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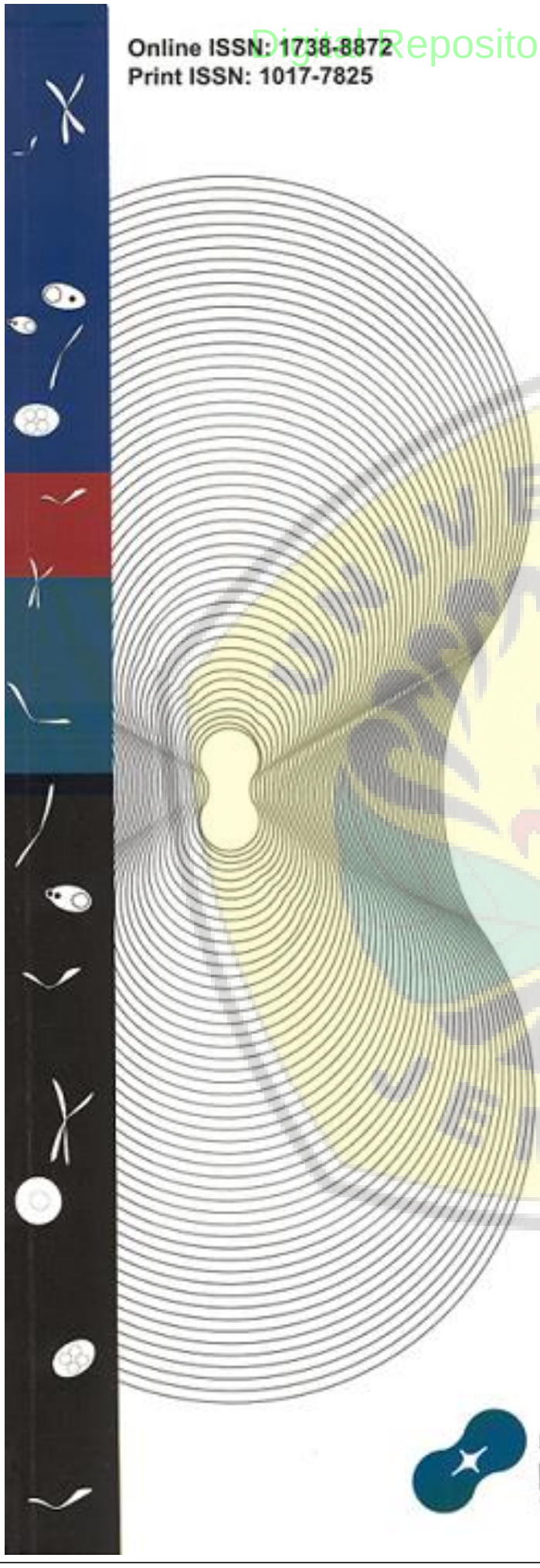

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JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

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3. Gershon AA, Russa P La, Steinberg SP. 1999. Varicella-zoster virus, pp. 900-911. *In* Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH (eds.), *Manual of Clinical Microbiology*, 7th Ed. American Society of Microbiology, Washington, D.C.

4. Kirkman TW. 1996. Statistics to Use. Available from http://www.physics.csbsju.edu/stats/. Accessed Nov. 20, 2011.

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- SI (Systeme International d'Unites) units and quantities should be used (see Tables below) (see http://www.bipm.fr/enus/3_SI/si.html) but Å, cal, p.p.m. can be used where appropriate.
- Leave a space between a number and its unit of measure. (Exception, do not leave a space between a number and the temperature degree, percent, angular degree, angular minute, or angular second symbols, 15°C, 50%, 90°, 75', 18").
- Use a slash (/) in units of measure (i.e., g/ml rather than g ml^{-1}).
- Use a slash (/) in spelled-out units of measure, not the word "per", before the abbreviation for a unit in complex expressions. e.g., 50 μg of peptide/ml, 25 mg of drug/kg of body weight, 10 counts/s, 12 domains/cm^3, 2×10^3 ions/min, 0.8 keV/channel, and 125 conversions/mm^2.
- Do not add an "s" to make the plural of any abbreviated units of measure. e.g., 50 mg (not 50 mgs), 3 mol (not 3 mols).
- Do not mix abbreviations and spelled-out units within units of measure.
- Do not capitalize surnames that are used as units of measure.
- Centrifugal force should be preferably expressed as ×g, rather than rpm.
- L-amino acid, D-amino acid (i.e., LD in small caps).
- Names for regional bioproducts should be written in non-italicized lowercase letters. The names should be explained in a parenthesis

when used first time in the abstract/text. (e.g., kimchi (Korean traditional fermented cabbages)).
• Usage should be consistent within a paper.

Table 1. Abbreviations

Name	memo	Name	memo
DNA	Deoxyribonucleic acid	$NADP^+$	Nicotinamide adenine dinucleotide phosphate, oxidized
cDNA	Complementary DNA	Poly(A) and poly(dT), etc.	Polyadenylic acid and polydeoxythymidylic acid, etc.
RNA	Ribonucleic acid	Oligo(dT), etc.	Oligodeoxythymidylic acid, etc.
cRNA	Complementary RNA	UV	Ultraviolet
RNase	Ribonuclease	PFU	Plaque-forming units
DNase	Deoxyribonuclease	CFU	Colony-forming units
rRNA	Ribosomal RNA	MIC	Minimal inhibitory concentration
mRNA	Messenger RNA	Tris	Tris[hydroxymethyl] aminomethane
tRNA	Transfer RNA	DEAE	Diethylaminoethyl
AMP, ADP, ATP, dAMP, ddATP, and GTP, etc.	For the respective 5' phosphates of adenosine and other nucleosides	EDTA	Ethylenediamine tetraacetic acid
ATPase and dGTPase, etc.	Adenosine triphosphatase and deoxyguanosine triphosphatase, etc.	EGTA	Ethylene glycol-bis[β-aminoethyl ether]-N,N,N',N'-tetraacetic acid
NAD	Nicotinamide adenine dinucleotide	HEPES	N-2-hydroxyethyl piperazine-N'-2-ethanesulfonic acid
NAD^+	Nicotinamide adenine dinucleotide, oxidized	PCR	Polymerase chain reaction
NADH	Nicotinamide adenine dinucleotide	AIDS	Acquired immune deficiency syndrome
NADPH	Nicotinamide adenine dinucleotide phosphate		

Table 2. Multiplying SI Prefixes

Factor	Prefix	Symbol
10^1	deca	da
10^2	hecto	h
10^3	kilo	k
10^6	mega	M
10^9	giga	G
10^{12}	Tera	T
10^{15}	peta	P
10^{18}	exa	E
10^{21}	zetta	Z
10^{24}	yotta	Y
10^{-1}	deci	d
10^{-2}	centi	c
10^{-3}	milli	m
10^{-6}	micro	μ
10^{-9}	nano	n
10^{-12}	pico	p
10^{-15}	femto	f
10^{-18}	atto	a
10^{-21}	zepto	z
10^{-24}	yocto	y

Table 3. SI-Derived Units

Name	Symbol	Quantity	In terms of other units	In terms of SI based units
becquerel	Bq	activity (of a radionuclide)	-	s^{-1}
coulomb	C	quantity of electricity, electric charge	-	s·A
farad	F	capacitance	C/V	$m^{-2}·kg^{-1}·s^4·A^2$
gray	Gy	absorbed dose, kerma, specific energy imparted	J/kg	$m^2·s^{-2}$
henry	H	inductance	Wb/A	$m^2·kg·s^{-2}·A^{-2}$
hertz	Hz	frequency	-	S^{-1}
joule	J	energy, work, quantity of heat	N·m	$m^2·kg·s^{-2}$
lumen	lm	luminous flux	cd·sr	$m^2·m^{-2}·cd=cd$
lux	lx	illuminance	lm/m^2	$m^2·m^{-4}·cd=m^{-2}·cd$
newton	N	force	-	$m·kg·S^{-2}$
ohm	Ω	electric resistance	V/A	$m^2·kg·S^{-3}·A^{-2}$
pascal	Pa	pressure, stress	N/m^2	$m^{-1}·kg·S^{-2}$
siemens	S	conductance	A/V	$m^{-2}·kg^{-1}·s^3·A^2$
sievert	Sv	dose equivalent	J/kg	$m^2·s^{-2}$
tesla	T	magnetic flux density	Wb/m^2	$kg·s^{-2}·A^{-1}$
volt	V	electric potential, potential difference, electromotive force	W/A	$m^2·kg·s^{-3}·A^{-1}$
watt	W	power, radiant flux	J/s	$m^2·kg·s^{-3}$
weber	Wb	magnetic flux	V·s	$m^2·kg·s^{-2}·A^{-1}$

Table 4. SI-Derived Compound Units

Name	Symbol	Quantity	In terms of other units
ampere per meter	A/m	Magnetic field strength	-
ampere per square meter	A/m^2	Current density	-
candela per square meter	Cd/m^2	Luminance	-
coulomb per cubic meter	C/m^3	Electric charge density	$m^{-3}·s·A$
coulomb per kilogram	C/kg	Exposure (X-rays and γ-rays)	-

Table 4. Continued

Name	Symbol	Quantity	In terms of other units
coulomb per square meter	C/m^2	Electric flux density	$m^{-2}·s·A$
cubic meter	m^3	Volume	-
cubic meter per kilogram	m^3/kg	Specific volume	-
farad per meter	F/m	Permittivity	$m^{-3}·kg^{-1}·s^4·A^2$
henry per meter	H/m	Permeability	$m·kg·s^{-2}·A^{-2}$
joule per cubic meter	J/m^3	Energy density	$m^{-1}·kg·s^{-2}$
joule per kelvin	J/K	Heat capacity, entropy	$m^2·kg·s^{-2}·K^{-1}$
joule per kilogram	J/kg	Specific energy	$m^2·s^{-2}$
joule per kilogram kelvin	J/(kg K)	Specific heat capacity, specific entropy	$m^2·s^{-2}·K^{-1}$
joule per mole	J/mol	Molar energy	$m^2·kg·s^{-2}·mol^{-1}$
joule per mole kelvin	J/(mol K)	Molar entropy, molar heat capacity	$m^2·kg·s^{-2}·K^{-1}·mol^{-1}$
kilogram per cubic meter	Kg/m^3	Density, mass density	-
meter per second	m/s	Speed, velocity	-
meter per second squared	m/s^2	Acceleration	-
mole per cubic meter	mol/m^3	Concentration (amount of substance per volume)	-
newton-meter	N·m	Moment of force	$m^2·kg·s^{-2}$
newton per meter	N/m	Surface tension	$kg·s^{-2}$
pascal second	Pa·s	Dynamic viscosity	$m^{-1}·kg·s^{-1}$
radian per second	rad/s	Angular velocity	-
radian per second squared	rad/s^2	Angular acceleration	-
reciprocal meter	m^{-1}	Wavenumber	-
reciprocal second	s^{-1}	Frequency	-
square meter	m^2	Area	-
square meter per second	m^2/s	Kinematic viscosity	-
volt per meter	V/m	Electric field strength	$m·kg·s^{-3}·A^{-1}$
watt per meter kelvin	W/(m K)	Thermal conductivity	$m·kg·s^{-3}·K^{-1}$
watt per square meter	W/m^2	Heat flux density, irradiance	$kg·s^{-3}$
watt per square meter steradian	$W/(m^2 sr)$	Radiance	-
watt per steradian	W/sr	Radiant intensity	-

SEQUENCE DATA FORMATS

Diagrams of nucleotide and amino acid sequences should be prepared in the most effective layout. The layout should be designed to fit the journal page economically, i.e. to fill either the full width of the page (176 mm) or a single column (84 mm). The height of the characters should be about 1.5–2 mm (or 6–8 point). For sequence data at full-page width with this size of type, a layout with 80–100 nucleotides per line is appropriate (or 60–70 if there are spaces between the codons). A single-column layout would ideally fit 50–60 nucleotides per line. If possible, lines of nucleic acid sequence should be subdivided into blocks of 10 or 20 nucleotides by spaces within the sequences or by marks above it. There should not be too much space between the lines of sequence. Use of the single-letter amino acid code is preferred.

MICROARRAY DATA

Data from microarray gene expression studies must comply with the MIAME guidelines (see http://www.mged.org/Workgroups/MIAME/miame.html).

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Identification of Chinese Cabbage Sentrin as a Suppressor of Bax-Induced Cell Death in Yeast

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Studies into the cell death program termed apoptosis have resulted in new information regarding how cells control and execute their own demise, including insights into the mechanism by which death-preventing factors can inhibit Bax-induced caspase activation. We investigated high temperature stress-induced cell death in *Brassica rapa*. Using a yeast functional screening from a *Brassica rapa* cDNA library, the BH5-127 EST clone encoding an apoptotic suppressor peptide was identified. However, a phylogenic tree showed that BH5-127 clusters within a clade containing SUMO-1 (Small Ubiquitin-like Modifier-1). BH5-127 was confirmed similar to have function to SUMO-1 as Fas suppression. Expression of BH5-127 showed that substantial suppression of cell death survived on SD-galactose-Leu$^-$-Ura$^-$ medium. The results suggest that BrSE (*Brassica rapa* Sentrin EST, BH5-127) is one of the important regulatory proteins in programming cell death, especially in the seedling stage of Chinese cabbage.

Keywords: Apoptosis, *Brassica rapa*, sentrin, SUMO-1, yeast functional screening

Apoptosis is the process of programmed cell death (PCD) that may occur in cell organisms. The most downstream components of the cell death machinery identified to date are proteases known as caspases [11]. Although some of the interactions among known cell death components have been described, there are major gaps in the understanding of the cell death process. For example, the cellular targets of caspases that lead to cell death have not yet been fully described. Another example is the mechanism by which death-preventing members of the Bcl-2 family, such as Bax Inhibitor (BI-1), can inhibit Bax-induced caspase activation [13]. Overexpression of *Arabidopsis thaliana* BI-1 that is a suppressor of cell death increased resistance to Bax-induced cell death in this plant [6]. Genetic engineering and modern molecular breeding methods have been used to isolate key genes involved in stress responses in plants. Functional screening of microorganisms that express heterologous cDNA libraries is a powerful tool for identifying genes with a specific function. Functional screening in yeast to identify genetic determinants capable of conferring abiotic stress tolerance in *Jatropha curcas* has been reported [3].

Recently, PCD in plant cells under abiotic stresses has also been investigated. Expression of BI-1 in *Arabidopsis* is induced under high temperature and reactive oxygen stress condition. Heat shock has been implicated in inducing abiotic stress factors including expression of BI-1 genes [5, 17]. The yield losses due to high temperature are large and are often combined with losses from other environmental stress. Chinese cabbage (*Brassica rapa*) is more sensitive to heat stress than other *Brassica* species. Although observation did not indicate the exact developmental phase at when the reproductive organs are susceptible to heat stress, pods that had passed the critical threshold developmental phase tolerated heat stress. The temperature effect on reproductive organs appeared to be responsible for the reduction in yield [1]. Expression profiling of *Brassica rapa* showed that heat treatment triggered significant accumulation of heat shock proteins, and some cell-wall-modifying genes related to thermotolerance were up-regulated. With respect to temperature shift, severe heat stress induces an alarming situation leading to irreversible

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injury and cell death, whereas moderate heat stress triggers thermotolerance [20].

It has been demonstrated that abiotic stresses cause a significant reduction in the potential yield of crops, and this loss will only be worsened with the dramatic climate change expected in the near future [11]. Abiotic stress is widely considered a detrimental factor in crop production. In this study, we report the existence of a *Brassica rapa* EST encoding the peptide as an apoptotic suppressor otherwise known as an antiapoptotic factor under high temperature stress.

Materials and Methods

Plant Material and Stress Test

Seeds of the *Brassica rapa* L. ssp. *pekinensis* inbred line "Chiifu" (289001) were received from the Brassica Resource Bank, Daejon, Korea. Seeds were germinated in Korean artificial soil and grown for 28 days in a growth chamber as previously described [10]. Three-week-old plants were subjected to a heat stress treatment, which was a growth chamber heated to 35°C (16 h day/8 h night, RH 50%) for one week. After the heat stress treatment, the leaves of the Chinese cabbage were sampled [1]. The plants that had no treatment were sampled as a control.

RNA Isolation and cDNA Library Construction

Total RNA was isolated using the RNeasy Plant Mini Kit (qiagen, Germany) according to the manufacturer's instructions, and the RNA quality and quantity were monitored visually using RNA gel electrophoresis and spectrophotometrically using a Nanodrop instrument (Nanodrop 2000 Thermoscientific, Wilmington, DE, USA). The cDNA library was created from total RNA using a TaKaRa cDNA Library kit (TaKaRa Code 6119 and 6130, Takara Bio Inc, Japan). After digestion with *Eco*RI and *Xho*I, cDNA derived from Chinese cabbage was inserted into the *Eco*RI and *Xho*I sites of vector pYX112 (Ingenius, Wisbaden, Germany), carrying an ARS/CEN replicon, the triose-phosphate isomerase promoter, and selectable marker *URA3*. The cDNA library was used to transform *Escherichia coli* (DH5α), and then 1.8×10^3 independent clones were pooled and amplified in a 10 ml culture to prepare plasmid DNA [15, 19].

Yeast Functional Screening

A plasmid-DNA-inserted Chinese cabbage cDNA library was transformed into yeast *Saccharomyces cerevisiae* strain QX95001 [18]. The transformation was performed using the lithium acetate method following heat shock for 5 min at 42°C. Transformants were grown on glucose-containing synthetic dropout medium lacking leucine and uracil at 30°C and screened for Bax-resistant transformants by plating on solid medium containing glucose and galactose with amino acids but lacking leucine and uracil. Plates were incubated at 30°C for 3 days. Yeast cell growth was determined by measuring the OD_{660} of a yeast suspension culture [11]. The clones were transferred to galactose-containing medium to induce cell death expression. The OD_{660} was measured after culturing for 48 h. The number of viable cells was counted by the addition of Evans blue. Evans blue penetrates only dead cells and results in a blue staining of the cellular contents. The percentage of dead cells generated by each treatment was determined by scoring the cells using a microscope [19]. Functional characterization of BH5-127 in suppression of cell death was shown by transformation of *S. cerevisiae* and heat stressed at 35°C for 72 h. Yeast cell growth was determined by measuring the OD_{660} of a yeast suspension culture.

Yeast Plasmid Isolation, RT-PCR, Construction of GFP, and Data Analysis

Isolation of yeast plasmid was performed using a QIAprep Spin Miniprep kit using a microcentrifuge (Qiagen, Germany) with modification in the membrane lyses stage. The yeast cell pellet was suspended in 250 µl of Buffer P1 containing RNase A by a TissueLyser (Qiagen, Germany). Cells were lysed by adding 250 µl of Buffer P2 from the Qiagen kit and mixed by inverting the tube, followed by 10 min incubation at 22°C. During this period, cell lysis causes release of DNA and the mixture in the tube becomes viscous. At this stage, 350 µl of Buffer N3 was added to the lysate, and after mixing by gentle inversion, the tube was incubated on ice for 30 min. Subsequently, the protocol of the QIAprep Spin Miniprep Kit using a microcentrifuge was followed [13]. Yeast plasmids were analyzed with PCR using pYX primers: 5'-end forward primer (5'-GGAGTTTAGTGAACTTGC-3') and 3'-end complementary strand reverse primer (5'-GGCATGTATCGGTCAGTC-3') with annealing temperature of 56°C for 30 s. The PCR product profiles were monitored by agarose gel electrophoresis.

Thereafter, 500 ng of purified RNA from *Brassica rapa* was analyzed by RT-PCR using SuperScript III One-step RT-PCR with Platinum *Taq* DNA Polymerase (Invitrogen Co., Carlsbad, CA, USA). RT-PCR was performed using 20 pmol of specific primers for *Brassica rapa* sentrin (BrS): 5'-end forward primer (5'-CGAGCGCGTGGTACCACGGT-3') and 3'-end complementary strand reverse primer (5'-TACTTAGTCGACTCTAGACC-3'). The cycling condition for PCR was 94°C for 2 min, 94°C for 15 s, 64°C for 30 s, and 72°C for 1 min. The amplification reaction was carried out for 40 cycles with a final extension at 72°C for 10 min.

The sequencing of Chinese cabbage cDNA conferring resistance high temperature stresses was performed by Solgent, Korea. We performed computational searches using BLAST sequence databases through the National Center of Biotechnology Information (NCBI; http://blast.ncbi.nlm.nih.gov/) to understand and assign functional classes the sequence information determined in this screening. Identification of amino sequence and homology was performed using GENETYX-WIN Version.3.1. The phylogenic tree was constructed by UPGMA using MEGA4 [7].

The indication of intracellular localization was analyzed by GFP construction. The indicated antiapoptotic gene from Chinese cabbage was constructed into vector pBin-GFP. The construction was performed by digestion with *Sal*I and *Nco*I and was inserted into the pBin-GFP vector. We confirmed the intracellular distribution of GFP-tagged BH5-127 by MitoTracker, a mitochondrion-selective probe (Molecular Probes, Inc. M-7513).

Results and Discussion

Brassica rapa was subjected to high temperature treatment (35°C) after the plant was 28 days old. The height, amount of leaves, and biomass were measured and compared

Table 1. Characteristics of growth to 35°C in *Brassica rapa*, "Chiifu".

Temperature treatment (°C)	Biomass		Leaf no.		Plant height (cm)	
	Fresh weight (mg)	Dry weight (mg)	27d[y]	40d	27d	40d
35	460.2±87.7[z]	35.0± 7.3	10.0±2.1	11.4±2.3	12.9±4.8	14.7±4.3
25	454.6±56.4	73.4±13.9	12.0±1.2	17.4±2.0	14.8±1.0	15.8±1.6

[z]mean±standard deviation, [y]d : days after treatment.

before being sampled. The leaves of *Brassica rapa* after high temperature stress treatment at 35°C showed that discoloration, quantity of height, amount of leaves, and biomass were lower than *Brassica rapa* control, which was incubated at 25°C (Table 1). High temperature can cause considerable harvest damages, including scorching of plant leaves, branches, and stems, root growth, and reduced yield [16]. Therefore, the discoloration of leaves and the less harvest had appeared in *Brassica rapa*.

Yeast cells containing pYX112-cDNA were used to screen for *Brassica rapa* genes that can inhibit yeast growth. The QX95001 contains a galactose inducible promoter (*GAL*1), whereas pYX112 contains a constitutive promoter (*TPI*). The clones that survived on a galactose medium were isolated. The clones contain the genes that inhibit yeast growth and whose activities are suppressed [8]. Screening of 528 clones of high-temperature-induced *Brassica rapa* cDNA identified about 39 clones that survived following Bax expression on galactose-containing medium, and 7 colonies were recovered that exhibited substantial suppression of cell death on galactose-containing medium (Table 2). Meanwhile, screening of 324 clones of Chinese cabbage cDNA resulted in 5 clones growing on galactose-containing medium, and no colony was recovered after suppression of cell death. Yeast clones that survived on galactose-containing medium were isolated and PCR performed using the pYX-forward and pYX-reverse primers. The gel analyses of amplification of the double-stranded cDNA library displayed large sizes over 300 bp that were assumed to be the insertions of the cDNA target in the vector. Analysis of DNA sequence information from yeast transformants demonstrated that 7 sequences encoded an expressed sequence tag (EST) as apoptosis suppressor. One of the EST clones, BH5-127 (Accession No. AB609070), contained SUMO-1 (Fig. 1). Nucleotide sequence analysis of SUMO-1 indicated that this clone is 293 nucleotides long and encoded a functional polypeptide of 98 amino acids (Fig. 1A). The predicted sequence was derived from the databases containing cDNA sequences at NCBI and are 63.9% identical to Sentrin mRNA from *Cervus nippon* (Accession No. AF242526.1; data not shown). As shown in Fig. 1B, Sentrin/SUMO-1 protein showed sequence homology to *Lycopersicon esculentum* (AJ012717), *Zea mays* (FJ515940.2), *Oryza sativa* (X99608), and *Arabidopsis* (X99609). This protein has homology with the *Cervus nippon* Sentrin protein (50%) and with *Homo sapiens* SUMO-1 protein (Accession No. NM003352.4; 16.7%). There was also weak homology with AtBI-1 (25%). A phylogenetic tree analysis of Sentrin/SUMO-1 protein from plant, animal, and human was constructed using the UPGMA (Unweighted Pair Group Method with Arithmetic Mean). A tree comprising Sentrin/SUMO-1 proteins in

Table 2. Identification of 39 clones that survived on selection galactose medium SD-galactose-Leu$^-$-Ura$^-$.

Putative characteristic	Clones
Prion protein	BH5-298
Proteinase inhibitor (serine)	BH5-289
Phosphoglucose isomerase PSI-1.1	BH5-294
Ferredoxin	BH5-287
Water stress inducible protein Rab21	BH5-127
Fe-superoxidase dismutase	BH5-25
SUMO (Small Ubiquitin like-Modifier)	BH5-127, BH5-132
Nothing significant found	BH5-2-1, BH5-14-2, BH5-10-3, BH5-11-5, BH5-277, BH5-362
No insert clones and small insert	BH5-18, BH5-275, BH5-207, BH5-12, BH5-13, BH5-32, BH5-91, BH5-94, BH5-118, BH5-216, BH5-222, BH5-223, BH5-251, BH5-268, BH5-277, BH3-274, BH3-290, BH3-299, BH3-362, BH3-244, BH3-245, BH3-246, BH3-271, BH3-272, BH3-283, BH3-285
Total	39 clones

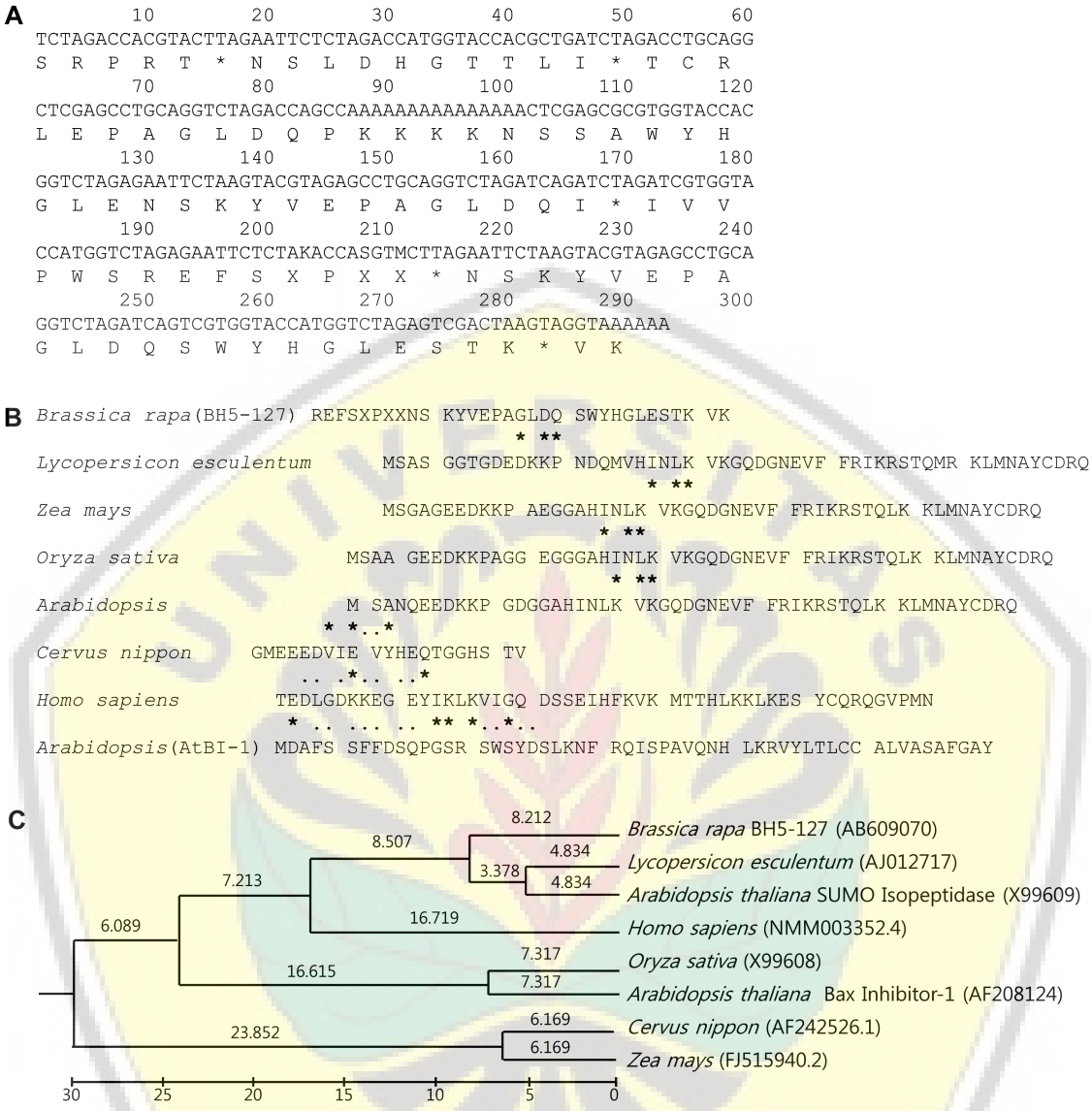


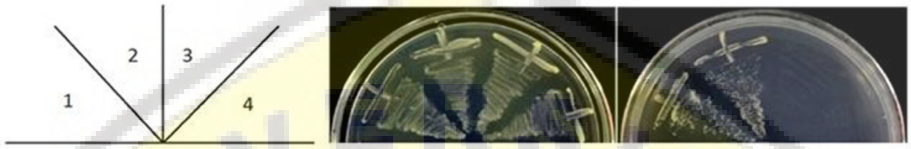
Fig. 1. Characteristics of BH5-127 screened from *Brassica rapa* "Chiibu" cDNA using yeast functional screening.
(**A**) DNA and amino sequence of coding region for BH5-127. (**B**) Homology of BH5-127. *,. **:** same and similar, respectively. (**C**) The phylogenic tree (neighbor-join, 100 bootstrap) from *Brassica rapa* EST (BH5-127), *Lycopersicon esculentum* Sentrin, *Arabidopsis thaliana* SUMO-1 Isopeptidase, *Homo sapiens* SUMO-1, *Oryza sativa* SUMO-1, *Arabidopsis thaliana* Bax Inhibitor-1, *Cervus Nippon* Sentrin, *Zea mays* SUMO-1.

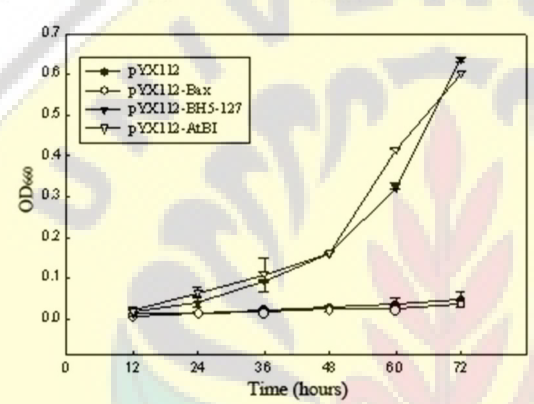
plant showed that *O. sativa*, *Arabidopsis*, and *Lycopersicon esculentum* are closely related to human Sentrin/SUMO-1. Otherwise, BH5-127 clusters within a clade contained SUMO-1 of *C. nippon*, which is implicated in suppressing cell death control (Fig. 1C). We named BrSE (*Brassica rapa* Sentrin EST) as Sentrin of Chinese cabbage.

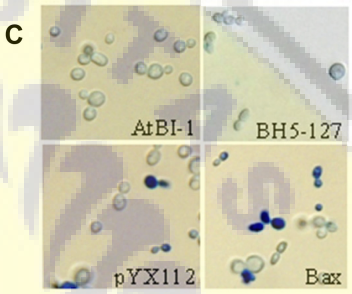
A cDNA library derived from *Brassica rapa* was constructed using a yeast expression vector, which was then screened for suppression of cell death in the *S. cerevisiae* strain QX95001 obtained by transforming wild-type yeast, BF264-15Dau, containing a Leu-marked plasmid with Yep51-Bax from which the full-length mouse Bax protein is expressed under the control of a galactose-inducible yeast *GAL1* promoter. Bax-induced cell death is initiated by transferring cells from glucose- to galactose-containing medium. As shown in Fig. 2A, yeast strain QX95001 transformed with the pYX112 vector, and pYX112-Bax did not grow when plated on galactose-containing medium. However, the clones AtBI-1 and BH5-127 survived and grew when plated on galactose-containing medium. The effect of apoptotic suppressor expression on yeast growth was determined by measuring the OD_{660} of the yeast culture suspension. The level of yeast growth was examined in SD-galactose-Leu^--Ura^- after 48 h at 30°C. The graph in Fig. 2B shows that AtBI-1 yeast culture growth reach 0.41, which was higher (0.15) than BH5-127

A

No.	Vector combination	Selection medium	
		SD-Glucose-Leu$^-$-Ura$^-$	SD-Galactose-Leu$^-$-Ura$^-$
1	pYX112-AtBI-1	+	+
2	pYX112-BH5-127	+	+
3	pYX112-Bax	+	-
4	pYX112	+	-







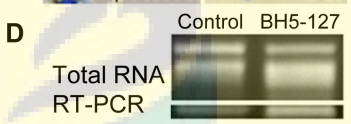


Fig. 2. Comparison of growth-suppression activities of BH5-127 in yeast.
(**A**) Yeast cells transformed with plasmids containing galactose-inducible BrSE (pYX112-BrSE), AtBI-1 (pYX112-AtBI-1), Bax (pYX112-Bax), and vector (pYX112) were cultured in glucose-containing SD medium. (**B**) Yeast growth culture is shown by OD_{660} measurements using galactose-containing medium. + : a clone showed on the medium. - : no clone showed on the medium. (**C**) The cell visualization of yeast growth culture after 14 h. Blue staining showed that the yeast cell was death. (**D**) BrSE (*Brassica rapa* Sentrin EST) was confirmed by RT-PCR.

yeast culture. The capability of yeast as an apoptotic suppressor was expressed by both AtBI-1 (0.41) and BH5-127 (0.15) yeast cultures. Strains transformed with the pYX112 vector or pYX112-Bax grew less than those expressing AtBI-1 or BH5-127, consistent with the fact that neither pYX112 nor pYX112-Bax contained the apoptotic suppressor.

These results were confirmed by visualizing yeast cells, where the BH5-127 clone prevented Bax-induced cell death. The number of dead cells was counted by Evans Blue staining. As shown in Fig. 2C, the cells began to die after 14 h of Bax-induced cell death expression. Visualization of strains expressing AtBI-1 or BH5-127 showed that the cells were still intact. The normal number of dead cells in yeast under these conditions was demonstrated by the pYX112 vector and pYX112-Bax samples. Bax-induced cell death of QX95001 is triggered by transferring cells from glucose- to galactose-containing medium [11]. Detection of ROS generation by Evans Blue staining was used. This reagent diffuses through cell membranes and is subsequently enzymatically deacetylated and then oxidized by ROS [19]. Expression of the SUMO-1 RNA from *Brassica rapa* was confirmed by RT-PCR using specific primers (BrS): 5'-end (5'-CGAGCGCGTGGTACCACGGT-3') and 3'-end complementary strand (5'-TACTTAGTCGACTCTAGACC-3'). As shown in Fig. 2D, the expression of SUMO-1 was confirmed. BH5-127 showed stronger expression than the *Brassica rapa* control. Expression of the proapoptotic gene Bax in yeast results in cell death with an apoptotic phenotype, which is suppressed by coexpression of anti-apoptotic genes. BH5-127 was tagged with GFP to explore the intracellular localization of BH5-127. This indicated that fusion of BH5-127–GFP was localized to mitochondria in the yeast cell (Fig. 3).

Using a similar screening system, several plant genes were isolated as suppressors of Bax-induced cell death in yeast [13, 18, 19]. Functional characterization of BH5-127 in the suppression of cell death was shown by transformation

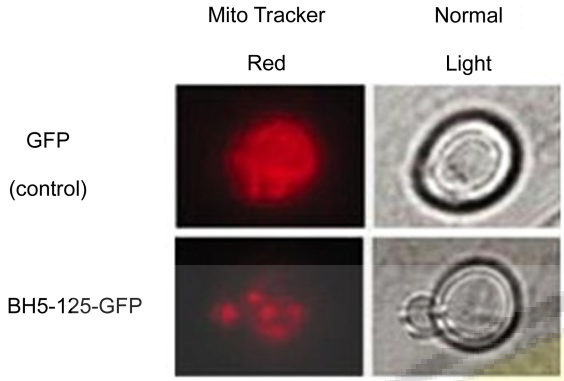


Fig. 3. Fluorescence distribution of GFP and GFP-tagged BH5-127 in yeast possessing Bax.
GFP and MitoTracker (left) are presented. Yeasts were cultured in glucose and galactose media until an OD_{660} of ~0.5 before observation.

of *S. cerevisiae* and heat stress at 35°C for 72 h. When exposed to heat stress conditions, the levels of ROS production also increased in transgenic and control yeasts, but only resulted in death of control yeast (transformed to pYX112). However, yeast expressing antiapoptotic protein was protected to a greater extent. Fig. 4. showed that transgenic yeast, including AtBI and BrSE, grew well under high temperature stress.

S. cerevisiae has been shown to be a useful model for apoptosis research. Features of metazoan PCD have been observed in yeast, where it has become evident that PCD occurs, exhibiting at least some of these features. Oxidative stress can cause cell death and occurs during heat-induced cell death in yeast. The use of yeast is a high throughput heterologous system for screening and identifying functionally relevant genes of interest that regulate apoptosis. Yeast may prove to be an attractive system to identify candidate plant genes with the functional ability to modulate plant cell death generated by ROS [2].

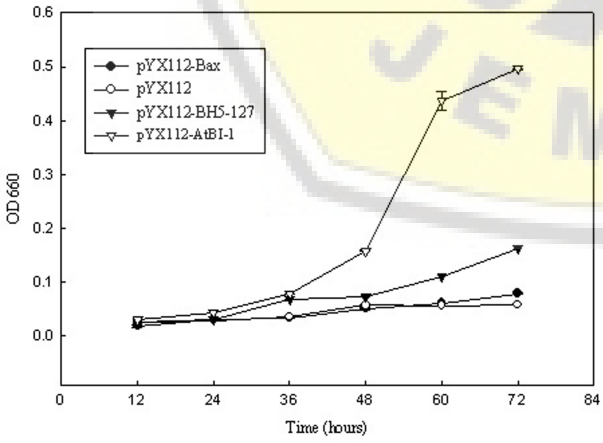


Fig. 4. Functional characterization of BH5-127 in suppression of cell death caused by high temperature.
Yeast cells were transformed to express AtBI-1 (pYX112-AtBI-1), BH5-127 (pYX112-BH5-127), vector (pYX112), or Bax (pYX112-Bax), and then high temperature stressed at 35°C for 72 h.

Another suppressor of Bax-induced cell death is dependent on the activation mechanism used to initiate programmed cell death. SUMO-1 is a protein that binds domains on the protein Fas but not FADD. SUMO-1 may inhibit FADD-dependent death by preventing aggregation of FADD on activated Fas and by inhibiting recruitment of caspase 8 [12]. The regulation of caspase and cell death by Bax is described in Fig. 5; Bax induces caspase activity. The most downstream components of the cell death machinery identified so far are proteins, known as caspases, a class of cysteine proteases that cleave substrates following aspartate residues. The activity of caspases can be regulated by a variety of cellular factors. Some, such as the *C. elegans* protein CED-4, the related mammalian protein Apaf-1, or the mammalian protein Fas, can activate caspases and are thus death promoters. Other proteins seem to promote cell death in part by inhibiting the activities of death-preventing proteins, such as BI-1 and SUMO-1. However, there are different mechanisms for inhibition by BI-1 and SUMO-1. BI-1 blocks cell death induced by Bax, but SUMO-1 inhibits the activation of caspases [13]. In mammalian cells, tumor necrosis factor (TNF) induces programmed cell death by binding to the Fas/APO-1 receptor. The presence of a "death domain" in the cytoplasmic region of the receptor is responsible for transducing the death signal. Using the death domain as bait in two-hybrid interaction assay, SUMO-1 can be isolated. SUMO-1 shows amino acid similarity to ubiquitin, Nedd8, and *S. cerevisiae* protein Smt3. SUMO-1 provides protection against both anti-Fas/APO-1 and TNF-induced cell death [4].

We have shown that the BrSE (*Brassica rapa* Sentrin EST) is a defense factor that can suppress a cell death inducible gene like Bax. By means of transient expression of individual proteins, we identified inhibition of plant PCD (programmed cell death) as the cell death mechanism

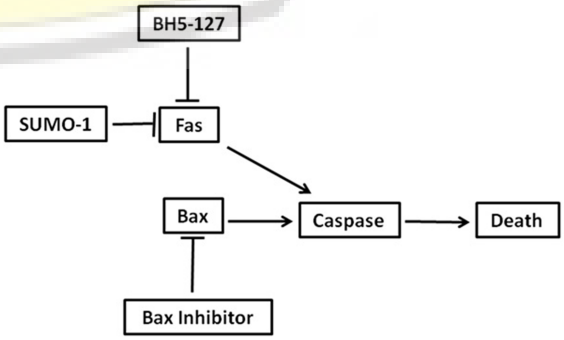


Fig. 5. A model for BH5-127-suppressed FAS in Chinese cabbage.
Blunt arrows indicate inhibition. Pointed arrows indicate activation.

of action of BrSE. Given the presumed importance of PCD in abiotic stress-based plant defense, it is logical that the AtBI-1 would target this process to cell death. It is possible that abiotic stress effectors that have been implicated in allowing plant growth to evade abiotic stress-based resistance also function using a similar mechanism. Kim *et al*. [9] reported the optimum concentration of difenoconazol for growth inhibition of Chinese cabbage for the summer production in the alpine region. This result reports that it is effective in adaptability elevation after implantation by making plug seedlings grow healthy in Chinese cabbage. Therefore, it is possible to develop a variety that can produce seedling of healthy Chinese cabbage if BrSE can be activated to control abiotic stress (high temperature) as investigated in this study.

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