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Review

Progress and Prospect of Breeding Utilization of Green Revolution Gene *SD1* in Rice

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Abstract: Rice (*Oryza sativa* L.) is one of the most important cereal crops in the world. The identification of *sd1* mutants in rice resulted in a semi-dwarf phenotype that was used by breeders to improve yields. Investigations of *sd1* mutants initiated the "green revolution" for rice and staved off famine for many people in the 1960s. The smaller plant height conferred by *sd1* allele gives the plants lodging resistance even with a high amount of nitrogen fertilizer. Guang-chang-ai-carrying *sd1* was the first high-yielding rice variety that capitalized on the semi-dwarf trait, aiming to significantly improve the rice yield in China. IR8, known as the miracle rice, was also bred by using *sd1*. The green revolution gene *sd1* in rice has been used for decades, but was not identified for a long time. The *SD1* gene encodes the rice Gibberellin 20 oxidase-2 (GA20ox2). As such, the *SD1* gene is instrumental in uncovering the molecular mechanisms underlying gibberellin biosynthesis There are ten different alleles of *SD1*. These alleles are identified by genome sequencing within several donor lines in breeding for semi-dwarf rice. Apart from breeding applications and the molecular mechanism of GA biosynthesis, the *SD1* gene is also involved in the molecular regulation of other important agronomic traits, like nitrogen fertilizer utilization. The dentification of new alleles of *SD1* can be obtained by mutagenesis and genome editing. These new alleles will play an important role in improving the resource diversity of semi-dwarf breeding in the future.

Keywords: rice; green revolution; *sd1*; molecular mechanism; breeding

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1. Introduction

Rice (*Oryza sativa* L.) is a cultivated cereal crop whose breeding species provide 35–60% of dietary calories to about 50% of the world's population [1]. The most significant milestone of rice breeding is the famous green revolution, which remarkably increased the rice yield worldwide in the 1960s. The term "green revolution" refers to the renovation of agricultural practices, which started in Mexico in the 1940s. Because of its success in producing more agricultural products, green revolution technologies spread worldwide in the 1950s and 1960s, and significantly improved the number of calories per acre of agriculture [2,3]. A major factor for the success of the green revolution was the introduction of high-yielding semi-dwarf varieties with the successful application of the nitrogen fertilizer. Nitrogen fertilizer is beneficial to the yield increase, but it leads to stem and leaf elongation and an increase plant height. This can easily result in lodging and yield losses. By contrast, the semi-dwarf varieties respond to fertilizer inputs properly with an increased yield because of their lodging resistance even under high nitrogen fertilization. This is the major reason why the green revolution can tremendously increase the yield in semi-dwarf wheat and rice [4,5].

This green revolution change in rice was caused in large part by introduction of semi-dwarf mutations, which led to a shortened culm with improved lodging resistance and a greater harvest index [5,6]. Strikingly, these short stature changes in the semi-dwarf lines were achieved mostly through mutations in a single gene, *Semi-dwarf 1* (*SD1*). This gene encodes an oxidase enzyme, GA20ox2, involved in the final steps of gibberellin synthesis [7–9]. Several mutations of *SD1* were identified and used in rice breeding for a long time.

This review aims to introduce the history of applying semi-dwarf gene *sd1* in rice breeding and the impact of semi-dwarf phenotype in higher yield during the green revolution in rice. Then, the variation allele of *SD1* gene was described which mutated *sd1* conferring the semi-dwarf phenotype in rice. The biological function and regulation mechanism of GA20ox in gibberellin biosynthesis was illustrated, and the advantage of semi-dwarf gene *sd1* allele diversity and molecular regulated mechanism of *sd1* in rice breeding was also emphasized. After explaining why *sd1* is used widely with a huge impact, and how to use *sd1* to achieve a new era of rice breeding, it can be seen that the semi-dwarf gene *sd1* still has tremendous vitality in rice breeding.

2. History of *sd1* Utilization in Rice Semi-Dwarf Breeding

Rice (*Oryza sativa* L.) is one of the most important cereal crops in the world. There have been many landmark achievements in rice improvement over the past 50 years, especially in the *indica* sub-species. Dwarf usually refers to the dwarf mutant whose plant height is equal to or less than half of the wild-type plant height at maturity, and semi-dwarf refers to the type of plant height between dwarf and normal height. In China, varieties with plant heights between 70 and 110 cm are generally classified as semi-dwarf, those below 70 cm as dwarf, and those higher than 110 cm as tall [10]. A major breakthrough resulted from the independent development of a series of semi-dwarf varieties in China and the International Rice Research Institute (IRRI) in the 1950s and 1960s, leading to the green revolution in rice [11–13]. In 1959, Yaoxiang Huang, the father of semi-dwarf rice in China, from Guangdong Academy of Agricultural Sciences, bred the world's first semi-dwarf *indica* rice variety Guang-chang-ai (85–100 cm) by crossing Ai-zai-zhan 4 with Guang-chang 13. Then, he popularized it on a large scale and initiated rice-dwarfing breeding [14]. In the same year, Qunying Hong and Chunli Hong found dwarf natural variants in the tall Nante 16, and bred the dwarf variety Ai-jiao-nan-te (70–80 cm), which was quickly popularized [15].

At the same time, Qiuzeng Hong, who came from an agricultural improvement farm in Taichung District of Taiwan, bred Taichung Native 1 (90–100 cm) by using Dee-geo-woo-gen and Cai-yuan-zhong, which was popularized in 1960s [13]. In 1966, the International Rice Research Institute used the Dee-geo-woo-gen as the dwarf source and the Indonesian high-quality tall rice variety Peta to breed IR8, which was called Miracle Rice [16]. Since then, a large number of semi-dwarf and high-yielding varieties carrying the semi-dwarf gene *sd1* increased rice yield by 20% to 30%, triggering a green revolution in rice breeding [17–19]. In the United States, semi-dwarf rice varieties accounted for 80% of the rice acres grown in Louisiana and 55% of the total US rice acreage. Two alleles were present in the US germplasm, one semi-dwarf variety was Calrose76 [20]. The reason why it is called rice green revolution is that almost all the traditional farm rice varieties are of high-stem type, showing low yield and no lodging resistance, while semi-dwarf rice varieties show excellent characteristics such as fertilizer tolerance and lodging resistance, sturdy leaves and more panicles, high harvest index and so on [3]. The *sd1* plays an essential role in this landmark rice-breeding revolution.

3. The Main Variation Types of *SD1* Allele Used in Rice Semi-Dwarf Breeding

Different alleles of *SD1* were used to achieve different heights of dwarfing breeding in rice. Reportedly, there were ten alleles of *SD1* (Table 1) that were marked in the structure of the *SD1* gene (Figure 1). Seven of them have been found in *indica* rice dwarf-breeding [21], and there was only one *SD1* haplotype in *japonica* rice [22]. Taking the *SD1* of *indica* rice variety Kasalath as wild-type, the *SD1* gene consists of three exons (contain 557 bp, 322 bp and 291 bp, respectively) and two introns (102 bp and 1471 bp). Seven main *SD1* alleles in *indica* rice include: semi-dwarf variety Deo-geo-woo-gen and its derivative IR8, Habataki and Minghui63 have 383 bp deletion from the middle of the first exon to the second exon, including the 278-bp sequence of the first exon, the 105 bp of the second exon and the intron; Ai-jiao-nan-te has 2-bp deletion of the first exon; The Proline (P) at position 240 of Zhai-ye-qing 8 changed to Leucine (L); The Leucine (L) at position 266 in the second exon was mutated to Phenylalanine (F) in Calrose76; The Glycine (G) at position 94 in the dwarf variety Jikkoku changed into Valine (V); The C base at position 1026 in 93–11 was mutated to G, resulting in early termination; The position 349 amino acid Aspartic (D) in dwarf variety Reimei changed into Histidine (H). In *japonica* rice, the Glycine (G) at position 100 encoded by *SD1* gene changes to Glutamate (E), and Arginine (R) at position 400 becomes to Glutamine (Q). Almost all *japonica* rice contains $sd1^{EQ}$, the nucleotide polymorphism near the *SD1* locus in *japonica* rice decreased significantly, but this phenomenon was not found in wild rice and *indica* rice. This indicates that the *SD1* locus was selected and used in the early domestication period of rice and preserved in *japonica* rice [22].

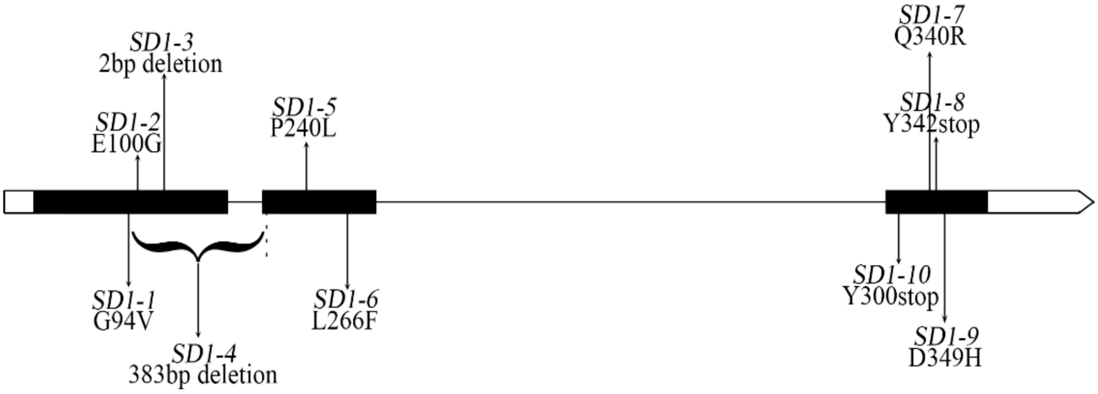

Figure 1. Structure of the *SD1* gene and mutant sites of the nine *sd1* alleles. The *GA20ox-2* gene consists of three exons and two introns. The sequence deleted in SD1-4 is denoted by the brace. The single nucleotide substitutions in *SD1-1*, *SD1-2*, *SD1-5*, *SD1-6*, *SD1-7*, *SD1-8*, *SD1-9* and *SD1-10*, and 2bp deletion in *SD1-3* are indicated by vertical arrows.

Due to the diversity mutation sites, the functional strength of each allele of *sd1* is also different. Compared with the *sd1* alleles of *indica* rice, $sd1^{EQ}$ in *japonica* rice is a weak functional allele. The $sd1^{EQ}$ has two mutants in the first exon and third exon. The G base at position 99 in the first exon was mutated to A, which led to Glycine (G) at position 100 becomes to Glutamic acid (E). The G base at position 1019 in the third exon was mutated to A which led to Arginine (R) at position 340 becomes to Glutamine (Q). (shown as *SD1-2* and *SD1-7* in Figure 1 and Table 1). The plant height of the single-segment substitution line contains *indica* rice variety 93–11 $sd1^{Y342stop}$ with Nipponbare background was significantly smaller than that of Nipponbare ($sd1^{EQ}$) [22,23]. The plant height of *japonica* rice Koshihikari ($sd1^{EQ}$) was also significantly taller than Jikkoku ($sd1^{G94V}$) and IR8 ($sd1^{383bp\ del}$) [24]. In addition to the polymorphism of the coding region, the polymorphism of the promoter region of *SD1* is also selected to adapt to the corresponding ecological environment.

There are 17 specific polymorphic sites in the promoter and second intron region of *SD1* in deep-water variety C9825, which is called deep-water rice-specific haplotype (DWH)). The polymorphic site of the haplotype promoter region can be bound and activated by the ethylene signal-related transcription factor *OsEIL1a* in a deep-water environment, resulting in the enhanced expression of *SD1*, increasing the plant height, keeping the leaves above the water surface, and ensuring the smooth progress of normal gas exchange and photosynthesis [25]. In addition to the natural variation of the *SD1* gene, new *SD1* alleles can be obtained using gene editing techniques (CRISPR/Cas9) and mutagenesis [26–28]. Thus, the *SD1* played a role in green revolution through reducing gibberellin synthesis by reducing enzyme activity, while it was selected by enhancing transcription in deep-water rice. Different alleles of the same gene played different functions in different cultivated rice, this adding great importance to the utilization value of *sd1*.

Table 1. Allele diversity of *SD1* and their representative varieties.

***SD1* Allele**	**Mutation Site in cDNA**	**Amino Acid Change**	**Represent Variety**	**Ref**
SD1-1	G281T	G94V	Jikkoku	[21,29]
SD1-2	G99A	G100E	Nipponbare, Pusa1652	[22,26]
SD1-3	C382 and G383 deletion	premature stop	Ai-Jiao-Nan-Te	[21]
SD1-4	deletion of 381bp in exon1 and 2bp in exon2	premature stop	Deo-geo-woo-gen, IR8, Habataki, Minghui 63, PA64s	[9,21,30,31]
SD1-5	C719T	P240L	Zhai-ye-qing 8	[21]
SD1-6	C796T	L266F	Calrose76	[21,29]
SD1-7	G1019A	R340Q	Nipponbare	[22]
SD1-8	C1026G	Y342stop	93-11	[21,32]
SD1-9	G1045C	D349H	Reimei	[21,29]
SD1-10	T900A	Y300stop	Pusa1652	[26]

4. Advantages of Semi-Dwarf Gene *sd1* in Rice Breeding

So far, more than 60 recessive dwarf mutants and 10 recessive semi-dwarf mutants have been identified in rice. However, only a few of them can be used in breeding. Most of the mutants are overly dwarfed or do not have practical agronomic characters. It has a negative effect on the yield components of rice, so it is of little value in breeding. At present, the main dwarf sources of *indica* rice used in production are Ai-jiao-nan-te, Dee-geo-woo-gen, Ai-zai-zhan, Hua-long-shui-tian-gu and Ai-zhong-shui-tian-gu, which are all controlled by *sd1* [15,33]. *SD1* can be widely used in rice breeding, especially in *indica* rice breeding, it has many advantages: (1) *sd1* promotes the moderate dwarfing of rice plants and enhanced the lodging resistance. Most of the local varieties are tall, and the introduction of *sd1* allele can reduce their plant height and breed semi-dwarf varieties. Due to the fact that GA20oxs in rice has multiple family members, it not only has the division of function, but also has the phenomenon of functional redundancy. In addition, many cloned dwarfing mutants lead to extreme dwarfing of rice plants, which are not conducive to mechanical harvesting and affects other agronomic characters. (2) *sd1* has little effect on yield traits. The agronomic characters, such as heading date, plant height, effective panicles, panicle length, grain type and 1000-grain weight of *sd1* near isogenic lines constructed from high plant height varieties and different *sd1* alleles of dwarf varieties were compared. The results shows that *sd1* only inhibits the growth of plant stem nodes, not affecting grain type, panicle type and other yield traits, but promotes the improvement of effective panicle number, seed setting rate and harvest index [34]. Most of the non-*sd1* allelic dwarfing materials identified have poor agronomic characters, weak growth potential and tilling ability, small panicle type and grain type, poor grain plumpness and difficulty in threshing [35]. As some dwarf sources have outcropping, high height, sterility and so on, they are difficult to use directly in production and breeding [36,37]. (3) It can be widely combined with other traits, and this is an important factor why *sd1* can be continuously used in breeding [38,39].

From the late 1950s to the mid-1970s, the main *sd1* type dwarf and semi-dwarf varieties were used to replace farm high-stem varieties, and through continuous renewal, the rice yield increased from 1.892 $t{\cdot}hm^{-2}$ in 1949 to 3.619 $t{\cdot}hm^{-2}$ in 1977, the total yield increased from 48.65 million t to 128.57 million t [14]. With the rise of hybrid rice from the mid-1970s to the mid-1980s, excellent semi-dwarf varieties are the basis of male sterile lines and restorer lines. Most male sterile lines and restorer lines in China contain semi-dwarf gene *sd1*. Hybrids with heterosis were obtained by crossing semi-dwarf varieties containing *sd1* with other varieties with excellent agronomic characters. The semi-dwarf gene *sd1* combined with heterosis have made an important contribution to the improvement of rice yield. Many excellent varieties containing *sd1* have been bred in China, such as Guang-lu-ai4, Er-jiu-qing, Xiang-ai-zao9 and so on [14].

In order to achieve another breakthrough in yield on the basis of dwarfing breeding and hybrid rice breeding, China launched a super rice research project in the 1990s, which requires the breeding of new varieties with high yield, high quality, multi-resistance and wide adaptability. Its core is the effective utilization of resources and the aggregation of favorable genes, and its key technology is the combination of the shaping of ideal plant type and the utilization of strong heterosis between *indica* and *japonica* subspecies. The combination of conventional technology and biotechnology was adopted in breeding. The ideal plant type of rice should have three basic conditions: strong lodging resistance, high optimum leaf area index and large number of filled grains per unit area. The high optimum leaf area index is the foundation, the large number of filled grains per unit area is the goal, and non-lodging is the guarantee. Proper plant height is the basic guarantee of lodging resistance and leaf area index. At present, several markers for different natural variants of *sd1* have been developed. Through the use of markers to quickly identify the combination of *sd1* and other excellent genes to achieve the purpose of molecular-assisted breeding, so as to quickly obtain super rice varieties with ideal plant type [21,39]. Through the implementation of the super rice breeding program with the combination of dwarfing and multi-objectives, so far, the Ministry of Agriculture and Rural Affairs of China has identified more than 100 super rice varieties, with an extension area of more than 27.6 million hm^2, accounting for about 25% of the total rice area.

The yield of super rice is about 15.0% higher than that of conventional varieties [14]. (4) The diversity of natural variation alleles enables *sd1* to be widely selected and used in rice breeding. In *indica* rice breeding, there are seven natural alleles of *sd1,* which are the most widely selected and used [21]. Based on the genetic analysis of *indica* rice varieties popularized in China from 1950 to 1985, there are four main dwarf sources of *indica* rice widely used in China, namely Ai-jiao-nan-te, Dee-geo-woo-gen, Ai-zai-zhan and Guang-chang-ai—all plant heights of which were controlled by *sd1* [33].

The derived varieties account for 83.3% of the total number of bred varieties. The representative varieties are Guang-chang-ai, Guang-lu-ai 4, Xiang-ai-zao 7 and Xiang-ai-zao 9, and so on [10]. The excellent semi-dwarf varieties carrying *sd1* abroad mainly include IR8 of International Rice Research Institute, Reimei and Akihikari of Japan, Calrose76 of USA and so on [40]. By 2012, the statistical analysis of 3656 conventional rice varieties showed that there were 19 most important core backbone parents of conventional *indica* rice in China, of which seven had the largest extension area and more than 100 derived varieties, of which six contained different alleles of the semi-dwarf gene *sd1* [14]. In sum, the wide application of *sd1* in *indica* rice breeding is not only related to its own gene function, but also to its widespread polymorphism in nature.

5. Contribution of *SD1* to Biological Studies in Rice

5.1. Main Biological Functions of SD1 in Gibberellin Biosynthesis

Gibberellins (GAs) play important roles in the regulation of plant growth and development, including seed germination, stem and leaf elongation, and flower and seed development. Although over 100 GAs have been identified, only a small number of GAs, such as GA1 and GA4, are bioactive in plants [41]. The ent-Kaurenoic acid is converted to GA12 by ent-Kaurenoic acid oxidase (KAO) in the endoplasmic reticulum (Figure 2). Then, GA12 is converted into the bioactive form GA4 by GA 20-oxidase (GA20ox) and GA 3-oxidase (GA3ox), both of which are 2-oxoglutarate-dependent dioxygenases. GA12 is also converted into the precursor GA53 by GA 13-oxidase (GA13ox) [42], which is then converted into the bioactive form GA1 and GA3 by GA20ox and GA3ox (Figure 2).

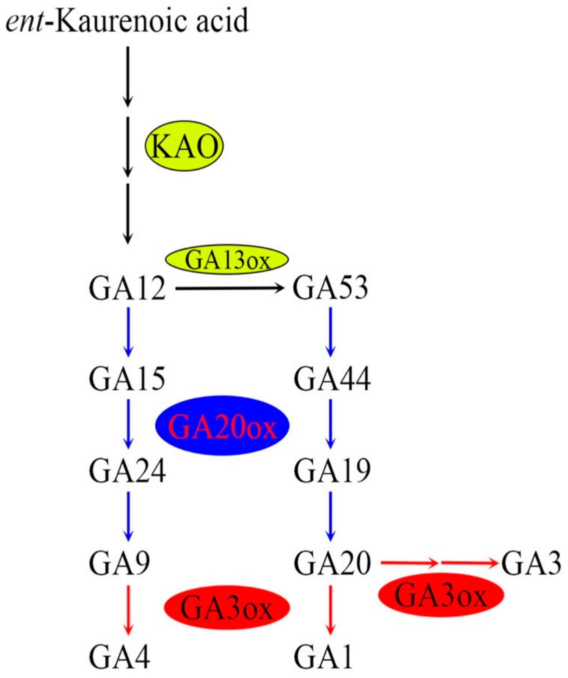

Figure 2. Gibberellin biosynthesis pathway related to gibberellin oxidase. The ent-Kaurenoic acid is converted to GA12 by ent-kaurenoic acid oxidase (KAO) in the endoplasmic reticulum. GA12 is converted into GA53 by GA13ox, and both GA12 and GA53 are converted into GA9 and GA20, respectively, by GA20ox. GA3ox catalyzes the biosynthesis pathway from GA9 and GA20 into bioactive GA1, GA3 and GA4, respectively.

There have been eight identified *OsGA20ox* genes in rice. The differences of *OsGA20ox1* (*GNP1, qEPD2*), *OsGA20ox2* (*SD1*) and *OsGA20ox3* in spatio-temporal expression patterns show that they have different functions [43–45]. The gene *SD1* encodes the key enzyme (GA20ox2) in gibberellin biosynthesis pathways (Figure 2), and is dominantly expressed in the stems with *OsGA20ox1* and *OsGA20ox4* [7–9,46]. GA20oxs can continuously catalyze gibberellin biosynthesis intermediate GA12/GA53 in the cytoplasm to form active gibberellin GA4/GA1 direct synthesis precursor GA9/GA20. *GA20ox2* (*SD1*) is mainly responsible for the synthesis of active gibberellin GA1, the product of C13 hydroxylation pathway, which is mainly expressed in leaves and stems, but only lower expressed in unopened flowers. After the *OsGA20ox2* (*SD1*) mutation, the concentration of GA53 increased while the content of main product GA19, GA20 and active gibberellin GA1 decreased significantly. These results showed that *GA20ox2* (*SD1*) mutation affected the biosynthesis of active gibberellin GA1 and led to rice dwarfing [8]. All the dwarf varieties bred by *sd1* have the deficiency of GA1 and the normal content of GA4. In addition, the cumulative biomass of seedlings in *sd1*-dwarf rice varieties was positively correlated with the content of GA4, but negatively correlated with the content of GA1, which means that *SD1* may be mainly responsible for the synthesis of GA1 in vegetative organs rather than the synthesis of GA4 in reproductive organs [47].

However, in the deep water rice variety C9285, ethylene content accumulated in the plant under deep water conditions, and *SD1* was activated by the transcription factor *OsEIL1a* related to ethylene signal transduction, which enhanced its transcriptional level, promoted the conversion of GA12 to GA9, and then increased the level of GA4, thus promoting rapid elongation of internodes, which made rice escape from anoxic environment and out of the water [25]. In addition, *SD1* was also highly expressed in rice seeds, its mutation can delay seed filling and maturation by affecting the accumulation of ABA and GA3, promote seed dormancy and avoid panicle sprouting [48]. The gibberellin C-20 oxidase gene *OsGA20ox1*, which is homologous to *SD1*, is mainly expressed in floral organs and panicles, but not as significantly in stems and roots. After the overexpression of *OsGA20ox1*, the contents of gibberellin precursor GA53 and GA19 decreased, while the contents of GA20 and active gibberellin GA1 increased compared with WT, resulting in the increase of plant height and the number of grains per panicle and the decrease of fertility [49]. *OsGA20ox1* is also involved in the synthesis of GA1 in the early C13 hydroxylation pathway. During the primary growth stage of rice, the expression of *OsGA20ox1* is higher than that of *OsGA20ox2* (*SD1*), which plays a major role in the synthesis of GA1 in the primary growth stage of rice, while *SD1* mainly regulates plant growth at jointing and heading stage [50]. *OsGA20ox3*, another homologous gene of *SD1*, is expressed in leaves of seedlings, roots, young panicles, anther and pollen, but not in mature vegetative organs [45]. *OsGA20ox3* can supplement the deficiency of *OsGA20ox1* and *OsGA20ox2* in the biosynthesis of GA in flower organs, and *OsGA20ox3* plays an important role in the synthesis of GA4 in reproductive organs. Low temperatures can inhibit the transcription of *OsGA20ox3* and *OsGA3ox1* in reproductive organs, thus reducing the level of endogenous active GA4, and affects the seed setting rate of rice [51].

5.2. Molecular Regulation Mechanism of SD1 in Gibberellin Biosynthesis

Gibberellin is found in almost all organs and tissues of higher plants. Gibberellin is synthesized mainly in immature seeds, young roots and buds. When the active gibberellin in the cytoplasm is synthesized, it is recognized and bound by the gibberellin receptor GIDs (Gibberellin Insensitive Dwarf) in the nucleus. DELLA is a kind of transcriptional regulatory factors located in the nucleus. As a repressor of GA signal transduction, DELLA protein accumulates in plant nuclei at rest and degrades rapidly after exogenous GA treatment. After binding to active GA, GID1 protein can interact with DELLA protein and initiate ubiquitin degradation of DELLA. When the content of GA decreases, DELLA protein accumulates, and feedback regulates gibberellin biosynthesis related enzymes to promote active gibberellin biosynthesis. This feedback regulation mechanism is very important to maintain the level of GA in plants.

In *Arabidopsis thaliana*, *AtGA20ox2* can be bound by the complex formed by DELLA and its interacting transcription factor *GAF1*, thus feedback regulating the level of GA [52]. In rice, when the content of GA in plants decreases, the increase of DELLA protein promotes the transcription of *OsGA20ox2* and accelerates the synthesis of GA [53]. In the semi-dwarf varieties carrying the *sd1* allele, the deficiency of active GA in plants led to the significant accumulation of DELLA protein, which blocked the signal transduction of GA and finally regulated plant growth and development. In addition to being self-regulated by plants, gibberellin C-20 oxidase is also regulated by the environment, such as photoperiod. The mRNA of GA5, a homologous gene of *SD1* in *Arabidopsis thaliana*, was obviously induced by long sunlight [54,55]. The mRNA and protein levels of *OsGA20ox* genes in spinach were induced by long sunlight, and the leaves, petioles and stems of spinach under long sunshine were significantly longer than those under short sunlight [56,57]. Transcription factors always regulated gene expression level, and then effected biological function. Protein contained zinc finger domain(s) were found to play important roles in eukaryotic cell regulating biological processes. ZFP207, a Cys2/His2 zinc finger protein, was reported as a transcriptional repressor of *SD1* expression. *ZFP207*-overexpression (*ZFP207OE*) plants displayed semi-dwarfism phenotype and small grains by modulating cell length [58].

5.3. Molecular Regulated Mechanism of SD1 in Rice Breeding

Semi-dwarf gene *sd1* can increased plant density, harvest index and lodging resistance. Most of cultivars developed by *sd1* showed increased tillers and solved the problems of plant lodging and yield reduction, and achieved a significant increase in rice yield per unit area. Recent studies have reported that *SD1* also has a partner gene, *HTD1*, which is involved in the synthesis of strigolactone and regulates rice tillers. The allele $HTD1^{HZ}$ can effectively increase the number of tillers and yield of rice [59]. $HTD1^{HZ}$ and $SD1^{DGWG}$ ($sd1^{383bp\ del}$) were selected and widely used by breeders at the same time during the green revolution in rice breeding and the subsequent breeding process of modern *indica* rice [59].

A newly identified NGR5 (Nitrogen-Mediated Tiller Growth Response 5) protein reveals its important role in controlling the balance between GA-regulated dwarfism and nitrogen-regulated tilling. The DELLA protein inhibits GID1–NGR5 interaction, thereby protecting NGR5 from degradation and enhancing nitrogen-induced tiller number [60]. However, breeders have also found that the semi-dwarf rice varieties containing *sd1* also showed a weakening of their growth and development response to nitrogen fertilizer, resulting in a decline in rice nitrogen use efficiency, which had to use a large amount of nitrogen fertilizer in order to ensure yield [30].

Data from the Food and Agriculture Organization of the United Nations (FAO, www.fao.org/statistics) show that worldwide nitrogen fertilizer consumption has increased significantly in the past decade, but food production has grown slowly. Excessive application of nitrogen fertilizer will not only increase the production cost of agriculture and decrease the economic benefit, but also causes series of eco-environmental problems such as air and water pollution. Nitrogen fertilizer can also increase ammonium ($NH4^+$) phytotoxicity, growth of *sd1* GA-deficient mutants was more tolerant to $NH4^+$ toxicity than that of their WT counterparts [30]. In semi-dwarf rice varieties, the deficiency of *SD1* will lead to the decrease of gibberellin level in plants, which leads to the high-level accumulation of DELLA protein SLR1 (a transcription factor that negatively regulates GA signal transduction), resulting in weak response to nitrogen fertilizer and decreased nitrogen use efficiency [31]. In addition, the varieties carrying the semi-dwarf gene *sd1* also showed a decrease in photosynthetic rate and carbon assimilation ability.

In order to solve the deficiency of nitrogen absorption and carbon assimilation of *sd1*, Chinese researchers recently compared several varieties carrying semi-dwarf gene *sd1* with Nanjing 6 (NJ6) carrying *SD1*, and screened a new strain NM73, with significantly increased nitrogen uptake rate of *sd1*. Through QTL analysis, a near-isogenic line was constructed and the key gene *GRF4* controlling nitrogen uptake in rice was cloned [31]. The *GRF4* gene encodes a positive regulator of the plant's carbon–nitrogen metabolism, which can promote nitrogen absorption, assimilation and transport pathways, as well as photosynthesis, carbohydrate metabolism and transport, thereby promoting plant growth and development. The protein GRF4 can interact with the DELLA protein to realize the synergistic regulation of photosynthetic carbon fixation in leaves and nitrogen uptake by roots, so as to maintain the balance of plant growth and carbon–nitrogen metabolism. The introduction of the excellent allele of GRF4 into the varieties carrying semi-dwarf gene *sd1* can not only improve its nitrogen use efficiency, but also maintain its excellent characteristics of semi-dwarfing and high yield. This study clarified the reasons for the low efficiency of nitrogen fertilizer utilization associated with dwarf breeding at the molecular level, and put forward a clear solution.

Thus, it can be seen that the potential utilization value of *sd1* in *indica* rice breeding is very considerable, but the application prospect in *japonica* rice breeding is not as clear as that in *indica* rice breeding. It is mentioned that almost all *japonica* rice contains $sd1^{EQ}$, the significant decrease of nucleotide polymorphism near the *SD1* locus in *japonica* rice is an important reason why *sd1* has not been further selected in *japonica* rice breeding [22].

Compared with the *sd1* alleles of *indica* rice, $sd1^{EQ}$ in *japonica* rice belongs to weak functional allele, which is another important reason its utilization effect is not significant in *japonica* rice breeding. The intersubspecific heterosis of *indica* and *japonica* rice is strong, but there are some problems such as semi-sterility, over-parent late maturity and over-parent plant height, which seriously affect the application of *sd1* allele in *indica* used in *japonica* rice breeding. At present, *japonica* rice dwarfing varieties are divided into two categories, one is controlled by a single dwarfing major gene, the other is controlled by multiple dwarfing minor genes, and the major genes controlling dwarfing in *japonica* rice are generally non-allelic. The dwarfism of *japonica* rice mainly comes from Nongken58 and Balilla [14].

6. Prospects of Utilization of *sd1* in Rice Breeding

In the past half-century, the use of *sd1* has greatly increased rice yield and set off the wave of a green revolution in rice breeding. Many alleles of sd1 have been used for decades in rice breeding across many different countries. Even now, *sd1* is still widely introduced into elite rice varieties, demonstrating the utility and importance of *sd1* in rice breeding. Interestingly, it is only one GA biosynthesis gene (*SD1*) mutation that determines the green revolution in rice. Therefore, the control of GA is important in cereal breeding for improved plant architecture.

With the arrival of the era of molecular design breeding, breeding objectives ranges from a single increase in yield to high quality, disease resistance and green health. Therefore, how to tap the new application value of *sd1* is a new challenge for breeders. The yield output potential of the varieties bred by *sd1* tends to be stable, and the response to the increasing nitrogen fertilizer input is weakened. It needs to be solved urgently to reduce the input and increase the output. Through the further study of the functions of *sd1* in nutrient element absorption, biotic stress, abiotic stress and so on, the combination of traditional breeding methods and modern molecular techniques to develop high-quality and multi-resistant semi-dwarf varieties is the direction of breeding in the future.

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