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Identification and Expression Analysis of Regulatory Genes Induced by Near-Ultraviolet Irradiation in *Bipolaris oryzae*

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Abstract

Bipolaris oryzae **is the causal agent of brown leaf spot disease in rice, and its asexual spore (conidium) formation is known to be induced by near-ultraviolet (NUV) irradiation. In order to reveal the photomorphogenic response and to identify new genes upregulated by NUV irradiation, suppression subtractive hybridization (SSH) was carried out in** *B. oryzae*. **To confirm the differential gene expression in NUV-irradiated mycelia, quantitative real-time PCR (qRT-PCR) analysis was performed among 301 genes arbitrarily chosen from 1170 cDNA clones. The expression of 46 genes (named NUV01 to NUV46) was found to be significantly enhanced (>4-fold) by NUV irradiation. Sequence analysis revealed that 23 out of the 46 sequences (50%) showed significant matches to known fungal genes. The 46 genes were categorized as either BLR1-dependent or BLR1-independent expression groups using the** *BLR*1-**deficient mutant, which presumably lacks the blue/UVA-absorbing photoreceptor. This finding demonstrates that NUV irradiation can induce gene regulation, and that this response may be mediated by both a blue/UVA-absorbing photoreceptor and an as-yet-unidentified photoreceptor in** *B. oryzae*.**

Keywords

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1. Introduction

Fungal life is greatly modulated by light, as observed in processes such as pigment biosynthesis, the formation of reproductive structures, phototropism, and so on [1] [2]. Blue light is most effective in fungal photomorphogenesis such as that in *Neurospora crassa* and *Trichoderma atroviride*, whereas red light and near-ultraviolet (NUV; 300 - 400 nm) can be effective for the induction of conidiation in *Aspergillus nidulans* [3] and *Bipolarisoryzae* [4] [5], respectively. The molecular bases of fungal photoreceptors as well as of photomorphogenesis by blue and red light have been widely studied, as detailed in a number of published reviews [2] [6] [7].

Bipolaris oryzae (Breda de Haan) Shoem. [anamorph of the ascomycetous fungus *Cochliobolus miyabeanus* (Ito et Kuribayashi) Drechsler ex Dastur] is the causal agent of brown leaf spot disease in rice, and NUV irradiation induces its asexual spore (conidium) formation. In previous studies, to reveal the mechanisms underlying the photomorphogenic response to NUV irradiation in *B. oryzae*, we focused on the regulatory genes induced by NUV irradiation, and reported that NUV irradiation specifically enhanced the expression of the novel NUV-inducible gene (*UVI*-1) [8], three melanin biosynthesis genes [9]-[11], the *BMR*1 gene encoding a transcriptional factor for these melanin biosynthesis genes [12], and the photolyase gene [13]. On the other hand, in *N. crassa* and *T. atroviride*, many blue light-inducible genes have been identified [14] [15]. Thus, in the present study, we constructed subtractive cDNA libraries from cultures of *B. oryzae* grown under NUV irradiation in order to perform a more comprehensive search for the regulatory genes induced by NUV irradiation in *B. oryzae*. Suppression subtractive hybridization (SSH) is a method based on suppressive PCR that allows the creation of subtracted cDNA libraries for the identification of genes differentially expressed in response to different experimental conditions. It requires relatively few steps to identify cDNAs corresponding to differentially expressed genes. SSH has been widely used to identify differentially expressed genes, as shown in the appressorium development of *Magnaporthe grisea* [16] and in the conidiation of *Exserohilum turcicum* [17] in phytopathogenic fungi. In this paper, we report 46 newly identified genes that were upregulated by NUV irradiation in *B. oryzae* using SSH methods.

2. Materials and Methods

2.1. Fungal Strains and Growth Conditions

Bipolaris oryzae strain D9/F6-69 (stock culture at the Laboratory of Plant Pathology, Shimane University) was used as the WT strain. The experimental fungus was grown on potato dextrose agar (PDA) in 9-cm petri plates (Iwaki, Tokyo, Japan). To peel off the mycelia from the agar medium, the agar surface of a petri plate (15 mL PDA) was overlaid with a single layer of dialysis tubing (8 cm in flat width; Eidia, Tokyo, Japan) that had been autoclaved at 121°C for 15 min. The plates were inoculated centrally with a mycelial plug. The cultures were incubated at 25°C ± 1°C for 4 days in continuous darkness and then subjected to light treatments. Black-light (BLB) lamps (FL-BLB; Toshiba Electric, Tokyo, Japan), which emitted wavelengths of 300 to 400 nm (mainly 352 nm), were used as the source of near-ultraviolet (NUV) radiation. The irradiance of NUV radiation was measured by an USB4000-UV-VIS miniature fiber optic spectrophotometer (Ocean Optics, Dunedin, FL). The average irradiance of NUV radiation was 280 mW·m^{-2}.

2.2. Isolation of Total RNA and mRNA

Colonies grown on a PDA medium for 4 days in darkness were exposed to BLB light as a source of NUV radiation for 1 h (light-treated mycelia). These mycelia were scratched from dialysis tubes by a sterilized stainless steel spatula, frozen in liquid nitrogen, and ground with a mortar and pestle to a fine frozen powder. Total RNA was isolated from the frozen mycelial powder by phenol/chloroform extraction, followed by LiCl precipitation according to the protocols [18]. Poly(A)$^+$ RNA was purified using an OligotexTM-dT30 <Super>mRNA purification kit (TaKaRa Bio, Otsu, Japan) according to the manufacturer's instructions. The DNA sequence data were analyzed using GENETYX (Software Development, Tokyo, Japan). The nucleotide sequence databases were searched using the BLASTX programs at the DDBJ website (http://www.ddbj.nig.ac.jp/).

2.3. SSH

SSH was carried out between NUV-irradiated mycelia (tester) and dark-grown mycelia (driver) using a PCR

Select cDNA subtraction kit (Clontech, Palo Alto, CA) according to the manufacturer's instructions. The subtracted cDNA fragments were cloned into the cloning vector pT7Blue T-vector (TaKaRa Bio) and sequenced. Sequencing reactions were performed using a Big Dye terminator cycle sequencing kit (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. DNA sequence analysis was performed on an ABI Prism 310 genetic analyzer (Applied Biosystems).

2.4. Quantitative Real-Time PCR Analysis

Total RNA was used as a template for cDNA synthesis. cDNA was prepared using Ready-To-Go You-prime First-Strand Beads (GE Healthcare UK, Ltd., Buckinghamshire, UK) with anoligo(dT)15 primer. DNA contamination was eliminated by treatment with RNase-free DNase I (TaKaRa Bio). Real-time PCR was performed using SYBR Premix Ex Taq (TaKaRa Bio) and a Thermal Cycler Dice Real-Time System TP800 (TaKaRa Bio). The primers used in the reaction (**Table S1**) were designed by Primer3 at the website http://frodo.wi.mit.edu. Post-PCR melting curves confirmed the specificity of single-target amplification, and the fold expression of each gene relative to the glyceraldehyde-3-phosphate dehydrogenase gene (GPD) was determined.For reverse transcriptase polymerase chain reaction (RT-PCR) analysis, PCR amplification was carried out using 25 cycles of denaturation at 94˚C for 1 min, annealing at 50˚C for 30 s, and elongation at 72˚C for 45 s. Amplification fragments were electrophoresed on a 1% agarose gel (Agarose L03; TaKaRa). The gel was stained with ethidium bromide, destained in water, and photographed under UV illumination (302 nm).

3. Results

3.1. Identification of and Sequence Analysis of NUV-Regulated Genes

We previously reported that NUV irradiation specifically enhances the expression of a novel NUV-inducible gene (*UVI*-1) [8], three melanin biosynthesis genes [9]-[11], the *BMR*1 gene encoding a transcriptional factor for these melanin biosynthesis genes [12], and a photolyase gene [13]. These transcripts accumulated to their maximum levels at 1 h after NUV irradiation in *B. oryzae*, suggesting that 1 h of NUV irradiation would be an appropriate condition under which to identify other genes upregulated by NUV irradiation in *B. oryzae*. Thus, total RNA was extracted from both dark-grown mycelia and mycelia irradiated with NUV for 1 h. SSH was carried out between dark-grown mycelia (driver) and NUV-irradiated mycelia (tester) in *B. oryzae* by using a PCR Select cDNA subtraction kit (Clontech). The PCR products of subtractive cDNA were then cloned separately into the cloning vector pT7Blue T-vector and stored at −20˚C before sequencing. We arbitrarily sequenced the inserts of 1170 randomly selected subtractive cDNA clones from the library and sorted these clones based on homology using the BLASTX program in the DNA Data Bank of Japan (DDBJ). These clones were candidates for transcripts specific to or upregulated in NUV-irradiated mycelia. In fact, the EST sequences of the three melanin biosynthesis genes and the photolyase gene mentioned above were included in these clones (data not shown). Furthermore, two opsin-like genes, *OPS*1 and *OPS*2, were identified from the subtractive cDNA library [19]. To confirm the differential gene expression in NUV-irradiated mycelia by the SSH strategy, quantitative real-time PCR (qRT-PCR) analysis was performed using each specific primer among 301 genes arbitrarily chosen from the 1170cDNA clones. The results showed that the expression levels of 46 (named NUV01 to NUV46) of the 301 genes were significantly enhanced (>4-fold) by NUV irradiation (**Table 1**). The average change among the 46 genes was 27.9-fold. NUV19 and NUV40 were particularly affected, showing 160- and 100-fold increases in mRNA levels after NUV irradiation, respectively. All 46 sequences (NUV01 to NUV46) have been submitted to the EST database of DDBJ andthe results of sequence matching with known genes are also summarized in **Table 1**. Sequence analysis revealed that 23 out of 46 sequences (50%) showed significant matches to known fungal genes. Putative photomorphogenesis-related genes were observed. NUV03 showed similarity to cryptochrome DASH as a putative blue light photoreceptor. NUV12 and NUV20 showed similarities to 1,3,6,8-tetrahydroxynaphthalene reductase and scytalone dehydratase, respectively, as melanin biosynthesis-related proteins. NUV36 showed similarity to deoxyribodipyrimidine photolyase. NUV19, NUV25, NUV39, and NUV46 showed similarity to several kinds of transporter/pump proteins. The remaining NUV-regulated genes showed similarity to cytochrome P450 (NUV01), acetyl-coA carboxylase (NUV08), monooxygenase (NUV10), linoleate diol synthase (NUV26), and so on. Sequence analysis also revealed that 21 out of 46 sequences (46%) were putative uncharacterized proteins, and the remaining 2 out of 46 sequences (4%) were novel sequences (NUV38 and NUV40).

Table 1. Genes upregulated >4-fold in NUV-irradiated mycelia compared to dark-grown mycelia.

Name	Accession no.	Length (bp)	Best BLASTX match (Species*)	Accession no.	E-value	Fold change (NUV/dark)
NUV01	AK424224	156	Cytochrome P450 (Ptri)	B2WMD6	2e−26	32.4
NUV02	AK424225	322	Putative uncharacterized protein (Ptri)	B2WIN2	2e−31	65.3
NUV03	AK424226	97	Cryptochrome DASH (Ptri)	B2W5Z1	2e−13	23.1
NUV04	AK424227	164	Putative uncharacterized protein (Pn)	Q0UKL3	7e−21	16.5
NUV05	AK424228	115	Putative uncharacterized protein (Pter)	E3S0E9	3e−15	86.9
NUV06	AK424229	126	Putative uncharacterized protein (Ptri)	B2WCB0	1e−18	33.2
NUV07	AK424230	198	Hypothetical protein (Pn)	Q0UXE1	1e−21	58.3
NUV08	AK424231	146	Acetyl-CoA carboxylase (Ptri)	B2VTF1	4e−17	4.7
NUV09	AK424232	259	Putative uncharacterized protein (Pter)	E3S3P6	1e−40	5.6
NUV10	AK424233	401	Monooxygenase(Ptri)	B2WMD7	2e−85	32.6
NUV11	AK424234	136	Putative uncharacterized protein (Pn)	Q0U4T9	3e−20	13.4
NUV12	AK424235	363	1,3,6,8-tetrahydroxynaphthalene reductase	A0SXQ8	2e−62	9.7
NUV13	AK424236	172	Similar to glycosyltransferase family protein (Lm)	E5A4Y9	1e−27	7.6
NUV14	AK424237	186	Putative uncharacterized protein (Pter)	E3S4X1	5e−12	57.7
NUV15	AK424238	170	Putative uncharacterized protein (Pter)	E3S0K1	7e−20	51.5
NUV16	AK424239	233	StcQ-like protein (Cl)	Q67FQ7	4e−42	82.8
NUV17	AK424240	276	Catechol O-methyltransferase (Ptri)	B2VZE6	3e−54	20.2
NUV18	AK424241	169	Putative uncharacterized protein (Pter)	E3REU5	2e−23	7.8
NUV19	AK424242	215	Opaque-specific ABC transporter CDR3 (Ptri)	B2VZ85	1e−29	160.1
NUV20	AK424243	182	Scytalone dehydratase (Gc)	F0XGI1	2e−12	7.4
NUV21	AK424244	211	Putative uncharacterized protein (Pn)	Q0UY63	2e−21	18.8
NUV22	AK424245	290	Putative uncharacterized protein (Pter)	E3RMJ1	3e−16	44.7
NUV23	AK424246	240	Similar to FAD binding domain protein	E4ZQX0	6e−27	58.0
NUV24	AK424247	338	Pentatricopeptide repeat protein (Ptri)	B2VQQ3	6e−52	4.8
NUV25	AK424248	274	Aflatoxin efflux pump (Ptri)	B2W0U0	4e−43	9.3
NUV26	AK424249	325	Linoleatediol synthase (Ptri)	B2VZV7	2e−60	9.3
NUV27	AK424250	311	NACHT domain containing protein (Ptri)	B2VYD6	1e−36	12.1
NUV28	AK424251	366	Putative uncharacterized protein (Pn)	Q0UBY6	5e−33	10.1
NUV29	AK424252	189	Putative uncharacterized protein (Ptri)	E3REK7	9e−14	13.6
NUV30	AK424253	315	Oxidoreductase domain containing protein (Ptri)	B2W869	3e−17	4.7
NUV31	AK424254	264	Putative uncharacterized protein (Lm)	E5ACX9	1e−12	7.6
NUV32	AK424255	207	Zinc finger protein (Ptri)	B2VV64	4e−37	15.1
NUV33	AK424256	241	Putative uncharacterized protein (Pter)	E3RCQ1	3e−37	25.2
NUV34	AK424257	97	Putative uncharacterized protein (Pter)	E3RX86	4e−14	5.5
NUV35	AK424258	116	Putative uncharacterized protein (Pter)	E3RN51	2e−09	10.7
NUV36	AK424259	230	Deoxyribodipyrimidine photolyase (Ptri)	B2WAE9	9e−34	12.2
NUV37	AK424260	230	Peptidyl-prolylcis-trans isomerase (Lm)	E5A619	3e−48	5.6
NUV38	AK424261	86	No hits found	-	-	60.1
NUV39	AK424262	236	Similar to tetracycline-efflux transporter (Lm)	E5A106	1e−32	20.9
NUV40	AK424263	304	No hits found	-	-	100.0
NUV41	AK424264	148	Mitochondrial ATPase (Ptri)	B2W1I0	6e−16	23.0
NUV42	AK424265	466	Putative uncharacterized protein (Ptri)	B2WP06	2e−60	5.7
NUV43	AK424266	133	Putative uncharacterized protein (Pn)	Q0U516	1e−08	6.2
NUV44	AK424267	155	Putative uncharacterized protein (At)	Q0CS86	5e−22	9.3
NUV45	AK424268	234	Putative uncharacterized protein (Pter)	E3RNT6	7e−40	6.0
NUV46	AK424269	128	Membrane zinc transporter (Ptri)	B2WBG1	6e−13	6.5

*At, *Aspergillus terreus*; Ch, *Cochliobolus heterostrophus*; Cl, *Cochliobolus lunatus*; Gc, *Grosmannia clavigera*; Lm, *Leptosphaeria maculans*; Pn, *Phaeosphaeria nodorum*; Pter, *Pyrenophora teres* f. *teres*; Ptri, *Pyrenophora tritici-repentis*.

3.2. BLR-Dependent NUV-Regulated Gene Expression

We previously isolated and characterized the *Blue-light regulator* 1 (*BLR*1) gene, which encodes a putative blue light photoreceptor similar to white collar 1 (WC-1) of *N. crassa* in *B. oryzae* [20]. In addition, it was suggested that the BLR1 protein is necessary not only for conidial formation but also for NUV radiation-enhanced photolyase gene expression in *B. oryzae* [20]. Thus, in the present work, we investigated whether or not the *BLR*1 gene is involved in NUV radiation-enhanced gene expression by qRT-PCR analysis using a BLR1-deficient mutant. **Figure 1** shows the results of the expression analysis of 36 NUV-regulated genes (NUV1-NUV36) selected from the 46 NUV-regulated genes in the WT and the *BLR*1-deficient mutant (Δ*BLR*1). Both genes with
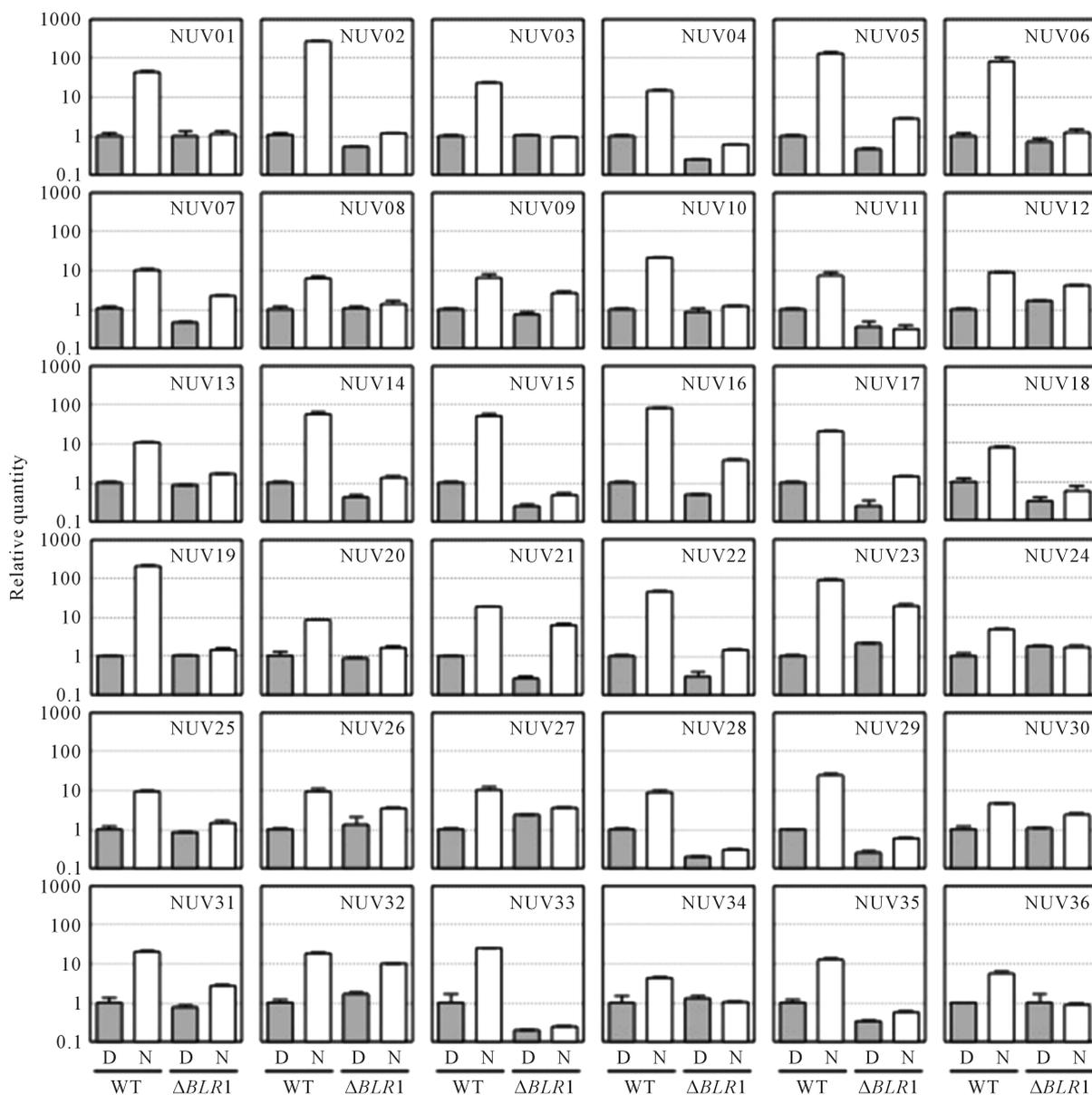

Figure 1. Expression analysis of 36 regulatory genes isolated from the NUV upregulated genes in the wild type (WT) and the *BLR*1-deficient mutant (Δ*BLR*1) [20]. Mycelia grown for 4 days on PDA medium in the dark (D, solid bar) were exposed to NUV radiation for 1 h (N, open bar), and total RNA was extracted. The expression was normalized by dividing the average value of the GPD as the internal control. The relative change in the expression of each mRNA was determined as a fold change relative to that of the WT control in the dark (=1). Vertical bars represent the SD. The primers used in this experiment are shown in **Table S1**.

BLR1-dependent and genes with BLR1-independent expression were recognized. In the BLR1-dependent expression group, which included NUV01, NUV06, and NUV35, the gene expression was significantly enhanced in mycelia exposed to NUV radiation in the WT, whereas the expression levels of these genes were the same as those under dark conditions in the *BLR*1-deficient mutant (**Figure 1**). On the other hand, in the BLR1-independent expression group, which included NUV07, NUV21, NUV23, and NUV32, the expression of genes was significantly enhanced in mycelia exposed to NUV radiation in both the WT and the *BLR*1-deficient mutant, suggesting that the expression of these genes could not be involved inBLR1 regulation. To confirm these differences in the expression profile between the two groups, RT-PCR analysis was performed using the same samples, and the amplified bands were visualized after electrophoresis (**Figure 2**). In the BLR1-dependent expression group, which included NUV01, NUV06, and NUV35, expression was significantly enhanced in mycelia exposed to NUV radiation, whereas NUV radiation did not enhance the expression levels of these genes in the *BLR*1-deficient mutant. On the other hand, in the BLR1-independent expression group, which included NUV07, NUV21, NUV23, and NUV32, gene expression was significantly enhanced in mycelia exposed to NUV radiation in both the WT and the *BLR*1-deficient mutant. In addition, the constitutively expressed GPD gene was observed in both the WT and the *BLR*1-deficient mutant.

4. Discussion

Generally, photomorphogenic responses in fungi are influenced by blue light, red light, and NUV radiation. The blue light responses have been best studied in *Neurospora crassa*, and White Collar-1 (WC-1) has been identified as a blue light photoreceptor protein [21] [22]. The red light responses have been best studied in *Aspergillus nidulans*, and phytochrome (FphA) has been identified as a red light-sensing photoreceptor protein [7] [23]. On the other hand, the effects of NUV irradiation on conidial induction have been studied in some phytopathogenic fungi, such as *B. oryzae* and *Alternaria solani*, and the action spectrum for conidial induction suggested that a putative UVB-absorbing photoreceptor would be involved in conidial induction [5]. A putative UVB-absorbing photoreceptor in fungi has not been identified thus far, although UVR8 as the UVB-absorbing photoreceptor in plantae was first identified and characterized in *Arabidopsis thaliana* [24] [25]. The possibility of a UVB-absorbing photoreceptor in *B. oryzae* has been suggested by the findings that the expression levels of *UVI*-1 [8] and of melanin biosynthesis genes [9]-[11] were enhanced by NUV irradiation but not by blue irradiation. In order to identify the putative UVB-absorbing photoreceptor in fungi, it is necessary to determine the global
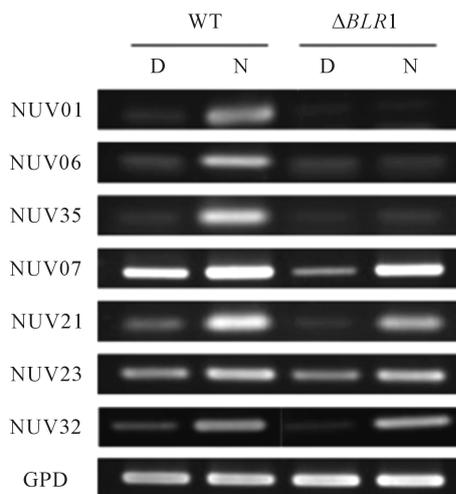

Figure 2. RT-PCR analysis of the NUV upregulated genes in the wild type (WT) and the *BLR*1-deficient mutant (Δ*BLR*1) [20]. Total RNA was extracted from mycelia growing in the dark (D) or exposed to NUV radiation for 1 h (N) from the WT and the *BLR*1-deficient mutant (Δ*BLR*1). The GPD gene was used as an internal control. The primers used in this experiment are shown in **Table S1**.

changes in gene expression induced by UVB irradiation. In this paper, we found that the expression of 46 newly identified genes was upregulated by NUV irradiation, based on the SSH procedure and quantitative real-time PCR analysis in *B. oryzae* (**Table 1**). NUV irradiation induced gene regulation mediated through both a blue/UVA-absorbing photoreceptor and a putative UVB-absorbing photoreceptor, because NUV radiation (300 - 400 nm) contains UVA radiation (320 - 400 nm) and little UVB radiation (280 - 320 nm). Thus, we tried to categorize the 46 genes as either blue/UVA-regulated genes or UVB-regulated genes using the *BLR*1-deficient mutant, which presumably lacks the blue/UVA-absorbing photoreceptor, similar to the blue light photoreceptor WC-1 of *N. crassa* [20]. As a result, BLR1-dependent and BLR1-independent expression groups were recognized (**Figure 1** and **Figure 2**). In the BLR1-dependent expression group, which included NUV01, NUV06, and NUV35, enhanced gene expression by NUV irradiation was not observed in the *BLR*1-deficient mutant, suggesting that the expression of these genes could be regulated by BLR1 as the blue/UVA-absorbing photoreceptor (**Figure 2**). On the other hand, in the BLR1-independent expression group, which included NUV07, NUV21, NUV23, and NUV32, enhanced gene expression by NUV irradiation occurred in both the WT and the *BLR*1-deficient mutant, suggesting that the expression of these genes could be regulated by a putative UVB-absorbing photoreceptor, which was distinct from the regulation by BLR1 as a blue/UVA-absorbing photoreceptor (**Figure 2**). Interestingly, NUV23 and NUV32 showed similarities to the zinc finger protein and the FAD-binding domain protein, respectively, suggesting that NUV23 and NUV32 could be regulatory elements controlled by a putative UVB-absorbing photoreceptor. The remaining products of the newly identified genes, NUV07 and NUV21, displayed similarities to hypothetical/putative uncharacterized proteins. BLR1-independent blue light-regulated gene expression was also reported in *Trichoderma atroviride*, indicating the existence of a blue light perception pathway independent of the blue/UVA-absorbing photoreceptor, BLR-1 [15].

It has previously been considered that NUV radiation emitted by black-light lamps is not a suitable radiation source for research into the photomorphogenic response in *B. oryzae*, because two photosensory pathways—a blue/UVA-absorbing photoreceptor and a putative UVB-absorbing photoreceptor—would be involved in the photomorphogenic response in *B. oryzae*, and because NUV radiation (300 - 400 nm) contains UVA radiation (320 - 400 nm) and little UVB radiation (280 - 320 nm). Thus, light-emitting diode (LED) emitting sharp UVB wavelengths would further characterize NUV-regulated genes in *B. oryzae*. The functions of NUV-regulated genes remain unclear. Further research, such as experiments investigating overexpression, gene disruption, or the introduction of antisense RNA, would help to reveal these roles in the NUV response of *B. oryzae*. Several kinds of photoreceptor proteins in fungi have been identified, including the White Collar protein complex (WC-1/WC-2), phytochromes, cryptochromes, and opsins [2] [26]. On the other hand, only BLR1 and BLR2 were thought to be blue light-absorbing photoreceptors in *B. oryzae* [20] [27], whereas a putative UVB-absorbing photoreceptor has not been identified. Thus, the NUV-regulated genes, especially BLR1-independent genes, will be helpful targets for the identification of unknown UVB-absorbing photoreceptors in *B. oryzae*.

Acknowledgements

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Supplement

Table S1. Nucleotide sequences of the primers used in quantitative real-time PCR.

Gene	Forward primer (5' to 3')	Reverse primer (5' to 3')
NUV01	CCGGAGACAGGGTTGATCTT	GGTCAGCGTCAATGTCTAGGTATG
NUV02	CAAACATGTCTTCTCCAATC	GTATGCATCATGGAAGGACT
NUV03	TCTCCGCCTACCTAGCACAAG	TCCTCGCCCCTCCTCAAAAT
NUV04	GAATGGCACTGCAGCAAACTAT	ACTCCTTTGATGGCTCTGGGT
NUV05	GCCCTTCGCTACGCAAACTG	ATACTTTCCGCGGCCCTGTG
NUV06	GTCTCAGGAGGTGCCTACGA	CATGAGAAGCAGGGTCAAGAA
NUV07	CATTGGCAAATGAGCGTGGT	GTTTGGCACGGCAGCACGAA
NUV08	TCTGCTGCCTCGCCAACAC	GCCATGTCGTCTTGCTTCCA
NUV09	GATTGTAGGCGGTGGAGGTA	GGATGCCGTCTAACAAAGCA
NUV10	CGCAGGGTCATTGACTACTTTG	TCAGCTGCAATCACAACATCTG
NUV11	TTATGTCAGGCGGGCTGTTC	TGCGGAAGCACCAAGAGTGG
NUV12	ATTGAGGATAACGGCCGTAT	AGCGTGGTCCTTGACACCAA
NUV13	GCCCAATTCCAGCTCCATTA	ACGCCATTCTCGTTGGAAGA
NUV14	CCAAAGCCGGCATATGGAA	TTGGTTCGAGGGCGTCAGAT
NUV15	GGCGAGTGGAAGAAGATGGT	CGCCCATACTAGGAACCTGATC
NUV16	CAACGACAATGTGCCTGGAT	CTGGCTTCCCTCAGACTTTG
NUV17	CACAAGAGGAAGAGGGAGCTT	AGACGCCCTCTGGTGAATCT
NUV18	CCAGGACATTGCAAAGATGA	TGCATCTTGAACGAGCTCACT
NUV19	GCAACGGCATCTACGACCTG	GACACCAGTGAGGTAATCTG
NUV20	TACAAGGGTCATGCGCATGG	ATCACTGTTCAAAAATCTTC
NUV21	ACTCGTGCCACAAGGTCACTT	TCAGATCAGTGGTGCACATGC
NUV22	CGCTCTTTGAAAGCACCATGT	CAAAGTCTTCGGTGAGGTGATG
NUV23	GGTCATGAACGGCAGAGTCG	CTCGGAAATCTCGGGGACAA
NUV24	TTGACGAGGCAACCGAAGCT	TTGCGCATTTCGGAGAAGTA
NUV25	GGCATGTGGATTGGCTACCA	TACGGCTCCACCCAATGTCT
NUV26	CCCACAACGAGTGGAAGCTT	AGCGTGTGGGCCTACTTCAA
NUV27	CTCTCTACGAGGCCGTTTCG	TCGTCAAGGCGCGTGAGATA
NUV28	CCTCGATGTCGCCTGTTGCT	CCATTTTCAACTCGCACCCATA
NUV29	CCACTCCTCAAACTTCTCGAAAT	CCAATCTTTCCATAGCCATCAT
NUV30	GACAGCGCCCGTTTTCATT	CATCTATCACCGGCTTCACCTT
NUV31	GCTCTGCTGGCATCTTTGC	ATCGGCTTGCCCAACCTTA
NUV32	CAAGTTTGTGCACCAACAGA	CCAGAGCTCCCTGTCTTCTT
NUV33	CGTCCTTCTTGCCGAGAAAG	TCCGAGACGGTTGTCACTGA
NUV34	TCCAGAGCATCAATTACCACA	GACCTTGCGGAGGATGAA
NUV35	AGTATGGCGCGGCTGGACTT	AGATGCTCTCAAGCGGATGCA
NUV36	GCATGTCGGCCTACTTCAGC	GTTGAATTTGAGGTTCTGCG
NUV37	CCAGCCTGGACTGCTGAGTAT	ACATGTTTTCCGTCCAGATGTG
NUV38	ATATGCGTGGAAGACGGTAGAG	GCTCCCAAACGTTGCGATA
NUV39	CCAATTCGACTGGCACGA	GCGGATGATGGAGAGATCAAAG
NUV40	ATCCCACAAAACAGACAGCATC	CACTACTCACAATCTACTCCCGACA
NUV41	TACCAAGATCTCAACTCTCCCTTTC	GGTTTGGCGGGTCATCTTC
NUV42	CATGAAGGCCTACCCCAAGA	CCTCAGTGTTGTAAGGGAACCAC
NUV43	GCAAAGTATCCCGTGGTCATC	GACCCAACAGCGTCTTGAAC
NUV44	GTGGGGTATTGTAAGAGACATTTGG	CAAAGCATCATGTCCCCAGTT
NUV45	TCAAGCCCAAATCCCTCAC	TGTTTCCGTCAACGACTTCATAC
NUV46	ACGCTTCTCTCCAACGGTATCT	CGCTACGGGCTAAACATGAAC
GPD	AACGGCAAGACCATCCGTT	GACGACGTAGTAAGCGCCAGT