Fifty Shades of SUMO: its role in immunity and at the fulcrum of growth-defense balance

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Summary

The sessile nature of plants requires them to cope with an ever changing environment. Effective adaptive responses require sophisticated cellular mechanisms at post-transcriptional and -translational levels. Post-translational modification by Small Ubiquitin-like Modifier (SUMO) proteins is emerging as a key player in these adaptive responses. SUMO conjugation can rapidly change the overall fate of target proteins by altering their stability or interaction with partner proteins or DNA.. SUMOylation entails an enzyme cascade that leads to the activation, conjugation and ligation of SUMO to lysine residues of target proteins. In addition to their SUMO processing activities, SUMO proteases also possess de-conjugative activity capable of cleaving SUMO from target proteins providing reversibility and buffering to the pathway. These proteases play critical roles in maintaining SUMO machinery in equilibrium. We hypothesise that SUMO proteases provide the all-important substrate specificity within the SUMO system. Furthermore, we provide an overview of the role of SUMO in plant innate immunity. SUMOylation also overlaps with multiple growth promoting and defense-related hormone signaling pathways and hence is pivotal for maintaining the growth-defense balance. This review aims to highlight the intricate molecular mechanisms utilized by SUMO to regulate plant defense and stabilize the growth-defense equilibrium.

Introduction

Global food security has emerged as an escalating challenge for mankind in the last few decades. The rapid decrease in arable land area, depleting groundwater table and aberrant climatic conditions are choking crop production efforts and posing serious threats to food security. The issue is further exacerbated by the exponential increase in crop losses due to plant pathogens. About ~15% of global crop production is lost every year due to plant diseases originating from fungi, oomycetes, bacteria and viruses (Dangl *et al.*, 2013). Therefore, an array of concerted and coordinated efforts is required to improve plant disease responses against different pathogens. This entails a deeper insight into plant defense mechanisms in order to boost plant resistance against various biotic stresses.

Evolutionary pressure over millions of years has equipped plants with intricate, yet sophisticated molecular mechanisms helping them to respond and defend against a wide array of stress conditions. Plants recognize conserved pathogen-associated molecular patterns (PAMPs) through pattern recognition receptors (PRRs) resulting in the activation of PAMP-triggered immunity (PTI) (Zipfel, 2014). To counteract PTI, some pathogens secrete 'effector' proteins in the host plant that interfere with plant processes including immune responses causing effector-triggered susceptibility (ETS) (Jones and Dangl, 2006). Nevertheless, the effectors can be recognized and blocked by distinct nucleotide-binding leucine-rich repeat (NB-LRR) resistance (R) proteins following the gene-for-gene resistance model by Harold Henry Flor (Chisholm *et al.*, 2006, Jones and Dangl, 2006) Flor, 1946; Such specific NB-LRR triggered plant responses elicit effector-triggered immunity (ETI) resulting in programmed cell death at the site of infection, also known as hypersensitive response (Bethke *et al.*, 2009).

PTI and ETI together form a complicated multi-layered defense phenomenon that involves rapid regulation of physiological events at transcriptional, translational and post-translational levels. Post-translational modification (PTM) of proteins has been identified as a critical process for quick adaptation of plants to environmental stresses (Yates *et al.*, 2016). PTM comprises the addition of small molecules or proteins to target proteins after translation, resulting in the modification of target proteins stability, localization or their interaction with other partners (Seo and Lee, 2004). There are several PTMs playing indispensable roles in mediating plant responses to different stress conditions. These include phosphorylation, acetylation, nitrosylation and glycosylation as the major small molecule PTM systems (Piquerez *et al.*, 2014). Besides these small molecule PTMs, a couple of small proteins ranging from 70 – 100 amino acids also serve as important post-translational modifiers.

Ubiquitination is a proteinaceous PTM system playing integral roles in controlling plant defense responses by altering target protein stability and activity (Kerscher *et al.*, 2006, Sadanandom *et al.*, 2012). One such example comes from the ubiquitination of leucine-rich repeat-receptor kinase FLAGELLIN-SENSING 2 (FLS2) by two E3 ligases Plant U-Box12 (PUB12) and PUB13 preventing constitutive activation of the immune receptor (Lu *et al.*, 2011). Biochemically, ubiquitin is a 76 amino acids peptide that binds to target proteins via the carboxyl terminus of Gly76 using three specific enzymes, namely, ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2) and ubiquitin ligase (E3) (Sadanandom *et al.*, 2012).

Monoubiquitination of target proteins affect cellular localization, activity or interaction with other proteins (Sadowski *et al.*, 2012), while polyubiquitination

determines the fate of target proteins depending upon the chain topology (Walsh and Sadanandom, 2014). For instance, polyubiquitination-mediated proteasomal degradation relies on Lys48-linkages (Jacobson *et al.*, 2009), whereas, Lys63-linked polyubiquitin chains recruit other binding partners and regulate kinase activation and DNA repair mechanisms (Jacobson *et al.*, 2009, Walsh and Sadanandom, 2014).

The removal of ubiquitin molecules is brought about by deubiquitinating enzymes (DUBs) that process poly-ubiquitin chains to mono-ubiquitin units or can completely remove ubiquitin from the substrate proteins (Isono and Nagel, 2014). The different enzymes participating in the process, besides catalyzing the respective reactions, are also responsible for providing substrate specificity. The *Arabidopsis* genome contains two genes encoding E1 enzymes, about 45 genes encoding for E2s and more than 1400 genes encoding E3 ligases (Kraft *et al.*, 2005). To further add to the specificity, there are about 50 genes for encoding DUBs (Isono and Nagel, 2014). Hence, the enormous permutations and combinations possible among the different ubiquitination enzymes provide selectivity and specificity towards different protein targets.

Similar to ubiquitination, Small Ubiquitin-like Modifier (SUMO), another potent post-translational protein modification system (Vierstra, 2012),,is also capable of modulating target protein stability, interaction with its partners and subcellular localization (Wilkinson and Henley, 2010). Since its discovery in plants, SUMO has been associated with many different biological processes, such as, growth, flowering, light signaling, abiotic stress responses and responses to pathogen infection (Bailey *et al.*, 2016, Conti *et al.*, 2008, Kurepa *et al.*, 2003, Lee *et al.*, 2007, Murtas *et al.*, 2003, Sadanandom *et al.*, 2015) . Notably, one of the main class of targets of SUMO

modification are transcription factors that coordinate transcriptional regulation during developmental and defense processes (Miller *et al.*, 2010). SUMO conjugation to transcriptional complexes facilitates precise regulation of gene expression networks.

The SUMO machinery

The process of SUMOylation, like ubiquitination, requires attachment of approximately 100-115 amino acid long SUMO proteins to target substrates via a series of enzymatic reactions (Fig. 1). In *Arabidopsis*, eight SUMO genes have been identified with only four of these eight genes encoding for SUMO proteins, *viz.* *At*SUMO1, *At*SUMO2, *At*SUMO3 and *At*SUMO5 (Hammoudi *et al.*, 2016). The SUMO proteins are highly divergent in their spatio-temporal expressions patterns and functions during development and defense (Saracco *et al.*, 2007, van den Burg *et al.*, 2010). *At*SUMO1 and *At*SUMO2 share high sequence identity (89%), whereas, *At*SUMO3 (48%) and *At*SUMO5 (35%) are less closely related to *At*SUMO1 (Hammoudi *et al.*, 2016). The evolutionary divergence among SUMO proteins is functionally relevant; since *At*SUMO1 and *At*SUMO2 together prevent salicylic acid accumulation in noninfected plants, while *At*SUMO3 promotes plant defense downstream of SA (van den Burg *et al.*, 2010).

The ubiquitin-like proteases (ULPs) via their SUMO peptidase activity initiate the process of maturation of SUMO precursor molecules (Park *et al.*, 2011). These ULPs recognize a carboxyl-terminal diglycine (GlyGly) motif in SUMO proteins and remove about 10 amino acids after the GlyGly (Johnson, 2004), thereby exposing the motif for conjugation to target proteins. The first step of SUMOylation is catalyzed by a SUMO activating enzyme, SUMO E1, consisting of two small subunits 1a and 1b

(SAE1a and SAE1b) and the large subunit SAE2 (Johnson, 2004). The reaction involves hydrolysis of an ATP molecule resulting in the formation of a high-energy thioester bond between the sulfhydryl group of a cysteine (Lucyshyn and Wigge) residue in SAE2 and the carboxyl group of a glycine (Gly) in SUMO (Park *et al.*, 2011). Activated SUMO is, subsequently, transferred from SAE2 to a Cys residue in SUMO conjugating enzyme, SCE1. The final step involves transfer of SUMO from SCE1 to the ε-amino group of a lysine (Lys) present in the SUMO attachment site of the target protein, largely, catalyzed by SUMO E3 ligases (Park *et al.*, 2011). The precise positioning of SUMO E3 ligases –between SCE1 and substrate- is facilitated by the presence of a SP-RING domain, which is central for interactions with SCE1 and substrate (Ishida *et al.*, 2012). Nevertheless, the scenario is contrasted in mammals where some E3 ligases do not possess the RING domain (Pichler *et al.*, 2004) and it is speculated that they exert their catalytic activity by altering E2's properties rather than by mediating interactions between E2 and substrate (Pichler *et al.*, 2004). The fact that SUMO conjugation to target proteins can also be directly catalyzed by E2 enzymes without the involvement of E3 ligases (Wilkinson and Henley, 2010) placed some doubt on the requirement of E3 ligases in the SUMOylation pathway. However, subsequently several proteins were reported to possess E3 ligase activity but the numbers are mainly restricted to yeast and mammalian systems (Miura and Hasegawa, 2010). Yeast possesses four E3 ligases, which include Siz1, Siz2, Mms21 and Zip3, whereas mammals have 10, such as PIAS, MMS21, Pc2 and TOPORS (Miura and Hasegawa, 2010).

So far in *Arabidopsis* only two E3 ligases, HIGHPLOIDY2 (HPY2) and SAP & Miz1 (SIZ1), have been identified (Ishida *et al.*, 2012). This is also in sharp contrast to the

plant ubiquitination system, which contains about 1400 E3 ligases (Kraft *et al.*, 2005). This clearly implies that plant SUMO E3 ligases are likely fail to provide the specificity component required for SUMOylation to participate in such a wide range of biological processes. However relative gene diversification observed in SUMO deconjugation enzymes,, in our opinion is likely to fulfill this gap.

Deconjugation of SUMO from SUMOylated protein substrates is also a crucial step of the SUMOylation process to maintain the equilibrium in SUMO signaling. This is termed deSUMOylation to differentiate it from SUMO maturation. However, similar to SUMO maturation, the deSUMOylation process is also brought about by the same set of ULPs via their isopeptidase activity (Saracco *et al.*, 2007). Nevertheless, a recent report by Castro *et al.* (2016) suggests that some ULPs function primarily as SUMO deconjugation/isopeptidase enzymes and do not have much of a role to play in SUMO maturation.

Intensive investigations into the functions of SUMO proteases in plants have identified seven SUMO-specific proteases in *Arabidopsis* so far (Yates *et al.*, 2016). These include ULP1a, ULP1b, ULP1c/OVERLY TOLERANT TO SALT 2 (OTS2), ULP1d/OTS1, ULP2a, ULP2b and EARLY IN SHORT DAYS 4 (ESD4) (Kurepa *et al.*, 2003, Miura and Hasegawa, 2010). Elegant physiological data have confirmed the involvement of SUMO proteases in various cellular processes of plants, such as, plant development (Castro *et al.*, 2016) cell cycle progression, hormone signaling and defense responses. Attempts to understand the diversification of the SUMO protease family in plants have highlighted the presence of more genes encoding SUMO proteases than SUMO E3 ligases (Yates *et al.*, 2016). This clearly indicates that

deSUMOylation is a key regulatory step in the entire SUMOylation process and SUMO proteases can provide the required specificity to substrate proteins, being functionally analogous to the role of E3 ligases in the ubiquitination process.

Some of the earlier reports highlighting the functioning and interaction of SUMO proteases offer valuable clues about their role in substrate specificity. A yeast two-hybrid screening with ESD4 as the bait protein identified 238 potential SUMO substrates (Elrouby and Coupland, 2010) implying that ESD4 SUMO protease activity is specific to the identified 238 interactors. Elegant biochemical experiments in the past exhibited striking features about the specificity of AtULP1 members for different SUMO substrates (Chosed *et al.*, 2006, Colby *et al.*, 2006). All AtULP1s process AtSUMO1 and AtSUMO2, whereas, only AtULP1a weakly processes AtSUMO3 and none of the AtULP1s cleave AtSUMO5 (Chosed *et al.*, 2006, Colby *et al.*, 2006). The functional divergence among different SUMOs (van den Burg *et al.*, 2010) and specificity of different AtULP1s for these SUMOs (Chosed *et al.*, 2006) provide substantial support to the claim that SUMO proteases play a key role in determining substrate specificity.

Besides covalent conjugation of SUMO to target proteins, SUMO also elicits its effects on target proteins via non-covalent interactions (Merrill *et al.*, 2010). In general, the SUMO interaction motifs (SIMs) consist of a hydrophobic core having the consensus sequence, V/I-x-V/I-V/I or V/I-V/I-x-V/I/L, where position two or three can be any amino acid (Merrill *et al.*, 2010). Acidic residues often flank the hydrophobic core. The SUMO-SIM interaction offers a vital point of control in regulating SUMO-mediated cellular processes (Conti *et al.*, 2014). Additionally, in

certain cases, SIMs are required to be present in the substrate for the covalent modification of the substrate by SUMO (Merrill *et al.*, 2010), thereby establishing SIMs as an integral part of the SUMO machinery. SUMO conjugation of SIM-bearing substrates is crucial for protein-group SUMOylation, an emerging concept proposed by (Jentsch and Psakhye, 2013). The model suggests that SUMOylation targets entire group of physically interacting proteins rather than individual targets. Protein-group SUMOylation is initiated by SUMO E3 ligases, which get recruited on preassembled protein complexes (Jentsch and Psakhye, 2013). The presence of SIMs in proteins targeted for protein-group SUMOylation facilitates physical interactions between proteins through multiple SUMO-SIM interactions.

SUMO in plant defense response

Elaborate genetic analyses of the mutants of the SUMO enzymatic machinery have highlighted the significance of SUMO conjugation in regulating plant defense (Gou *et al.*, 2017). For instance, modulation of SUMO protein levels has a pronounced impact on the plant defense system (van den Burg *et al.*, 2010). The *sum1sum2* knockdown mutant exhibited increased levels of SA and enhanced resistance to *Pst* DC3000, consequently (van den Burg *et al.*, 2010). Contrastingly, silencing of SCE1, the SUMO E2 conjugating enzyme, in *Solanum peruvianum* using virus-induced gene-silencing showed higher disease susceptibility to *Clavibacter michiganesis* subsp. *Michiganesis* (Esparza-Araiza et al., 2015). A recent work highlighted that besides SUMO machinery components, the process of SUMO conjugation also has a vital role to play in maintaining plant immunity (Castano-Miquel *et al.*, 2017). Disruption of SUMO E1-E2 interactions by expressing E1 $SAE2^{UFDCt}$ domain causing inhibition of SUMO conjugation, resulted in increased susceptibility to infection by *Botrytis*

cinerea and *Plectosphaerella cucumerina* (Castano-Miquel *et al.*, 2017). Furthermore, host SUMO conjugation was post-transcriptionally down regulated after fungal infection suggesting SUMOylation machinery as a target for fungal pathogencity, thereby reinforcing the role of SUMOylation in defense responses (Castano-Miquel *et al.*, 2017).

Null *siz1* mutants exhibited increased resistance to the bacterial pathogen *Pseudomonas syringae* pv. *tomato* (*Pst*) DC3000 (Lee *et al.*, 2007). The increased resistance was attributed to constitutive upregulation of disease response genes, such as, *PHYTOALEXIN-DEFICIENT4* (*PAD4*), *SALICYLIC ACID INDUCTION DEFICIENT2* (*SID2*) and *ENHANCED DISEASE SUSCEPTIBILITY1* (*EDS1*) and pathogenesis-related genes, which was due to elevated SA levels in the *siz1* mutant (Lee *et al.*, 2007). Recently, Gou *et al.* (2017) showed that *siz1*-regulated plant immunity is partially mediated by the NB-LRR immune receptor SNC1. The autoimmune phenotype showed by *siz1* mutant is dependent on SNC1 and *SIZ1* overexpression partially rescues the mutant phenotype by attenuating the SNC1 protein amounts (Gou *et al.*, 2017).

Being the key regulatory step and the specificity-determining factor, the deSUMOylation process has proved to be a prime target for undermining plant immunity (Hotson *et al.*, 2003, Orth *et al.*, 2000). One of the first pieces of evidence for of the role of deSUMOylation in phytopathogen infection comes from the finding of bacterial effector proteins functioning as cysteine proteases with plant-specific SUMO substrate specificity (Hotson *et al.*, 2003). XopD, a type III effector protein from *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*), encodes an active SUMO

protease that is injected into the plant cells by the type III secretion system during *Xcv* pathogenesis (Hotson *et al.*, 2003). Subsequently, the protein is translocated to the plant cell nucleus and subnuclear foci. XopD mimics an endogenous plant SUMO isopeptidase with SUMO-conjugated proteins as their substrates (Hotson *et al.*, 2003).

XopD$_{Xcc8004}$, a shorter version of XopD lacking the N-terminal domain is also a type III effector from *X.c.* pv. *campestris* (*Xcc*) acting as a SUMO protease (Tan *et al.*, 2015). XopD$_{Xcc8004}$ expression in *Arabidopsis* elicited host defense response genes solely dependent on its SUMO protease activity; in transgenic plants harbouring XopD$_{Xcc8004}$ (C355A) no elicitation was noticed (Tan *et al.*, 2015). A recent report has highlighted that besides having SUMO protease activity, XopD also possesses deubiquitinase activity (Pruneda *et al.*, 2016). This clearly implied that *Xanthomonas* uses PTM deconjugation as a general mechanism to attenuate plant defense signaling pathways.

YopJ and YopJ-like effectors, such as, AvrBsT and AvrXv4, from *Xanthomonas* were also, initially, proposed to possess SUMO protease activity based on the limited sequence homology with ULPs (Orth *et al.*, 2000, Roden *et al.*, 2004). However, interestingly, later studies revealed that YopJ functions as an acetyltransferase (Mukherjee *et al.*, 2006). A similar report surfaced for AvrBsT and showed the effector to be an acetyltransferase (Cheong *et al.*, 2014). It was surprising, at least for AvrXv4, because its expression *in planta* had resulted in a reduction of SUMO-conjugated proteins exhibiting its SUMO isopeptidase activity (Roden *et al.*, 2004). Therefore, it could be speculated that YopJ-like effectors possess the dual function of SUMO deconjugation and acetylation, equivalent to XopD functioning as a SUMO

protease and deubiquitinase (Hotson *et al.*, 2003, Pruneda *et al.*, 2016). Alternatively, AvrXv4 could be a stand-alone YopJ-like effector functioning as a SUMO protease, whereas, all other YopJ-like effectors are acting as acetlytransferases, because to date, no study has shown the acetyltransferase activity of AvrXv4.

In addition to the phytopathogen-injected effector proteases that mediate deSUMOylation in order to alter plant defense responses, plant endogenous SUMO proteases are also known to play integral roles in regulating defense responses (Bailey *et al.*, 2016). Increased amounts of global SUMO conjugation in the *ots1ots2* double mutant coincided with enhanced resistance to *Pst* DC3000 and elevated levels of SA, compared to wild-type plants (Bailey *et al.*, 2016). OTS1 and -2 limit SA biosynthesis by suppressing *ISOCHORISMATE SYNTHASE1* (*ICS1*) expression. As a feedback, SA promotes degradation of OTS1 and -2 (Bailey *et al.*, 2016). Taken together, SUMO proteases form a critical aspect of plant defense signaling and hold the key for regulating defense responses at transcriptional, translational and post-translational levels. Given that novel SUMO targets are being identified lately, newer SUMO proteases are likely to be discovered to account for substrate specificity. Hence, the total number could be ten-fold or greater, compared to SUMO E3 ligases.

Identification of chromatin remodeling proteins, DNA repair proteins and transcription factors involved in plant defense responses as SUMO targets have further substantiated the role of SUMOylation in controlling defense processes in plants (Elrouby and Coupland, 2010, Miller *et al.*, 2010). Identification of histone H2B, HISTONE DEACETYLASE19 (HDA19), a transcriptional repressor of defense-related genes and the transcriptional corepressors SIN3 and TOPLESS as

SUMO targets was a big step forward (Miller *et al.*, 2010). Recognizing transcription factors as potential SUMOylation targets has opened an entire new avenue of research (van den Burg and Takken, 2010). Transcription factors are deemed responsible for controlling the expression of thousands of genes required to regulate cellular processes of plants under specific conditions. Therefore, SUMOylation of the transcription factors would provide an additional level of control for boosting or suppressing biological events (van den Burg and Takken, 2010). Identification of key stress-responsive transcription factors, such as, WRKY33, ETHYLENE RESPONSE FACTOR 104 (ERF104) and ETHYLENE INSENSITIVE 3 (EIN3) as SUMOylation substrates clearly indicated that involvement of SUMO in plant disease resistance is deep-rooted (Miller *et al.*, 2010). WRKY33 is an important component of the plant defense response against necrotrophic pathogens, such as, *Botrytis cinerea* and *Alternaria brassicicola* (Zheng *et al*., 2006). *wrky33* mutants showed enhanced susceptibility to these necrotrophs, whereas *WRKY33* overexpression resulted in increased resistance (Zheng *et al.*, 2006). On the contrary, overexpressing WRKY33 supported enhanced growth of *Pseudomonas syringae*. This highlighted that WRKY33 mediates cross-talk between defense responses to pathogens with different mechanisms of pathogenesis. ERF104, another identified putative SUMO target is also a vital regulator of basal immunity (Bethke *et al.*, 2009). Both overexpression of *ERF104* and *erf104* mutant exhibited enhanced susceptibility to a non-adapted bacterial pathogen and more growth inhibition by flg22 (Bethke *et al.*, 2009). Moreover, it has also been observed that the list of identified SUMO targets overlaps significantly with targets for phosphorylation by MITOGEN ACTIVATED PROTEIN KINASEs (MAPKs), which are protein kinases phosphorylating transcription regulators controlling defense gene expression (van den Burg and

Takken, 2010) (Bethke *et al.*, 2009, Mao *et al.*, 2011). For example, both WRKY33 (Mao *et al.*, 2011) and ERF104 (Bethke *et al.*, 2009) are phosphorylated by MAPK cascades in response to pathogen stimulus. This implies that SUMOylation and MAPK-mediated phosphorylation converge to regulate the same transcription factor target for modulating expression of defense genes.

SUMO as the pivot of growth-defense balance

Plant development is a multifaceted phenomenon with several processes taking place simultaneously. At the same time environmental signaling has to be integrated into the growth and development of plants (Lorrain and Fankhauser, 2012, Lucyshyn and Wigge, 2009). However, since plants are assumed to possess a limited pool of resources, one process is activated at the expense of another (Huot *et al.*, 2014, Karasov *et al.*, 2017); for instance, stress responses in crop plants results in massive yield losses. Therefore, it is imperative for plants to maintain a proper balance between growth and defense processes for sustained development.

Noting the extensive involvement of SUMO modification in plant defense responses (Lee *et al.*, 2007) one can envision that it must play an equivalent role in regulating plant growth to maintain the growth-defense equilibrium. Such beliefs are substantially supported by strong expression profiles of OTS1 and OTS2 in different developmental stages of plant growth (Castro *et al.*, 2016) and late germination, leaf growth defects and early flowering phenotypes in their loss-of-function mutant (Castro *et al.*, 2016, Conti *et al.*, 2008). Furthermore, with its diverse range of target proteins associated with different physiological processes, such as, growth, light signaling, flowering and defense responses (Murtas *et al.*, 2003, Sadanandom *et al.*, 2015), SUMO appears to be playing a central role in coordinating the growth-defense equilibrium.

SUMOylation of DELLA proteins provides an example of how SUMO is maintaining the growth-defense balance by modifying growth regulators during stress (Conti *et al.*, 2014). DELLA proteins are known to function as key repressors of molecular pathways regulated by the growth-promoting gibberellic acid hormone (Ikeda *et al.*, 2001, Peng *et al.*, 1997). However, studies have highlighted that growth restraint via DELLA proteins is being utilized as a strategy by plants to survive adverse conditions by accumulating DELLAs (Achard *et al.*, 2008, Navarro *et al.*, 2008). Recently, Conti *et al.* (2014) demonstrated that DELLA accumulation during stress (salt stress) is brought about by SUMOylation-mediated stabilization of DELLA proteins. The covalent conjugation of SUMO to DELLA proteins blocks their direct access to the GA receptor GA INSENSITIVE DWARF 1 (GID1), responsible for promoting DELLA degradation. However, DELLA proteins still recognize GID1 but via the SUMO-SIM (SUMO Interaction Motif) interaction (Conti *et al.*, 2014). This GA-independent interaction between GID1 and SUMOylated DELLAs results in sequestration of GID1, thereby allowing free DELLAs to accumulate and inhibit growth. The SUMO site mutant of GA INSENSITIVE (GAI) supported this notion, because GAI^{K49R} plants had increased root growth in salt stress (Conti *et al.*, 2014). Thus, SUMOylation provided a hormone bypass mechanism to block growth during stress conditions by allowing a fast sequestration of GID1 before GA signaling is downregulated.

Targeting proteins involved in growth as well as defense could be another strategy employed by SUMOylation to keep the growth-defense balance (Fig. 2). AtMYB30, an R2R3-type transcription factor, was earlier reported to be a positive regulator of the hypersensitive cell death response (Vailleau *et al.*, 2002). *MYB30* overexpression

in *Arabidopsis* and tobacco accelerated and intensified the appearance of HR against different bacterial pathogens (Vailleau *et al*., 2002). Contrastingly, in *AtMYB30* antisense lines HR cell death was significantly reduced, thereby diminishing the resistance against different bacterial pathogens (Vailleau *et al.*, 2002). However, recently AtMYB30 was found to be SUMOylated by SUMO E3 ligase SIZ1 and its SUMOylation is critical for the regulation of Abscisic Acid (ABA) signaling during germination and seedling growth (Zheng *et al.*, 2012). *myb30* mutants are hypersensitive to ABA during germination, but expression of wild-type *MYB30* completely complements the mutant phenotype. However, the SUMO site mutant of MYB30 ($MYB30^{K283R}$) only partially rescues the ABA-hypersensitive phenotype of *myb30* (Zheng *et al*., 2012).

The intricate association of SUMOylation with different growth promoting and defense-related hormonal pathways provides further support to the role of SUMO in regulating growth and defense simultaneously. The aforementioned examples of DELLA (Conti *et al.*, 2014) and MYB30 (Zheng *et al.*, 2012) SUMOylation have already offered glimpses of SUMO intervention in GA and ABA signaling, respectively. However, the relationship between SUMO and hormone signaling is far more intense and inherent. Another point of intersection for SUMO and GA signaling is the F-box protein SLEEPY1 (SLY1) (Dill *et al.*, 2004). SLY1 mediates GID1 interaction with DELLA proteins facilitating degradation of DELLA proteins (Dill *et al.*, 2004). Recent work by Kim *et al.* (2015) has shown that SLY1 is a SUMO target and its modification resulted in increased stabilization and interaction with DELLA proteins. This, consequently, caused more DELLA degradation, thereby promoting growth. The SUMOylated SLY1-mediated DELLA degradation compared to

SUMOylated DELLA stabilization gives an image of how different substrates can cause SUMO to have antagonistic impacts on the same protein. This also reinforces the point that substrate specificity has a vital role in determining SUMO functioning. ABA INSENSITIVE 5 (ABI5), one of the key transcription factors involved in ABA signaling (Vishwakarma *et al.*, 2017), is also a SIZ1 substrate for SUMOylation (Miura *et al.*, 2009). The hypersensitive response of the SUMO mutant version of ABI5 ($ABI5^{K391R}$) to ABA clearly implicated that SUMOylation (including SIZ1) functions as a negative regulator of ABA signaling (Miura *et al.*, 2009).

Besides overlapping with GA and ABA signaling pathways, the SUMO system is also known to associate closely with salicylic acid signaling (Bailey *et al.*, 2016). As discussed above, SA, a key player in biotic stress responses, has increased biosynthesis and more active signaling in the SUMO protease mutant *ots1 ots2*, which enhanced resistance to *Pst*DC3000 (Bailey *et al.*, 2016). On the contrary, the SUMO E3 ligase mutant *siz1* has enhanced resistance to *Pst* with elevated levels of *SID2*, *EDS1* and *PAD4 (Lee et al., 2007)*. *sum1 sum2* knockdown mutants also exhibited enhanced resistance to *Pst* DC3000 associated with increased SA levels (van den Burg *et al.*, 2010). Furthermore, NON EXPRESSOR OF PATHOGENESIS-RELATED PROTEIN 1 (NPR1), a key regulator of basal immunity and of SA signaling was, recently, reported to undergo SUMOylation upon immune activation (Saleh *et al.*, 2015). It is noteworthy that NPR1 is only SUMOylated by *At*SUMO3 (Saleh *et al*., 2015), which is unique to *Arabidopsis*, because it is not present in other Brassicaceae members (Hammoudi *et al*., 2016). SUMOylation of NPR1 alters its interaction with partner proteins by promoting interaction with TGA3, which is

required for PR gene expression and blocks its interaction with WRKY70, a repressor of PR genes (Saleh *et al.*, 2015).

Conclusions and Future Directions

From the foregoing discussion, it is evident that SUMO has elaborate molecular and cellular functions in regulating plant growth, development and defense responses. Its ability to fine-tune different signaling cascades, including the hormone signaling pathways brands SUMO as the master regulator of plant adaptation processes. SUMO also fortifies the robustness of defense responses by offering strategies for quick adaptations of hormone signaling pathways in a hormone-independent manner. Furthermore, the concurrent functioning of SUMO in growth and defense processes makes it a critical molecule for maintaining the growth defense balance. However, despite the depth of information being added to the SUMO pool recently, the exact molecular mechanisms underlying the role of SUMO in mediating plant defense responses is still unclear. For example, key chromatin remodeling proteins, such as, HDA19 and defense-related transcription factors, such as, WRKY33 and ERF104 have been identified as SUMO substrates upon stress by large-scale proteome analysis, but how the SUMO conjugation of these proteins are modulating the responses are yet to be understood. Therefore, an elegant set of studies aimed at exploring molecular processes altered by SUMOylated WRKY and ERF proteins, such as, protein-protein interactions, protein-DNA interactions and protein stability should be conducted. This will help to unravel the precise molecular mechanisms utilized by SUMO to modulate defense responses in plants.

The involvement of SUMO in managing the growth defense equilibrium is evident, but how it is suppressing growth and promoting defense, simultaneously, during a stress condition is still not clear. This demands a comprehensive analysis of the SUMO-mediated signaling pathways modulated during growth and defense. Identification of key SUMO target proteins functioning in both pathways will offer valuable entry points of control to start to uncouple growth and defense. It is also possible that SUMO itself is acting as the link between growth and defense and is coordinating the balance by modifying growth-related targets at one end and defense-related targets at another. Uncoupling the two major pathways could be a breakthrough discovery, because it will allow plant growth and defense to progress hand in hand, rather than one at the expense of another. The information can also allow us to manipulate the plant for favouring one pathway over another. Hence, plants will be able to afford sustained growth even under stressed conditions. Therefore, a deeper insight into the SUMO-mediated regulatory processes and identification of novel SUMO targets can open up new avenues of research in the field of plant stress biology.

In addition, adequate emphasis also needs to be laid on the discovery of different classes of SUMO proteases because they hold the key for SUMOylation-mediated regulation of different plant processes. Acting as the determining factor for substrate selectivity during the process adds further onus on the SUMO proteases. The SUMO machinery, unlike the ubiquitin system, lacks the vast number of E3 ligases implying that SUMO proteases, rather than SUMO ligases, are vested with the responsibility of providing the much-needed substrate specificity. Therefore, attempts to uncover newer classes of SUMO proteases can have a far-reaching impact, since they can lead

to the discovery of new SUMO targets. Such exciting outcomes can be exploited to benefit crop plants by improving their tolerance to different stress conditions.

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Figure Legends

Figure 1: A schematic representation of target protein SUMOylation. It starts with SUMO maturation, then SUMO conjugation via E1, E2 and E3 enzymes and finally, SUMO de-conjugation by SUMO proteases. 'K' is the Lysine in the target protein where SUMO is conjugated. Protein X is the target substrate.

Figure 2: A hypothetical model illustrating the role of SUMO in mediating the growth-defense balance. Under normal circumstances, a growth promoting transcription factor (TF-G) is binding to a/the promoter of growth-related genes and enhancing their expression. In the meanwhile, a defense-responsive transcription factor (TF-D) is sequestered by a SUMO target protein (STP) preventing it from activating defense-responsive genes. However, during a stress condition the STP is SUMOylated causing perturbation in its interaction affinity with its partners. Consequently, it now adheres to TF-G and blocks growth, whereas, TF-D is released for promoting defense responses. Encircled 'S' represents SUMO molecule. Red cross is depicting inhibition of transcription.