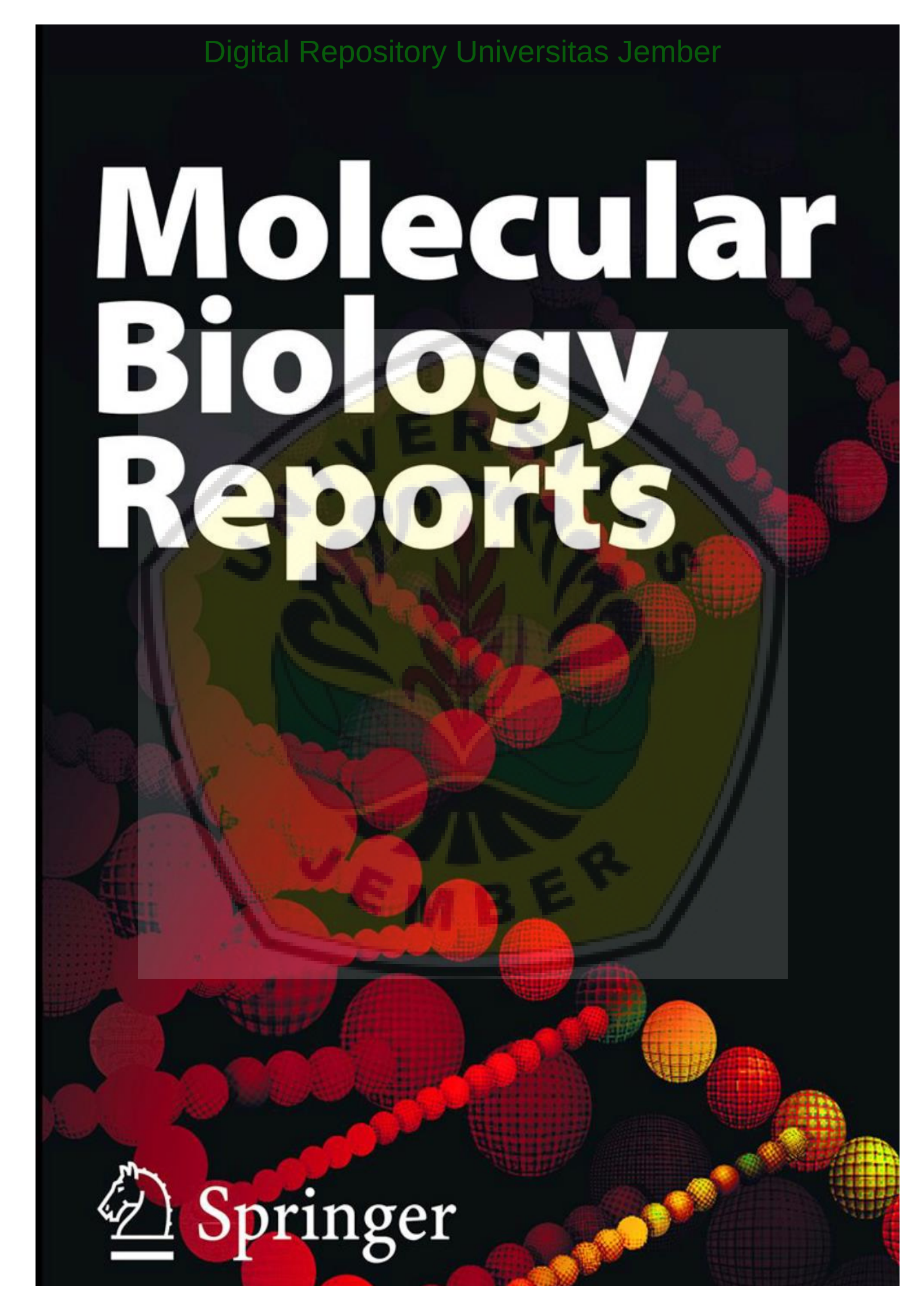
Molecular Biology Reports



Springer

Molecular Biology Reports publishes original research papers and review articles that demonstrate novel molecular and cellular findings in both eukaryotes (animals, plants, algae, funghi) and prokaryotes (bacteria and archaea).The journal publishes results of both fundamental and translational research as well as new techniques that advance experimental progress in the field and presents original research papers, short communications and (mini-) reviews.

Molecular Biology Reports focuses on:

All aspects of Molecular and Cellular Biology

All aspects related to the different structures and functions of the cell (eukaryotic and prokaryotic) and to its components (DNA, RNA, protein) are relevant to the scope of this journal

DNA replication, transcription, nucleic acid-protein interaction, RNA processing, intracellular transport, protein biosynthesis are examples of topics within the Molecular Biology field

Nuclear function, cytoskeleton and cell membrane interactions, transport of cellular products between different organelles fits in the Cellular Biology category.

Related subjects » Animal Sciences - Biochemistry & Biophysics

Abstracted/Indexed in

Science Citation Index, Science Citation Index Expanded (SciSearch), Journal Citation Reports/Science Edition, SCOPUS, EMBASE, Chemical Abstracts Service (CAS), Google Scholar, AGRICOLA, ASFA, Biological Abstracts, BIOSIS, CAB Abstracts, CNKI, Current Abstracts, Current Contents/ Life Sciences, EBSCO Biomedical Reference Collection, EBSCO Discovery Service, EBSCO STM Source, EBSCO TOC Premier, Elsevier Biobase, EMBiology, Gale, Gale Academic OneFile, Gale InfoTrac, Global Health, OCLC WorldCat Discovery Service, Pathway Studio, ProQuest Biological Science Database, ProQuest Biology Database, ProQuest Central, ProQuest Health & Medical Collection, ProQuest Health Research Premium Collection, ProQuest Medical Database, ProQuest Natural Science Collection, ProQuest Pharma Collection, ProQuest Science Database, ProQuest SciTech Premium Collection, ProQuest-ExLibris Primo, ProQuest-ExLibris Summon, Reaxys, Vitis - Viticulture and Enology Abstracts

Arit Ghosh
a6ghosh@ucsd.edu
University of California, San Diego
Expertise: ubiquitin biology, ribosome, E3 ligases, protein homeostasis, G protein signaling, GPCRs

Dan Zhao
danzhao@wakehealth.edu
Wake Forest School of Medicine,Winston-Salem, NC
Expertise: basic cancer research, breast cancer research, transcription factors, transcriptional regulation, microRNAs, long noncoding RNAs

Deeksha Gambhir Chopra
Deeksha.Gambhir@ucsf.edu
University of California, San Francisco
Expertise: diabetes and its complications (mainly diabetic retinopathy), age-related macular degeneration, inflammation, G-protein coupled receptor signaling, angiogenesis, unfolded protein response (UPR) and mitochondrial biology

Deepak Parashar
dparashar@mcw.edu
Medical College of Wisconsin
Expertise: the mechanism of tumorigenesis, Apoptosis and EMT in cancer cells, molecularly targeted and cellular therapies for breast , ovarian, and cervical cancer

Hadi Maazi
maazi@usc.edu
UCLA, Keck School of Medicine
Expertise: immunology, allergy, asthma, adaptive immunity, T cells, Innate Lymphoid Cells, IL-5, IL-13

Hagop Atamian
atamian@chapman.edu
Schmid College of Science & Technology
Chapman University, Orange, California
Expertise: molecular mechanisms of plant interactions with various biotic stresses and daily environmental fluctuations

Harikanth Venkannagari
havenkan@utmb.edu
University of Texas Medical Branch at GalvestonExpertise: structural biology, x-ray crystallography, assay development, high throughput screening, small molecule drug discovery, neurobiology, and ADP-ribosylation; molecular biology related research

Huiwen Wang
whw@moon.ibp.ac.cn
Institute of Biophysics of Chinese Academy of Sciences, China
Expertise: molecular cell biology, especially disease-associated RNA metabolism regulation

Kasturi Pal
kaspal@scripps.edu
The Scripps Research Institute
La Jolla, California
Expertise: cellular information processing pathways, focusing on signal transduction by G-protein coupled receptors (GPCRs); orphan GPCR, Gpr161, which localizes to a specialized sub-cellular compartment called the primary cilia

Liming Li
limingli@princeton.edu
Princeton University, NJ
Expertise: human population genetics and forensic genetics; population structure; Archaic Introgression

Mahsa Zarei
mzarei@bwh.harvard.edu
Brigham and Women's Hospital, Harvard University, MA
Expertise: inflammation, cancer biology and biochemistry; cellular metabolism and targeted therapy in disease

Mohammad Najaf-Panah
mjnp@nmsu.edu
New Mexico State University
Expertise: Bioinformatics and Computational Biology; Gene expression and transcriptomics; Molecular Biology; Nucleic Acids; Regulation of Gene Expression; Biotechnology, Cell Biology, Animal Genetics, Biochemistry, Omics; Human/Mouse/Plants/Mammalian Genetics

Narisimham Parinandi
Narasimham.Parinandi@osumc.edu
College of Medicine
Dorothy M. Davis Heart & Lung Research Institute
The Ohio State University Wexner Medical Center
Expertise: Lipidomics and Lipid Signaling; Vascular Biology; Oxidative Stress and Antioxidants; Membrane Biology; Mitochondrial Regulation; Trace Metal Biochemistry; Lung Biology and Pulmonary Diseases

Sarah Abou Alaiwi
sarah_aboualaiwi@dfci.harvard.edu
Dana Farber Cancer Institute
Matthew L. Freedman and Toni K Choueiri's Labs
Expertise: Liver disease, Liver function, Liver cell failure, End-stage liver disease, Bilirubin, Biliary disease, Chronic hepatitis, Viral hepatitis, Cirrhosis, Ascites, Chronic liver disease, Liver transplantation, primary biliary cholangitis, primary sclerosing cholangitis, Non Alcoholic Fatty Liver Disease, Alcoholic hepatitis, Liver Failure, Shock Liver, and Jaundice

Shuzhen Men
shuzhenmen@nankai.edu.cn
Department of Plant Biology and Ecology

Nankai University
Expertise: Auxin, root development, embryo, gametogenesis, lipids

Sourav Roy
sourav.roy@ucr.edu
Center for Disease Vector Research
and Institute for Integrative Genome Biology
University of California Riverside
Expertise: Gene regulation, Functional Genomics, Sequence Analysis, Motif Discovery, Expression Profiling, Protein-protein interaction, Regulatory Networks, Insect Hormones and miRNAs

Suranjana Goswami
sgoswam1@kent.edu
Kent State University
Kent, Ohio
Expertise: reproductive physiology, Immunology (specifically immune activation by infectious agents), metal induced immunotoxicity and biochemistry

Swetha Parvathaneni
swetha.parvathanen@Howard.edu
Howard University
Washington, D.C.
Expertise: DNA repair, genome stability, cancer predisposition syndromes, cancer initiation and progression; gene regulation and chemoresistance/drug targets/chemotherapy

Vikas Koundal
vikas.koundal@wsu.edu
Washington State University
Expertise: plant molecular biology, biotechnology, and plant pathology
plant diseases, plant pathogens, their genomics, management, and prevention, miRNAs, siRNAs, RNAi, Small RNAs, CRISPR

Table Contents

Isolation of microsatellite loci for the endangered vermetid gastropod Dendropoma lebeche using Illumina MiSeq next generation sequencing technology
Violeta López-Márquez, Ricardo García-Jiménez, Marta Calvo…

Original Article
Effect of explant type and plant growth regulators on callus induction, growth and secondary metabolites production in Cnidium officinale Makino
Muhammad Adil, Xiuxia Ren, Dong Il Kang, Luc The Thi…

Original Article
Genetic characteristics of the P1 coding region of Coxsackievirus A16 associated with hand, foot, and mouth disease in China
Li Xu, Dawei Cui, Lei Wang, Jun Cheng, Changgui Sun…

Original Article
A preliminary study of the relation between IL-4 and hypertension in type II diabetes mellitus
Eman Badr, Mohamed Assar, Elsayed I. Elshayeb…

Original Article
Neuregulin-1β modulates myogenesis in septic mouse serum-treated C2C12 myotubes in vitro through PPARγ/NF-κB signaling
Li Liu, Xueru Liu, Yiping Bai, Ni Tang, Jie Li, Yingying Zhang…

Original Article
Relationship between CETP gene polymorphisms with coronary artery disease in Polish population
Joanna Iwanicka, Tomasz Iwanicki, Paweł Niemiec…

Short Communication
Proteomic investigation of liver from beef cattle (Bos indicus) divergently ranked on residual feed intake
W. A. Baldassini, S. F. M. Bonilha, R. H. Branco…

Original Article
Identification of hub genes and analysis of prognostic values in pancreatic ductal adenocarcinoma by integrated bioinformatics methods
Yi Lu, Chujun Li, Honglei Chen, Weijie Zhong

Original Article
The clinical significance of miR-335, miR-124, miR-218 and miR-484 downregulation in gastric cancer
Ali Zare, Alireza Ahadi, Pegah Larki, Mir Davood Omrani…

Short Communication
Full sequence of the coat protein gene is required for the induction of pathogen-derived resistance against sugarcane mosaic virus in transgenic sugarcane
Retnosari Apriasti, Suvia Widyaningrum, Weny N. Hidayati…

Original Article
Isolation of a feather-degrading strain of bacterium from spider gut and the purification and identification of its three key enzymes
Fang Qu, Qingwang Chen, Yiying Ding, Zihao Liu, Yan Zhao…

Original Article
Polymorphisms in the 3′-UTR of SCD5 gene are associated with hepatocellular carcinoma in Korean population
Gyeong Im Yu, Kwang Ho Mun, Seon Hee Yang, Dong Hoon Shin…

Original Article
Milk fatty acid variability and association with polymorphisms in SCD1 and DGAT1 genes in White Fulani and Borgou cattle breeds
Isidore Houaga, Anne W. T. Muigai, Fredrick M. Ng'ang'a…
Download PDF (1155KB) View Article

Original Article
Insight into Arthrospira platensis Δ9 desaturase: a key enzyme in poly-unsaturated fatty acid synthesis
Faten Ben Amor, Hajer Ben Hlima, Slim Abdelkafi, Imen Fendri

Original Article
Introgression of UfCyt c6, a thylakoid lumen protein from a green seaweed Ulva fasciata Delile enhanced photosynthesis and growth in tobacco
Sweta K. Yadav, Kusum Khatri, Mangal S. Rathore, Bhavanath Jha

Original Article
Vitamin D receptor gene polymorphisms (Apa1 and Taq1) in temporomandibular joint internal derangement/osteoarthritis in a group of Turkish patients
Ayça Dilara Yilmaz, Duygu Yazicioglu…

Original Article
WUS and PIN1-related genes undergo dynamic expressional change during organ regeneration in response to wounding in Zoysia japonica
Linkun Li, Xusheng He, Fangdong Zhao, Chen Zhu, Huiming Zeng

Short Communication
Genetic diversity and maternal lineages of south Indian goats
Ranganathan Kamalakkannan, Jesna Jose, Subhash Thomas…

Review
Regulation and modulation of PTEN activity
Elahe Naderali, Amir Afshin Khaki, Jafar Soleymani Rad…

Original Article
Development of bovine embryos in vitro in coculture with murine mesenchymal stem cells and embryonic fibroblasts
Ivan J. Ascari, Sávio C. Martins, Luiz S. A. Camargo…

Original Article
Investigate of AQP gene expression in the liver of mice after ischemia–reperfusion
Solmaz Karimi, Saeid Reza Khatami, Negar Azarpira…

Original Article
Curcumin confers hepatoprotection against AFB1-induced toxicity via activating autophagy and ameliorating inflammation involving Nrf2/HO-1 signaling pathway
Ishfaq Muhammad, Xinghe Wang, Sihong Li, Rui Li, Xiuying Zhang

Original Article
A l-proline/O2 biofuel cell using l-proline dehydrogenase (LPDH) from Aeropyrum pernix
Aina Tonooka, Tomohiro Komatsu, Shino Tanaka, Hiroaki Sakamoto…

Original Article
177Lu-DOTA-coupled minigastrin peptides: promising theranostic agents in neuroendocrine cancers
Syed Faheem Askari Rizvi, Syed Ali Raza Naqvi, Samina Roohi…

Original Article
Development and characterization of a novel monoclonal antibody that recognizes an epitope in the central protein interaction domain of RapGEF1 (C3G)
Zareena Begum, Ch. Varalakshmi, Divya Sriram, Vegesna Radha

Original Article
Identification and analysis of dominant negative mutants of RIP1 DD that disrupt RIPoptosome core formation
Hyun Ji Ha, Hyun Ho Park

Original Article
Uncoupling Warburg effect and stemness in CD133+ve cancer stem cells from Saos-2 (osteosarcoma) cell line under hypoxia
Pavani Koka, Reddy Sailaja Mundre, Rohini Rangarajan…

Short Communication
ABI4 regulates the floral transition independently of ABI5 and ABI3
Kai Shu, Feng Chen, Wenguan Zhou, Xiaofeng Luo, Yujia Dai…

Original Article
Molecular dynamics simulation as a tool for assessment of drug binding property of human serum albumin
Meenu Narwal, Deepak Kumar, Tapan Kumar Mukherjee…

Original Article
Relationship between epigenetic marks and the behavior of 45S rDNA sites in chromosomes and interphase nuclei of Lolium–Festuca complex
Marco Tulio Mendes Ferreira, Laiane Corsini Rocha…

Original Article
Transgenic creeping bentgrass plants expressing a Picea wilsonii dehydrin gene (PicW) demonstrate improved freezing tolerance

Molecular Biology Reports (2018) 45:2749–2758
https://doi.org/10.1007/s11033-018-4326-1

SHORT COMMUNICATION

Full sequence of the coat protein gene is required for the induction of pathogen-derived resistance against sugarcane mosaic virus in transgenic sugarcane

Retnosari Apriasti[1] · Suvia Widyaningrum[1] · Weny N. Hidayati[1] · Widhi D. Sawitri[1,2] · Nurmalasari Darsono[3] · Toshiharu Hase[4] · Bambang Sugiharto[2,5]

Received: 11 July 2018 / Accepted: 20 August 2018 / Published online: 31 August 2018

Abstract

Sugarcane mosaic virus (SCMV) is a plant pathogenic virus of the family *Potyviridae* that causes chlorosis, stunting and significantly reduced sugar productivity in sugarcane. Pathogen-derived resistance is a method used to develop SCMV-resistant sugarcane by overexpression of viral DNA. In this study, the gene encoding the coat protein (CP) of SCMV was amplified by reverse transcriptase PCR from symptomatic sugarcane leaves and used to generate transgenic sugarcane. Nucleotide sequence analysis of amplified cDNA indicated that the 998-bp-long cDNA, termed *ScMVCp* cDNA, codes for the CP of SCMV from the PS881 isolate. The *ScMVCp* cDNA was inserted into the binary vector pRI101-ON with two constructs, a full nucleotide sequence (p927) and a sequence coding for N-terminally truncated protein (p702). The constructs were then introduced into sugarcane using *Agrobacterium*-mediated transformation. Southern blot analysis showed a single hybridized DNA copy inserted into the genome of transgenic sugarcane lines. The inserted genes were expressed at both the RNA transcript and protein levels in the transgenic sugarcane. The highest expression was found in transgenic lines 10, 11 and 13 from the p927 construct. Artificial infection by the virus showed that p927 generated a higher resistance to virus compared with p702. This resistance was passed on to the second generation of transgenic sugarcane with 100 and 20–40% levels of resistance in the p927 and p702 transgenic lines, respectively. This report shows that the full sequence of the CP gene is required to disrupt viral assembly and packaging, thereby generating resistance to SCMV infection.

Keywords Sugarcane mosaic virus · Pathogen derived resistance · Coat protein · Full DNA sequence · N-terminal deletion · SCMV-resistant sugarcane

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11033-018-4326-1) contains supplementary material, which is available to authorized users.

✉ Bambang Sugiharto
sugiharto.fmipa@unej.ac.id

1 Postgraduate Program for Biotechnology, University of Jember, Jember, Indonesia

2 Center for Development of Advanced Science and Technology (CDAST), University of Jember, Jl. Kalimantan No 37, Kampus Tegalboto, Jember 68121, Indonesia

3 Division of Biotechnology, PT. Perkebunan Nusantara XI, Surabaya, Indonesia

4 Division of Protein Chemistry, Institute for Protein Research, Osaka University, Suita, Osaka, Japan

5 Department of Biology, Faculty of Mathematic and Natural Science, University of Jember, Jember, Indonesia

Introduction

Sugarcane (*Saccharum* spp. hybrids) is an important sugar-producing plant grown in warm sub-tropical to tropical regions. The growth and production of sugarcane is affected by various environmental conditions, such as abiotic and biotic stresses. One of the most serious biotic stresses is sugarcane mosaic, a disease caused by the sugarcane mosaic virus (SCMV). Infection by the virus causes chloroplast damage, decreased photosynthesis and a sugar productivity loss of approximately 20–50% [1, 2]. Recently, we have found that the mosaic symptom is spread widely among sugarcane plants in Indonesia, with 78 and 65% disease incidence and severity, respectively [3]. SCMV has also been reported as the dominant viral pathogen in several countries, such as Argentina [4] and Cuba [5], while in China, SrMV (*Sorghum mosaic virus*) is a major sugarcane mosaic

disease, rather than SCMV [6]. Thus, several strategies have been developed to overcome SCMV infection, such as viral elimination using meristematic culture or antivirus and hot water treatments [7–9]. However, these methods are not able to fully protect against the spread of SCMV infection in sugarcane fields [10].

There are a range of strategies to obtain virus-resistant plants, either through introducing genes that confer natural resistance in susceptible plants through conventional breeding programmes or through genetic engineering. Expression in transgenic plants of viral genes or nucleotide sequences derived from a virus is referred to as pathogen-derived resistance (PDR), and this expression can induce resistance [10, 11]. The gene coding for coat protein (CP) has been widely used to induce resistance in plants since the first reported CP was able to mediate resistance against tobacco mosaic virus (TMV) in transgenic *Nicotiana tabacum* [12]. Since that time, many transgenic plants resistant to the virus have been developed using CP-mediated resistance (CPMR) in cases such as soybean mosaic virus (SMV) [13] and soybean dwarf virus (SbDV) [14] in soybeans and cucumber mosaic virus (CMV) in *N. tabacum* [15]. In addition, a transgenic sugarcane line resistant to SCMV has also been examined for agronomic performance and yield characteristics in the field [16]. However, the report of transgenic sugarcane resistance to SCMV lacked evidence from molecular analyses and only focused on the field performance of transgenic SCMV-resistant sugarcane.

SCMV belongs to the *Potyvirus* subgroup and is a positive sense single-stranded RNA virus with an open reading frame and an approximately 10 kb genome encoding for 10 functional proteins, including a CP that is located at the C-terminus [17]. The complete genomes of SCMV isolated from several geographic regions, such as from Argentina (JX237862.1), Australia (AJ278405), China (AF494510.1, JN021933.1), Iran (KT895081.1), and Mexico (GU474635.1), have been published in the NCBI GenBank database. The CPMR mechanism postulates that CP expression in host plants involves blocking disassembly of the infecting virus [10]. However, it has been suggested that the effectiveness of resistance is determined by the level of amino acid sequence similarity of CP between the transgenic plant and the infecting virus [11, 18]. For example, a mutation of the TMV virus containing the amino or carboxyl termini was unable to overcome CPMR in transgenic plants that contained TMV CP [19]. A similar result was reported in barley plants, where deletion of the N-terminus of the CP from bromo mosaic virus (BMV) failed to either package viral RNA in protoplasts or affect systemic infection [20]. In this study, we cloned the cDNA for the CP of SCMV from sugarcane cultivar PS-881 and constructed a binary vector with either the full nucleotide sequence (p927) or the sequence coding for N-terminally truncated CP (p702) to induce resistance in transgenic sugarcane. We found that expression of the full sequence induced resistance against SCMV in the transgenic sugarcane, unlike the sequence encoding N-terminally truncated protein. A possible role of the N-terminal CP sequence in the binding of viral RNA and encapsulation is discussed. The results suggest that overexpression of the full-sequence CP cDNA is more efficacious compared to the N-terminally truncated CP cDNA.

Materials and methods

Cloning of coat protein cDNA and its binary vector construct

Cloning of cDNA coding for CP of SCMV was conducted by reverse transcriptase PCR (RT-PCR) using total RNA isolated from symptomatic leaves of sugarcane cultivar PS-881, and the cDNA was named *ScMVCp*-cDNA [21]. It was reported that asp-ala-gly (DAG) amino acid residues located at the N-terminus were identified as the conserved motif for SCMV transmission by aphids [22, 23]. To study the effect of N-terminal truncation, two binary constructs were prepared since the *ScMVCp*-cDNA contained two deduced amino acid DAG sites. The first DAG was located at the N-terminus, and the second was located 74 amino acids downstream of the first (Fig. S1). The first construct was prepared by amplification of the cDNA using a forward primer containing an additional *Nde*I site (F1) and a reverse primer with a *Bam*HI site (R1) that contained start and stop codons, respectively (Table 1). The second construct was prepared using the same approach, with a forward primer containing an *Nde*I site (F2) and a *Bam*HI site (R1). The amplified cDNAs were digested with the corresponding restriction enzymes and inserted in a pRION101 binary vector (Takara, Japan). The resulting first and second constructs were named as full sequence (p927) and N-terminal deletion (p702) constructs, respectively (Fig. 1). The correct cDNA insertions were confirmed with restriction enzyme digestions (Fig. S2) and nucleotide sequence determinations.

Agrobacterium co-cultivation and transformant selection

Sugarcane in vitro shoots were used as explants for *Agrobacterium*-mediated transformation. The sugarcane shoot was prepared by the micropropagation of meristematic apical tissue isolated from 4 to 5-month-old field growth of the commercial sugarcane cultivar Bululawang (BL). The transformation was conducted using *Agrobacterium tumefaciens* strain GV 3101 that harboured the constructs, either p927 (pRION-927) or p702 (pRION-702), according to the method previously described [24]. After *Agrobacterium*

Table 1 Oligonucleotide primers used in this study

Primer names	Sequence (5′–3′)	Product (bp)	Target genes
F1	GACATATGGATGTAGATGCTGGTACGACA	927	*ScMVCp*-p927
F2	CCCCATATGACAGTCGATGCAGGTGCTC	725	*ScMVCp*-p702
R1	ATGGATCCTAGTGGTGCTGCTGCACTCCC	950–725	*ScMVCp*-p927 and *ScMVCp*-p702
F3	TGAATGAACTGCAGGACGAG	550	*npt II*
R3	AGCCAACGTATGTCCTGAT	550	*npt II*
F4	GCAACTGGGATGACATGGAG	568	*Actin*
R4	ATGGCTGGAAGAGGACCTCAG	568	*Actin*
F5	GCCATACTCGAGTGGGATCG	483	*Nib-Cp*
R5	CCTTGTCTCTTTGGCCTCCTG	483	*Nib-Cp*

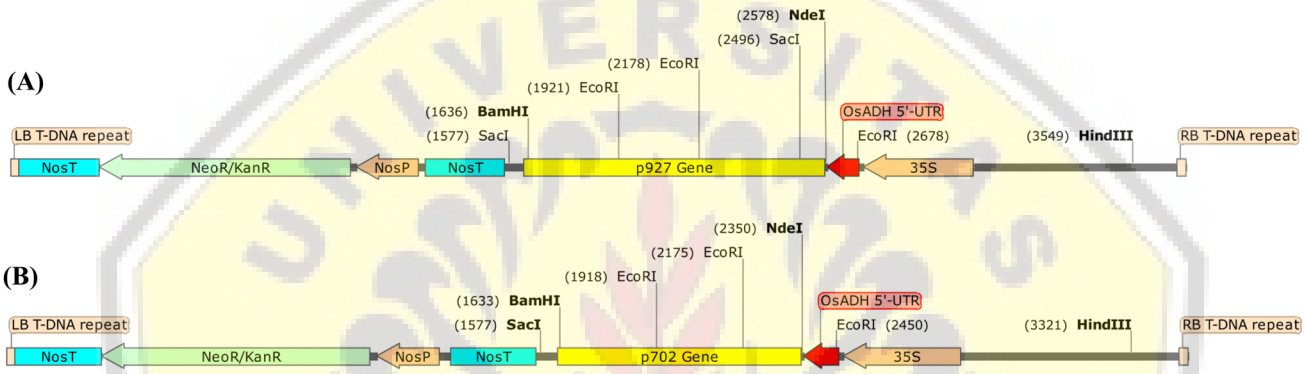


Fig. 1 Schematic diagrams representing the p927 and p702 constructs. **a** Construct p927 and **b** Construct p702 containing 35S, Cauliflower mosaic virus promoter; NosT, nopaline synthase gene terminator; NosP, nopaline synthase gene promoter; *npt*II, neomycin phosphotransferase II gene (kanamycin resistance gene); OsADH, 5′-UTR rice ADH; RB and LB, T-DNA right and left border, respectively

co-cultivation for 3 days, the infected explant was incubated on selection MS basal medium containing 75 ppm kanamycin. The selection was performed for 3 weeks, and after five successive cycles in the selection medium, the explant was acclimated in a growth chamber under illumination for 4 weeks. The transformed sugarcane was transplanted into a greenhouse for the analysis and evaluation of resistance against SCMV.

Genomic analysis by PCR and Southern blotting

Genomic DNA was extracted from 4 g of leaves of the transformed sugarcane grown in the greenhouse according to the method previously described [25] with minor modifications. The sugarcane leaves were pulverized under liquid nitrogen in a mortar and pestle, and then the frozen leaf powder was continuously ground in 8 mL of extraction buffer containing 100 mM Tris–HCl (pH 8), 50 mM EDTA, 500 mM NaCl, 1% SDS and 5 mM 2-mercaptoethanol. After incubation at 65 °C for 10 min, 4 mL of 5 M potassium acetate was added, and the sample was incubated on ice for 10 min. The pellet debris was separated by centrifugation at 12,000×*g* at 4 °C for 10 min, the DNA was precipitated by adding 0.8-fold isopropanol to the supernatant, and the sample was then incubated at − 20 °C for an hour. The DNA was recovered by centrifugation at 12,000×*g* at 4 °C for 10 min, and the DNA pellet was dissolved in 0.5 mL of 10 mM TE buffer. The RNA was removed by the addition of RNAse, and DNA was precipitated by ethanol precipitation. The DNA was recovered by centrifugation at 12,000×*g* at 4 °C for 10 min, and the pelleted DNA was dissolved in 100 µL of TE buffer and stored at − 20 °C until analysis. The DNA amount was measured using a NanoVue spectrophotometer (GE Healthcare, USA) at 260 nm.

To detect the presence of the introduced *ScMVCp* gene in the genomic DNA, PCR analysis was carried out using a master mix kit (Roche, Germany) and pairs of primers, F1–R1 and F2–R1 for detection of p927 and p702 genes, respectively. In addition, a pair of primers, F3 and R3, was applied for detection of the *npt*II gene. The PCR reaction was performed in a T100 thermal cycler (Bio-Rad, USA) using pre-denaturation at 95 °C for 3 min, followed by 30

cycles of denaturation at 95 °C for 30 s, annealing at 63 °C for 30 s, and extension at 72 °C for 1 min, and a final extension at 72 °C for 5 min. The PCR product was run on 1% agarose gel, stained with ethidium bromide and documented on GelDoc.

Southern blot analysis was performed by digestion of 20 µg of the genomic DNA with the restriction enzymes *Hin*dIII or *Bam*HI at 37 °C overnight. The digested DNA was separated on a 1% agarose gel and transferred to an Amersham Hybond N+ membrane (GE Healthcare, UK). The gel was soaked in 0.25 M HCl for 15 min for depurination and then rinsed with autoclaved H_2O. The DNA was denatured with a solution containing 0.5 N NaOH and 1.5 M NaCl; then, after washing, the DNA was transferred to a Hybond N+ nitrocellulose membrane using capillary transfer. The DNA was hybridized with a DIG-labelled DNA probe (Roche, Germany) overnight at 42 °C with gentle agitation. The DNA probe was prepared by amplification of *ScMVCp*-cDNA by PCR using the primers F2 and R1; it was then labelled with DIG. The processes of probe preparation and washing of the membrane to remove the unbound probe were performed according to the method described in the manufacturer's instructions (Roche). Hybridization was visualized by exposing the membrane to X-ray Fuji Film.

Semi-quantitative RT-PCR and western blot analysis

The expression of the transgene CP (*ScMVCp*) was determined in the transgenic sugarcane leaves using semi-quantitative RT-PCR. The sugarcane leaves (0.5 g) were pulverized under liquid nitrogen, and total RNA was isolated using an RNA isolation kit according to the manufacturer's instructions (Tiangen, China). The total RNA was dissolved in 50 µL of autoclaved pure H_2O, and the content was measured using a NanoVue spectrophotometer (GE Healthcare, USA) at 260 nm. Then, 1 µg of total RNA was converted into cDNA using RT and an oligo-dT primer (Bio-Rad, USA). The resulting cDNA was used for PCR amplification at 25 cycles with the primer pairs F1–R1 or F2–R1, as described in the previous section. To differentiate between the transgene and viral RNA, the F5 and R5 primer pair was used for RT-PCR to amplify corresponding cDNA from the *Nib* gene, located in the upstream region of the *Cp* gene. This pair of primers only amplified the viral RNA and not RNA resulting from transgene expression. The PCR products were run on a 1% agarose gel and visualized with GelDoc. To ensure that the same amount of total RNA was used, *Actin* was used as the reference gene, and expression was determined using primer pairs F4 and R4 (Table 1).

The expression protein levels of CP in the transgenic sugarcane were determined by western blot analysis. Approximately 2 g of the transgenic sugarcane leaves was pulverized in liquid nitrogen and continuously ground in the extraction buffer containing 50 mM Tris–HCl (pH 7.5), 2.5 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 10 mM 2-mercaptoethanol and 2% polyvinylpolypyrrolidone (PVP). Soluble and insoluble protein fractions were separated by centrifugation at 14,000×*g* at 4 °C for 10 min. The insoluble protein was then solubilized in a buffer containing 50 mM Tris–HCl (pH 8.5), 1 mM EDTA, 2% SDS, and 30% sucrose, and the protein fraction was collected by centrifugation at 12,000×*g* for 10 min. The soluble and insoluble proteins were separated by SDS-PAGE (12.5% acrylamide) and transferred onto Immobilon-P transfer membrane (Millipore) using a semi-dry trans-blotter (Bio-Rad, USA). The membrane was washed with Tris-buffered saline (TBS) three times, followed by blocking of the membrane with 0.5% skim milk. The membrane was then incubated with antiserum against the recombinant CP protein [20] diluted in TBS containing 0.5% skim milk (1:3000) overnight at room temperature with gentle agitation. After washing three times with TBS, the membrane was incubated with a secondary antibody goat anti-rabbit IgG alkaline phosphatase (AP)-conjugate (Bio-Rad) at 1:3000 dilution for 60 min at room temperature. The CP band was visualized by incubation of the membrane with a mixture of the substrate BCIP (5-bromo-4-chloro 3-indolyl-phosphate) and NBT (nitro blue tetrazolium) (Bio-Rad, USA).

Mechanical inoculation

The viral resistance of the transgenic sugarcane was evaluated by mechanical inoculation according to the method previously described [3]. Approximately 2 g of sugarcane leaves of the cultivar PS881 with typical mosaic SCMV symptoms was harvested and ground in 10 mL of 0.1 M phosphate buffer (pH 8.0) containing 2% PVP (polyvinylpyrrolidone) using a mortar and pestle. The homogenate plant sap was filtered and used for mechanical inoculation by rubbing it, along with carborundum as an abrasive material, on healthy, fully expanded 2-month-old transgenic sugarcane leaves grown in a greenhouse, and then rinsing the leaves with sterile water to remove unnecessary materials. To maximize viral infection, the inoculated sugarcane plants were incubated 24 h before and after mechanical inoculation in a dark room. The development of newly emerging symptomatic leaves was observed daily for 40 days post-inoculation (dpi). The sugarcane was classified as resistant or susceptible, according to the degree of development of symptomatic leaves [3].

Results

Cloning of the coat protein gene and generation of transgenic sugarcane

Cloning of the cDNA for CP of SCMV was conducted to develop transgenic sugarcane resistant to the virus. Amplification of CP cDNA by RT-PCR obtained a single cDNA band with a molecular size of approximately 1.0 kb. The sequence analysis revealed that the cDNA contained 998 bp nucleotides and had a high homology with the cDNAs for CPs of the virus isolates from Argentina (AGR-130: JX237868.1 and AGR-345: JX2378651), Australia (Brisbane: AJ278405.1), China (FZ-C1: KR108212.1), Brazil (BR08: DQ315492.1), Mexico (VER1: EU091075) and Vietnam (VN/AR1: DQ925432.1) (Fig. S1). The nucleotide sequences of the cDNA for CP have been submitted to the NCBI GenBank database and named *ScMVCp*-cDNA isolate Jember PS881.

Confirmation of transgene integration by PCR and Southern blot analysis

To examine the effect of the CP N-terminal deletion on viral resistance, *ScMVCp*-cDNA was used to construct two binary vectors containing either the full nucleotide sequence (p927) or the sequence coding for N-terminally truncated protein (p702), which encoded 309 and 234 amino acid residues, respectively. These constructs were then introduced into sugarcane cultivar BL using the *Agrobacterium*-mediated transformation method previously reported [24]. As expected, the utilization of sugarcane shoots as explants resulted in a high transformation efficiency (4–10%). PCR analysis using the primers F1–R1 and F2–R1 revealed the amplification of the corresponding DNA in transgenic, but not wild-type (WT), sugarcane. The *Agrobacterium* harbouring the p927 and p702 constructs resulted in DNA bands with molecular sizes of 950 and 725 bp, respectively, in transgenic sugarcane (Fig. 2a). These results showed that the DNA resulted from the integration of the transgenic DNA into the sugarcane genome and was not the viral RNA genome of SCMV.

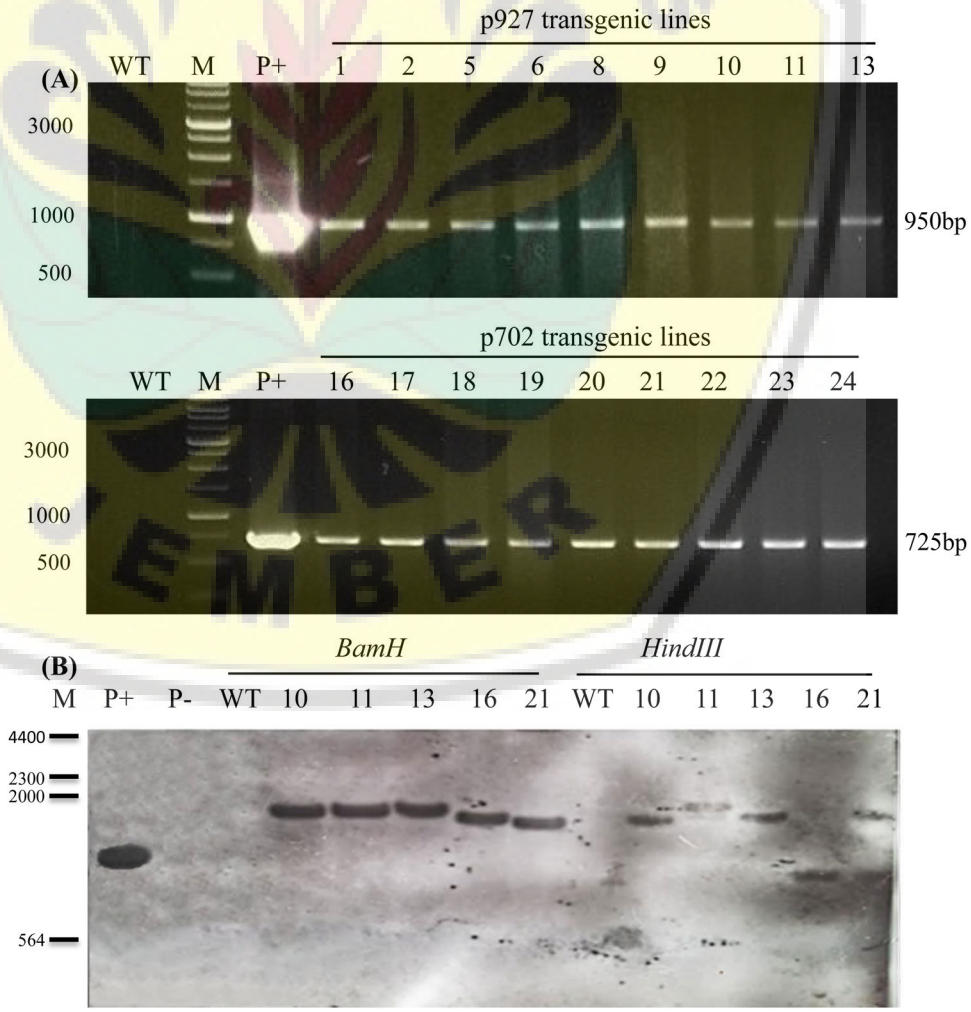


Fig. 2 PCR amplification and Southern blot analysis of sugarcane leaf genomic DNA. The genomic DNA was isolated from the leaves of transgenic and wild-type (WT) sugarcane and used for analysis. **a** Nucleotide primer pairs F1–R1 and F2–R1 were used for PCR to amplify the p927 (upper panel) and p702 (lower panel) genes, respectively. **b** Southern blot analysis was carried out according to the method described in "Materials and methods" section. Lines 10, 11, and 13 and lines 16 and 21 are digested genomic DNA from p927 and p702 transgenic sugarcane, respectively. *WT* wild-type sugarcane, *P*+ p702 DNA construct, *P*− pRION plasmid without insert, *M* molecular marker for DNA

To confirm the insertion of the transgene *ScMVCp* DNA, Southern blot analysis was conducted using a DNA genome isolated from leaves of five lines of transgenic sugarcane. The DNA was digested by restriction enzymes and hybridized with DIG-labelled *ScMVCp* DNA. The Southern blot analysis showed that all five lines of the transgenic sugarcane displayed a single hybridized DNA copy with a difference in molecular size. In contrast, the hybridized DNA was not found in the genome of the WT plant (Fig. 2b). These results confirmed that a single copy of the *ScMVCp* gene was integrated into the genome of the transgenic sugarcane.

Detection of *ScMVCp* gene expression in transgenic sugarcane

To observe whether the inserted *ScMVCp* genes were expressed in the transgenic sugarcane, semi-quantitative RT-PCR was conducted to detect *ScMVCp* expression at the RNA transcript level. *Actin* gene transcription was used as an internal control to normalize variations in the total RNA amount. The results show that the inserted *ScMVCp* was expressed in the transgenic sugarcane, but not in the WT sugarcane. The expression of the transgene was clearly observed in the transgenic lines generated from the p927 and p702 constructs (Fig. 3a). The highest expression levels were found in transgenic sugarcane lines 10, 11, and 13

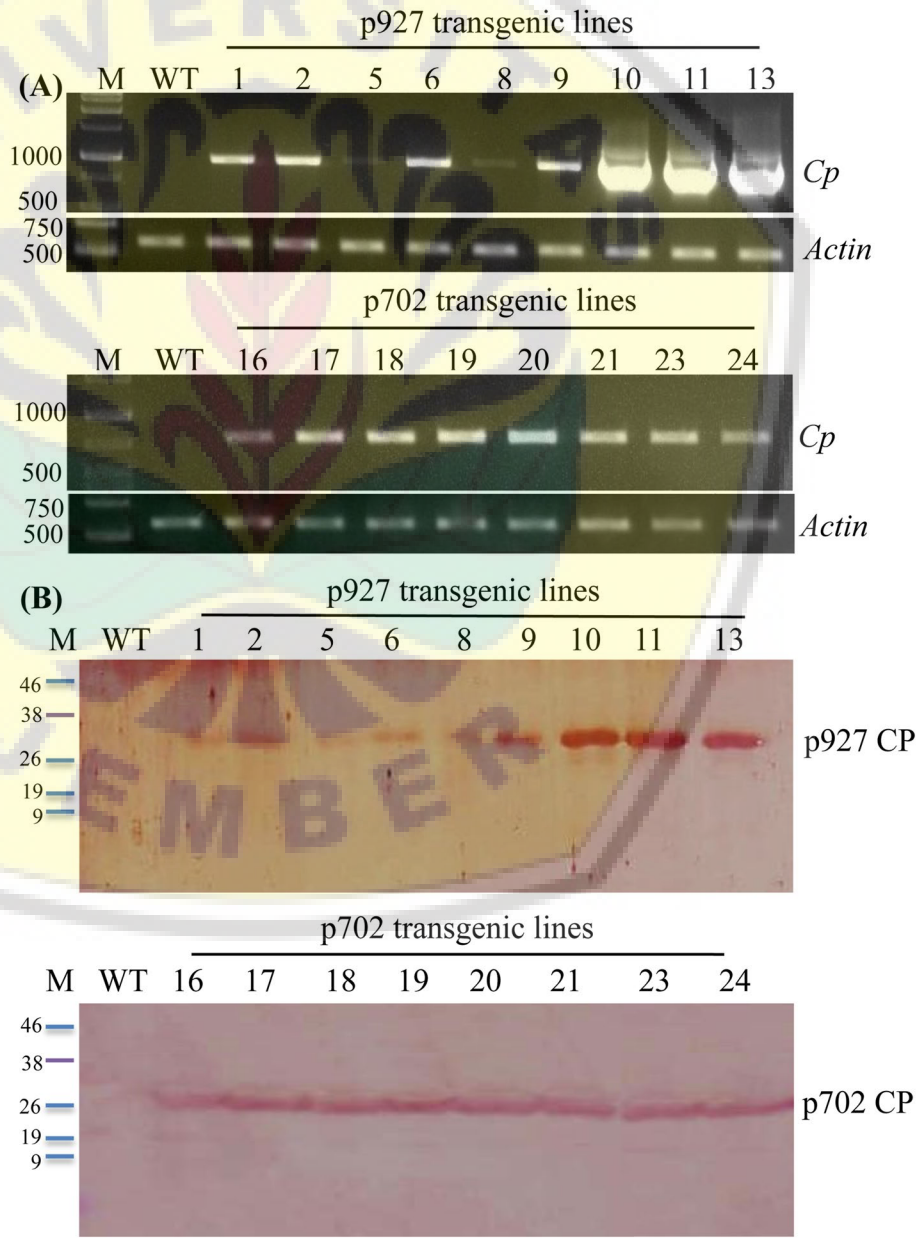


Fig. 3 Expression of the p927 and p702 genes at the RNA transcript and protein levels. **a** Total RNA was isolated from the leaves of transgenic and wild-type (WT) sugarcane, and the RNA transcript levels of the p927 and p702 genes were determined by RT-PCR for 25 cycles (see "Materials and methods" section). *Actin* gene expression was used as the reference. **b** Insoluble and soluble proteins were extracted from the leaves of transgenic and WT sugarcane, and the protein (30 μg) was subjected to western blot analysis using a polyclonal antibody against recombinant CP, as described in "Materials and methods" section. *M* molecular marker for DNA or protein

generated from the p927 construct and were increased to a lesser extent in lines 5 and 8. These increases in expression levels resulted from the insertion of the *ScMVCp* genes and not from variations in RNA content. The expression of *Actin* as the control of gene expression was at the same level in all of the sugarcane examined (Fig. 3a).

The CP levels expressed from the *ScMVCp* genes in the leaves of transgenic sugarcane were determined by western blot using a polyclonal antibody against the recombinant CP protein [21]. Soluble and insoluble proteins were extracted from the leaves and subjected to immunodiagnostics. The predicted CP molecular sizes using the online software Expasy (http://web.expasy.org) were matched with CP bands with molecular sizes of 33.3 and 26.9 kDa in the transgenic sugarcane, which resulted from the p927 and p702 constructs, respectively. CP levels increased according to mRNA transcript levels in transgenic sugarcane and were not found in the WT sugarcane (Fig. 3b). As expected, the highest CP levels were found in transgenic sugarcane lines 10, 11, and 13 generated from p927, and sharply increased in p702. Interestingly, the CP generated from the full p927 sequence was expressed as insoluble protein, but the CP from the N-terminal truncation p702 produced a soluble protein (Fig. S3). The insolubility of transgene protein p927 may be due to the presence of basic amino acid residues at the N-terminal sequence (Fig. S1), that play a role in binding encapsidated RNA [20]. These results imply that the N-terminal sequence of *ScMVCp* may play an important role in the encapsulation and packaging of viral RNA.

Resistance of transgenic sugarcane to SCMV infection

Five lines of the transgenic sugarcane generated from p927 and p702 were grown in a greenhouse and used for the viral infection challenge. The response to viral infection was judged according to the appearance of mosaic symptoms, as previously described [3], and responses were classified into resistant and susceptible groups. After viral infection, mosaic and yellowing symptoms appeared in newly emerging leaves of susceptible plants around position number 4 from the inoculated leaf after 25 and 35 dpi but were not observed in the resistant sugarcane lines. The symptoms were clearly observed on young stage leaves and gradually disappeared during the ageing stage. Based on the appearance of the symptoms, all of the transgenic sugarcane lines generated from the p927 gene were classified as resistant, but not all of the transgenic lines from the p702 gene were resistant because some of the lines were susceptible to viral infection (Table 2). The results indicate that the full-sequence CP gene produced more resistant transgenic sugarcane compared to the N-deletion CP gene.

Table 2 Resistance to SCMV Inoculation of the wild-type and transgenic sugarcane at 25 and 35 dpi

Sugarcane plants	Symptoms		Resistance
	25 dpi	35 dpi	
WT	Symptoms	Symptoms	Susceptible
Line 2	NS	NS	Resistance
Line 9	NS	NS	Resistance
Line 10	NS	NS	Resistance
Line 11	NS	NS	Resistance
Line 13	NS	NS	Resistance
Line 16	NS	NS	Resistance
Line 19	NS	NS	Resistance
Line 20	Symptoms	Symptoms	Susceptible
Line 21	NS	NS	Resistance
Line 23	Symptoms	Symptoms	Susceptible

The resistance was judged according to visibility of mosaic symptoms
NS no symptoms

Viral challenge in vegetative propagated transgenic sugarcane lines

To further characterize the resistance of the transgenic sugarcane, the viral infection challenge was performed in vegetative propagated sugarcane. Five lateral buds from the first generation of the resistant transgenic sugarcane lines were grown in a greenhouse, and their resistance was evaluated by mechanical inoculation. PCR analysis using primers for *npt*II gene detection confirmed the presence of the inserted gene in all DNA genomes in the propagated transgenic sugarcane lines (Fig. S4). Second-generation plants generated from p927 did not show mosaic symptoms and were resistant to viral infection. On the other hand, the transgenic sugarcane p702 lines were mostly susceptible and showed mosaic symptoms to a high degree (60–80%) in the second generation (Table 3). These results demonstrate that the full sequence of the CP gene produced viral resistance that was inherited in the second generation using vegetative propagation.

To confirm the resistance of the second generation of transgenic sugarcane, RT-PCR analysis was conducted for detection of the transgene RNA transcript as well as viral RNA in the sugarcane leaves. The results showed that a 483-bp cDNA band corresponding to the viral RNA was observed in the mosaic symptomatic WT (WTs lane) and transgenic leaves generated from p702, but was not apparent in the resistant transgenic lines of p927. However, the RNA transcript was observed in all resistant (927 bp) and susceptible lines (702 bp), except for the non-symptomatic healthy (WT lane) leaves, when the RT-PCR was designed to amplify the RNA transcript (Fig. S5). These results indicated the resistance of transgenic sugarcane p927 and the presence

Table 3 Degree of resistance and incidence of the wild-type and second-generation transgenic sugarcane after inoculation with SCMV at 25 and 35 dpi

Sugarcane plants	Number of plants	Number of symptoms		Resistance (%)
		25 dpi	35 dpi	
WT	5	5	0	0
Line 10	5	0	0	100
Line 11	5	0	0	100
Line 13	5	0	1	80
Line 16	5	2	1	40
Line 19	5	2	2	20
Line 21	5	1	3	20

Resistance was determined in percentages of the total number of symptomatic plants with the number of plants at 25 and 35 dpi

of viral infection in the susceptible transgenic sugarcane generated with the N-terminal deletion p702 construct.

Discussion

The gene coding for virus CP has been widely used to engineer virus-resistant plants. For plant RNA viruses, PDR has been applied to generate virus-resistant plants by transforming the CP gene into host plants [10]. Resistance has also been demonstrated by the transgene-mediated RNA silencing and generation of RNA interference (RNAi) [26]. A range of engineering strategies is currently used to obtain virus-resistant plants, including strategies for sugarcane. Recently, transgenic sugarcane lines resistant to SCMV have been generated with the CP gene using both PDR [16] and RNAi techniques [27], which were achieved using biolistic bombardment. The field performance of the transgenic lines generated using the PDR technique showed that they were highly resistant to SCMV and had significantly greater cane production [16]. In this study, we confirmed that the resistance of sugarcane to SCMV infection can be developed using PDR techniques. In addition, our results included molecular analysis at the transcript and translation levels of the *ScMVCp* gene in transgenic sugarcane, which have not previously been reported.

The viral infection challenge of the transgenic sugarcane indicated that the full nucleotide sequence of the CP gene (p927) induced higher resistance than did the sequence of the N-terminally truncated CP gene (p702). This indicates that the N-terminal amino acid residues play a pivotal role in viral resistance. It was reported that the N-terminus of BMV CP contains basic amino acid residues that are implicated in binding encapsidated RNA [20]. By analogy, the basic N-terminal amino acid residues in *ScMVCp* might interact with the viral RNA during assembly. The mutation of the amino or carboxyl termini of the CP of TMV was unable to overcome CPMR in transgenic tobacco [19]. Recently, it was reported that the peptides containing the N-terminal arm of the CP from BMV preferentially bind to viral RNA [28]. Thus, the N-terminus of CP contributes amino acid residues for interaction with encapsidated viral RNA. Missing this N-terminal domain causes disruption and failure in the replication and packaging of viral particles [29].

In this study, it is clear that the full nucleotide sequence of *ScMVCp* (p927) induced resistance against SCMV in transgenic sugarcane. These results suggest that the transgenic CP generated from the p927 construct is structurally similar to the viral CP and blocks the specific step of viral assembly and replication. It has been reported that similarities and specific interactions between transgenic CP and viral CP are a necessary component of CPMR against the virus [11]. On the other hand, the CP generated from p702, which lacks the N-terminus, may be dysfunctional in the viral assembly and, hence, does not disturb viral assembly and does not produce plants resistant to the virus.

The CP molecular size induced by SCMV in plant tissues can vary between isolates, with sizes ranging from 34.4 to 39.7 kDa [30]. A previous analysis showed that authentic CP with a molecular size of 36.7 kDa was observed by serological analysis in SCMV symptomatic sugarcane leaves. In addition, expression of the full nucleotide sequence of *ScMVCp* DNA produced a 44 kDa recombinant CP in prokaryotic *Escherichia coli* [21]. However, expression of the full nucleotide sequence of p927 generated a 33.3 kDa CP in transgenic sugarcane (Fig. 3b). These discrepancies may reflect the presence of post-translational modifications due to differences in host cell systems.

Agrobacterium-mediated transformation produces plants with low copy numbers of transgenes, and in this case, we found a single copy of the *ScMVCp* gene inserted in the genome of the transgenic sugarcane (Fig. 2b). Depending on the method employed, the particle bombardment technique produces more gene insertions that may cause multiple gene arrangements and expression silencing [31]. However, the transgene produced by both methods was transmitted to progeny according to Mendelian rules; in some cases, non-Mendelian inheritance was observed in generative propagated plants [32]. In the case of the vegetative propagation of sugarcane, it has been reported that the phenotypic expressions of transgenes remained unchanged over three generations [24, 33]. Thus, the results from propagated transgenic sugarcane showed that the resistance against SCMV was stable and inherited in the second generation (Fig. S5). Therefore, this SCMV-resistant transgenic sugarcane could be incorporated into future sugarcane breeding programmes.

Conclusion

The CPMR method is a workable strategy for obtaining plants that are resistant to viruses. In this study, we succeeded in producing transgenic sugarcane resistance to SCMV infection. Based on our molecular analysis, this resistance was caused by introducing the full nucleotide sequence of the *ScMVCp* gene.

Acknowledgements The research was supported by the Ministry of Research, Technology, and Higher Education through a Research Grant from the Islamic Development Bank (IDB) Project of The University of Jember and PUSNAS 2017.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Research involving human and animal participants

This article does not contain any studies with human participants or animals performed by any of the authors.

References

1. Putra LK, Kristini A, Achadian EM, Damayanti TA (2014) Sugarcane streak mosaic virus in Indonesia: distribution, characterisation, yield losses and management approaches. Sugar Tech 16:392–399. https://doi.org/10.1007/s12355-013-0279-9
2. Akbar S, Tahir M, Wang M-B, Liu Q (2017) Expression analysis of hairpin RNA carrying sugarcane mosaic virus (SCMV) derived sequences and transgenic resistance development in a model rice plant. BioMed Res Int. https://doi.org/10.1155/2017/1646140
3. Addy H, Nurmalasari D, Wahyudi A et al (2017) Detection and response of sugarcane against the infection of sugarcane mosaic virus (SCMV) in Indonesia. Agronomy 7:2–11. https://doi.org/10.3390/agronomy7030050
4. Perera MF, Filippone MP, Ramallo CJ et al (2009) Genetic diversity among viruses associated with sugarcane mosaic disease in Tucumán, Argentina. Phytopathology 99:38–49. https://doi.org/10.1094/PHYTO-99-1-0038
5. Puchades Y, La OM, Montalván J et al (2016) Genetic and symptomatic characterization of sugarcane mosaic virus (SCMV) in Cuba. Sugar Tech 18:184–191. https://doi.org/10.1007/s12355-015-0375-0
6. Luo Q, Ahmad K, Fu H-Y et al (2016) Genetic diversity and population structure of *Sorghum* mosaic virus infecting *Saccharum* spp. hybrids. Ann Appl Biol 169:398–407. https://doi.org/10.1111/aab.12310
7. Seker MG, Süzerer V, Elibuyuk IO, Çiftçi Y (2015) In vitro elimination of PPV from infected apricot shoot tips via chemotherapy and cryotherapy. Agric Biol 17:1066–1070. https://doi.org/10.17957/IJAB/15.0024
8. Danci M, Oana D, Luiza M et al (2012) Production of virus free potato plantlets. Hortic For Biotechnol 16:232–238
9. Dewanti P, Widuri LI, Ainiyati C et al (2016) Elimination of SCMV (sugarcane mozaik virus) and rapid propagation of virus-free sugarcane (*Saccharum officinarum* L.) using somatic embryogenesis. Procedia Chem 18:96–102. https://doi.org/10.1016/j.proche.2016.01.016
10. Goldbach R, Bucher E, Prins M (2003) Resistance mechanisms to plant viruses: an overview. Virus Res 92:207–212. https://doi.org/10.1016/S0168-1702(02)00353-2
11. Bendahmane M, Fitchen JH, Zhang G, Beachy RN (1997) Studies of coat protein-mediated resistance to tobacco mosaic tobamovirus: correlation between assembly of mutant coat proteins and resistance. Virology 71:7942–7950
12. Abel P, Nelson R, De B et al (1986) Delay of disease development in transgenic plants that express the tobacco mosaic virus coat protein gene. Science 232:738–743. https://doi.org/10.1126/science.3457472
13. Furutani N, Hidaka S, Kosaka Y et al (2006) Coat protein gene-mediated resistance to soybean mosaic virus in transgenic soybean. Breed Sci 56:119–124. https://doi.org/10.1270/jsbbs.56.119
14. Tougou M, Yamagishi N, Furutani N et al (2007) Soybean dwarf virus-resistant transgenic soybeans with the sense coat protein gene. Plant Cell Rep 26:1967–1975. https://doi.org/10.1007/s00299-007-0404-x
15. Dubey VK, Chandrasekhar K, Srivastava A et al (2015) Expression of coat protein gene of Cucumber mosaic virus (CMV-subgroup IA) *Gladiolus* isolate in *Nicotiana tabacum*. J Plant Interact 10:296–304. https://doi.org/10.1080/17429145.2015.1101496
16. Yao W, Ruan M, Qin L et al (2017) Field performance of transgenic sugarcane lines resistant to sugarcane mosaic virus. Front Plant Sci. https://doi.org/10.3389/fpls.2017.00104
17. Zhu M, Chen Y, Ding XS et al (2014) Maize Elongin C interacts with the viral genome-linked protein, VPg, of Sugarcane mosaic virus and facilitates virus infection. N Phytol 203:1291–1304. https://doi.org/10.1111/nph.12890
18. Lindbo JA, Dougherty WG (1992) Pathogen-derived resistance to a *Potyvirus*: immune and resistant phenotypes in transgenic tobacco expressing altered forms of a *Potyvirus* coat protein nucleotide sequence. Mol Plant Microbe Interact 5:144–153
19. Clark WG, Fitchen J, Nejidat A et al (1995) Studies of coat protein-mediated resistance to tobacco mosaic virus (TMV). II. Challenge by a mutant with altered virion surface does not overcome resistance conferred by TMV coat protein. Gen Virol 76:2613–2617
20. Sacher R, Ahlquist P (1989) Effects of deletions in the N-terminal basic arm of brome mosaic virus coat protein on RNA packaging and systemic infection. Virology 63:4545–4552
21. Darsono N, Azizah N, Putranty K et al (2018) Production of a polyclonal antibody against the recombinant coat protein of the sugarcane mosaic virus and its application in the immunodiagnostic of sugarcane. Agronomy 8:93. https://doi.org/10.3390/agronomy8060093
22. Atreya PL, Lopez-Moya JJ, Chu M et al (1995) Mutational analysis of the coat protein N-terminal amino acids involved in *Potyvirus* transmission by aphids. Gen Virol 76:265–270. https://doi.org/10.1099/0022-1317-76-2-265
23. Lopez-Moya JJ, Wang R, Pirone T (1999) Context of the coat protein DAG motif affects *Potyvirus* transmissibility by aphids. J Gen Virol 80:3281–3288
24. Sugiharto B (2008) Biotechnology of drought-tolerant sugarcane. In: de Oliveira AB (ed) Sugarcane technology and research, InTech Open, London, pp 139–165
25. Dellaporta SL, Wood J, Hicks JB (1983) A plant DNA minipreparation: Version II. Plant Mol Biol Rep 1:19–21. https://doi.org/10.1007/BF02712670
26. Sudarshana MR, Roy G, Falk BW (2007) Methods for engineering resistance to plant viruses. In: Ronald PC (ed) Plant-pathogen interaction. Humana Press Inc, New Jersey, pp 183–195
27. Aslam U, Tabassum B, Nasir IA et al (2018) A virus-derived short hairpin RNA confers resistance against sugarcane mosaic virus in transgenic sugarcane. Transgenic Res. https://doi.org/10.1007/s11248-018-0066-1

28. Jacobs A, Hoover H, Smith E et al (2018) The intrinsically disordered N-terminal arm of the brome mosaic virus coat protein specifically recognizes the RNA motif that directs the initiation of viral RNA replication. Nucleic Acids Res 46:324–335. https://doi.org/10.1093/nar/gkx1087
29. Sivanandam V, Mathews D, Garmann R et al (2016) Functional analysis of the N-terminal basic motif of a eukaryotic satellite RNA virus capsid protein in replication and packaging. Sci Rep: https://doi.org/10.1038/srep26328
30. Jensen SG, Long-Davitson B, Seip A (1986) Size variation among protein induced by sugarcane mosaic viruses in plant tissue. Mol Plant Pathol 76:528–532
31. Vaucheret H, Béclin C, Elmayan T et al (1998) Transgene-induced gene silencing in plants: transgene-induced gene silencing. Plant J 16:651–659. https://doi.org/10.1046/j.1365-313x.1998.00337.x
32. Yin Z, PL W (2004) Transgene inheritance in plants. J Appl Genet 45:127–144
33. Gallo-Meagher M, Irvine J (1996) Herbicide resistant transgenic sugarcane plants containing the bar gene. Crop Sci 36:1367–1374. https://doi.org/10.1007/s004250050369