

Review and Progress

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Research Status and Prospect of QTL Mapping for Tillers and Other Traits in Forage Sorghum

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Abstract Forage sorghum is an important raw material for winemaking and livestock feed. It has many excellent agronomic traits. Its genome-wide sequencing work has been completed. Molecular marker-assisted selection technology is widely used in sorghum breeding work. The number of genetic loci affecting the sorghum agronomic traits has been being positioned. Tillering, as an important plant type trait, has an important influence on the physiological characteristics of sorghum, such as the tolerance, lodging resistance and light absorption efficiency. At present, considerable progress has been made in gene mapping of plant height, ear length, leaf morphology and other traits of forage sorghum. However, the progress of gene mapping for tillering is relatively slow due to the fact that tillering traits are easily affected by various factors. This study summarized the recent research progress of QTL mapping for tillering traits and other agronomic traits in sorghum, and proposed that some conserved sequences with high homology were retained during the evolution of different crop varieties, which proved that there was correlation between different traits of the same species. In sorghum breeding research, combining genetic engineering breeding with experimental statistics and quantitative genetics can better reveal the contribution of various factors to sorghum agronomic traits, improve breeding efficiency and reduce the loss of human and material resources. **Keywords** Forage sorghum; Tillering; QTL mapping; Genetic trait correlation

Sorghum (*Sorghum bicolor* (L.) Moench) is an important food crop in Asian and African countries. It is grown in large areas in Asia and Africa and has high edible and nutritional value (Kotla et al., 2019). Sorghum is the fifth largest food crop in the world, and the research on its breeding is very important. Sorghum can be divided into feed sorghum and grain sorghum according to its uses (Cui et al., 2012). Forage sorghum can be divided into different types such as grain type, forage type, and sweet sorghum (Ma, 2017, Modern Animal Husbandry Technology, (7): 67). The development of forage sorghum has made a great contribution to the breeding industry (Xu et al., 2006, Coarse Crops, (2): 152-153).

Forage sorghum has many excellent characteristics such as high-quality drought resistance and salt-alkali tolerance, good stress resistance, and strong regenerability. Its nutrient content, protein, fat and biological yield are similar to corn, and it has strong palatability (Duan et al., 2016). Some varieties have high sugar content in stalks and good feeding properties. They have become an excellent forage crop. They play an important role in some large-scale pastures abroad and play a very important role in the adjustment of agricultural industry and the improvement of animal husbandry. Potential (Luo et al., 2013, Tianjin Agricultural Sciences, 19(2): 6-8). As a comprehensive manifestation of morphological structure and physiological efficacy, plant type has an extremely important impact on yield (Wang et al., 2015). In the current sorghum production, in order to adapt to mechanized harvesting, the concept of perfect plant type breeding is deeply rooted in the hearts of the people. Ideal plant type breeding is the most ideal plant type development that is conducive to plant growth and development, photosynthesis, and biological yield, which maximizes the utilization of light energy and maximizes biological yield (Li et al., 2018). Sorghum has high edible value, and its grains are rich in starch, protein, fat, tannins, trace elements and dietary fiber (Kotla et al., 2019). As the biological content and proportion of sorghum grains are different from other gramineous crops,



sorghum has unique nutritional value and is a typical coarse grain (Xiao et al., 2019). The application of feed sorghum in raising livestock can effectively reduce the use of traditional feed corn and sorghum and save costs.

Tillering is an extremely important trait of forage sorghum, which determines the yield and quality of sorghum. Tillering is affected by the interaction of genetic environment and it is difficult to make a clear explanation at the molecular level. At present, the research on forage sorghum tillering is still in the exploratory stage. There is no clear understanding of the physiological characteristics of sorghum tillers. It is difficult to control the number of forage sorghum tillers in the actual production process. There is a strong randomness. Agronomic traits that are easy to locate, such as plant height and ear length, have made great progress, but the correlation between different agronomic traits has not been well explained. Root traits are difficult to investigate due to their field traits. Research on QTL mapping of roots is relatively rare. At present, there are many limitations in the QTL mapping of forage sorghum tillering traits, and the overall location is still in the primary mapping stage, and the environmental error has a greater impact in the experimental process. In addition, in traditional QTL mapping research, there are problems of singularity, restriction, low contribution rate of gene mapping, and insufficient innovation. Therefore, this study considers the research progress of forage sorghum tillering and summarizes the gene mapping of other agronomic traits, laying the foundation for the future research progress of forage sorghum.

1 Physiological Characteristics of Forage Sorghum Tillering Traits and Other Agronomic Traits

Tillering is an important feature of crops adapting to environmental conditions, and is the product of interaction between environment and genes. Different fertility factors will affect the development of leaf area, which in turn affects photosynthetic efficiency and ultimately yield. Depending on the plant density, fertile tillers may account for 60% of the total plant leaf area and 5% to 80% of the grain yield. Generally, it is believed that high tiller genotypes tend to have an environment with sufficient water and high resource availability, because the increase in tillers increases the number of grains per unit area, which leads to the maximum conversion of available resources to grains. On the contrary, in an environment with limited water, grain yield is closely related to the water supply after flowering, and the low-divided type is more suitable for such an environment, because its smaller canopy will reduce the water usage before flowering (Jahanzad et al., 2013). Therefore, the optimization of tillers is essential for breeders who aim to match their genotype to their target environment.

Tillering is a very complex trait, controlled by a variety of environmental and physiological factors, including hormone regulation and assimilation competition. Increasing the length and width of the leaves, thereby increasing the leaf area, will increase the vitality of the main stem, and affect the carbon supply and demand balance of the plant, thereby affecting tillering. The resource competition in plants affects the genetic regulation of tillers. QTL analysis is a common method to explain the genetic basis of complex trait variation. The understanding of the plant quantitative genetic development system is a gradual process. It was first thought that QTL was controlled solely by minor polygenes, and later it was discovered that some plant quantitative traits were also controlled by major genes; a large number of studies found that the gene ability of controlling traits was different, and they were divided into major genes and minor polygenes. The main polygene mixed inheritance proposed by Gai et al. (2000) is a universal model for quantitative gene mapping. Taking simple main genes or polygenes as an example, a set of QTL genetic model analysis methods developed on the basis of predecessors. Extension of Mendelian general genetics research methods to general models of quantitative traits.

The agronomic traits of sorghum are the key factors to determine the ideal plant type breeding. Studies on agronomic traits such as plant height, ear length, panicle stalk length, stem diameter, leaf scattering and tiller number have greatly promoted the development of Sorghum Breeding (Zhang et al., 2018). In the process of sorghum production, there is a great correlation between various agronomic traits, such as flag leaf sheath coating can enhance panicle stalk strength (Sun et al., 1998); panicle length has a negative correlation with yield per plant; longer panicle will lengthen plant flowering period (Bai et al., 2019); photosynthesis plays a significant role in Sorghum filling period



(Sun and Ma, 2000). Perfect plant type breeding pursues good agronomic traits, dwarf plant type breeding to enhance plant lodging resistance; longer panicle length, more tillers to increase economic yield; reasonable number of tillers can effectively increase photosynthesis and lodging resistance; reasonable flowering period and leaf chlorophyll content of leaves are beneficial to the improvement of drought resistance (Ni et al., 2012). The leaf morphology of sorghum has a great influence on photosynthesis, such as the height of the last two leaves, the height of the last three leaves, the length of flag leaf and the area of flag leaf. There is a correlation between the number of tillers and the stem strength of sorghum. At present, it has been studied that removing the tillers of Sweet Sorghum at heading stage will increase the stem strength (Zhao et al., 2012). There are many varieties of sorghum agronomic traits. Tillering plays an important role in increasing crop yield, improving plant density tolerance and stem yield, and panicles per unit area (Shao et al., 2017).

The crossbreeding between parents who are far apart is more vigorous than between parents who are closer. Information about genetic diversity also allows parents to be classified into hybrid groups, adding a new dimension to cross-breeding methods. In the past, indirect estimation of genetic diversity based on morphological information and lineage has been widely used in many species, including sorghum. Morphological characteristics are believed to help identify hybrid patterns of combinations between hybrid breeding parents. In fact, breeders usually select parents by combining morphological traits and pedigree information that are easy to observe and inherit the desired traits. Because the interaction between genetic environment and the genetic control of polygenic genetic morphology and agronomic traits are still unclear, morphological variation cannot reliably reflect actual genetic variation.

The agronomic traits of sorghum are under the coordinated control of genetics and environment. Genetic markers are used to identify QTLs related to target traits and the environment, to monitor the recombination of progeny and favorable alleles formed by progeny crossing. Therefore, the selection of parents is not guided by the goal of maximizing the number of QTLs detected, but by the good interoperability of the parents, that is, crossing. The purpose is to concentrate on the genetic material to isolate QTL useful to breeders. Genetic marker-assisted selection improves the superiority of breeding selection and is used to guide variety selection and improvement (Guindo et al., 2019).

2 Application of QTL Mapping in Sorghum Breeding

Since the 1990s, the knowledge of sorghum genetics has made significant progress. A variety of genetic studies have been conducted on quality traits and important agronomic traits of grains by drawing population and genetic maps. With the development of molecular genetic technology, people use various types of molecular markers and molecular markers of DNA markers to evaluate genetic diversity. These have proven to be powerful tools for evaluating multiple genetic variations, including sorghum. Molecular markers can reveal genetic differences between plants at the DNA level, eliminate environmental impacts, and effectively evaluate the genetic diversity of germplasm in breeding programs (Zhu et al., 2018).

2.1 Molecular markers based on DNA molecular hybridization

Genetic markers reflect the characteristics of genetic diversity and are the general term for morphological, cellular, and molecular markers used in genetic analysis. In classical genetics, genetic diversity is the difference between alleles; now genetics extends it to the difference at any locus in the genome. Contains two basic characteristics: heritability and recognizability. General molecular markers include five types: Morphological marker, Cytological marker, Biochemical marker, Immune genetic markers, and Molecular marker (You, 2013).Determining the location of various genes on a bunch of homologous chromosomes by drawing genetic markers is the basis for the isolation and identification of target genes (Ren et al., 2019). DNA molecular markers are genetic markers developed based on morphological markers, cytological markers, and biochemical markers, which are based on the close linkage of DNA polymorphisms and traits. They are the true reflection of genetic variation on traits. Its advantage lies in the location of the entire genome, rich genetic markers, and due to the codominance of most molecular markers, the identification of homozygous and heterozygous genotypes can provide more complete genetic information. DNA molecular



markers are widely used in biology, agronomy and medicine. Among DNA molecular markers, SSR markers are widely used in current breeding research because of their high polymorphism, general requirements for DNA quality and low quantity, and simple operation. In recent years, the third-generation marker SNP has a wide range of application prospects due to its abundant quantity and good genetic stability, combined with large-scale and efficient detection of DNA chip technology (Zhang, 2008). Near-allelic lines make other traits the same, only the target gene shows different traits, which is convenient for screening the target traits to be marked. The marker band type is consistent with near-isogenic traits. This marker can be applied to assisted selection.

2.2 QTL location statistical analysis method

The analysis of QTL location results often uses statistical model estimation methods. Since the 1980s, there have been more than 20 methods of mapping statistical methods. The following are commonly used mapping statistical methods.

2.2.1 Single label analysis

As the earliest QTL mapping method, two markers are used in a single interval to detect QTL, and the difference of a single quantitative trait gene marker is compared by using methods such as analysis of variance. If the difference is significant, it means that the QTL controlling the quantitative trait is linked to the marker (Li and Zheng, 1998).

2.2.2 Interval mapping method

Due to the shortcomings of the single-marker mapping method, Lander and Botstein proposed the interval mapping method in 1989. It is based on the genetic assumption that phenotypic variation is controlled by genetic effects and residual errors, and there is no interaction between genetic environment. The principle is to use a complete molecular marker genetic map to establish a linear regression relationship between the observed values of individual quantitative traits and the indicator variables of the two-sided marker genotype, so as to separate the recombination rate and QTL effect in the test statistics, and the genetic sequence is adjacent The molecular markers are analyzed one by one according to a certain genetic distance.

2.2.3 Composite Interval Mapping Method (CIM)

CIM is a QTL compound interval mapping method proposed in 1994 by combining interval mapping method and multiple regression characteristics, that is, "compound interval mapping method". It introduces markers to introduce marker covariates to eliminate the influence of markers outside the current interval, avoids the influence of the current detection interval in simple interval mapping from the QTL of other intervals, and eliminates the "phantom QTL". At the same time, compound interval mapping is suitable for the situation where there is more than one QTL on a single chromosome, which greatly improves the accuracy of mapping compared to simple interval mapping. The essence is to use some or all of the remaining markers outside the checked interval to eliminate the influence of other QTLs on the checked interval.

2.2.4 Compound interval mapping method based on mixed linear model (MCIM)

This method is a method proposed by Zhu Jun in 1999 that includes additive effects, dominant effects and interaction effects with the environment. This method takes the population mean and the main genetic effects of QTL as fixed effects, and takes environmental effects, QTL locus-environmental interaction effects, and molecular marker effects as random effects. By combining effect estimation and positioning analysis, multi-environmental Joint QTL mapping analysis under. It can overcome the failure of the above several QTL mapping methods to correctly analyze the interaction between genotype and environment and various genetic effects (Kao and Zeng, 1997).

3 Research Progress of Forage Sorghum Tillering and Other Traits

3.1 Analysis of tillering traits of feed sorghum

Tillering is an important agronomic trait of forage sorghum. The number of effective tillers is closely related to the yield of sorghum. Increasing the number of tillers of forage sorghum can effectively increase the production of grains and stalks. Sorghum tillering is controlled by genes and environment, and is a quantitative trait controlled by



multiple genes with minor effects. Environmental factors such as planting density, soil nutrients, light, and temperature all affect the number of tillers. It is difficult to reduce the influence of environment on tillers in the experiment, and the progress of tiller positioning is relatively slow. Hart et al. (2001) obtained 7 QTLs influencing tiller number through the F6~8 generation population study, located on chromosomes 1, 6, and 8; Shehzad et al. (2009) analyzed representative samples from Asia and Africa The sorghum cultivars conducted a mapping study and found that 7 QTLs controlling tillering were located on chromosomes 1, 2, 7, and 10. Feltus et al. (2006) used F6~F8 generation populations and detected 9 QTLs related to tillering; Shirin-gani et al. (2010) used the F6 generation recombinant inbred line of the hybrid combination of SS79 and M71 to detect 6 QTLs with a contribution rate of 6.7% to 15.7%, located on chromosomes 1, 2, 5, 6, and 10 on. Wang et al. (2019) used BSA and SLAF technology to map sorghum tiller and main stem plant height consistency genes and found that 4 SNP sites are located in the correlation region of chromosome 9 with a length of 1.95 Mb. These 4 SNP sites the dots correspond to 3 genes, which may be functional genes directly related to the trait. The research on sorghum tillering can be considered from the environmental and gene contribution rate to tillering traits. The comparison of the tillers of different lines in the environment is carried out, and the agronomic traits of different tillers of the same line are compared. At the molecular level, QTL mapping can only be presumed to be related to the corresponding hybridization and environment, and the QTL positions can be compared under different genetic backgrounds and environmental conditions. For example, a comparative study of QTLs for height and maturity of gramineous plants revealed that there are corresponding QTLs among sorghum, rice and maize.

3.2 Research progress on other agronomic traits of forage sorghum

With the completion of the whole genome sequencing of sorghum, certain progress has been made in its QTL mapping in yield traits, plant type traits, growth period traits, quality traits, and stress resistance, which provides a scientific explanation for the growth and development of sorghum. From the publication of the first article on QTL mapping of sorghum in 1995 to today, there have been more than 40 articles on QTL mapping of sorghum, mapping nearly 200 quantitative traits in 32 mapping groups (Mace and Jordan, 2011).

In terms of grain morphology, using the BTx623/IS3620C RIL population, six QTLs that control the morphology of sorghum glumes were detected, located on chromosomes 1, 2, 3, 6, and 7 (Feltus et al., 2006); Murray et al. (2008) Using the BTx623/RIO population, 2 QTLs for controlling grain protein, 1 QTL for controlling grain water content, 2 QTLs for controlling fat content and 1 QTL for controlling starch content were mapped; QTLs related to sorghum ear side On the other hand, the predecessors used the CK60/PI229828 F2 generation population and used the genetic linkage map of 111 RFLP loci to map 6 QTLs for ear length and 2 QTLs for ear type (Pereira and Lee, 1995). Rami et al. (1998) mapped 13 QTLs related to ear length, and Klein et al. (2001) mapped 6 QTLs related to ear stalk length. In the research on sorghum roots, Mace et al. (2012) identified 4 QTLs related to root meristem angle, 3 QTLs related to plant area, 2 OTLs related to stem weight and 4 OTLs related to root dry weight Related OTL: In terms of disease resistance, sorghum head smut is widespread and seriously affects the yield of sorghum. The resistance to sorghum head smut is controlled by major genes and minor polygenes. Li et al. (2008) used the segregating population grouping analysis method to analyze two segregating populations using SSR markers to determine 1 QTL; Zou et al. (2010) used different materials to locate resistance genes on chromosome B. Fine mapping and cloning of chromosome B. Sorghum is susceptible to aphids and attracts flies. Aruna et al. (2011) used the 27B/IS2122 combination of RIL populations to identify 25 QTLs related to flies; in terms of drought resistance, keeping green after flowering is an important indicator of drought resistance, Kassahun et al. (2010) identified 4 QTLs related to the green pass. The results of QTL mapping research on other traits of sorghum are as follows (Table 1).



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Group	Trait	Number of QTL	Chromosome	Contribution ratio
CK60/PI229828	Hundred-grain weight	3	1, 9, 10	9%~11%
BTx623/IS3620C		3	4, 8, 9	12.3%~18.4%
Red Kafir/Takakibi		9	1, 2, 3, 4, 5, 7	<15.7%
296B/IS18551 BTx623/IS3620C RIL	Grain weight	3	1, 4, 6	7%~14.8%
E36-1/SPV570		11	Except of 5	
		3	2, 3, 4	<7.82%
IS2807/379 RIL	Germination rate	3	1, 7, 10	13.9%~32%
KS115/Macia	Carotenoid content	16	1, 7, 10	7.13%~25.4%
296B/IS18551	Number of blades	3	1, 3, 7	7.8%~14.1%
S. bicolor/S. propinquum	Plant height	5	1, 3, 6, 7, 9	
IS8525/R931945-2-2		4	1, 7, 9	>10%
SC56/Tx7000		2	1, 6	12.4%~24.2%
T70/P607 RIL		6	2, 3, 5	1%~10%
IS8525/R931945-2-2	Flag leaf height	3	1, 1, 7	6.25%~14.23%
SC56/Tx7000	Lodging	3	1, 5, 6	15%
R9188/r9403463-2-1	Total dry matter	2	1, 6	<15%
RTx7078/B35 RIL	Drought resistance	6		
B35/Tx7078 RIL		6	2, 3, 7	
SC56/Tx7000 RIL		4	2, 3, 4	11.9%~37.7%
Tx7078/B35 RIL	Green trait	7		53%
B35/Tx430 RIL		7		42%, 25%
Sureno/RTx430 RIL	Resistant to granulosis	5	2, 3, 4, 5	10%~24%
IS2807/379 RIL	Anti-grain mold	4	1, 2	13.9%~28.3%
IS8525/R931945-2-2	Spike length	3	2, 6	5.91%~6.85%
R9188/r9403463-2-1	Fructose content	2	6, 7	<15%
R9188/r9403463-2-1	Sucrose content	4	1, 3, 5, 6	<15%
R9188/r9403463-2-1	Total sugar content	4	1, 3, 5, 6	<15%
296B/IS18551 RIL	Resistance to leaf blight	17	3, 4, 6	6.9%~44.9%
BTx623/IS3620c	Blade angle	3	1, 6, 7	<45.3%
BTx623/IS3620c	Inflorescence length	4	1, 3, 7	10.6%~13.8%
M71/SS79	Fresh weight	7	Except of 3, 9, 10	<12.9%
B35/Tx430	Days from germination t	to2	1, 10	20.3%, 47.5%
	flowering			

Table 1 Progress of QTL mapping for some sorghum traits

In recent years, sorghum whole genome sequencing and high-density genetic linkage maps covering the whole genome have been completed, and effective sites have been mapped for plant type traits, yield traits, quality traits and resistance traits of sorghum. The quantitative traits located are distributed on each chromosome of sorghum, and the gene loci in the table are analyzed by statistical software (Spss 22) (Table 2). According to the analysis of statistical software, the located QTL loci are widely distributed on chromosomes No. 1, No. 3, No. 6 and No. 7, and contain numerous agronomic traits, which provide a reference for future fine mapping and gene cloning.



Chromosomal location	Total occurrence times of QTL sites	Percentage of chromosome occurrence
1.00	19	22.9
2.00	8	9.6
3.00	12	14.5
4.00	7	8.4
5.00	6	7.2
6.00	11	13.3
7.00	11	13.3
3.00	1	1.2
9.00	4	4.8
10.00	4	4.8
Total	83	100.0

Table 2 QTL distribution frequency on chromosome

4 Discussion and Prospect of QTL Mapping for Agronomic Traits of Forage Sorghum

At present, the research of sorghum breeding has made great progress. Sorghum has obtained whole genome sequencing. Sorghum has become a model plant for breeding work because of its simple molecular sequence and high level of crop resistance. In the genetic mapping of agronomic traits of sorghum, morphological characteristics and molecular markers are used to analyze genetic diversity. In the current breeding research of sorghum, forage sorghum and grain sorghum have been studied extensively, and the research on ear traits and plant type traits is perfect and mature. Tiller research that has a greater impact on plant type traits is less taught because it is affected by genes and the environment. Interaction adjustment is more sensitive to the environment and the research is more complicated. Stem research focuses on the determination of various substances, such as the measurement of sugar content. Stem is a typical green energy source. Good research is of great significance to future environmental crises.

Efficient breeding research is a combination of molecular breeding and traditional breeding; sorghum, as a self-pollinated crop, has lower genome sequence polymorphism than maize genome, and its genome linkage disequilibrium range is much larger than that of maize (Wang, 2015). To reduce the impact of environmental factors on agronomic traits of sorghum, selection can be made in the early generations of breeding; while traits with high heritability can be selected in the high generation (Li et al., 2018). For the study of molecular markers of various shapes of sorghum, SSR molecular markers for F2 generation populations are often used. The limitation is often that the population is not stable enough, the contribution rate of the marked genes is low, and the marker population is not large enough due to the influence of workload. The SSR marker coverage is not large enough, but some genes are not marked. The stable RIL group is less used because of its long formation period and complicated process. The current research on various traits is relatively single, the marked gene verification work is lacking, the fine positioning work cycle is long, and the work is complicated and cannot be further verified, and the contribution rate to the actual sorghum breeding work is low. For sorghum QTL trait mapping research, different traits can be combined to analyze the relationship between different shapes; different crops should also be related to each other. Data research shows that 1/3 of the sorghum genome sequence is a conservative sequence, and its gene sequencing and gene distribution density are similar to those of rice genome. Sorghum and rice genomes have almost the same amount of euchromatin. Therefore, the research on rice can be linked with sorghum. In the process of crop domestication, the existence of the same characteristics in the crops is beneficial to human development. The phenotypes are also similar (Liu, 2016). In previous studies, Lin et al. (2012) cloned the SH1 gene from sorghum. The homologous gene of SH1 can also control the shatter of rice and maize. In addition, there are many other genes and their homologous genes involved in the evolution of crops. Increasing the differences in sample populations makes the sorghum breeding QTL mapping research more accurate, and the same population can be planted in a very different environment. Currently, with the widespread use of molecular technology, the genetic mechanism of sorghum is gradually being uncovered (Lu et al., 2007).



The tiller research of forage sorghum is still in a relatively vague stage. The environmental and genetic factors that affect the tiller of forage sorghum have yet to be thoroughly explored. Molecular marker technology also needs to be further refined in experiments to study the genetics of sorghum. Explain the dominant effect, additive effect, and epistatic effect of different genes. The research on forage sorghum tillering and other agronomic traits should not be limited to the statistics of field phenotypic traits and the exploration of gene location, and the ways of influencing the forage sorghum tillering and other agronomic traits should be carried out from the perspective of metabolomics and proteomics. Explore. The physiological regulation of plants affects the growth and development of sorghum. Tillering is a trait that is greatly affected by the environment, and its physiological characteristics should be revealed. At present, different forage sorghum varieties should be screened to determine the changes in the number of tillers in different physiological periods, and to further reveal the distribution of nutrients between the main stem and the tillers, so as to realize the phenotypic changes, physiological changes, and gene regulation of sorghum. The explanation of the mechanism of tillering traits prepares for the future field production to be able to artificially control the number of tillers, and ultimately maximize production efficiency.

Authors' contributions

Kang Chen is the main writer of the review, completing the collection and analysis of relevant literature and writing the first draft of the paper; Luo Feng participates in the analysis and sorting of the literature and data; Sun Shoujun is the project designer and person in charge, guiding the writing of the paper. All authors read and approved the final manuscript.

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