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Structural and functional characterization of Tomato *SUMO1* gene

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ABSTRACT

Small ubiquitin-related modifier (*SUMO*) genes regulate various functions of target proteins through post-translational modification. The SUMO proteins have a similar 3-dimensional structure as that of ubiquitin proteins and occur through a cascade of enzymatic reactions. In the present study we have cloned a new SUMO gene from Tomato (*Solanum lycopersicum* L.), cv Saudi-1, named *SlS-SUMO1* gene by PCR using specific primers. This gene has SUMO member's features such as C-terminal diglycine (GG) motif as processing site by ULP (ubiquitin-like SUMO protease) and has SUMO consensus ΨKXE/D sequence. Phylogenetic analysis showed that *SlS-SUMO1* gene is highly conserved and homologous to Potatoes *Ca-SUMO1* and *Ca-SUMO2* genes based on sequence similarity. Expression protein of *SlS-SUMO1* gene found to be localized in the nucleus, cytoplasm, and nuclear envelop or nuclear pore complex. SUMO conjugating enzyme SCE1a with SlS-SUMO1 protein co-expressed and co-localized in nucleus and formed nuclear subdomains. This study reported that the *SlS-SUMO1* gene is a member of SUMO family and its SUMO protein processing using GG motif and activate and transport to nucleus through Sumoylation system in the plant cell.
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1. Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crops grown worldwide and forms an essential industry for food (Rukhsar et al., 2012). Tomato contains natural minerals, vitamins, and high level of antioxidants, which suppress cancer formation and reduce many of serious diseases particularly the cardiovascular diseases (Tambo and Gbemu, 2010; Saad et al., 2016). Tomato plants have ability to grow under different ecosystems. However, biotic and abiotic stresses are the main factors for limiting their production (Radzevicius et al., 2013). Therefore, it is necessary to develop high yielding tomato variety with improved quality traits to meet the highly demand of tomato consumers.

Small-ubiquitin related modifier (SUMO) is a type of ubiquitin-like proteins that regulates several functions of cellular proteins through post-translational modification to various substrate proteins (Elrouby and Coupland, 2010; Lois, 2010). This process is calling Sumoylation and it is highly similar to the Ubiquitination pathway. Sumoylation occurs through a cascade of similar enzymatic reactions including E1 SUMO activation enzymes, E2 SUMO conjugation enzymes and E3 SUMO ligation enzymes (Miura et al., 2007a). It's often happens in lysine (K) within SUMO consensus sequence CKXD/E (C is a hydrophobic, K is lysine, X is any amino-acid and D/E is glutamic or aspartic acids) (Schmidt and Muller 2003; Hickey et al., 2012). Sumoylation process regulates many of eukaryotic cellular processes such as sub-nuclear localization, enzymatic stability and activity, cell cycle regulation, and DNA repair (Miura et al., 2007b). Hence, SUMO genes are considered as the key regulators of many environmental responses. It was found that SUMO genes regulate a number of plant biological processes such biotic (Flick and Kaiser, 2009) and abiotic stresses tolerance (van den et al., 2010; Robert and Augustine, 2018); root development (Zhang et al., 2010); plant reproduction (Augustine et al., 2016); and plant development (Pedro et al., 2018). Under abiotic stress, the SUMO system affects plant development through the accumulation of SUMO conjugates (Conti et al., 2008). SUMO E3 ligase SIZ1 was found to regulate the heat stress and plant growth in Arabidopsis (Hammoudi et al., 2018), and enhanced drought tolerance in tobacco (Song et al., 2017). The Esd4 is a type

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of SUMO specific protease regulates the flowering time and processes a precursor SUMO protein in Arabidopsis (Golebiowski et al., 2009). Also, it was reported that SUMO protease OTS1is a positive regulator of rice seed germination and root system development under salinity (Anjil et al., 2016). Over-expression of maize ZmSCE1e gene increased SUMO conjugation level and improved drought and salinity tolerance in transgenic tobacco (Huanyan et al., 2019). In addition, SUMO system modulates ABA signaling (Lois et al., 2003), and mediates bacterial and viral pathogenesis resistances (Lee et al., 2007). Although, the SUMO system is well understood at molecular level in Arabidopsis, but its roles still remains poorly understood in many food crops including Tomato. Therefore, this study reported that the structure and functional characterization of *SlS-SUMO1* gene from Tomato.

2. Material and methods

2.1. RNA extraction and gene amplification

Experimental analysis was carried out in the CEBR Lab, Science College, King Saud University (KSU) and Rice Research and Training Center (RRTC), Field Crops Research Institute, Egypt. *Solanum lycopersicum* L., cv Saudi-1 was used for gene cloning. The total RNA was extracted from leaves of three weeks old seedlings by the method described by (Yuta, et al., 2012), and used for first strand cDNA synthesis using SuperScriptII (Invitrogen, currently Life-technologies Co.). The coding region of *SlS-SUMO1* was amplified using specific primers: 5′-ATATGTCAGGCGTCACTCAACA-'3 (forward) and 5′-TCTAAGACAAAGATCCACCAGT-'3 (reverse) primer, which have designed based on cDNA of SUMO region of Potatoes genome (*solanum tuberosum* L.). The PCR amplification reaction was done as following: 10X PCR buffer 2.5 μL, 3.5 mM dNTP 2.5 μL, 2.5 μM primers (forward and reverse primer mix) and 0.2 μL of Taq DNA Polymerase (10U/μL) (genshung bio). Gene amplification was carried out following the PCR protocol with initial denaturation 94 °C for 4 min, annealing temperature 55 °C for 1 min., PCR cycle was 35 cycles of 94 °C 1 min; and final extension was 72 °C for 7 min. Electrophoresis was done using 2% Agarose gel with 100 bp DNA ladder to determine the expected band size.

2.2. Sequences analysis and phylogenetic tree

Sequencing of *SlS-SUMO1* region: The PCR product was purified using QIAquik Gel Extraction kit (Qiagon Inc., Chatsworth, California) and both strands were sequenced with an ABI377 automated DNA sequencer (Applied Biosystems Inc., Foster City, California). The sequences for individual components of Sumoylation from different organisms were obtained from the NCBI database. Sequences were aligned using ClustalW program and were searched for conserved domains using the Pfam database (Finn et al., 2008). The evolutionary history was inferred by using the Maximum Likelihood method based on Finn-2008 model. Phylogenetic tree was obtained using Neighbour-Joining method to a matrix of pairwise distances estimated using a JTT model. *SlS-SUMO1* coding region was analyzed to investigate the sequence features of Sumoylation binding domain.

2.3. Construction of the expression vector harboring SlS-SUMO1

To analyze cellular localization of Tomato SUMO proteins, the constructed vector, which expresses fluorescent maker DsRed, and SlS-SUMO1 fusion protein, named *pDsRed:SlS-SUMO1* has been designed (Fig. 1a). The gene was driven by the cauliflower mosaic virus 35S promoter (CaMV35S). Additionally, the SUMO conjugation enzyme gene (SCE1a) has cloned into *pGFP:SCE1a* vector (Fig. 1b) to study the co-expression and co-localization with SlS-SUMO1 proteins.

2.4. Transformation and subcellular localization of SlS-SUMO1 protein

Onion epidermal cells were bombarded with 3 μg of DNA constructs using a helium biolistic gun (Biolistic® PDS-1000/He Particle Delivery System, BioRad). After keeping the onion epidermal cells in the dark at room temperature for 24 hrs, cellular localization of DsRed and GFP fusion proteins have observed using confocal laser scanning microscopy (FV300-BX61, Olympus) according to method described by Kitajima et al., 2009.

3. Results

3.1. Sequence analysis of SlS-SUMO1 gene

In this study, PCR results amplified single band around 400bps (Fig. 2a) using the specific primers: 5′-ATATGTCAGGCGTCACT CAACA-'3 (forward) and 5′-TCTAAGACAAAGATCCACCAGT-'3 (reverse). This target band has sequenced using an ABI377 automated DNA sequencer and then cloned into pUC18 vector (Fig. 2b). This gene named *SlS-SUMO1*and was found to have the full-length coding sequences (CDS) 340 bp with 115 amino acids (aa) full length protein contains 99 aa active and conserved regions for precursor SUMO protein. The protein sequence of this gene has the features of SUMO members, which has C-terminal diglycine

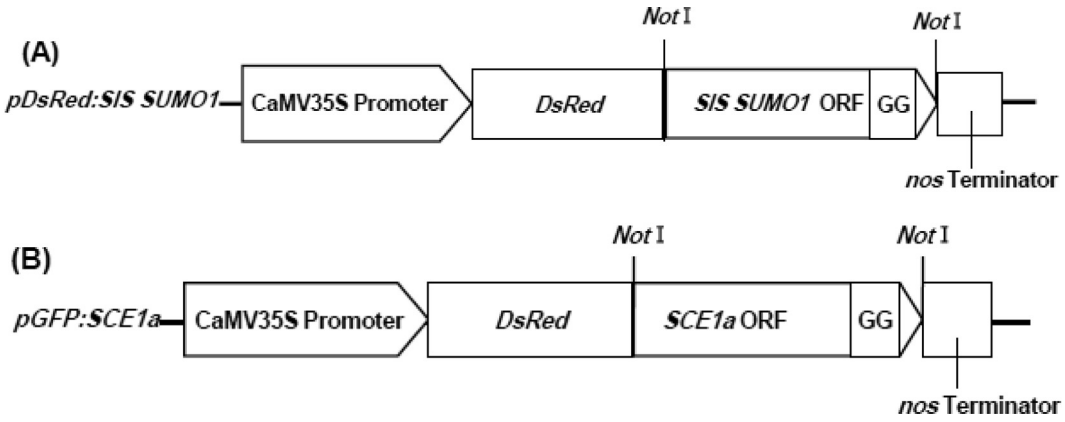

Fig. 1. Construction of expression plasmids harboring SlS-SUMO1 gene (A), and SCE1a gene (B). Fluorescent maker DsRed associated with SlS-SUMO1 protein, while GFP marker associated with SCE1a protein.

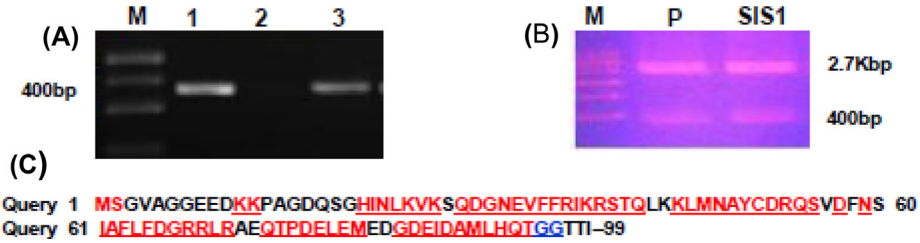

Fig. 2. PCR amplification of *SlS-SUMO1* gene (A) M: marker, 1, 3 represent positive band of the gene, 3 negative sample. (B) Re-Digestion of recombinant pUC18 plasmid containing *SlS-SUMO1* gene, P: positive control. (C) The active length protein (99 aa) of the *SlS-SUMO1* gene, the conserved regions marked with red color, C-terminal diglycine (GG) motif marked with blue color as processing site by ULP.

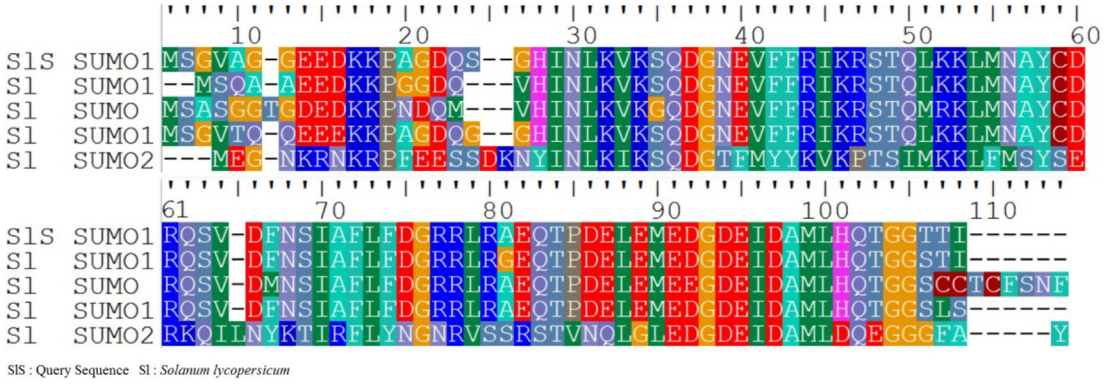

Fig. 3. Alignment of SUMO genes in Tomato. The genes have C-terminal diglycine (GG) motif as processing site. SlS-SUMO1 query protein has 90% identity to all Sl-SUMO1 proteins.

(GG) motif as processing site by ULP (Fig. 2c). The protein alignment analysis of *SlS-SUMO1*gene with Tomato *SUMO* gene family, showed higher identity with the proteins of all Tomato *SUMO1* genes especially in the features of Sumoylation and conserved regions. While it showed similarity with *SlSUMO2* in Sumoylation features and some conserved sites (Fig. 3).

3.2. Phylogenetic analysis of SlS-SUMO1 gene

Phylogenetic tree was performed to elaborate the genetic relationship between Tomato SUMO family and SUMO members of different plant families including Solanacea, Asteraceae, Chenopodiaceae, Pedaliaceae, Cucurbitaceae, Pedaliaceae, Chenopodiaceae, Fabaceae, and Asparagaceae. *SlS-SUMO1* was analyzed with twenty-six *SUMO* genes collected from Genbank database and evolutionary analyzed using MEGA7 program (Kumar et al., 2016). The phylogenetic tree indicated that all putative and identified SUMO proteins classified in the clades (Fig. 4). In addition, the results recorded five putative SUMO proteins in Tomato genome and are conserved inside plant genome of different species. This suggested the conserved nature of the homology Tomato SUMO member's transcription factors regardless of their origins. SlS-SUMO1 protein is homologous to Potato Ca-SUMO1 and Ca-SUMO2 based on sequence similarity. The other Tomato SUMO proteins classified in anther sub-cluster, however, they are SUMO members but their function still unknown.

3.3. Cellular localization and expression analysis of SlS-SUMO1 protein

The expression vector; *DsRed:SlS-SUMO1* was transiently expressed in onion epidermal cells and the fluorescent DsRed signals were observed using confocal laser scanning microscopy (Fig. 5). The results showed that *DsRed:SlS-SUMO1* signals are detected in both cytoplasm and nucleus but not in nuclei (Fig. 5a). Control DsRed signal also detected in both cytoplasm and nucleus only (Fig. 5d). Moreover, control DsRed showed clear gap at the border zone around nucleus, whereas *DsRed:SlS-SUMO1* did not showed any gap around nucleus. The result suggested that the *DsRed:SlS-SUMO1* also located in nuclear envelop or nuclear pore complex.

3.4. Sumoylation activity of DsRed:SlS-SUMO1 protein

To investigate the Sumoylation activity of SlS-SUMO1 protein, the expression vectors *DsRed:SlS-SUMO1* and *GFP:SCE1a* have constructed (Fig. 1). The Onion cells have transformed to study the co-expression and co-localization of SlS-SUMO1with SUMO conjugation enzyme. The results showed that both signals of *GFP:SCE1a* and *DsRed:SlS-SUMO1* co-expressed and co-localized in nucleus and formed nuclear subdomains (Fig. 6a). The high magnification image of this result also confirmed the co-expressed DsRed:SUMOs with GFP:SCE1a formed nuclear subdomains (Fig. 6b). These subdomains seem to be speckles. These results indicated that *DsRed:SlS-SUMO1* is processed and activated by Onion Sumoylation system, and conjugated to the unknown target proteins of Onion cells within *SCE1a:GFP* localized in nuclear foci.

4. Discussion

This study reported the cloning and functional characterization of *SlS-SUMO1* gene from Tomato. The *SlS-SUMO1* gene has C-terminal diglycine (GG) motif as processing site, and it is homologous to Potatoes *Ca-SUMO1* and *Ca-SUMO2* based on sequence similarity. So far, there are five putative *SUMO* genes have been

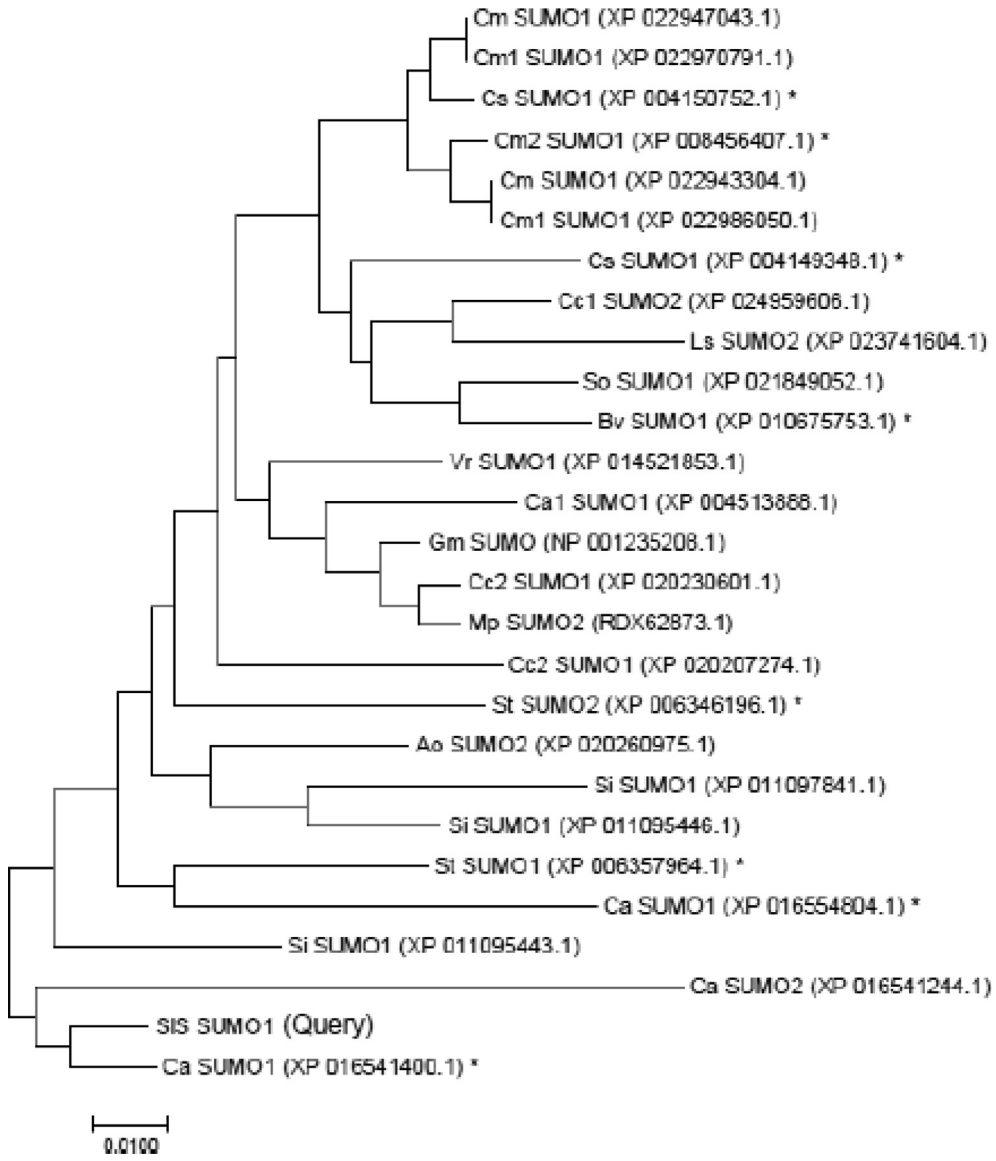

Fig. 4. The phylogenetic tree of Tomato SUMO family with different 26 SUMO members from plant species. SlS-SUMO1 protein is homologous to Potato Ca-SUMO1 and Ca-SUMO2. The other Tomato SUMO proteins classified in anther sub-cluster. Multiple sequence alignment result of SUMOs was done using ClustalW program.

cloned from Tomato genome including *SlS-SUMO1* gene (Song et al., 2017; Saad et al., 2016). Data from phylogenetic tree showed that Tomato SUMO proteins classified in different sub-clusters, however, they are SUMO members but their function still unknown. Alignment sequence of SlS-SUMO1 protein with 23 SUMOs like proteins from different organisms depict all the SUMOs like proteins share higher level homology at DNA binding domain. This suggested such SlS-SUMO1 protein regions have been conserved during evolution due to their vitality in sequence conformation and function. The DsRed signals from *DsRed:SlS-SUMO1* and *35S::DsRed* (Control) were detected in both nucleus and cytoplasmic location. Moreover, control *35S::DsRed* showed clear gap at the border zone around nucleus, whereas *DsRed:SlS-SUMO1* did not showed any gap around nucleus. This indicated that the expression of DsRed:SlS-SUMO1 protein located in nucleus, cytoplasm, and nuclear envelop or nuclear pore complex. In addition, the results demonstrated the rule of GG motif for processing and localization of SlS-SUMO proteins in the plant cell. This data was in agreement with the data reported by (Conti et al., 2008). Our data revealed the co-expression of DsRed:SlS-SUMO1 protein with E2-congjugation enzyme (GFP:SCE1a) was co-localized into the nucleus and showed sub-nuclear localization. This suggested that the SlS-SUMO1 precursor is processed, activated and transported to nucleus through the Onion Sumoylation system, and the SlS-SUMO1 possibly bind to substrate proteins of Onion cells.

Recent studies on SUMO rice reported that the DsRed signals from *DsRed:SUMO1* and *DsRed:SUMO2* of rice were detected both in nucleus and cytoplasmic location, but not in nucleoli (Yuta et al., 2012). While *DsRed:SUMO3* signal was detected mainly in nucleus, and formed sub-nuclear domain structure (Lois, 2010, Yuta et al., 2012). In addition, deletion of GG motif suppressed the accumulation of the rice SUMO proteins in the nucleus (Yuta, et al., 2012). These data indicated that the C-terminal processing of rice SUMO precursor proteins are necessary for SUMO localization to nucleus in plant cells (Masayuki et al., 2008). It has been demonstrated that SUMO conjugation on target proteins is one such mechanism that plays an important role in regulating *Arabidopsis* growth under abiotic stress (Conti et al., 2014; Robert

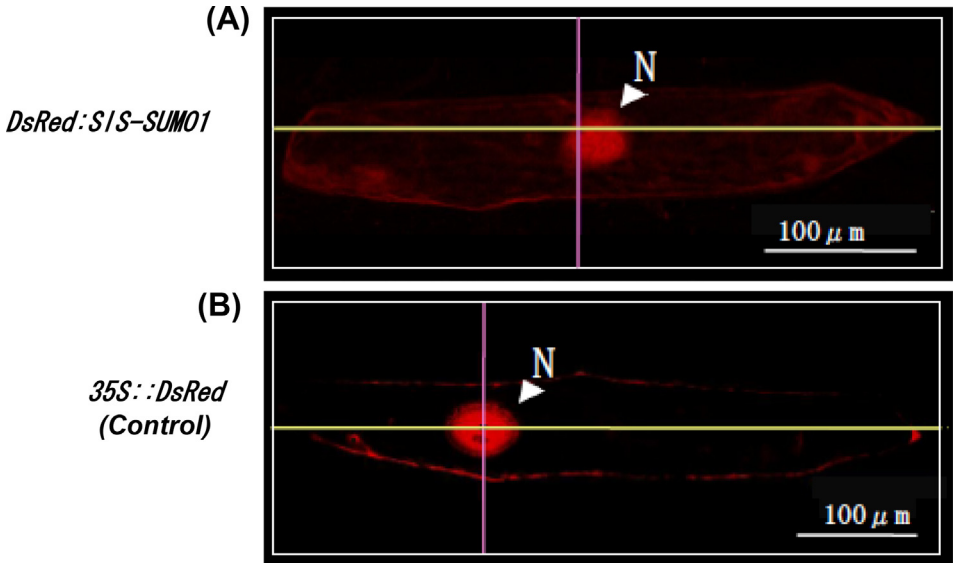

Fig. 5. Transient Expression of *DsRed:SlS-SUMO1* in Onion Cells. (A) *pDsRed:SlS-SUMO1* was transiently expressed in onion epidermal cells, and the DsRed signals were detected by confocal scanning laser microscopy. (B) Distribution of DsRed signals by expression of 35S::DsRed (control). White arrow heads showed nucleus.

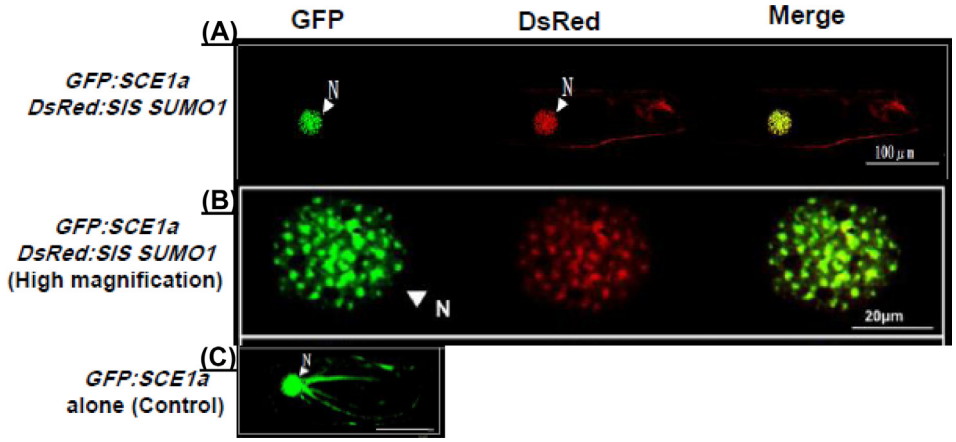

Fig. 6. Transient co-expression and co-localization of *DsRed:SlS-SUMO1* and *GFP:SCE1a* in onion cells. (A) *GFP:SCE1a* with *DsRed:SlS-SUMO1*, (B) *GFP:SCE1a* with *DsRed:SlS-SUMO1* in higher magnification image. (C) *GFP:SCE1a* control. Signal from GFP, DsRed and the merge of both signals are shown in the left, center and right, respectively. N indicates the nucleus in each panel.

and Augustine, 2018). However, till now a few reports exist on the role of SUMO in crops especially Tomato. So that further study should be done to study the functional expression of *SUMO* genes in Tomato under biotic and abiotic stress.

5. Conclusion

This study concluded that the *SlS-SUMO1* gene from Tomato is a member of Tomato SUMO system. The *SlS-SUMO1* gene has GG motif and SUMO consensus ΨKXE/D sequence. It is homologous to *Ca-SUMO1* and *Ca-SUMO2* based on sequence similarity. The expression protein of this new gene was localized in the nucleus, cytoplasm. The SlS-SUMO1 protein of this new gene with SUMO conjugating enzyme SCE1a co-expressed and co-localized in nucleus and formed nuclear subdomains. This data demonstrated that the *SlS-SUMO1* gene is a member of SUMO family and its SUMO protein activate and transport to nucleus through Sumoylation system in the cell.

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