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REVIEW ARTICLE

The SPL gene family in rice: Master regulators of abiotic stress tolerance and prospects for crop resilience

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Abstract

Rice production faces severe challenges from abiotic stresses, including drought, salinity, heat, cold and nutrient imbalance, causing yield losses of 30-70 % depending on stress severity and developmental stage. SQUAMOSA promoter binding protein-LIKE (SPL) genes, regulated primarily by miR156 and miR529, have emerged as key transcriptional regulators of abiotic stress tolerance in rice. This review synthesizes current research on SPL gene functions in stress adaptation, examining their roles in hormone signalling, ion homeostasis and developmental regulation during both vegetative and reproductive stages. We analyse functional genomics and reverse genetics studies demonstrating SPL contributions to yield improvement and stress tolerance and evaluate recent advances in CRISPR/Cas9 and base editing technologies for precise SPL gene modulation. The findings reveal distinct tissue specific and developmental stage specific functions of miR156/miR529-SPL regulatory modules, with miR156 predominantly controlling vegetative development while miR529 regulates reproductive processes. This review provides a framework for leveraging SPL gene networks in developing climate-resilient rice varieties through targeted genome editing approaches.

Keywords: abiotic stress, CRISPR/Cas9, miR156, rice, SPL gene

Introduction

Rice is one of the major crops for global food security, with more than half of the world population using it as a primary calorie source (1). Major abiotic stresses such as drought stress, heat stress, cold stress, salt stress and nutrient stress affect the normal physiological functions of the plant and reduce the crop yield (1). Drought and salt stress are the major contributors among these stresses; the yield loss may reach up to 65 % or more (2). Drought is considered the most dangerous stress that has the potential to cause up to 100 % yield loss under extreme conditions, mainly in drought-prone areas (3). Due to salinity stress, crop yield loss is calculated at approximately 64.5 % (4). Different abiotic stresses acting together causes loss of spikelet fertility, leading to massive yield loss (5). The current average yield is 10 to 15 % lower than the actual crop potential because of both biotic and abiotic stresses, with abiotic stress covering the bigger portion (6). In addition to reduced yield, abiotic stress also reduces grain quality and affects the milling and cooking characteristics (5, 7). Due to the salinity chalkiness of rice increased, meanwhile the rate of head rice decreased, which affects the market value of grains (4). Enhancing the rice tolerance to these stresses is very important because of the increasing food demand and climate change.

Transcription factors (TF) are the main regulators of gene networks. They play a vital role in abiotic stress tolerance, such as drought, salinity and extreme heat, thereby enhancing crop resilience and yield stability (8). Major TF families- including bZIP, NAC, AP2/ERF, MYB and WRKY regulate gene expression in response to various stresses, often by different hormonal signalling pathways such as abscisic acid (ABA) and jasmonic acid (JA); they are crucial for adapting to stress and yield stability (9, 10). The *SPL*, one of the major TF families, encodes plant-specific transcription factors that play a major role in growth, development and responses to environmental stimuli. *SPL* genes are the main target for certain miRNAs, mainly miR156 and miR529, which modulate the *SPL* gene expression post transcriptionally through translational repression or mRNA cleavage (11, 12). In fact, both miRNAs target *SPL* genes; their spatial temporal expression pattern varies, which means they control SPL activity in different tissues at different developmental stages (13-15). *SPL* genes influence various biological processes, such as phase transition, shoot and root development (16), panicle architecture (17), grain formation (17), flowering time control, male sterility (18) and different stress responses (19).

The ability of *SPL* genes to integrate different developmental

cues with stress signalling marks them as important targets for crop improvement strategies. Many *OsSPL* gene family members, including *OsSPL8* and *OsSPL10*, are known for negative regulation of abiotic stress tolerance. Their suppression can increase rice's tolerance to withstand salinity, drought, heat and cold extremes and nutrient deficiency, offering potential strategies for crop improvement (19, 20). Despite progress, most OsSPL genes remain weakly characterized, with functional roles beyond OsSPL14, OsSPL10 and OsSPL7 largely unclear. Their regulation under simultaneous stresses, interactions with hormonal and transcriptional networks and context-dependent expression add further complexity. Functional redundancy among SPLs complicates gene editing and hampers gene-specific characterization, highlighting the need for precise, stage and tissue specific approaches to dissect their roles in stress tolerance. This review aims to convey current knowledge on the *SPL* gene family in rice with a focus on their molecular functions, miRNA regulation, interaction with hormonal and transcriptional networks and their potential applications in biotechnology for climate resilient rice varieties.

Overview of *SPL* gene family in rice

Nineteen SPL genes have been identified in the rice genome. These genes encode specific transcription factors characterised by a highly conserved *SQUAMOSA* promoter binding protein, which contains two zinc finger motifs and a nuclear localisation signal (NLS), allowing DNA to bind and transcriptional regulation. The zinc finger motifs are structural domains that stabilise protein folds and help DNA, RNA, or other proteins to bind. In plants, various gene families are present with these motifs and they control growth, development and stress responses (21). The C_2H_2 type is the most common zinc finger motif in plant transcription factors, but other motifs like RanBP2 and ZHD (zinc finger homeodomain) contribute to functional diversity (22).

In *OsSPL*, a functional NLS is typically located within the C-terminal region of the SBP domain. It is required for the nuclear localisation and biological roles of transcription factors (23). By interacting with importin proteins, these short amino acid sequences direct *SPL* proteins to the nucleus, which mediate their passage through the nuclear pore complex (24). Nuclear localisation gets disrupted by a mutation in the NLS of *SPL* genes (e.g. *SPL9* in Arabidopsis), resulting in loss of function. For example, a single amino acid change in the NLS of *SPL9* prevents its entry into the nucleus and delays developmental transitions, proving that the NLS is indispensable for normal *SPL* function (23). NLSs can also influence higher order protein assembly, organelle dynamics and different cellular stress responses, highlighting their regulatory importance (25). The NLS often overlaps with the DNA-binding domains in many DNA-binding proteins, including *SPL*, suggesting t an evolutionary adaptation for efficient nuclear targeting and function (26, 27).

The *SPL* SBP domain binds to cis-regulatory elements that possess the GTAC core motif within the promoters of the target genes and thus modulates downstream expression (28). The conservation of the GTAC motif as the SBP domain binding site in various plant species including rice, Arabidopsis and Petunia indicates the existence of a basic regulatory mechanism (28, 29). Chromatin immunoprecipitation sequencing (ChIP-seq) and other molecular studies have confirmed that *OsSPL* proteins like *OsSPL3* and *OsSPL12* use their SBP domains to directly bind to the GTAC cis-element in the promoters of their target genes like *OsMADS50* and thus regulate developmental processes like crown root development (16).

Phylogenetic analysis classifies *OsSPL* genes into different subfamilies according to sequence similarity and evolutionary origin. Gene expression profiling revealed that *SPL* genes follow tissue specific and developmental stage specific expression profiles (30-32). The genes are also regulated by microRNAs like miR156 and miR529, which regulate their expression to control important agronomic traits. For example, *OsSPL3* and *OsSPL12*, which are targets of OsmiR156, control crown root development by activating *OsMADS50*. *OsSPL3* mutations suppress this pathway, resulting in reduced crown roots. *OsSPL14* is also directly targeted by OsmiR156f and controls plant height and tiller outgrowth, which are crucial for rice architecture and yield (16, 33). *OsSPL16* and *OsSPL17*, controlled by miR156, control male fertility under temperature stress. *OsSPL17* controls flavonoid biosynthesis and reactive oxygen species (ROS) homeostasis in the tapetum, which influences pollen development and fertility in PTGMS rice (18). *OsSPL7* is required for ROS homeostasis under heat and pathogen stress. Overexpression and knockout of *OsSPL7* modify disease resistance and stress tolerance, which suggests its role in balancing growth and defence (34). Some other *SPL* genes, i.e., *OsSPL2*, *OsSPL7*, *OsSPL14*, *OsSPL16*, *OsSPL17* and *OsSPL18* are controlled by miR529a at different developmental stages, which control the plant height, tiller number, panicle architecture and grain size. This regulation is stage specific and controls the overall rice yield (11).

Comparative genomics identified that SPL gene family is highly conserved among major plant species like wheat, maize and Arabidopsis. Orthologous relationships between rice and wheat *SPLs* have been reported (Table 1) (32). In rice, several *SPL* genes are found in clusters that have been conserved for at least 50 million years, reflecting their general evolutionary conservation (35). Chromosomal synteny and sequence similarity comparisons have also yielded evidence for the conservation of *SPL* genes and their regulatory miR156 elements between rice and other grass plants like maize and sorghum. These data support the hypothesis of common ancestral origin of *SPL* genes and their regulatory elements like miR156 in the grass family (14, 36).

Even with worldwide conservation, there is divergence of *SPL* gene structure, expression pattern and regulatory element between and even within species. Different temporal and spatial expression of some of the *SPL* genes in rice and wheat indicates functional divergence following duplication events (32). Segmental duplication and positive selection in maize have created new evolutionary features, with some *SPL* gene pairs evolving under positive selection, unlike the predominantly purifying selection in rice and sorghum (36). In the Oryza genus, *SPL* genes are classified into separate lineages and clades with

Table 1. Conservation and divergence features of *SPL* gene in major cereal crops

Species	SPL gene count	Conservation features	Divergence features	References
Rice	19	Ancient gene clusters, miR156 targets	Expression divergence, lineage specificity	(14, 32, 35-37)
Wheat	56	Orthology with rice *SPLs*	Spatial-temporal expression differences	(32)
Maize	31	Synteny with rice, miR156 targets	Segmental duplication, positive selection	(36)

varying exon counts, intron lengths and miR156 regulation, indicating ongoing evolutionary diversification (37).

Regulation of SPL genes in rice

OsSPL genes code for the plant specific transcription factors that are crucial for transitions between developmental stages, organ development and environmental responses. Their regulation is strictly controlled at the transcriptional and post transcriptional levels, with the miR156-*SPL* module being a key regulatory hub. Developmental signals, like phase transitions, organogenesis and reproduction and environmental cues, such as temperature, oxidative stress and pathogen infection, influence *OsSPL* gene expression directly or indirectly through miR156 and associated microRNAs (Fig. 1) (12, 18, 30, 38-42).

The miR156-*SPL* module exhibits a high level of conservation and is responsible for merging intrinsic development signals and environmental external stimuli, thus defining complex phenotypic outcomes involving plant structure, fertility, stress resistance and yield (18, 30, 38-41). miR156 acts as a master regulator of *OsSPL* gene expression, allowing for either transcript cleavage or translational repression of target *OsSPLs* (12, 16, 30, 33, 41, 42). miR156 levels are high in juvenile tissues and decline with age, thereby enabling phase changes and organ differentiation (30, 42). Both miR156 and the highly similar counterpart miR529 exhibit conserved as well as stage specificity, withmiR156 being dominant in vegetative tissues and miR529 being more active in reproductive tissues (11-13). Recent studies have also shed light on the interactions between miR156, miR529 and other regulatory elements, indicating both conserved and divergent functionalities between rice and other plant species (11-13, 39, 42). Overexpression or suppression of miR156/miR529 causes significant phenotypic changes, including changes in plant height, number of tillers, panicle structure and stress responses(11-13, 16, 30, 33, 41, 43).

OsSPLs are grouped into subfamilies based on sequence and motif analysis and have unique exon-intron structures and regulatory motifs. Some of the OsSPLs, including *OsSPL14, OsSPL3, OsSPL4, OsSPL17* and *OsSPL18*, play a central role in regulating tillering, panicle architecture, grain size and fertility(12, 17, 18, 30, 40-43). *OsSPL* gene expression is controlled by developmental cues like phase transitions, organogenesis and reproductive development and environmental cues like temperature, oxidative stress (e.g., H_2O_2), pathogen attack and heavy metal stress. For instance, hydrogen peroxide inhibits miR156, resulting in upregulation of *OsSPL2* and *OsTIFY11b*, which control plant defence (38). *OsSPLs* (especially *OsSPL2, OsSPL4, OsSPL16, OsSPL17*) control temperature sensitive male fertility in rice through the miR156-SPL module, affecting flavonoid biosynthesis and ROS homeostasis (18). Pathogen induced downregulation of miR156/529 enhances *OsSPL7/14/17* mediated resistance through jasmonic acid and salicylic acid pathways (39, 44). Likewise, during cadmium stress, a peptide encoded by pri -miR156e (miPEP156e) enhances the concentration of miR156, preventing cadmium uptake and ROS accumulation and improving cadmium tolerance in rice (45). Also, stress induced upregulation of miR156 maintains the plant in a juvenile state, promoting development and stress tolerance, whereas in the absence of stress, low concentrations of miR156 allow normal development. This adaptive regulation of miR156 is critical to balance growth, survival and reproduction under fluctuating environments (46-48).

miR156-SPL module interacts with various regulatory networks, such as hormonal pathways including gibberellin, auxin; epigenetic regulators such as *OsLHP1*; and other microRNAs such as miR172, miR535 (40-42, 44). *OsSPL14* influences auxin transport by regulating *OsPIN1b* and *PILS6b*, regulating tiller outgrowth (41). Epigenetic marks, i.e., *H3K27me3* deposition, regulate miR156 expression and thereby *OsSPL*-mediated developmental transitions (42).

Functional roles of SPL genes in plant development

The *OsSPL* gene family is a master switch for numerous developmental transitions and morphogenic processes that involve vegetative to reproductive phase change, flowering time regulation, plant architecture transition, inflorescence and floral development and grain development. Numerous OsSPL genes are direct miR156 targets, which regulate their spatiotemporal expression to coordinate organ differentiation, phase changes and developmental timing (16, 17, 30, 49). *OsSPLs* control the transcription of downstream genes such as *OsMADS50* (phase

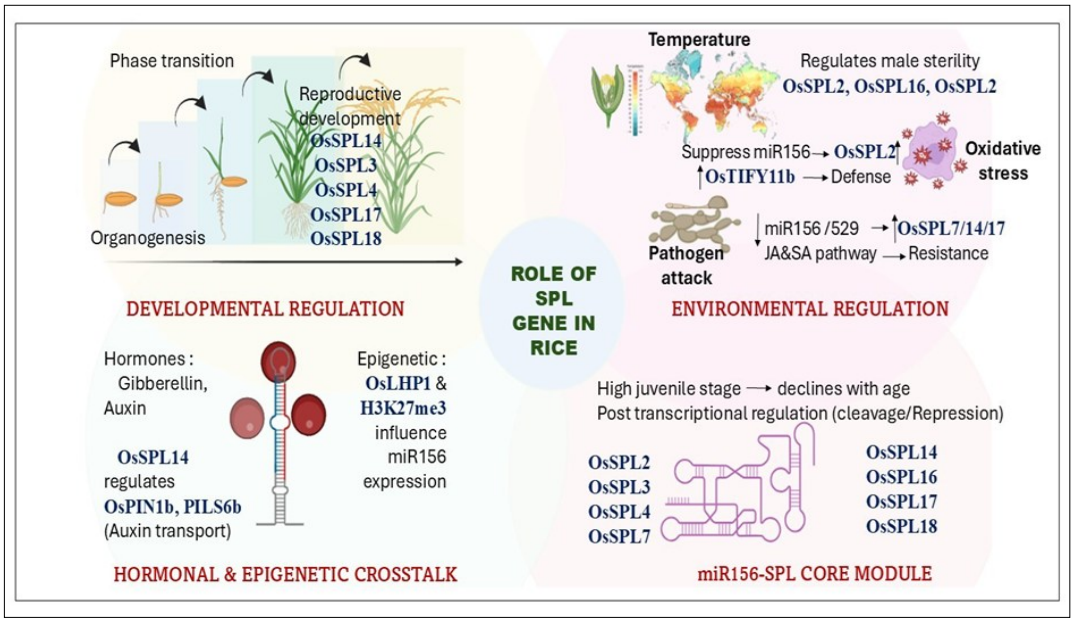

Fig. 1. Role of SPL gene families in rice.

change), *DEP1* (grain/number of panicles) and *RCN1* (branching of panicles) (16, 17). Specialized *OsSPLs*, which involve some redundancy, exert tight control over development and adaptation (Table 2) (30, 42).

SPL genes in specific abiotic stress responses

Drought stress

In rice, drought stress causes decreased growth, yield and spikelet fertility but also elevates plant oxidative stress. Rice thus responds to adjustments such as osmotic adjustment, ROS scavenging and stress- inducible gene expression to reverse the harmful impacts of stress (50, 51). Drought stress elevates miR156 expression and suppresses SPL transcription factors by mRNA cleavage or translational repression (51). Suppression of *SPLs* causes alterations in plant architecture (e.g., tillering, branching), phase transition delay and altered stress response, which enable the plant to conserve resources during drought (52). *OsSPL10* and *OsSPL14* are key regulators of drought tolerance in rice within the *SPL* gene family. *OsSPL10* promotes drought tolerance by modulating genes for the production of ROS and stomatal function, thus helping rice plants preserve water and reduce cell death caused by stress (53). OsSPL10 is a transcription factor that directly regulates *OsNAC2,* a key gene that is part of drought-responsive gene networks. Remarkably, reduced expression of *OsSPL10* (notably the Hap1 allele) leads to reduced activity of *OsNAC2*, which in turn reduces *OsAP37* (a pro-death gene) and increases *OsCOX11* (a protective gene), leading to reduced ROS accumulation and reduced programmed cell death (PCD) (53). Knockdown or knockout lines of *OsSPL10* show increased stomatal closure, which reduces water loss and increases drought tolerance (53). The *OsSPL10* Hap1 allele, which is common in upland and improved lowland rice, is associated with increased drought tolerance due to its regulatory functions (53). The *OsSPL10* Hap1 allele is an important genetic target for the breeding of drought resistant rice cultivars and offers a viable solution to enhancing crops in water scarce environments.

OsSPL14 promotes drought tolerance by activating SNAC1 gene, a master regulator of a stress-response pathway and ROS detoxification. This results in the up-regulation of SNAC1 expression, essential for drought tolerance (54). *OsSPL14*-deficient plants have high hydrogen peroxide (H_2O_2) levels, reflecting high oxidative stress levels and high drought sensitivity. *OsSPL14* is also regulated by drought signals like abscisic acid, salt and oxidative stress, indicating it is a part of a bigger stress response network (Table 3) (54).

The miR156-SPL regulatory module presents another area of controversy, although most studies consistently report miR156 upregulation under drought stress, leading to SPL suppression, the downstream outcomes vary considerably. Some reports suggest that this shift promotes survival through resource conservation, while others argue that it compromises stress adaptation by delaying development (55, 56). These contradictory interpretations are further compounded by the fact that most drought stress studies rely on single gene knockout or overexpression approaches, without accounting for the complex regulatory networks involving multiple SPL genes and their collective influence on drought tolerance mechanisms.

Salt stress

Salinity stress damages rice by reducing yield, panicle number and grain quality. Excess sodium (Na^+) causes ion imbalance, osmotic stress and promotes ROS accumulation, which causes cellular function and growth damage (57, 58). Osmotic and ionic toxicity caused by salt stress led to growth suppression and oxidative stress. Rice plants react by preserving ionic homeostasis, enhancing antioxidant activity and regulating osmotic balance in order to counteract damage (59).

When subjected to salt stress, miR156 is upregulated, leading to downregulation of SPL gene expression. This reduces phase transition and extends the juvenile phase, thus favouring survival over precocious development (47). This type of developmental stage regulation favors energy conservation and increased tolerance to stress. The overexpression of miR156 enhances stress tolerance, whereas suppression renders plants hypersensitive to salt (46). This regulatory module is strictly conserved in plant species such as rice and is linked to the regulation of downstream genes for protective metabolic processes such as anthocyanin biosynthesis, which can act to prevent damage caused by stress (46, 47).

Functional studies have confirmed that *OsSPL10* negatively affects salt tolerance in rice (19). Loss-of-function knockout of *OsSPL10* renders the plants more resistant to salt, as evident from higher survival

Table 2. Functional role of *OsSPL* genes during different development stages of rice

Developmental process	Genes involved	Functional insights	References
Vegetative to reproductive transition	*OsSPL3, OsSPL12, OsSPL14*	*OsSPL3/12* regulates crown root and shoot development; *OsSPL14* influences growth period and phase change	(16, 30, 71)
Regulation of flowering	*OsSPL3, OsSPL12, OsSPL14*	*OsSPL3/12* activate *OsMADS50,* promoting phase transition and flowering	(16, 30, 71)
Shoot and plant architecture	*OsSPL14, OsSPL3, OsSPL10*	*OsSPL14* shapes ideal plant architecture; *OsSPL3/10* affect plant height, tillering and leaf traits	(16, 19, 35, 71)
Inflorescence & floral development	*OsSPL9, OsSPL18, OsSPL13*	*OsSPL9/18/13* regulates panicle branching, spikelet formation and floral organ size	(17, 30, 49, 87)
Organ size & grain development	*OsSPL13, OsSPL16, OsSPL18*	*OsSPL13/16/18* controls grain size, weight and number by modulating cell proliferation and hull size	(17, 49, 69, 87)

Table 3. Key *OsSPL* genes involved in abiotic stress tolerance

OsSPL gene	Stress type	Regulatory role	Key mechanism/target	References
OsSPL14	Drought, cold, nutrient stress	Promotes ROS scavenging, activates SNAC1	ABA pathway, NAC TF, miR156 downregulation	(64)
OsSPL10	Drought, salt	Regulates root traits and salt sensitivity	Ion homeostasis, Trichomes	(53)
OsSPL9	Cu uptake under high Fe	Activation of Cu transporter gene	miR156 repression	(68)
OsSPL13	General stress	Influences grain traits under stress	miR156 repression	(57)

rates, better photosynthetic activity and increased stomatal conductance compared to the wild type under salt stress (60). The knockout plants also have reduced growth of trichomes, resulting in glabrous glumes and leaves (60). Molecularly, salt stress response genes like *OsNHX2* and *OsIDS1* are upregulated, while *OsGASR1* is downregulated in knockout lines (60). On the other hand, overexpression (gain-of-function) of *OsSPL10* leads to poor salt tolerance and reduced survival rates under stress (Table 3) (19, 60).

Despite this well-established negative regulatory role, the mechanistic basis of *OsSPL10* action remains poorly defined. Although studies cite involvement in regulating ion transport and developmental genes, direct transcriptional targets and regulatory pathways have not been definitively established. The absence of chromatin immunoprecipitation (ChIP) data or comprehensive transcriptomic analyses represents a major gap, limiting our understanding of how *OsSPL10* integrates developmental regulation with salt stress signalling. Notably, this negative regulatory role in rice contrasts with findings in other plant species, where certain SPL genes act as positive regulators of salt tolerance, highlighting possible species-specific functional divergence within the SPL gene family.

Heat and Cold Stress

Heat exposure in rice induces poor germination of seeds, pollen sterility and loss of grain filling, hence reduced yield and quality (50, 61). Rice plants, in response to heat stress, produce heat shock proteins, initiate antioxidant defence and experience hormonal modification as part of the stress response (61). During heat stress periods, miR156 levels are reduced, hence up regulating its target genes such as OsSPL2 and *OsTIFY11b*. These proteins are engaged in protein-protein interactions in the nucleus to regulate defence related gene expression such as *OsRBBI3-3* (38). Under stress, high levels of miR156 delay the juvenile stage of the plant, hence retarding development but increasing tolerance to stress. Under non-stress, miR156 levels are lowered and normal development continues (46, 47).

OsSPL7 is necessary for ROS homeostasis during heat stress. Loss-of-function of *OsSPL7* produces growth defects and lesion mimic spots, whereas overexpression produces ROS accumulation but increases disease resistance. *OsSPL7* is a transcription activator and plays a role in the regulation of plant growth and stress response (34). Epigenetic research indicates that the repression of *SPL* transcription factors is necessary for heat stress memory so that plants can remember and react more effectively to future heat stress (62).

Cold stress in rice results in delayed heading, sterility of pollen and smaller grain and plant yield. Stress is resisted by plants through increased ROS scavenging activity, overexpression of *DREB1* genes and metabolic reorganizations to continue cellular activity at low temperature (61, 63, 64). Overexpression of *OsSPL7* enhances cold tolerance, whereas knockout mutants are cold-sensitive, suggesting that *OsSPL7* has a protective function against cold stress (34). Overexpression of miR535 suppresses the expression of *OsSPL14, OsSPL11 and OsSPL4*, resulting in reduced cold tolerance, most likely by repressing the CBF-mediated cold signalling pathway (65). Overexpression of miR156, targeting *OsSPL3*, promotes cold tolerance by regulating downstream transcription factors and stress-response genes (66). Epigenetic regulation, i.e., *SPL* gene expression modification, is involved in cold stress memory, enabling plants to react to repeated cold stress (Table 3) (62).

The field still lacks comprehensive studies on temperature stress, with most reports relying on limited expression analyses rather than functional validation through genetic approaches. While some studies suggest epigenetic regulation of SPL genes in temperature stress memory, these claims remain speculative, as no direct evidence linking DNA methylation or histone modifications to SPL gene expression under temperature stress has been demonstrated (67).

Nutrient stress

Sustainability and yields of rice have a direct connection with its capacity for optimal utilization of nutrients, especially nitrogen (N), phosphorus (P), copper (Cu) and iron (Fe). Nutrient stress also inhibits photosynthesis and changes the metabolic activity of the plant, resulting in stunted growth. Rice plants respond by changing the patterns of gene expression and adjusting their metabolic activities in an effort to correct the imbalance of nutrients (63).

Recent studies have elucidated the roles of some of the *OsSPL* genes, *OsSPL2*, *OsSPL3*, *OsSPL9*, *OsSPL14* and *OsSPL16*, in regulating N, P, Cu and Fe deficiency responses and Nitrogen use efficiency (NUE) under stressed N conditions. *OsSPL9* triggers Cu uptake and transport under Fe-high conditions through the activation of Cu transporter genes, enabling rice to adapt to red soils with high Fe and low Cu (68). *OsSPL3* and *OsSPL12*, miR156 targets, regulate crown root growth, which is crucial for nutrient uptake (16). *OsSPL14* and *OsSPL16* are associated with panicle branching, grain number and grain size, traits sensitive to nutrient availability (12, 69, 70). Under stress, miR156 is downregulated, which enhances the expression of its targets *OsSPL2* and *OsTIFY11b*, which interact and regulate defence-related genes (38).

Some of the *OsSPL* genes directly or indirectly participate in NUE. *OsSPL14* expression is associated with grain yield components and N % under low N conditions, indicating a role in N metabolism and their trans-conversion to yield (70). Genome editing of *OsSPL16* using CRISPR/Cas9 enhances grain yield through regulation of metabolic pathways, which indicates its potential for enhancing NUE (69). The miR156-*SPL* module also affects root development and tillering; characteristics associated with efficient N uptake and utilization. Most, however, target developmental implications with fewer directly connecting *OsSPLs* with molecular mechanisms of NUE (Table 3) (12, 16, 47, 48).

Despite these insights, the role of SPL genes in nutrient stress responses remains the weakest area of current knowledge. While connections between *OsSPL9, OsSPL14 and OsSPL16* with nutrient uptake and utilization have been reported, most of these relationships are correlative rather than mechanistic, as the underlying regulatory pathways and direct target genes remain unidentified. For instance, although *OsSPL9* has been linked to copper uptake under iron-high conditions, its downstream effectors are still unknown and the associations of *OsSPL14* and *OsSPL16* with nitrogen use efficiency likewise lack molecular validation (67). Moreover, most studies inferring SPL involvement rely heavily on expression profiling rather than functional validation and knockout or overexpression studies specifically addressing nutrient stress responses are largely absent. To date, no systematic comparisons have been made across different

nutrient stress conditions and the interactions between nitrogen, phosphorus and other nutrient limitations on SPL regulation remain unexplored.

Crosstalk with other transcription factors and pathways

SPL transcription factors function as central hubs within multi-layered regulatory networks that coordinate plant development and stress responses through systematic crosstalk with hormonal signalling pathways and diverse transcription factor families. This hierarchical organization enables SPLs to integrate multiple environmental and developmental signals, positioning them as master regulators of plant adaptation. Furthermore, the miR156-SPL regulatory module is strongly embedded in hormonal signalling pathways. *OsSPL14* exemplifies the sophisticated integration of SPL transcription factors with hormonal regulatory systems, particularly auxin signalling networks. As a tiller outgrowth repressor, *OsSPL14* directly activates auxin transport genes, including *OsPIN1b* and *PILS6b*, which regulate auxin distribution in axillary buds and suppress their outgrowth (11, 40, 41). It regulates tillering and panicle development through its own pathway to control auxin flow and thus bud outgrowth (40, 41). The regulatory influence of OsSPL14 extends beyond auxin to encompass broader hormonal networks essential for stress adaptation. Overexpression of OsSPL14 induces systematic alterations in gene expression within abscisic acid and gibberellin signalling pathways, both critical components of stress response mechanisms. This multi-hormonal regulation enables plants to adjust their architecture dynamically under stress conditions by modulating hormone pathways and auxin flow simultaneously (71).

OsSPLs interact with several families of transcription factors, most prominently MYB, PIL/PIF, TCP and MADS-box proteins, to regulate rice development and stress responses. *OsSPL10* physically interacts with *OsJAmyb*, a MYB transcription factor that plays a role in jasmonic acid signalling. This interaction increases blast disease resistance by stabilizing *OsJAmyb* and enhancing immune responses like ROS burst and callose deposition (72). The crosstalk between SPL and PHYTOCHROME-INTERACTING FACTOR-LIKE (PIL) transcription factors represents another critical regulatory axis. *OsSPL3* and *OsSPL17* physically interact with PIL transcription factors to suppress tillering and branching in both rice and wheat through regulation of dominant architectural genes like *OsTB1*. This interaction demonstrates the evolutionary conservation of SPL-mediated plant architecture control across cereal species (73). *OsSPL3* and *OsSPL12* directly regulate the MADS-box transcription factor *OsMADS50*, which is a major regulator of crown root development in rice. This regulatory relationship illustrates how SPLs coordinate above-ground and below-ground developmental programs through targeted transcriptional control (16). The interaction between SPL and TEOSINTE BRANCHED1/CYCLOIDEA/PCF (TCP) transcription factors adds another layer of regulatory complexity. OsSPL proteins interact with TCP transcription factors like *OsTCP5* through specific protein domains, with these interactions being particularly important in processes such as meiotic fate acquisition (74).

The integration of SPL networks with diverse transcription factor families enables plants to fine-tune developmental programs in response to environmental challenges while safeguarding essential growth processes. Through multi-level interactions, these networks coordinate stress responses across physiological systems, linking hormonal signalling, metabolic adjustment and architectural modification. The conserved interactions of SPLs with MYB, PIL/PIF, TCP and MADS-box families across cereal species further highlight the fundamental importance of these regulatory networks in plant adaptation and evolution.

Biotechnological applications and genetic manipulation of SPL genes for climate resilience

Advances in genome editing and molecular breeding tools have made it possible to specifically manipulate SPL genes to improve stress tolerance and agronomic traits in rice. *OsSPL* genes were targeted to enhance yield traits and abiotic stress tolerance. CRISPR/Cas9, prime editors and base editors are versatile tools to design precise genetic modifications to improve crop tolerance to drought, heat and salinity. These tools allow targeted modification of regulatory genes of importance in stress response to enhance traits like root development, water use efficiency and stress tolerance pathways (75, 76). Gene editing and genomics-assisted breeding are employed to find and manipulate genes and quantitative trait loci (QTLs) involved in stress adaptation in rice. This enables the improvement of climate-resilient varieties through high-throughput sequencing and phenotyping integration (77). Genetic diversity enhancement by biotechnology is the gold standard for climate resilience, but practical uses outside the laboratory for research purposes are yet to be achieved.

Gene markers harbouring alleles of major genes like *OsSPL14* have been discovered to increase the precision of genome-wide association studies as well as the precision of genomic prediction of quantitative traits like grain weight and grain yield. Removal of major gene-based markers from prediction models brings precision down to a very low level, which proves their value in GS programs (78). The development of molecular markers linked to beneficial *SPL* alleles has facilitated their integration into marker-assisted selection (MAS) programs. These markers enable rapid identification of desirable genotypes during early breeding stages, accelerating the development of climate-resilient varieties. The combination of *SPL*-based markers with high-throughput genotyping platforms has enhanced breeding efficiency and selection accuracy (78).

CRISPR/Cas9 genome editing has been most useful to regulate *SPL* gene function. For example, *OsSPL14 (IPA1)* targeted editing enhanced grain size, panicle architecture and drought tolerance without a yield penalty and *OsSPL16* targeted mutagenesis enhanced grain yield by improving pyruvate metabolism and cell cycle proteins (69). These edits also have the added advantage of improving productivity and stress tolerance. *OsSPL13*, *OsSPL7* and *OsSPL12* are regulators of plant development. CRISPR-Cas9 has been utilized to edit these genes, i.e., by targeting microRNA156 recognition elements (MREs) to regulate gene expression (79). Targeted editing of the MRE in *SPL* genes suppresses microRNA binding, leading to increased transcript levels and altered plant traits (79). Cross-species validation has demonstrated the effectiveness of this approach, with CRISPR-induced mutants in the *TaSPL13* (ortholog of *OsSPL13*) MRE in wheat producing increased transcript levels, shortened flowering time, decreased tiller number and plant height and increased grain size and number. This approach offers precise control over *SPL* gene expression without disrupting protein-coding sequences (79).

Overexpression approaches have also been employed to

investigate SPL function. Overexpressing *OsSPL14* increases antioxidant enzyme activities such as superoxide dismutase (SOD), catalase (CAT) and peroxidases. This leads to higher scavenging of ROS generated during stress (such as drought or salinity), protecting plant cells from oxidative damage and aiding in the maintenance of increased physiological function under stress (54). Repression or Downregulation of *OsSPL14* tends to compromise the plant's antioxidant defences by reducing the activities of crucial antioxidant enzymes, rendering the plant susceptible to oxidative damage when stressed. This can result in increased damage and loss of cellular integrity (54). The antioxidant role of *OsSPL9* is less well established than that of *OsSPL14*, but the indication is that *OsSPL9* has a role in stress response signalling. Overexpression may tip the balance between oxidative stress and antioxidant enzyme expression. However, excessive *OsSPL9* expression will also enhance leaf senescence and increase ROS levels, potentially if antioxidant systems are not activated in synchrony (80). Repression of *OsSPL12* has been linked with increased environmental stress resistance, perhaps through increased antioxidant pathway activation. Plants with downregulated *OsSPL12* expression have a tendency to show increased tolerance to stresses and increased ROS scavenging ability, suggesting a function in modulating the antioxidant defence system (81).

In addition to single-gene alterations, pyramiding of genes and multiplex CRISPR approaches have been suggested to modulate multiple *SPL* targets simultaneously. Combination of genotype or alteration of miR156-binding sites can fine-tune expression dynamics for improved stress acclimatization. For example, alteration of the miR156-binding site of *OsSPL14* has been reported to introduce semi-dominant drought tolerance with maintenance of major yield qualities (82, 83). Furthermore, combination of *SPL* gene alteration with other stress regulators such as *NACs*, *MYBs* and *WRKY*s can facilitate multi-trait enhancement. In combination with MAS and genomic selection (GS) platforms, SPL-based breeding may facilitate the fast tracking of climate-resilient rice varieties (84). These biotech platforms, strategically applied, can unleash the complete potential of *SPL* genes in generating next-generation rice varieties that can thrive under extreme abiotic stresses.

Research gaps and future direction

Despite significant progress in the elucidation of SPL gene functions in rice stress response, several gaps and challenges remain to be overcome. One major limitation is the weak functional characterization of the majority of *OsSPL* genes. While *OsSPL14, OsSPL10* and *OsSPL7* are well characterized, the functions of other *OsSPL* genes, particularly those with expression in reproductive organs and roots, are still weakly characterized (32, 34, 37).

A second challenge relates to context-dependent regulation of *SPL*s. The underlying mechanisms of the transcriptional, post-transcriptional and epigenetic regulation of *SPL*s in response to simultaneous or sequential stressors are not well understood. In addition, the interactions of *SPL*s with other transcription factors and hormonal networks are complex and often oscillate with regard to developmental stage, tissue type and stress intensity (37, 45). Functional redundancy of *SPL* gene family members imposes layers of complexity on genetic studies, thus precluding functional specificity of individual genes. The redundancy also presents formidable challenges to gene editing approaches, as compensatory expression of other *SPL*s may preclude detectable phenotypic effects (32). While informative results have been derived from overexpression and knockout experiments, more studies are needed using tissue-specific, inducible, or environmentally responsive promoters to regulate *SPL* expression with specificity. This approach strives to circumvent deleterious pleiotropic effects on growth as well as reproduction (34, 85).

Future research should also include the application of integrated omics platforms like transcriptomics, proteomics, metabolomics, epigenomics and chromatin accessibility profiling together with natural variation analysis and field phenotyping, to identify *SPL*-centred regulatory networks and further delineate their downstream targets (32, 37). Examination of *SPL* interactions in abiotic stress acclimatized natural rice landraces or wild relatives could uncover new alleles or regulatory variants for improvement (37). This diversity remains underutilized and could give untapped resilience mechanisms for abiotic stress. A deeper understanding and precise regulation of SPL gene expression will ultimately expedite the development of next generation, climate resilient rice varieties that ensure global food security.

Finally, application of CRISPR-mediated base editing and prime editing technologies also promises site specific *SPL* gene editing without the generation of double-stranded breaks. These technologies may further speed up the improvement of rice varieties with stable, multi-stress tolerance without sacrificing major agronomic traits (86). Addressing these knowledge gaps will be critical to translating *SPL* research into field level innovations in rice agriculture.

Conclusion

This review represents the comprehensive synthesis of *SPL* gene functions in rice abiotic stress tolerance, providing a unique integration of developmental regulation and stress response mechanisms that has been lacking in the fragmented literature. By consolidating research across multiple stress types of droughts, salinity, heat, cold and nutrient deficiencies we establish a unified framework for understanding how the miR156/miR529-*SPL* regulatory networks coordinate plant adaptation under diverse environmental challenges.

Our analysis reveals several novel insights that advance the field significantly. We demonstrate for the first time the tissue specific and developmental stage specific functions of *SPL* genes in stress responses, with miR156 predominantly controlling vegetative stress adaptation while miR529 regulates reproductive stress tolerance. The identification of key regulatory hubs particularly *OsSPL14*, *OsSPL10* and *OsSPL7* as master integrators of hormonal pathways, ROS detoxification and transcriptional networks provides a roadmap for targeted crop improvement strategies.

The review's contribution to biotechnological applications is particularly significant. We bridge the critical gap between basic *SPL* research and applied crop improvement by evaluating CRISPR/Cas9, prime editing and base editing technologies for precise *SPL* gene manipulation. The documentation of successful genome editing approaches, including targeted alteration of miRNA recognition sites and allele pyramiding strategies, provides practical guidance for developing climate resilient rice varieties.

Looking forward, several transformative opportunities

emerge from this synthesis. Advanced genome editing technologies, including prime editing and base editing, offer unprecedented precision for *SPL* gene modulation without off-target effects. Multi-omics integration combining transcriptomics, proteomics, metabolomics and epigenomics will unlock comprehensive *SPL*-centred regulatory networks currently hidden from view. Natural variation mining in wild rice relatives and landraces represents an untapped reservoir of stress-resilient *SPL* alleles that could revolutionize breeding programs. The development of tissue specific and inducible expression systems will enable fine-tuned *SPL* regulation that avoids detrimental pleiotropic effects while maximizing stress tolerance. Multiplex CRISPR approaches targeting multiple *SPL* genes simultaneously, combined with other stress regulators like *NACs*, *MYBs* and *WRKYs*, promise to create rice varieties with robust multi-stress tolerance.

As climate change intensifies abiotic stress pressures on global rice production systems, the *SPL* gene family emerges as a critical nexus for next-generation breeding programs. The regulatory principles and biotechnological strategies outlined in this review extend beyond rice to other major cereal crops, potentially transforming how we approach crop resilience in an era of environmental uncertainty. The integration of *SPL*-based improvements with marker-assisted selection and genomic selection platforms will accelerate the development of climate smart rice varieties essential for feeding a growing global population while maintaining agricultural sustainability.

This comprehensive framework positions *SPL* genes at the forefront of climate-resilient agriculture, offering both immediate applications for rice improvement and a foundational understanding that will guide future research toward achieving global food security in the face of climate change challenges.

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Authors' contributions

SPAB performed the data curation and writing the original draft. Conceptualization, visualization, formal analysis, Ssupervision and editing were done by RP, SPR, SAR, SM and RM. Editing the resources were performed by KE, RS, PR and RM. All authors read and approved the final manuscript.

Compliance with ethical standards

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