


## REVIEW ESSAY

## Prospects &amp; Overviews

# Research progress on plant stress-associated protein (SAP) family: Master regulators to deal with environmental stresses

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## Abstract

Every year, unfavorable environmental factors significantly affect crop productivity and threaten food security. Plants are sessile; they cannot move to escape unfavorable environmental conditions, and therefore, they activate a variety of defense pathways. Among them are processes regulated by stress-associated proteins (SAPs). SAPs have a specific zinc finger domain (A20) at the N-terminus and either AN1 or C2H2 at the C-terminus. SAP proteins are involved in many biological processes and in response to various abiotic or biotic constraints. Most SAPs play a role in conferring transgenic stress resistance and are stress-inducible. The emerging field of SAPs in abiotic or biotic stress response regulation has attracted the attention of researchers. Although SAPs interact with various proteins to perform their functions, the exact mechanisms of these interactions remain incompletely understood. This review aims to provide a comprehensive understanding of SAPs, covering their diversity, structure, expression, and subcellular localization. SAPs play a pivotal role in enabling crosstalk between abiotic and biotic stress signaling pathways, making them essential for developing stress-tolerant crops without yield penalties. Collectively, understanding the complex regulation of SAPs in stress responses can contribute to enhancing tolerance against various environmental stresses through several techniques such as transgenesis, classical breeding, or gene editing.

## KEYWORDS

abiotic and biotic stress, development, gene expression, redox regulation, stress-associated protein, tolerance, ubiquitination

## INTRODUCTION

Environmental pollution significantly hampers plant growth, development, and overall yield, posing a threat to global food security.<sup>[1–3]</sup> However, plants constantly perceive environmental changes and activate specific responses to protect themselves from a variety of biotic and abiotic stress factors. These mechanisms involve stimulus perception, signal transduction, and the activation of end-stage components.<sup>[4–6]</sup> Recently, stress-associated proteins (SAPs), a class of zinc finger transcription factors, have emerged as crucial molecular players in regulating both plant development and stress responses.<sup>[7–9]</sup> Plant SAPs contain A20, AN1, or both A20/AN1 zinc-finger domains at the N- or C-terminus. Some SAP proteins also contain extra Cys2-His2 RING motifs at the C-terminus.<sup>[10]</sup>

The A20 zinc-finger domain is characterized by multiple Cys2-Cys2 finger motifs,<sup>[11]</sup> whereas the AN1 zinc-finger domain is characterized by the presence of multiple Cys and His residues. The Cys- and His-residues in these zinc-finger domains are arranged in specific orders that also form typical metal-binding domains. To further elucidate SAP diversity, the SAP family in plants can generally be divided into eight patterns and two categories based on the type and number of domains present. These patterns include: A20+AN1, 2A20+AN1, A20, AN1, 2AN1, 2AN1+C2H2, AN1+2C2H2, and 2AN1+2C2H2. Among them, the most typical A20+AN1-type SAPs are the most widespread and exist in various plants. The 2A20+AN1 and AN1+2C2H2 types are the least common, with OsSAP12 and RcSAP9 currently being the only representatives of these two patterns (Figure 1).<sup>[12,13]</sup> Therefore, SAP proteins can be further classified into two major groups: Type I and Type II.<sup>[10,14]</sup> Type I genes contain the traditional pattern of cysteine- and histidine-rich motifs, such as CX2CX9-12CX1-2CX4CX2HX5 HXC, whereas Type II SAP genes contain the expanded domain CX4CX2CX9-12CX1-2CX4CX2HX5HXC, where X represents any amino acid.<sup>[15,16]</sup> Most Type I genes lack introns and contain one intact A20-type domain and/or one AN1-type zinc finger domain; most Type II genes have a single intron but do not contain an A20 domain.<sup>[17,18]</sup> Research indicates that these two types of plant SAPs existed before the emergence of higher plants and expanded in different ways during biological evolution, suggesting that the mechanisms of action of group I and group II SAPs differ.<sup>[19]</sup>

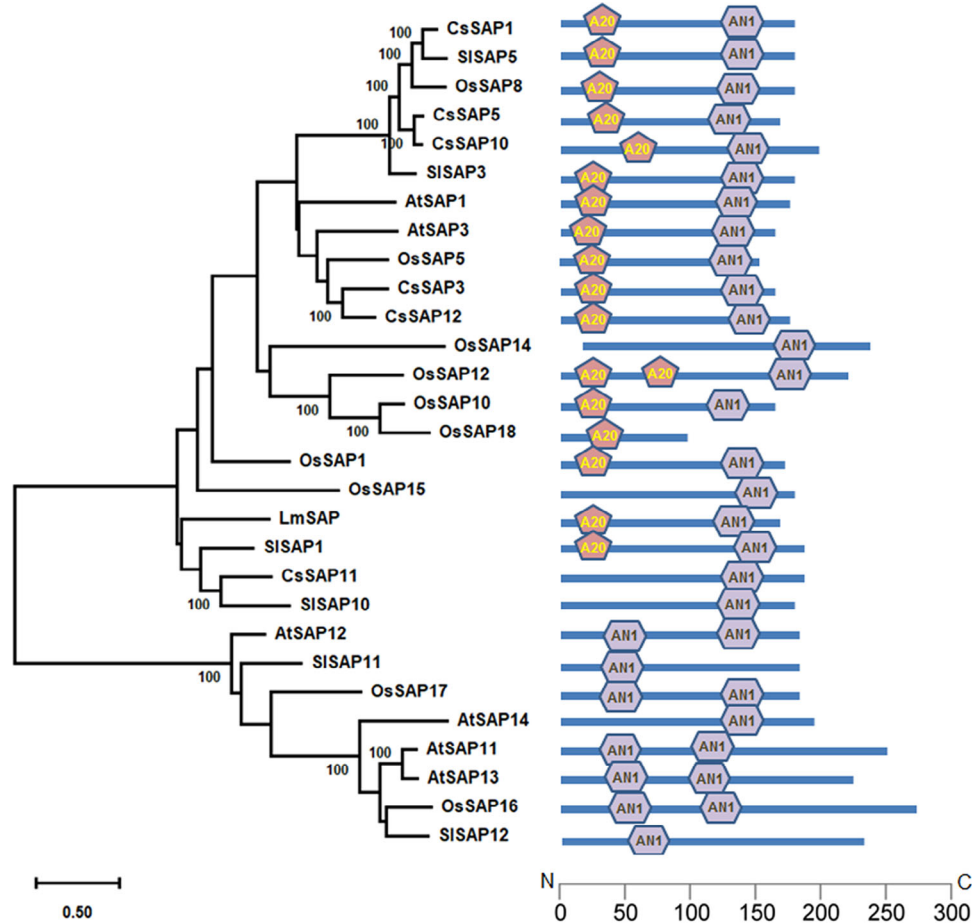
Genes encoding proteins containing A20/AN1 domains are usually differentially expressed in response to diverse environmental stresses, a phenomenon observed in both animals and plants. The precise biochemical role of the AN1 domain remains elusive. The A20 and AN1 domains have been demonstrated to play roles in immune responses, involving protein–protein interactions and contributing to the transcriptional regulation of stress- and pathogenesis-related genes.<sup>[20–24]</sup>

Moreover, some SAP proteins with A20 and AN1 domains have been reported to possess ubiquitin ligase activity.<sup>[15,16,25]</sup> In 2004, Mukhopadhyay et al.<sup>[26]</sup> identified the first SAP gene in rice (*Oryza sativa*), *OsSAP1*, and demonstrated that its presence is related to enhanced tolerance against cold and osmotic stresses. Since then, numerous homologs of SAPs have been discovered and characterized across a wide array of plants, including model species, agricultural and horticultural crops, woody plants, and herbaceous halophytes (for examples, please refer to Table 1). Owing to extensive studies, these genes family and their encoded proteins were established as novel regulators involved in plant responses to either abiotic or biotic stress.<sup>[7]</sup> Numerous examples of SAP involvement in plant biology are presented in detail in this review.<sup>[27–31]</sup> Given the acknowledged ability of SAPs to orchestrate both plant development and stress response pathways, this review highlights the importance of SAP genes in mitigating abiotic or biotic stresses across diverse plant species.<sup>[13,17,32–35]</sup> Understanding the mechanisms of action and potential roles of SAP family genes could be useful in increasing crop productivity through genetic modification, particularly in agricultural crops. This includes investigating their role in phytohormone regulation and the maintenance of redox balance in plants under unfavorable conditions. By compiling data on the structure, subcellular localization, and biological function of the SAP genes family, this review enumerates the progress made in understanding the evolution and interactions of SAPs during stress responses, as well as the mechanisms underlying plant defense.

## THE FEATURES OF SAP PROTEINS IDENTIFIED IN PLANTS

Large gene families increase diversity and promote organismal adaptation by providing opportunities for both neo- and sub-functionalization, thereby increasing adaptability. In the case of the SAP family, the first extensive study examining the genomes of 22 organisms representing a wide range of Eucaryota revealed variability in the number of gene homologs, ranging from 1 (in some protists) to 19 (in *Populus trichocarpa*).<sup>[36]</sup> The greater abundance of the larger SAP genes family in plants than in animals is likely attributed to genome duplication processes during evolution.<sup>[10]</sup> Whole-genome and gene duplications are significant evolutionary mechanisms that ensure the survival of organisms. Particularly in plants, including flowering ones, genome duplications are more widespread, and all sequenced plant species have been reported to have undergone at least one whole-genome duplication event.<sup>[7]</sup>

SAPs, which are classified as zinc-finger proteins and belong to a diverse multigene family, have been found to play roles in diverse stress



**FIGURE 1** Phylogenetic analysis of different plant stress-associated proteins (SAPs) along with their domain architecture. The MEGA 11 software was used build the phylogenetic tree based on Maximum Likelihood method with a bootstrap value of 1000 replicates. Bootstrap support values are indicated above each node. The domains on the right were predicted using NCBI conserved domains search (<https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi>) and visualized via TBtools software. The prefix At assigned for *Arabidopsis thaliana*, Os: *Oryza sativa*, Cs: *Camellia sinensis*, Sl: *Solanum lycopersicum*, and Lm: *Lobularia maritima*.

responses in numerous plant species.<sup>[7,36–38]</sup> These studies have been complemented by genome-wide analyses of gene family composition, revealing significant variability in the number of gene members.<sup>[39]</sup> In plants, the number usually ranges from 10 to 20 genes; for example, *Zea mays* L. has 10 ZmSAP genes,<sup>[40]</sup> *A. thaliana* –14 AtSAPs,<sup>[12]</sup> and *O. sativa* –18 OsSAPs<sup>[26]</sup> (refer to Table 1 for more details). A significantly greater number of SAP genes have been identified in *Glycine max* (27 GmSAPs),<sup>[35]</sup> 30 MdSAPs in *Malus domestica*,<sup>[41]</sup> 37 GhSAPs in *Gossypium hirsutum*,<sup>[31]</sup> and even 57 BnSAPs in *Brassica napus*.<sup>[42]</sup> In turn, the lowest number of SAP-encoding genes was found in *Ricinus communis* L., with 9 RcSAPs<sup>[13]</sup> Currently, the list of vascular plants in which SAPs have been identified includes agricultural and horticultural crops, medicinal species, and model woody and herbaceous plants (Table 1). SAP proteins are generally small and rarely exceed the 292 amino acid mark. The smallest SAP recorded thus far is 50 aa in *B. napus*.<sup>[42]</sup> whereas the largest is CsSAP6, which is 579 aa in *Camellia sinensis*.<sup>[43]</sup>

SAP genes are distinguished by their high prevalence of introns lacking sequences, a trait commonly associated with housekeeping genes of evolutionarily conserved function. Variations in the number of exons

and introns are significant factors in the evolution of transcription factor protein families.<sup>[44]</sup> A significant number of SAP genes lack introns in the majority of the examined plants.<sup>[28,45]</sup> For example, in *Malus domestica*, 25 out of 30 SAP genes were found to be intronless,<sup>[41]</sup> whereas in *G. max*, 18 out of 27 SAP genes were intronless.<sup>[35]</sup> Similarly, in maize only ZmSAP9 possesses a single intron and two exons.<sup>[40]</sup> Indeed, studies have indicated that genes with a low number of introns are more frequently associated with responses to drought and salt stress than are intron-rich genes, suggesting their involvement in the cell reaction to osmotic imbalance.<sup>[46]</sup> The predominance of intronless genes allows for the rapid accumulation of transcripts without the need for extensive post-transcriptional modifications, which may particularly benefit during the early stress response.<sup>[47,48]</sup>

Despite the amino acid composition variability of the plant SAP proteins, their predicted three-dimensional structure is conserved, particularly in the A20 and AN1 domains.<sup>[19,29]</sup> The A20 domain adopts a zinc finger fold, consisting of a compact structure with a zinc ion coordinated by cysteine and histidine residues. This domain is involved in deubiquitination and the regulation of immune and stress

**TABLE 1** Overview on the characterized SAP gene family in plant species.

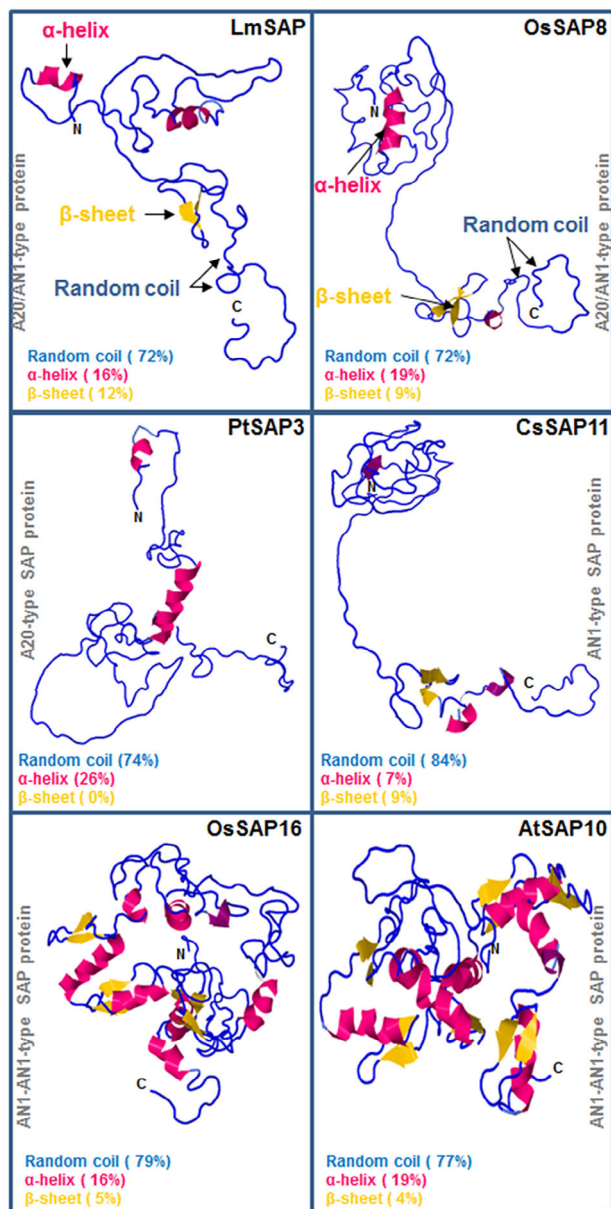
Species	Gene family	Characterized members	Intronless members	Protein length (aa)	Ref.
<i>Arabidopsis thaliana</i>	AtSAP	14	3	126–280	[12]
<i>Artemisia annua</i>	AaSAP	16	NA	AaSAP1:154 AaSAP2:101	[108]
<i>Brassica napus</i>	BnaSAP	57	35	50–292	[42]
<i>Camellia sinensis</i> var. <i>sinensis</i>	CsSAP	14	11	145–579	[4]
<i>Capsicum annuum</i>	CaSAP	14	11	127–277	[14]
<i>Cucumis sativus</i>	CsSAP	12	9	137–289	[55]
<i>Glycine max</i>	GmSAP	27	18	123–292	[35]
<i>Gossypium hirsutum</i>	GhSAP	37	30	92–295	[31]
<i>Hordeum vulgare</i>	HvSAP	17	NA	NA	[107]
<i>Ipomoea batatas</i>	IbSAP	20	11	142–294	[81]
<i>Ipomoea trifida</i>	ItrSAP	26	14	141–294	[81]
<i>Ipomoea triloba</i>	ItbSAP	23	14	141–293	[81]
<i>Jatropha curcas</i>	JcSAP	11	11	133–288	[116]
<i>Malus domestica</i>	MdSAP	30	25	136–293	[41]
<i>Medicago truncatula</i>	MtSAP	17	7	111–287	[9]
<i>Oryza sativa</i>	OsSAP	18	11	72–356	[12] [12]
<i>Populus euphratica</i>	PeuSAP	18	15	147–325	[33]
<i>Populus trichocarpa</i>	PtSAP	19	NA	79–294	[61]
<i>Prunus dulcis</i>	PdSAP	9	9	152–292	[44]
<i>Ricinus communis</i>	RcSAP	9	4	124–265	[13]
<i>Solanum lycopersicum</i>	SISAP	13	8	151–272	[32]
<i>Salix purpurea</i>	NA	19	NA	134–292	[33]
<i>Salix suchowensis</i>	NA	15	NA	148–375	[33]
<i>Solanum melongena</i>	SmSAP	21	12	66–368	[]
<i>Solanum tuberosum</i>	StSAP	17	13	124–448	[106]
<i>Sorghum bicolor</i>	SbSAP	18	NA	SbSAP14:180	[36,101]
<i>Tamarix hispida</i>	ThSAP	11	NA	167–293	[42]
<i>Vitis vinifera</i>	VvSAP	15	5	67–293	[118]
<i>Zea mays</i>	ZmSAP	10	9	152–290	[40]

Abbreviation: NA, not available.

responses in plants. The AN1 zinc finger domain typically forms a beta-beta-alpha zinc finger structure, with conserved cysteine and histidine residues coordinating a zinc ion.<sup>[49]</sup> This domain is responsible for DNA binding and protein-protein interactions, regulating the transcription of stress-responsive genes in plants.<sup>[49]</sup> Generally, SAP protein amino acids are arranged into three major secondary structures, especially  $\alpha$ -helices,  $\beta$ -sheets, and random coils.<sup>[19,50]</sup> Notably, random coils and  $\alpha$ -helices are predominant in A20/AN1 domain-containing SAP proteins (Figure 2). Further studies on the x-ray crystal structure of SAPs will provide highly accurate relevant structural information for unraveling the structural changes associated with the molecular functions of SAP proteins involved in plant stress response mechanisms.

## SPATIO-TEMPORAL, ORGAN- AND TISSUE-SPECIFIC EXPRESSION OF SAP GENES

Researchers have explored the functions of SAPs in diverse physiological processes. Multiple studies have investigated the expression of SAP genes across various tissues and organs, revealing differential expression patterns influenced by spatial and temporal factors. Gimeno-Gilles et al. (2011)<sup>[17]</sup> reported that, in *Medicago truncatula*, the expression of *MtSAP1* was induced up to 8-fold during seed maturation, contributing to increased desiccation survival. After a few hours of seed rehydration, the expression significantly decreased. Additionally, *MtSAP1* expression is associated with the accumulation of storage proteins such as globulin, vicilin, and legumin, facilitating efficient



**FIGURE 2** Illustration of three-dimensional structures of different plant SAPs predicted via the protein homology/analogy recognition engine tool V2.0 (<http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index>).

seed germination.<sup>[17]</sup> Analysis of castor bean SAP gene expression via high-throughput RNA-seq data from various tissues revealed that the majority of these genes were expressed at low levels under typical conditions. Among these genes, the *RcSAP4* gene presented relatively high expression, suggesting a potential role for *RcSAP4* in seed development and growth regulation.<sup>[13]</sup> Liu et al. (2011)<sup>[51]</sup> reported that *OsDOG* is a gene constitutively expressed in rice tissues, with increased expression observed in rapidly growing plants. However, despite being induced by gibberellin, *OsDOG* negatively regulates cell elongation, as evidenced in dwarf rice overexpressing this gene.<sup>[51]</sup> The inhibition of cell elongation within the leaf sheath and internodes was attributable to a decrease in the GA1 concentration. This study unveiled that *OsDOG*

modulates GA homeostasis by influencing the expression of genes involved in the metabolism of these phytohormones. Deeper insights into the molecular roles of SAPs across diverse developmental processes can be achieved through transcriptional analyses. In this way, the expression of *GhSAPs* is elevated in the stamens and pistils of cotton plants, suggesting their involvement in the regulation of flower development.<sup>[31]</sup> Similarly, *AtSAP9* in *Arabidopsis*, *PagSAP11* in poplar, and *PpSAP1* in *Prunus persica* are involved in the regulation of flowering time, shoot branching, and cell growth.<sup>[34,52,53]</sup> Differential expression of *Prunus dulcis PdSAP* was also reported during different stages of fruitlet development following flowering, specifically at days 12, 17, 22, 27, 32, and 37.<sup>[44]</sup> Zhang et al. (2016)<sup>[54]</sup> reported that the *ZFP185* gene is constitutively expressed across different rice tissues and plays a role in regulating plant habits by modulating gibberellic acid (GA) synthesis. Overexpression of the gene resulted in a dwarf phenotype associated with decreased GA content. Lai et al. (2020)<sup>[55]</sup> demonstrated that cucumber *CsSAP* genes presented different expression patterns across different tissues, with notable changes observed during fruit development. The majority of the *CsSAP* genes lacked introns and contained several stress- and hormone-regulatory elements within the promoter sequence. Previously significant transcriptional activity of *CsSAP* genes at specific time points throughout fruit development was reported.<sup>[56]</sup> Additionally, a number of identified *CsSAP* genes were expressed in unfertilized ovaries, roots, and basal tendrils. All of these studies suggest that these genes may have diverse roles depending on the tissue context. When subjected to abiotic stresses, *ALSAP* transcripts reach their peak accumulation in roots, whereas *OsSAP9* transcripts tend to accumulate predominantly in leaves.<sup>[57]</sup>

The role and mode of action of SAP genes may also be recognized by investigating spatio-temporal expression patterns. Studies have revealed diverse response times for SAP gene expression across plant species under stress conditions. In cotton, nearly all SAPs are upregulated within a single hour of salt exposure.<sup>[31]</sup> However, other species display different timeframes. For instance, *LcSAP* reaches its peak expression level within 6 h of salt stress, and this level is maintained for 24 h.<sup>[58]</sup> Similarly, *TaSAP17D* displayed an early response with increased expression within 30 min of salt treatment.<sup>[59]</sup> Conversely, *LmSAP* requires a longer period, showing an increase only after 12 h of exposure to heavy metals.<sup>[60]</sup> *P. trichocarpa* also exhibits a rapid SAP response, with seven *PtSAP* genes induced within an hour of salt treatment, reaching their maximum expression level within 6 h.<sup>[61]</sup> Different transcript levels and spatio-temporal expression patterns in response to environmental stimuli have also been reported for *C. sinensis* SAPs.<sup>[43]</sup> Experiments involving *AtSAP13* promoter—GUS fusion revealed that expression prevailed in vascular tissue, hydathodes, and root regions with actively growing cells in response to cold and drought stress.<sup>[62]</sup> Similarly, Hou et al. (2022)<sup>[63]</sup> reported that *GmSAP5* from soybean was expressed primarily in roots and stems. However, according to Zhang et al. (2019),<sup>[35]</sup> the expression of soybean SAP genes may be an important regulator of diverse tissue-dependent networks. Therefore, analyzing the promoter region of SAP genes can offer deeper insights into their transcriptional induction. Several studies have investigated the sequences of the regulatory

regions of numerous SAP genes and revealed the existence of various cis-acting regulatory elements associated with their expression in response to stress.<sup>[8,12,24,64–67]</sup> The fusion of the *PrLmSAP* sequence with the *gusA* gene resulted in tissue-specific expression in transgenic rice. The staining was abundant in most of the root tissues, coleoptiles, leaves, stems, and floral organs but was absent in the seeds and the root elongation zone.<sup>[68]</sup> Likewise, the *PrAISAP* promoter was found to be age-dependent, tissue-specific, and inducible under abiotic stress.<sup>[64,65]</sup> Taken together, these results show that the expression of SAP genes is organ/developmental stage-specific. Most of these genes are highly expressed in areas of intense growth or in reproductive and storage organs but are also involved in the first line of stress defense. This provides a compelling route for future exploration with the purpose of enhancing plant tolerance and regulating plant development under unstable environmental conditions.

## SUBCELLULAR LOCALIZATION OF SAP PROTEINS

The potential function of proteins can be predicted through the determination of their subcellular localization. In the case of the SAP family, the most frequent cytosolic and nuclear localization was proposed for different SAP sequences.<sup>[69–71]</sup> The nuclear localization of SAP genes may play a significant role in regulating plant reactions to a variety of abiotic and biotic stresses.<sup>[71,72]</sup>

The rice proteins OsiSAP8 and ZFP177/OsSAP9 are localized solely in the cytosol.<sup>[67,71]</sup> On the other hand, AtSAP10 is localized in both the nucleus and the cytosol.<sup>[62]</sup> Although the SAPs discovered did not contain a typical nuclear localization signal (NLS), the NLS in *Arabidopsis* AtSAP5 and banana MusaSAP1 consisted of two parts. However, AtSAP5 is localized primarily to the nucleus, whereas MusaSAP1 is detected throughout the cell, including the nucleus.<sup>[25]</sup> Sreedharan et al. (2012)<sup>[45]</sup> discovered that the MusaSAP1-GFP fusion protein is present in both the cytosol and nucleus. Sharma et al. (2015)<sup>[73]</sup> demonstrated a dual localization pattern of the OsiSAP7 protein in entire cells or in nuclear bodies in the onion epidermis. Other examples of SAPs detected in both the cytoplasm and nucleus included the SAP-GFP fusion proteins AtSAP13, GmSAP5, and GmSAP16,<sup>[8,35,63]</sup> as well as TaSAP7-A.<sup>[74]</sup> *Arabidopsis* COP1 is responsible for ubiquitination and degradation and accumulates its target-protein HY5 in the nucleus.<sup>[75,76]</sup> Additionally, Zhang et al. (2017)<sup>[77]</sup> indicated that the TaSAP5 protein is a nuclear/cytoplasmic protein that exhibits E3 ubiquitin ligase activity. In addition to those two most frequent localizations, SAP proteins can also be incorporated into cellular membranes, as in the case of castor bean,<sup>[13]</sup> wheat TaSAP17-D protein<sup>[59]</sup> and group II and IV CaSAP proteins of pepper.<sup>[14]</sup> In *B. napus* and cotton, SAPs have been reported to be present in the cytoplasm, nucleus, and endoplasmic reticulum.<sup>[31,42]</sup> In several species, one specific location prevails, such as in *P. dulcis*, where the majority of PdSAP proteins are localized in the nucleus region.<sup>[44]</sup> The *Lobularia maritima* LmSAP protein was localized only in the nucleus, which may be related to its interaction with other NLS-containing proteins or to its small size, facilitating transport to the nucleus.<sup>[29]</sup> Later, Ben Saad et al. (2019)<sup>[22]</sup>

reported that the A20 domain on the LmSAP $\Delta$ AN1-truncated protein is crucial for its stability and nuclear localization, which may contribute to its potential function as a transcriptional activator. In turn, Su et al. (2022)<sup>[78]</sup> demonstrated that maize ZmSAP8 was located in the nucleus but exhibited no transactivation activity in yeast. In essence, the members of the SAP family usually occur in the cytoplasm, nucleus, and cellular membranes, including the plasmalemma and endoplasmic reticulum. Their translocation from the external parts of the cell to the nucleus could play a critical role in regulating various abiotic and biotic stresses. As indicated by previous studies, SAP proteins may also function through protein–protein interactions, in addition to their anticipated role as transcription factors in the nucleus.

## MULTIPLE SAPs ACTION MODES: UBIQUITINATION AND REDOX REGULATION

In the botanical domain, the influence of ubiquitin-mediated protein modifications is unequivocally established, exerting profound effects across diverse cellular functions.<sup>[79,80]</sup> These functions encompass photomorphogenesis, defense against pathogens, responsiveness to phytohormones, and orchestration of acclimation to abiotic stressors.<sup>[81–85]</sup> SAPs have been subjected to extensive scrutiny in plants, emerging as pivotal contributors to elevated resilience against abiotic stresses. A comprehensive exploration of SAPs has unveiled their multifaceted roles in plants, ranging from functioning as ubiquitin ligases<sup>[34,72]</sup> to serving as redox sensors.<sup>[24,86]</sup> Furthermore, SAPs play crucial roles in the regulatory network governing stress-responsive genes.<sup>[57,61]</sup> This complex web of interactions highlights the integral role of SAPs in modulating diverse molecular pathways that collectively contribute to a plant's adaptive response to environmental challenges.

In the context of post-translational modification, ubiquitination is a reversible process regulated by the coordinated actions of ubiquitin ligases and deubiquitinases. In both plants and animals, these molecular players serve as critical regulators of stress responses. In mammals, the A20 protein assumes a central role in its cytoplasmic localization, performing both ubiquitination and deubiquitination functions within NF- $\kappa$ B signaling pathways.<sup>[24,87]</sup> The sophisticated regulatory interplay of ubiquitin modifications serves as a conserved mechanism shaping cellular responses in both the plant and animal kingdoms.<sup>[88]</sup> Parallel functionalities akin to those observed in the A20 protein are documented in plants, primarily owing to the presence of A20 domains positioned at the N-terminus of SAPs. At the molecular level, certain SAPs assume the role of ubiquitin E3 ligases, orchestrating the linkage of substrate ubiquitination with subsequent proteasomal degradation events. These proteins frequently contribute significantly to boosting both abiotic and biotic stress tolerance within the plant kingdom. In contrast, other SAPs play pivotal roles in the ubiquitination process by interacting with proteins that are integral to ubiquitination pathways. Kang et al. (2011)<sup>[25]</sup> delineated the ligase activity of AtSAP5 in *Arabidopsis*. It was found that the ligase activity was specifically localized within the AN1 domain of AtSAP5. This enzymatic activity was attributed to the strategic replacement of cysteines 6 and 7 by histidine

and of histidines 4 and 5 by cysteines.<sup>[25]</sup> This complex orchestration highlights the nuanced regulatory roles of SAPs in plant cellular processes, particularly in the interplay of ubiquitination events that govern stress responses.

The structural alteration observed in the AN1 domain, akin to RING finger proteins acting as ligase enzymes in ubiquitination, signifies a noteworthy conformational shift.<sup>[25]</sup> Notably, an investigation revealed a highly conserved diaromatic patch in animal orthologs of AtSAP5, including the A20 protein and Rabex-5, which play pivotal roles in polyubiquitin binding.<sup>[89]</sup> However, the diaromatic patch of AtSAP5 assumes a distinctive role by facilitating linkage-specific polyubiquitin binding, specifically recognizing K-63 linked polyubiquitin to finely regulate the downstream signaling of target proteins.<sup>[89]</sup> In the context of rice, the protein OsSAP7 has been shown to exhibit ubiquitin E3 ligase activity, functioning as a negative regulator of stress signaling through targeted degradation of its substrates.<sup>[73]</sup> This ligase activity of SAPs extends beyond *Arabidopsis* and rice; for instance, in wheat, *in vitro* ubiquitination assays conducted with TaSAP5 resulted in the formation of polyubiquitin chains, exemplifying the broad spectrum of ligase functions within the SAP family across diverse plant species.<sup>[77]</sup> The role of TaSAP5 in the ubiquitination of DRIP (DREB2A—interacting protein) has been reported, leading to the destabilization of proteins associated with drought tolerance. Through this ubiquitination process, TaSAP5 enhances the degradation of DRIP, consequently resulting in the increased accumulation of drought-related proteins. This finding implies a pivotal role for wheat TaSAP5 in regulating drought tolerance.<sup>[77]</sup> In the case of *Arabidopsis* AtSAP5 and *Prunus* PpSAP1, their binding to different polyubiquitin chains facilitates their biological functions, as they target substrate proteins for degradation.<sup>[34]</sup> In the context of tomato, Liu et al. (2019)<sup>[90]</sup> demonstrated that SISAP4 and SIRAD23d interact with each other. This interaction was made possible by the A20 domain of SISAP4 binding to the UBA domain of SIRAD23d. Although SISAP3 lacks ubiquitin E3 ligase activity, it is speculated to carry out immune-related functions by cooperating with other proteins associated with the ubiquitin-proteasome system.<sup>[72]</sup> A study by Chang et al. (2018)<sup>[15]</sup> demonstrated that Pha13, an analog of AtSAP5, harbors both E3 ligase and ubiquitin-binding activities within its A20 domain. The interaction of Pha13 with several ubiquitinated proteins was validated, demonstrating the involvement of Pha13 in ubiquitination processes.<sup>[15]</sup> Additionally, investigations involving deletions and mutants of the Pha13 AN1 and A20 domains underscore the principal importance of the A20 domain for its activity.<sup>[15]</sup> Indeed, structural analyses of the A20/AN1 domains revealed their coordinated role in ubiquitination processes. Performing functional analyses of these domains across various plant species will provide valuable insights into the observed functional divergence within SAP domains in the plant kingdom.

The cellular redox state, which is responsive to diverse environmental stresses, plays a pivotal role in cellular stress signaling, as extensively noted in studies.<sup>[91,92]</sup> Redox-dependent reversible protein conformational changes contribute to a swift response to environmental fluctuations, enabling an adaptive mechanism.<sup>[93]</sup> In the context of plants, Ströher et al. (2009)<sup>[94]</sup> elucidated the involve-

ment of AtSAP12 in redox homeostasis. AtSAP12 is distinguished by its 16 cysteine residues, which are located across two AN1 domains. Among these residues, 12 are notably involved in coordinating zinc ions. Through 2D SDS-PAGE analysis, researchers observed the formation of monomers, dimers, and oligomers under both oxidizing and reducing conditions. This observation suggested that the conformation of AtSAP12 is influenced by redox conditions, likely due to the formation of intra- or inter-molecular disulfide bonds. Moreover, Giri et al. (2011)<sup>[24]</sup> demonstrated that OsSAP1/11 proteins in rice undergo redox-dependent conformational changes, influencing their interaction with OsRLCK253 (receptor-like cytoplasmic kinase) and subsequently activating stress response mechanisms in transgenic rice. In the context of animals, the functionality of A20 domain in regulating redox processes is considered crucial for NF- $\kappa$ B activity, although additional experimental confirmation is necessary to firmly substantiate this concept.<sup>[95]</sup> These findings highlight the role of redox-dependent mechanisms in shaping the functional dynamics of SAPs across various biological contexts.

## RESPONSES OF SAP GENES TO ABIOTIC STRESS: SALINITY, DROUGHT, METAL EXPOSURE AND MULTIPLE STRESS

Nowadays, it is evident that plant SAPs are associated primarily with responses to abiotic stresses and metal exposure. To date, many SAP genes conferring tolerance to a range of abiotic stresses have been overexpressed in transgenic plants (Table 2). The specific regulatory mechanisms remain unclear and need further investigation. So far, the studies have provided valuable information on the potential effects exerted by SAPs under exposure to various abiotic stressors. It can be seen that the main modes of action presented above, that is, ubiquitination and redox regulation are often activated in response to drought, salinity, or excess toxic metals.

Yet Mukhopadhyay et al. (2004)<sup>[26]</sup> demonstrated that the overexpression of OsSAP1 in rice enhanced tolerance to cold and osmotic imbalance. Another study revealed that the overexpression of OsSAP8 in rice resulted in a 50% yield penalty under control conditions but improved salt tolerance. These findings suggest that while OsSAP8 may confer stress tolerance, it may also have negative effects on plant growth and development under optimal conditions.<sup>[71]</sup> Li et al. (2022)<sup>[96]</sup> demonstrated that OsSAP8 regulates rice drought response via interaction with the rice lectin protein r40c1 (Osr40c1). Moreover, transgenic tobacco plants overexpressing the OsSAP9 gene were tolerant to both cold and heat stress but became sensitive to salinity and drought. These findings suggest that rice OsSAP9 may be involved in mechanisms controlling tolerance to a wide range of temperatures but may not be efficient in ameliorating tolerance to osmotic status-related stresses.<sup>[67]</sup> Similarly, constitutive expression of the rice gene *ZFP177* in tobacco resulted in enhanced tolerance to high and low temperatures as well as to peroxide stress. However, it also renders plants more susceptible to dehydration and salinity stress.<sup>[67]</sup> In turn, another rice gene, *OsSAP16*, enhanced tolerance to drought, suggesting a specific

**TABLE 2** Update on the Characterized SAP genes in plant species and their responses to environmental factors.

Species (plants)	Gene name	Target specie	Approaches	Functions	Ref.
<i>Artemisia annua</i>	AaSAP1	<i>A. annua</i>	OE	Positively regulating glandular trichomes development	[23]
<i>Aeluropus litoralis</i>	AISAP	<i>O. sativa</i>	OE	Tolerance to cold, salt, and drought	[57]
				Enhancing susceptibility to <i>Magnaporthe oryzae</i>	[20]
		<i>N. tabacum</i>	OE	Tolerance to salt and drought	[28]
		<i>T. turgidum</i>	OE	Tolerance to salt and drought	[98,99]
<i>Asparagus officinalis</i>	AoSAP8-P	<i>N. sylvestris</i>	OE	Tolerance to salt and drought	[119]
<i>Arabidopsis thaliana</i>	AtSAP5	<i>A. thaliana</i>	OE	Tolerance to salt and drought	[25]
	AtSAP9	<i>A. thaliana</i>	OE	Increased sensitivity to ABA and osmotic stress	[52]
			OE	Susceptibility to infection by <i>Pseudomonas syringae</i> pv. <i>Phaseolicola</i>	
	AtSAP10	<i>A. thaliana</i>	OE	Tolerance to heat stress and heavy metals	[62]
	AtSAP12	<i>A. thaliana</i>	OE	Tolerance to cold and salt stresses and low	[94]
	AtSAP13	<i>A. thaliana</i>	OE	Tolerance to heavy metals, salt, and drought stresses	[8]
<i>Capsicum annuum</i>	CA01g16220	<i>C. annuum</i>	S	Tolerance to drought stress	[14]
	CA01g19950	<i>C. annuum</i>	S	Tolerance to drought stress	
	CA10g20690	<i>C. annuum</i>	S	Tolerance to drought stress	
	CA02g10410	<i>C. annuum</i>	S	Tolerance to low temperature	
	CA03g17080	<i>C. annuum</i>	S	Increasing sensitivity to low temperature	
<i>Camellia sinensis</i>	CsSAP12	<i>S. lycopersicum</i>	OE	Tolerance to drought stress	[]
<i>Gossypium hirsutum</i>	GhSAP17A/D	<i>G. hirsutum</i>	S	Increasing resistance to <i>Vertillium dahliae</i>	[31]
<i>Glycine max</i>	GmSAP5	<i>A. thaliana</i>	OE	Tolerance to drought stress	[63]
		<i>G. max</i>	OE	Tolerance to drought stress	
		<i>A. thaliana</i>	OE	Tolerance to salt drought	[35]
		<i>G. max</i>	OE	Tolerance to salt drought	
<i>Ipomoea batatas</i>	IbSAP16	<i>A. thaliana</i>	OE	Tolerance to salt stress	[81]
<i>Leymus chinensis</i>	LcSAP	<i>S. cerevisiae</i>	OE	Tolerance to salt stress	[58]
<i>Lobularia maritima</i>	LmSAP	<i>N. tabacum</i>	OE	Tolerance to abiotic stresses and heavy metals	[22,29,60,68]
<i>Malus domestica</i>	MdSAP15	<i>A. thaliana</i>	OE	Tolerance to osmotic and drought stresses	[41]
<i>Medicago truncatula</i>	MtSAP1	<i>N. tabacum</i>	OE		[103]
					Tolerance to cold, heat, salt and osmotic stresses
<i>Musa</i>	MusaSAP1	<i>Musa</i>	OE	Impaired stress tolerance to abiotic stress	[45]
<i>Oryza sativa</i>	OsSAP1	<i>O. sativa</i>	OE	Tolerance to water-deficit	[120]

(Continues)



TABLE 2 (Continued)

Species (plants)	Gene name	Target specie	Approaches	Functions	Ref.
		<i>N. tabacum</i>	OE	Tolerance to cold, dehydration, and salt stress Enhanced resistance against <i>Pseudomonas syringae</i>	[26,114]
	OsSAP7	<i>A. thaliana</i>	OE	Insensitivity to ABA and sensitivity to drought stress	
	OsSAP8	<i>O. sativa</i>	OE	Tolerance to cold, drought, and salt stress	[72]
	OsSAP11 ( <i>OsDOG</i> )	<i>O. sativa</i>	OE	Shorter cells	
		<i>A. thaliana</i>	OE	Tolerance to drought and salt	[24]
	OsSAP4 ( <i>ZFP185</i> )	<i>O. sativa</i>	OE	Decreasing of endogenous GA <sub>3</sub> content and reducing cell size	[62]
	OsSAP6	<i>O. sativa</i>	OE	Tolerance to salt stress	[121]
	OsSAP8	<i>O. sativa</i>	OE	Semi-dwarfism and reduced endogenous GA <sub>3</sub> content Tolerance to salt and drought	[71,96]
			KO	Higher endogenous GA <sub>3</sub> content and slightly increased plant height	
	OsSAP9 ( <i>ZFP177</i> )	<i>N. tabacum</i>	OE	Tolerance to low and high temperature Increased sensitivity to salinity and drought	[67]
	OsSAP16	<i>O. sativa</i>	OE	reduced stomatal conductance and biomass	[97]
Hybrid poplar ( <i>Populus gladiosa</i> X <i>P. alba</i> )	PagSAP1	h. poplar	S	Tolerance to salt stress	[109]
			OE	Increasing salt sensitivity	
	PagSAP11	h. poplar	KO	Tolerance to drought stress	[53]
<i>Prunus persica</i>	PpSAP1	<i>P. domestica</i>	OE	Tolerance to drought stress	[34]
<i>Populus trichocarpa</i>	PtSAP13	<i>A. thaliana</i>	OE	Tolerance to salt stress	[34]
<i>Sorghum bicolor</i>	SbSAP14	<i>O. sativa</i>	OE	Tolerance to salt stress	[101]
<i>Saccharum officinarum</i>	ShSAP1	<i>N. tabacum</i>	OE	Tolerance to salt and drought	[105]
<i>Triticum aestivum</i>	TaSAP17-A	<i>A. thaliana</i>	OE	Tolerance to salt stress	[74]
	TaSAP17-D	<i>A. thaliana</i>	OE	Tolerance to salt stress	[1]
	TaSAP5	<i>A. thaliana</i>	OE	Tolerance to drought stress	[77]
		<i>T. aestivum</i>	OE	Tolerance to drought stress	
<i>Tamarix hispida</i>	ThSAP6	<i>T. hispida</i>	OE	Tolerance to salt stress	[102]
<i>Vitis amurensis</i>	VaSAP15	<i>V. amurensis</i>	OE	Tolerance to cold stress	[122]
<i>Zea mays</i>	ZmSAP2	<i>S. cerevisiae</i>	OE	Tolerance to salt stress	[40]
	ZmSAP7	<i>S. cerevisiae</i>	OE	Tolerance to salt stress	
	ZmSAP8	<i>A. thaliana</i>	OE	Tolerance to drought stress	[78]
	ZmAN13	<i>A. thaliana</i>	OE	Tolerance to cold and drought	[23]
<i>Phalaenopsis orchid</i>	PhaSAP4	<i>P. aphrodite</i>	OE	Increasing antiviral immunity	[15]
	PhaSAP5	<i>A. thaliana</i>	OE	Increasing resistance to <i>P. syringae</i> pv. Tomato DC30000	[15]

(Continues)

TABLE 2 (Continued)

Species (plants)	Gene name	Target specie	Approaches	Functions	Ref.
		<i>P. Aphrodite</i>	OE	Increasing resistance to CymMV infection	
<i>Solanum lycopersicum</i>	SISAP3	<i>S. lycopersicum</i>	OE	Increasing resistance to <i>Pst</i> DC3000	[72]
			S	Reducing resistance to <i>Pst</i> DC3000	
	SISAP4	<i>S. lycopersicum</i>	S	Reducing resistance to <i>Botrytis cinerea</i>	[90]
		<i>N. benthamiana</i>	TE	Increasing resistance to <i>Botrytis cinerea</i>	

Abbreviations: KO, knockout; OE, overexpression; S, silencing/knockdown; TE, transient overexpression.

role for *OsSAP16* in response mechanisms to this stress.<sup>[97]</sup> The stress-responsive promoter of *OsSAP11* had no adverse effects on yield under normal conditions, whereas its overexpression in *Arabidopsis* resulted in a reduction in plant height through the suppression of gibberellin levels.<sup>[51]</sup> Overexpressing *OsZFP185* (also known as *OsiSAP4*) also led to dwarf phenotypes characterized by reduced GA levels, causing inadequate cell elongation.<sup>[53]</sup> Considering phytohormonal crosstalk, *OsSAP7* negatively influences ABA signaling, increasing the sensitivity of transgenic *Arabidopsis* plants to drought and salt stress.<sup>[54,73]</sup>

An insight into the mechanisms by which SAP family proteins respond to abiotic stresses was achieved in studies on the model species *Arabidopsis thaliana*. Kang et al. (2017)<sup>[52]</sup> reported that boosted tolerance to osmotic stress is an effect of the ubiquitinase activity of *AtSAP9*, whereas the overexpression of another gene, *AtSAP12*, has been shown to increase tolerance to salt and cold stress by balancing the redox state.<sup>[94]</sup> Furthermore, *AtSAP10* has been identified as a regulator of *A. thaliana* response to heavy metals (Ni, Mn, Zn) and high-temperature stress.<sup>[60,62]</sup> Particular interest has been given to SAP genes in halotolerant plants. Halophytes are equipped with adaptive genes, such as the *AISAP* gene, which was isolated from the halophytic grass *A. littoralis*.<sup>[28]</sup> The overexpression of the *AISAP* gene in transgenic tobacco, durum wheat, and japonica rice cv. Nipponbare<sup>[28,57,98,99]</sup> has been shown to increase plant tolerance to osmotic and oxidative impairments. Notably, *AISAP* rice lines exposed to drought stress during the reproductive stage yielded more efficiently than did the wild-type controls, and no yield penalty occurred when irrigation was applied.<sup>[100]</sup> Transcriptomic analysis of two *AISAP*-expressor rice lines unveiled a significant number of deregulated stress-related genes.<sup>[20]</sup> Another gene encoding SAP was isolated from the halotolerant plant *L. maritima*. This gene, *LmSAP*, is induced by salt and ionic stresses,<sup>[29]</sup> and when it is overexpressed in transgenic tobacco, it enhances tolerance to Cd and Mn toxicity.<sup>[60]</sup> Ben Saad et al. (2019)<sup>[22]</sup> subsequently demonstrated that plants overexpressing *LmSAP*, irrespective of its form (full-length or truncated) exhibited tolerance to osmotic-related stresses, including salinity. This effect was attributed to the modulation of the expression of genes encoding enzymes involved in ROS scavenging, which ameliorated oxidative damage. *LmSAP* is also capable of reducing the intracellular

levels of free metal ions by influencing the accumulation of metal-binding proteins in the cytosol.<sup>[30]</sup> Another study demonstrated that the overexpression of the *LcSAP* gene from the halophyte *Leymus chinensis* improved tolerance to salt, ionic, and osmotic stresses in yeast cells.<sup>[58]</sup> Taken together, these studies revealed that *SAP* genes from halophytic species are promising tools for enhancing stress tolerance in crops through genetic engineering. Considering other crops and economically important species, representatives of the *SAP* gene family have also been studied for their impact on plant behavior under unfavorable conditions. There is a wide range of evidence supporting their involvement in modulating plant reactions to osmotic and ionic-related disturbances. Most highlighted is the ability of SAPs to regulate the plant response to salinity and drought. Investigations in *Sorghum bicolor* L. revealed that the expression of *SbSAP14* in rice helps shield plants against oxidative disturbances induced by salinity.<sup>[101]</sup> Su et al. (2022)<sup>[78]</sup> reported that the overexpression of the maize *ZmSAP8* gene enhanced drought tolerance in *Arabidopsis*, increasing seed germination and root elongation. In *Tamarix hispida*, *ThSAP6* is significantly induced by salinity and positively regulates the response to this stress, and its overexpression enhances salt tolerance compared with that of wild-type plants.<sup>[102]</sup>

Recently, substantial evidence has shown that SAPs are crucial regulators of plant responses to multiple stresses and their spatio-temporal expression may act as a switch, controlling the level and direction of defense reactions. In wheat (*Triticum aestivum* L.), the *TaSAP17-D* gene was upregulated in response to salt, drought, and cold stresses, and its overexpression improved salt stress tolerance in transgenic *Arabidopsis*.<sup>[59]</sup> Similarly, soybean (*G. max*) *GmSAP16* exhibited increased transcript levels in response to drought, salt, and ABA, while its overexpression in both *Arabidopsis* and soybean resulted in enhanced tolerance to a range of abiotic stresses.<sup>[35]</sup> Compared with control plants, tobacco plants expressing the sugarcane *ShSAP1* or *M. truncatula* *MtSAP1* transgenes were shown to exhibit greater tolerance to both salinity and drought.<sup>[103–105]</sup> Transcriptomic studies of cucumber *CsSAP* genes demonstrated that only *CsSAP5/6/9/10* were involved in response to salt and cold treatments, whereas all *CsSAP* genes were upregulated under drought stress.<sup>[55]</sup> An analysis of *CaSAP* gene expression by Bae et al. (2021)<sup>[14]</sup> revealed differential induction

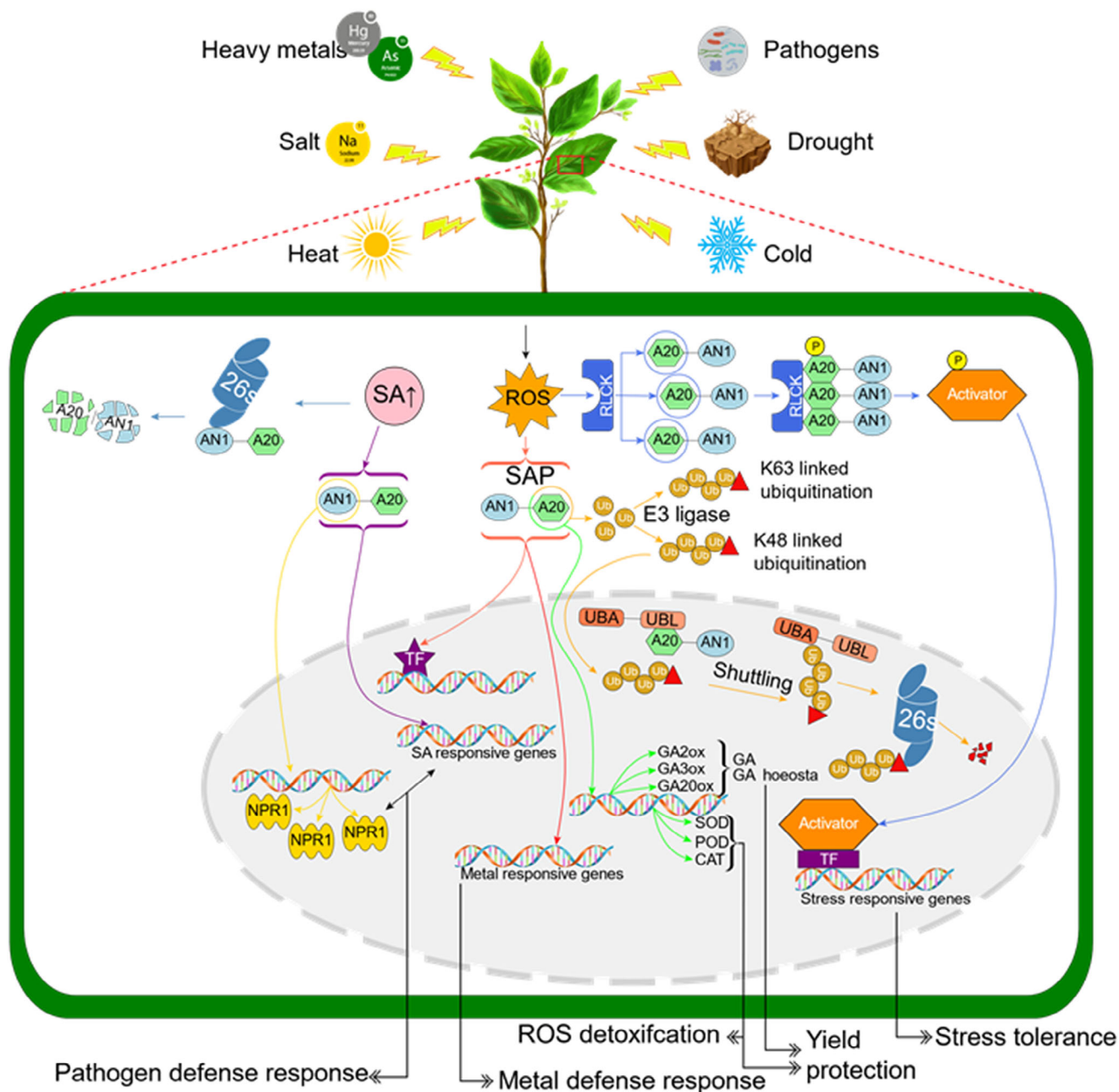
patterns. Low temperature, dehydration stress, and exogenous ABA treatment triggered *CaSAP* gene expression but to varying degrees. Notably, Group II genes exhibited a strong response to low temperature, whereas Group IV genes presented significant upregulation under dehydration stress conditions. Additionally, an *in silico* transcriptomic analysis approach was applied to study the expression of potato *StSAP* genes in response to heat, mannitol, and salt.<sup>[106]</sup> Studies investigating barley under salt stress conditions have revealed that five specific *SAP* genes (*HvSAP5/6/11/12/15*) exhibit increased expression levels.<sup>[107]</sup>

In addition to controlling defense responses to stress, *SAPs* may contribute to alterations in the metabolic pathways of secondary metabolite synthesis, as well as to anatomical rearrangements. The overexpression of *AaSAP1* in *Artemisia annua* increased the content of artemisinin and positively regulated the development of glandular trichomes.<sup>[108]</sup> In *P. trichocarpa*, the ability of *PtSAP13* gene overexpression to counteract the effects of salt stress was associated with the stimulation of flavonoid biosynthesis,<sup>[61]</sup> whereas in apple, the overexpression of the apple *MdSAP15* gene was related to the maintenance of chlorophyll content and osmolyte concentration.<sup>[41]</sup> The overexpression of the *P. persica PpSAP1* gene resulted in leaf blade modifications, ultimately ameliorating water status under drought stress.<sup>[34]</sup> On the other hand, studies have suggested that increased salt tolerance may be associated with the downregulation of *SAP* gene expression, as in the case of *PagSAP1* in poplar (*Populus alba* × *P. glandulosa*).<sup>[109,110]</sup> Fu et al. (2022)<sup>[40]</sup> demonstrated that in maize (*Zea mays* L.), the expression of *ZmSAP2* and *ZmSAP7* was suppressed by salt stress. Li et al. (2021)<sup>[74]</sup> reported that the wheat *TaSAP7-A* gene, characterized as a 2AN1-type stress-associated protein-encoding gene, had a negative effect on abiotic stress responses. Collectively, the findings discussed here highlight the significant and versatile roles of *SAP* proteins in modulating stress tolerance and improving plant yield, as well as the need for further investigations of their mechanistic action in various plant species.

## THE PLANT SAP GENES INVOLVEMENT IN PHYTOHORMONE-MEDIATED PATHOGEN DEFENSE

A universally acknowledged variety resides in the fundamental role that *SAP* genes assume, not only in reacting to abiotic stress, but also in orchestrating the expression of genes integral to the plant's immune response against a spectrum of pathogens, thus imparting resistance.<sup>[111]</sup> The plant innate immune system relies upon intracellular nucleotide-binding/leucine-rich repeat proteins, extracellular transmembrane-anchored receptor-like kinases, or receptor-like proteins, which, in turn, activate subsequent signaling pathways.<sup>[112,113]</sup> Moreover, several *SAP* genes have been identified as regulators of the plant's defense against pathogens, thereby emphasizing the role of *SAP* genes in crop–pathogen interactions (Figure 3). The functional dynamics of *OsSAP11* and *OsRLCK253* hinge upon their interaction through the A20 domain, thereby governing their role in plant innate

immunity.<sup>[24]</sup> Moreover, a plethora of plant *SAP* genes have been identified as pivotal regulators of phytohormone biosynthesis, thereby augmenting the defense mechanisms against pathogen attack. Notably, *MusaSAP1* has been shown to be activated by both wounding and methyl jasmonate (MeJA) application, indicating its involvement in the plant response to biotic stressors.<sup>[45]</sup> In rice, the responsiveness of *OsSAP1* to diverse biotic stresses is evident, with its overexpression in tobacco fortifying resistance against the formidable pathogen *Pseudomonas syringae* pv. *tabaci*.<sup>[114]</sup> Likewise, the induction of *GhSAP17A/D* in response to salicylic acid (SA) and MeJa has been documented.<sup>[31]</sup> A homolog of *AtSAP5*, *Pha13*, from the orchid *Phalaenopsis aphrodite* was induced in response to exogenous SA treatment. Intriguingly, its overexpression in both orchid and *Arabidopsis* plants imparted resistance to various viruses, and in *Arabidopsis*, it enhanced resistance to *P. syringae* pv. *tomato* DC3000. Additionally, a study on *Pha13* mutants underscored the importance of the AN1 domain in the expression of pathogenesis-related genes, while both the A20 and AN1 domains proved to be essential for conferring resistance to virus infection.<sup>[15]</sup> Conversely, the overexpression of *AtSAP9* has been associated with heightened sensitivity to *P. syringae* pv. *phaseolicola*, indicating a counterproductive role of *AtSAP9* in immunity.<sup>[52]</sup> The exploration of tomato *SisSAP* genes unveiled their inducibility by SA, MeJa, and the precursor of ethylene synthesis-1-aminocyclopropane-1-carboxylic acid (ACC). Notably, the expression of these genes peaked within 24 h of *Botrytis cinerea* infection, culminating in heightened immunity against this necrotrophic fungus. The expression of these genes boosted within the first day of infection with *Botrytis cinerea*, and the underlying mechanism involved the interaction of these genes with RAD proteins via ubiquitinating activity and the activation of jasmonic acid-ethylene signaling. Silencing experiments targeting *SisSAP4* and *SisSAP10* have underscored the critical role of *SisSAP4* in bolstering tomato resistance to *B. cinerea*.<sup>[90]</sup> The overexpression of another tomato gene, *SisSAP3*, has emerged as a strategy to augment basal resistance against *Pseudomonas syringae* pv. *tomato* DC3000.<sup>[72]</sup> Transcripts encoding the *SAP* of *L. maritima* (*LmSAP*) are upregulated in response to various stimuli, including wounding and treatment with MeJa, picolinic acid, and ethephon.<sup>[115]</sup> Purified *LmSAP* exhibited remarkable antioxidant properties, as evidenced by its strong DPPH scavenging ability, significant reducing power, and remarkable reduction in thiobarbituric acid reactive substances (TBARS). In addition, *LmSAP* showed remarkable antimicrobial activity against a range of pathogenic strains, ameliorating a number of parameters, such as the inhibition zone (IZ), minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MCB). The application of *LmSAP* effectively inhibited the growth of *Listeria monocytogenes* in ground beef while improving product characteristics. This study underlined the potential of *LmSAP* to reduce bacterial abundance, especially *L. monocytogenes*, during the storage of ground beef. Furthermore, this study highlights the central role of *LmSAP* in the modulation of defense signaling pathways under different biotic stress situations. Although the exact biochemical mechanisms underlying the action of *LmSAP* at the cellular level remain elusive and require further investigation, its proven positive regulation of stress responses and its successful direct use against pathogens



**FIGURE 3** Schematic diagram of the abiotic and biotic stress responses of the SAP gene in plants. In this model, various stress signals disrupt the cellular redox balance, leading to the generation of ROS, resulting in the activation of RLCK receptors that subsequently interact with SAPs (represented by the domains A20 and AN1) through their A20 domain. This interaction induces the formation of SAP oligomers and the phosphorylation of activators. Notably, SAPs possess E3 ubiquitin ligase activity and can participate in both K63 and K48-linked ubiquitination. Particularly, K48-linked ubiquitination targets proteins for degradation via the 26S proteasome, potentially involving shuttling factors such as Rad23b (represented by the domains UBL and UBA) in the nucleus. Furthermore, SAPs play a critical role in regulating the expression of stress-responsive genes. This regulation can occur through direct interaction with DNA or indirectly via interactions with transcription factors. The A20 domain specifically upregulates genes such as GA20ox, GA3ox, and GA2ox, which are involved in GA homeostasis and ROS scavenging genes (CAT, SOD, and POD) resulting in yield protection and ROS detoxification. Additionally, stress triggers the production of salicylic acid (SA), which activates the AN1 domain of SAPs and subsequently NPR1. Activated NPR1 promotes the expression of SA-responsive genes, ultimately leading to pathogen defense. SA also promotes the degradation of SAPs by the 26S proteasome leading to post-transcriptional regulation of SAPs. P, phosphate group; RLCK, receptor-like cytoplasmic kinase; TFs, transcription factors; Ub, ubiquitin moiety.

make it a promising candidate for industrial applications, particularly in the area of ground beef preservation.<sup>[115]</sup>

## TOWARDS UNRAVELING MECHANISM OF ACTION OF SAPs IN PLANTS

To date, the complex mechanism of action of plant SAP proteins has not been fully characterized. However, recent research has suggested that these proteins can participate in stress responses through various mechanisms, primarily by acting as E3 ubiquitin ligases to ubiquitinate target proteins or by interacting with the ubiquitin/26S proteasome system (UPS) to participate in the ubiquitination pathway, by acting as redox sensors involved in the redox pathway, or by acting as transcription factors (TFs) involved in transcriptional regulation. Figure 3 explains these different mechanisms of action of plant SAP proteins corresponding to tolerance to abiotic or biotic stresses. According to the proposed model, external stresses induce redox instability, leading to the release of reactive oxygen species (ROS). This triggers the activation of receptors such as receptor-like cytoplasmic kinases (RLCKs). These RLCKs then interact with SAPs through their A20 domain, leading to the formation of multi-protein complexes (oligomers). The precise mechanism by which RLCKs activate SAPs remains under investigation. One potential model involves RLCKs that target specific amino acids within SAPs. This targeting could have two key functions: (1) degradation of specific repressors of stress signaling pathways via the 26S proteasome or (2) regulation of stress-responsive signaling pathways themselves. Alternatively, RLCK activation might lead to the phosphorylation of SAPs or other regulatory proteins. This phosphorylation could then trigger further downstream changes, ultimately influencing the expression of stress-responsive genes. Interestingly, our analysis of conserved amino acids within the A20 domain of SAPs suggested a possible preference for protein modification by RLCKs, rather than degradation through the ubiquitin-proteasome system.

Beyond their interaction with RLCKs, SAPs exhibit additional regulatory functions. They can act as E3 ubiquitin ligases, facilitating the attachment of ubiquitin molecules to target proteins. This ubiquitination process can involve different types of linkages, including K63 and K48 linkages on lysine (K) residues of the target protein. Specifically, K48-linked ubiquitination by SAPs might involve collaboration with shuttling factors such as nuclear Rad23b. This cooperation could lead to the degradation of specific proteins via the 26S proteasome, potentially contributing to stress response regulation. Furthermore, SAPs play a critical role in regulating the expression of stress-responsive genes. They can achieve this regulation through two main mechanisms: direct interaction with DNA or interaction with transcription factors. By utilizing these mechanisms, SAPs can induce the expression of various genes involved in stress responses, including those encoding signaling molecules (such as *GA20ox*, *GA3ox*, and *GA2ox*), metallothionein (*Met1–5*), and enzymatic antioxidants (*SOD*, *POD*, and *CAT*).

Additionally, phytohormone signaling cascades, including those mediated by jasmonic acid (JA), SA, ethylene (ET), and abscisic acid

(ABA), are also regulated by SAPs. Interestingly, *AtSAP5* and *AtSAP9* from *Arabidopsis*, as well as *OsSAP7* and *OsSAP11* from rice, tend to regulate phytohormone signaling pathways negatively.<sup>[51,52,73]</sup> In the abovementioned *Phalenopsis*, *Pha13* induced the expression of two SA-responsive genes, *PhaRdR1* and *PhaGRX*.<sup>[15]</sup> More recently, the A20 domain of *LmSAP* in *L. maritima* was identified as a regulator of GA homeostasis in plants. Overexpressing *LmSAP* or *LmSAPΔAN1* in tobacco lines resulted in the upregulation of GA biosynthetic genes, leading to an increase in endogenous GA levels, which subsequently influenced the expression of GA-responsive genes.<sup>[68]</sup> Moreover, SA might regulate SAPs not only at the transcriptional level but also at the post-transcriptional level, activating other SA-responsive genes and *NPR1* that contribute to pathogen defense mechanisms. These observations highlight the diverse roles that SAPs play in plants, emphasizing the need for further research to elucidate the molecular mechanisms underlying these novel functions of SAPs. Collectively, further research is needed to explore the precise involvement of SAPs in stress responses, with the aim of gaining functional insights that could facilitate the genetic manipulation of these proteins for the development of climate-resilient crop species.

## CONCLUSIONS AND FUTURE PROSPECTS

In this review we highlighted numerous studies that have underscored the critical role played by SAPs during biotic and abiotic stresses. The SAP protein family has been the subject of significant attention due to its contributions to plant development and responses to both biotic and abiotic stresses, as it participates in intricate networks. As extensively reviewed and in this work, SAPs are involved in plant reactions to pathogen attack and wounding, as well as to salinity, drought, other osmotic imbalances, suboptimal temperatures, metal toxicity, etc. SAPs are particularly important in the agricultural context, where cultivated species are often simultaneously exposed to challenging actions of multiple stresses. Plant responses to combined stresses cannot be predicted on the basis of their reactions to a single stress; therefore, the characterization of biomolecules that are capable of co-regulating the entire defense system is desirable. Establishing a collaborative network of experts may help elucidate the mechanisms of the regulatory action of SAPs and facilitate the development of crops resistant to multiple stresses. Nevertheless, the specific functions of SAPs require insightful analyses. Various SAP isoforms may exhibit distinct functions under specific environmental conditions, with both positive and negative effects on responses to stress. Current investigations into the roles of plant SAPs have focused primarily on model plants, and future research should prioritize examining the functions of these proteins in economically important plants.

Nonetheless, there remains a need to identify the precise number of SAPs in sequenced plant species and analyze their phylogenetic relationships. A deeper understanding of SAP functions also requires a focus on functional genomics in future research. Rapid progress in omics technologies offers a convenient avenue for unraveling the molecular evolution mechanism of SAPs across different plant species.

This knowledge will empower the scientific community to develop innovative strategies for sustainable agriculture and food production, which are essential for boosting crop productivity amidst ever-evolving environmental challenges.

#### AUTHOR CONTRIBUTIONS

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#### CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

#### DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated during the current study.

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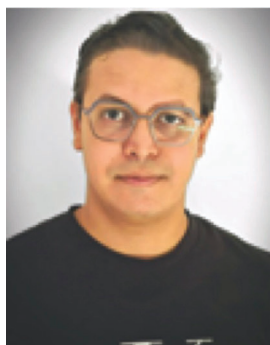
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