene expression level was induced under UV-A, -B, and -C exposure, but it was reduced by drought stress. Taken together, the evidence shows that BraNUDX15 can be expected to play a role in accumulating Ap_4A to induce drought-stress-relieving genes under drought stress and decreasing long-chain (Ap_nA) before attaining a potentially toxic concentration under UV irradiation in chloroplasts.

Conclusions

Results of this study demonstrated that *Brachypodium* (Ap_nA) hydrolase BraNUDX15, which showed homology with Arabidopsis long-chain (Ap_nA) hydrolase and conserved glycine tripeptide motif, was a unique (Ap_nA) hydrolase that has different substrate specificity from those of Arabidopsis (Ap_nA) hydrolases. The expression level of BraNUDX15 gene was increased by UV irradiation and decreased by drought stress. Moreover, the protein was localized in chloroplasts. These results suggest that BraNUDX15 is a unique (Ap_nA) hydrolase with different substrate specificity from those of Arabidopsis (Ap_nA) hydrolases. It might play a role in regulating (Ap_nA) levels in chloroplasts under drought stress and UV irradiation.

Acknowledgments

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