Progress in Molecular Genetic Breeding of Rice Spike Development

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Abstract. Rice (*Oryza sativa* L.) is one of the most important food crops in the world, belonging to the Gramineae family of the basal monocotyledonous group of angiosperms. It has become a model plant for Gramineae and even monocotyledons. Rice flower/spike is the first major element of rice yield composition, and the study of the molecular genetic mechanism of flower/spike development in rice is of great significance in guiding the yield improvement of rice and other gramineous crops. However, there were fewer reviews on rice flower/spike development. This paper summarized the related gene regulation of rice spike development, gene editing, the genetic regulatory network of spike development, and the application of high-yield breeding. This will provide a theoretical basis and technical support for the research on the molecular genetic improvement of rice in flower/spike.

Keywords: Rice flower spike; molecular genetics; gene regulation; gene editing; regulatory network; high-yield breeding.

1. Introduction

The Neolithic Revolution was propelled by the domestication of wild species into cultivated plants [1]. The "domestication syndrome," which includes larger and more frequent fruits or seeds, more robust plants, hardier stems and roots, and more prolonged seed dormancy, has been applied to all crops [2]. Humans have made effective domestication and breeding choices for crops. Following human domestication and breeding selection, crops have significant inflorescence morphological modifications, most often relating to spikelet number, morphology, location, and floret fertility [3]. By enhancing branching and grain number, an elite allele of SOUAMOSA promoter-binding protein-like 14 (OsSPL14), also known as Ideal Plant Architecture 1 (IPA1,) boosts rice yield. By using molecular marker-assisted selection (MAS), the Institute of Genetics and Developmental Biology of the Chinese Academy of Sciences (CAS) created the Jiayou Zhongke cultivar, a novel variety with improved yield performance [4]. In order to create five high-yielding rice varieties, the OsSPL14WFP allele was inserted into superior indica rice cultivars by the International Rice Research Institute (IRRI) [5]. Furthermore, in order to increase the number of florets per spike, Crispr-cas9 mediated mutation (OsDEP1), a crucial regulatory gene for the construction of spike architecture, has now also been exploited in rice breeding [6]. This has aided in the global advancement of high-yielding rice breeding. In this article, the molecular genetic regulatory network of rice spike structure creation and the localization and gene editing of genes relevant to rice spike development are reviewed. The relevance of these findings to high-yield rice breeding is also highlighted.

2. Advances in gene regulation and gene editing controlling spike development in rice

Cultivated rice (Oryza sativa) was domesticated from wild rice (Oryza rufipogon). The main traits of domestication include grain-falling, awn length and presence or absence, heterosis rate, and yield. The genes controlling these traits were *LABA1* and *GAD1* for long awns to short or no awns [7], *sh4* and *SHA1* for very easy to drop grains to not easy to drop grains, *OsLG1* for loose to tight ears, *Rc* and *Rd* for grain color, and genes controlling the traits of grain width (*chr1*), grain mass (*chr6*), and stigma exsertion (*chr8*) [8]. Only a few genes played a role in the long evolution of rice over

thousands and tens of thousands of years, and in the last hundred years or so of rice breeding, the results of the study had tentatively shown that only a few key genes also play an important role.

Transcriptome studies had shown that 357 genes out of 22,000 played different roles in spike development, indicated that there were still many genes that had not yet been identified. Among the genes identified so far, the *Gn1a* (Os01g0197700) gene was the first cloned locus for the primary quantitative trait controlling grain number in rice spikes, which was highly homologous to the *OsCKX2* (*Os01g0197700*) gene for cytokinin oxidase/dehydrogenase. When the expression level of *Gn1a* was reduced, cytokinin (CTK) accumulated in large quantities on the inflorescence meristematic tissues, increased the number of pedicels and grains, whereas the active CTK synthesis-deficient mutant *log1* showed a significant decreased in the number of pedicels as well as grains in the spike [9]. The *DST* (*Os03g0786400*) gene can affect the overall number of pedicels and grains of the rice spike directly through the modulation of the expression of *Gn1a* [10]. In addition, overexpression as well as *cgal*, a GATA transcription factor that interfered with the CTK response, reduced the number of pedicels as well as the number of grains in the rice spike [11].The *GNP1* (*Os03g0856700*) gene encoded a gibberellin 20-oxygenase, which was involved in the signaling regulation of gibberellin(GAs). This gene enhanced CTK activity in rice spike meristematic tissues by regulated the transcriptional feedback loop of the *KNOXs* (*Os07g0129700*) gene, which in turn promoted the transcription of *GNP1* encoding a gibberellin 20-oxidase and inhibited the accumulation of active GA1 and GA3, thus increased the number of spikes [12]. These results also indicated, on the other hand, that the expression of CTK-related genes played a crucial regulatory role in the formation of grain number per spike.

Gene editing allows the study of the interactions function of multiple genes, *hd1* (*Os06g0275000*), *Ghd7* (Os07g0261200) and *GHD8* (*Os08g0174500*) can simultaneously control three traits of rice plant height, number of grains per spike, and tasseling stage, which were the three main effector quantitative trait loci associated with rice tasseling stage photoreceptor. Single *HD1* (knockdown of *Ghd7* and *GHD8*) promoted spike number formation, whereas single *Ghd7* (knockdown of *HD1* and *GHD8*) suppressed spike number formation. The length of sunlight also had an important effect on the expression levels of the above three genes. Under long sunlight, *Hd1* promoted the expression of *Ghd7* and was recruited by *Ghd7* and *GHD8* to form different repressor complexes, resulted in different degrees of delayed spiking or no spiking. While under short sunlight, the expression of *Ghd7* was very low, and *Hd1* showed a competitive relationship with the inhibitory complexes, and the increased of *Hd1* expression could promote tassel twitching and spike grain number formation to different degrees [13-14].*Ghd7* was mainly expressed in young tissues, and its high expression could significantly increase the number of primary peduncles and the number of secondary peduncles, especially the number of secondary peduncles.

It has also been observed that *DEP1* (*Os09g0441900*) promotes cell division and increased the number of branching peduncles and the number of grains per spike, which in turn promotes increased rice yield [15]. In addition, the number of grains per spike was also regulated by genes such as *IPA1* (*Os08g0509600*), *PAY1* (*Os08g0407200*), *APO* (*Os04g0598300*), and *TAW1* (*Os10g0478000*) [16].

3. Genetic regulatory network of rice spike development

In the early 1990s, it was proposed that the development of floral organ characters was mainly regulated by the combination of homozygous genes A, B and C. Class A genes mainly controled calyx characters; class A+B genes jointly determined petal characters; class B+C genes jointly determined stamen characters; class C genes determined carpel characters; class D genes controled ovule development; and class E genes acted in synergy with the BCDE genes to regulate the characteristics of the four rounds of floral organs. In addition, class a genes and class C genes antagonized each other, and when the function of class C genes were lost, class a genes were expressed in the whole floral organ and vice versa. In *Arabidopsis*, all ABCDE genes excepted *AP2* belong to the MADS-box family of genes.

The research on the molecular mechanism of floral organ development was mainly based on the ABCE model for dicotyledonous plants such as *Arabidopsis thaliana*, and the most classical model of floral organ development is the ABCE model, which is as follows: Floral organ development in angiosperms is synergistically controlled by four types of genes, namely, A, B, C, and E, which encode the majority of the MADS-box transcription factors, and the Mads-box proteins encoded by these four types of genes can be homologous or homologous to each other, and can also form a homologous or homologous organ. The mads-box proteins encoded by the four ABCE genes can regulate the development of floral organs in each cycle by forming homo- or heterodimers and then heterotetramers. Mutations in any group of genes result in homozygous heterozygous shifts in flower morphology [17]. The current regulatory mechanisms identified in the gene regulatory network of floral organ features are mainly focused on the chromatin level, transcriptional level and microRNA regulation, while those at the translational level are extremely rare.

A functional genes in *Arabidopsis* mainly determined the characteristics of floral organs in the 1st and 2nd whorls, while suppressed the ectopic expression of C functional genes in the 1st and 2nd whorls. A functional genes in *Arabidopsis* include *AP1* (*APETALA1*) and *AP2* (*APETALA2*), and *AP1* had a two-fold function: it acted as a gene for the characteristics of the floral meristem, and as a gene for the characteristics of the floral organs. B-functional genes in *Arabidopsis* include *AP3* and *PI*, which mainly specialized the characteristic development of the 2nd and 3rd whorls of floral organs (petals and stamens). In mutants of ap3 and pi, petals were transformed into calyx and stamens into carpels.

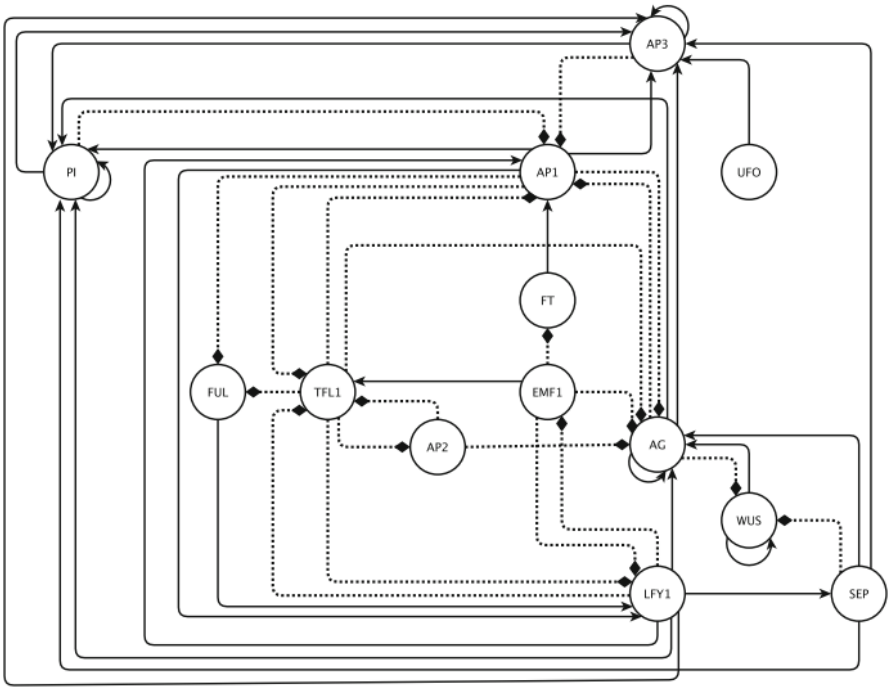

Fig 1. Arabidopsis thaliana flower development gene regulatory network.

All of the genes found to exercise B function belonged to the *DEF*/*GLO* (*AP3*/*PI*) gene family, and the relatively conserved functions of B-function genes in angiosperms were basically confined to the characteristic development of petals and stamens, although some differentiation of their specific functions had occurred.

The main role of C-functional genes was to mainly determine the characteristics of the 3rd and 4th whorls of floral organs (stamens and carpels) and to regulate the definiteness of floral meristems, while repressed the expression of A-functional genes in these two whorls. In angiosperms, C-functional genes havd been divided into three subclasses: *AG*, *PLE*, and *AGL*.11 Although C-functional genes were basically restricted to the reproductive organs, their specific functions had

diverged considerably. D-functional genes were mainly responsible for determining ovule formation and development (Fig. 1).

As early as 1995, two MADS-box genes, *FBP7* (Floral Binding Protein 7) and *FBPI11*, were isolated from Petunia, as well as *AGLl*l/*STK* (*AGAM0US-LIKE11*/*SEEDSTICK*), which were later discovered in *Arabidopsis*, and which were decisive for ovule formation.

In angiosperms, members of the *SEP* (*E*)-like gene family were quite abundant and were involved in the regulation of the development of floral organ characters. First, the SEP gene family was divided into two major subclasses, *AGL9* and *AGL2/3/4*, and then the *AGL2/3/4* subclass of genes was subdivided into smaller groups such as *AGL2/4*, *AGL3*, and *FBP9*. In *Arabidopsis*, there were four E-functional genes, *SEP1/2/3/4*, which had similar expression patterns and regulate the development of floral organ features in combination with ABCD-like genes.

In the monocotyledon rice, the ABCDE model was also partially applicable. The characteristic development of lemma, plasma segments, stamens and pistils in the four whorls of floral organs of rice was also regulated by the combined action of five classes of genes, namely, A, B, C, D, and E. The functions of genes in the classes B, C, and D were relatively conserved, but the functions of genes in the classes A and E seem to be more clearly differentiated.

The rice spikelet consisted of a pair of paraconservative glumes, a pair of guard glumes and a fertile floret, which consisted of an inner lemma, a plastron, a stamen and a pistil from the outside to the inside. Currently, related studies showed that more and more homologous genes had been identified in rice, demonstrated that the ABCE model was partially applicable in rice. However, the homology between palea and sepals was still controversial [18-21]. Therefore, it had been proposed that the same and different factors together determine the specific development of the palea and lemma [22-24]. Among them, the *Arabidopsis* AP1-like genes *OsMADS14* and *OsMADS15/DEGENERATIVE PALEA* (*DEP*) were involved in regulating the specific development of the palea and guardian glume [25]; and the SEP-like gene *OsMADS1*/*LEAFY HULL STERILE* (*LHS1*) regulated the determining attributes of the palea and the lemma development. In addition, B-functional genes in rice included *OsMADS2*, *OsMADS4*, and *OsMADS16*, which regulated the development of plastid and stamen, among which, OsMADS4 and *OsMADS16* were mainly expressed in plastid and stamen primordia. Class C genes in rice include *OsMADS3* and *OsMADS58*, which were homologs of AG genes and regulate carpel and stamen development. *OsMADS3* played a dominant role in stamen and ovule development, whereas *OsMANDS58* was associated with the determinacy of rice floral organs, and may share the same role of regulating carpel development as *OsMADS3*, which were both associated with the *OsMADS6* gene and *OsMADS16* gene interact [26-28]. The *DL* (*DROOPING LEAF*) gene was also present in rice, which encoded a class of YABBY transcription factors and, therefore, was not a MADS-box gene family protein and, unlike the monofunctionality of gynoecium characterization genes in the traditional ABCE model, the *DL* gene regulates gynoecium and lemma expression [29-30].

In recent years, scientists had successively cloned some genes from monocotyledonous plants such as rice that regulated the development of floral organ features. Since the structures of monocotyledonous and dicotyledonous plants were not exactly the same, the *Arabidopsis* ABCDE model was not fully applicable to the regulation of the development of floral organ features in monocotyledonous plants such as rice. It was found that most of the B, C, D, and E functional genes in rice had conserved functions in flower development, but some genes, especially A and E functional genes, had obvious differences in their functions relative to *Arabidopsis*. However, some genes, especially the A and E genes, have different functions compared with Arabidopsis thaliana. The understanding of the ABCE genes in rice and other monocotyledonous plants is not in-depth, and it is urgent to clone and discover new genes for the development of flower organs in rice, so as to lay a foundation for the enrichment of the regulatory network of genes characterizing the development of flower organs in monocotyledonous plants.

4. Progress in the application of genetic improvement of rice flower spike for high-yield breeding

Rice breeding is to breed varieties with new or improved traits. And the essence of breeding is to aggregate favorable genes or a collection of favorable genotypes. Looked back at China's rice breeding for more than a hundred years, every time there was a breakthrough, it was some core genes that play a key role. Looked back at a hundred years of rice breeding, the semi-dwarf gene sd1, the wild septic cytoplasmic male sterility gene *WA352* and the restoration genes *Rf3* and *Rf4*, and the light and temperature-sensitive nuclear sterility gene pms3 or the temperature-sensitive nuclear sterility gene *tms5* had led to three leapfrog breakthroughs in China's rice breeding, respectively--dwarf rice, three-lineage method hybrid rice, and two-line hybrid rice. Meanwhile, the rice blast resistance gene Pi also caused another major breakthrough in Chinese rice breeding.

Rice yield is determined by three important factors: the number of effective spikes, the number of grains per spike, and the quality of 1,000 grains. Among them, the effective number of spikes was determined by the tillering capacity of rice, the number of grains per spike depended on the number of primary and secondary branching pedicels and the fruiting rate, and the quality of thousand grains was influenced by grain size traits [33]. It had been shown that rice grain size-related traits were quantitative traits controlled by multiple genes, and their genetic mechanisms were complex. However, in rice spike development research, increasing the number of grains per spike and promoting the formation of large spikes in rice had been an important goal of high-yield rice breeding. Currently, a large number of studies had been conducted in this area, and significant progress had been made. In 2023, the Rice Research Center of China Agricultural University (CAU) made another important progress in rice yield trait research - they discovered a key gene *COG1* (encoding the transcription factor *OsMADS17*), which can synergistically increase the number of grains per spike and the grain weight, as well as increase the yield of rice. In cultivated rice, a 65-bp deletion of the 5' UTR of *OsMADS17* reduced the translation efficiency of its mRNA, leaded to increase in the number of grains in a spike, grain weight and yield in rice [32].

The innovation team of rice excellent germplasm resource discovery and innovative utilization at the institute of crop science, Chinese Academy of Agricultural Sciences, in March 2023, discovered a new gene locus from wild rice that can increase grain length and grain weight in cultivated rice, revealed a new pathway of grain length regulation in rice, and finely localized it to a gene locus related to grain length, *GL12*. This gene was found in the indica population only in a small number of wild rice genotypes In the indica rice population, there were only a few wild rice genotypes with this gene, the average grain length increased significantly, the grain weight increased, and the increase of grain length and grain weight were positively correlated, and the grain length and grain weight recovered to the parental level after knocked out this gene. Knockouted of this gene increased the grain length and grain weight to the parental level, which can improve the grain length and grain weight of cultivated rice, and revealed a new pathway for the regulation of grain length in rice.

In recent years, the large-spike rice varieties represented by the Yongyou series have been widely used in production, showed high and stable yields, and the representative varieties Yongyou 1540, Yongyou 2640 and Yongyou 12 had more than 300 grains per spike [33-34], much higher than the number of grains per spike of the commonly used high-yield rice varieties in large-area production.

5. Conclusion

With the development of modern molecular biology and RFLP (Restriction fragment length polymorphism), RAPD (Random amplified polymorphic DNA) and SSR (Simple sequence repeats) markers, the number of grains per spike was much higher than the number of grains per spike of commonly used high yielding rice varieties in large scale production. With the progress and development of marker technologies such as RAPD (Random amplified polymorphic DNA), RAPD (Random amplified polymorphic DNA) and SSR (Simple sequence repeats), more and more genes related to grain number and grain weight will be finely localized and cloned, and by using molecular

marker-assisted selective breeding, we will polymerize the genes controlling various resistance and good performances of grain number, grain weight, and grain shape, so we can breed rice varieties of high quality and high yield.

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