

# Research Progress on Application of Molecular Markers in Breeding of *Camellia oleifera*

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**Abstract** *Camellia oleifera* is an important woody oil tree species unique to China. It is known as the world's four major woody oil crops along with olive, oil palm and coconut. It is known as the 'king of oil' because of its high oil content. With the increase of people's attention to the yield of *Camellia oleifera*, its high yield has become the focus. In traditional breeding model, judgment is performed by phenotypic traits, but this method is single and easily affected by the environment, and can no longer meet the demand. In contrast, molecular marker breeding is not affected by the environment, and is stable and efficient and capable of accurately mapping target genes, so it has attracted much attention. In this paper, the research progress on *C. oleifera* germplasm resources diversity, DNA fingerprinting construction, genetic linkage map construction and QTL mapping was summarized, and the application of SSR molecular marker technique combined with association analysis in *C. oleifera* breeding in recent years was discussed, in order to provide new ideas for high-yield breeding of *C. oleifera*.

**Key words** *Camellia oleifera*; Diversity; SSR molecular marker technology; Correlation analysis

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*Camellia oleifera*, as one of woody oil tree species, has an ideal healthy proportion of fatty acids and unique quality components, such as squalene, tea polyphenols and camelliaside, and it thus shows rich nutritional effects. Camellia seed oil is listed as a kind of healthy edible oil promoted by FAO, and *C. oleifera* is as famous as olive, oil palm and coconut<sup>[1]</sup>. The content of unsaturated fatty acids in camellia seed oil is as high as 90%, which can effectively prevent cardiovascular diseases such as hypertension, hyperlipidemia and arteriosclerosis<sup>[2]</sup>. Camellia seed oil also contains many bioactive substances, such as theasaponin, tea polyphenols and VE, and has the functions of sterilization, itching relieving, skin metabolism promotion and immunity improvement, and it can be widely used in blood pressure reduction, body fat reduction and agricultural production<sup>[3-5]</sup>. Meanwhile, *C. oleifera* shell can also be used to make high-quality activated carbon and electrodes<sup>[6]</sup>. In recent years, with the improvement of people's understanding of the value of *C. oleifera*, China's support for *C. oleifera* industry has been continuously enhanced, making *C. oleifera* industry flourish<sup>[7]</sup>. By 2019, the planting area of *C. oleifera* in China has reached 4.533 million hm<sup>2</sup>, and it continues to grow at a rate of 125 000 hm<sup>2</sup> per year<sup>[8]</sup>. At present, the annual output of camellia seed oil in China can reach 627 000 t, with an annual output value exceeding 116 billion, which plays an important role in forestry industry and economy in hilly and mountainous areas<sup>[9]</sup>. However, with the increase of *C. oleifera* planting area, some problems that cannot be ignored have also

appeared. Although the planting area has been expanded, there are differences in output among different regions, and the varieties in the market are mixed. Therefore, finding high-yield and high-quality *C. oleifera* varieties has become one of the urgent problems for scholars to solve. Although the traditional breeding technique has cultivated a number of varieties with excellent characters, the number is limited and the cultivation period is long. As a result, it is easily affected by external environment and other factors and has fallen into a bottleneck period<sup>[10]</sup>. Therefore, it is urgent to find new breeding methods. Molecular marker technique has the advantages of being unaffected by the environment, rapid response, and high accuracy, reliability, stability and efficiency, and can be considered as an important research direction of *C. oleifera* breeding<sup>[11]</sup>. These factors together constitute a comprehensive understanding of the development of *C. oleifera* industry, and also suggest the development direction and challenges of *C. oleifera* industry in the future.

## Comparison of Common Molecular Markers

With the rapid development of molecular biology, there is an urgent need to cultivate new varieties with high oil content, high quality and strong adaptability in *C. oleifera* breeding<sup>[12]</sup>. Compared with traditional methods, molecular breeding can significantly shorten the breeding cycle, obtain high-yield and high-quality varieties with strong adaptability, and achieve more accurate and efficient breeding goals. Therefore, molecular marker technique has become an indispensable key tool. Molecular markers are detectable genetic DNA sequences or proteins, which reflect the differences in the genomes of individuals or populations. Through DNA molecular marker technique, scholars can analyze the diversity of genetic materials, diagnose the rules of gene arrangement and external traits, and compare the composition of genomic DNA of different varieties, so as to realize variety

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identification<sup>[13]</sup>. Commonly used molecular marker techniques include random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), sequence-related amplified polymorphism (SRAP), microsatellite (SSR), and simple repetitive sequence marker (ISSR)<sup>[14]</sup>. These techniques provide researchers with rich tools to better understand the genetic variation and genome characteristics among *C. oleifera* varieties and guide the breeding of better *C. oleifera* varieties.

### Restriction fragment length polymorphism (RFLP)

Restriction fragment length polymorphism (RFLP) is a technique that uses restriction endonucleases to identify specific nucleotide sequences and cut DNA. When enzyme recognition sequences have point mutation and deletion, insertion or inversion of some DNA fragments, the DNA fragments produced by cleavage change, showing polymorphism among different varieties<sup>[15]</sup>. Restriction endonucleases produced by different varieties have different lengths, quantities and sizes, so they can be used to distinguish varieties. Since 1980s, RFLP technique has been widely used in variety identification, and it is considered as the earliest molecular marker technique for this purpose. However, although RFLP can detect the allelism of loci, it has large demand for DNA, high cost, and cumbersome and time-consuming operation, which limit its popularization and application<sup>[15]</sup>. In addition, the detection process of RFLP requires the use of radioactive isotopes, which has potential hazards to operators and the environment, further limiting its application scope. Therefore, although RFLP technique is of great significance in variety identification, its shortcomings limit its wide popularization in practical application.

### Random amplified polymorphic DNA (RAPD)

Random amplified polymorphic DNA (RAPD) is a technique for PCR amplification of target genomic DNA by using one or more synthetic random primers (usually 10 bases) to produce discontinuous DNA products. Through electrophoretic separation and staining, due to the differences in the length of amplified regions of primers in genomic DNA of different species, polymorphism is observed<sup>[16]</sup>. The advantage of this technique is that fingerprints can be constructed and genetic diversity can be analyzed without any molecular research foundation, and meanwhile, a set of primers can be used for the analysis of multiple genomes, with a low requirement for purity. However, RAPD technique also has some shortcomings. First of all, it can't distinguish homozygotes from heterozygotes, which leads to poor repeatability of results<sup>[17]</sup>. Secondly, the stability of amplification results is quite different, and the polymorphism level is relatively low. These factors need to be considered in practical application, especially for research that requires high accuracy and repeatability.

### Amplified fragment length polymorphism (AFLP)

Amplified fragment length polymorphism (AFLP) was founded by Dutch scientists Zabeau and Vos in 1995<sup>[18]</sup>. This technique combines RFLP technique with PCR technique, which has both the reliability of the former and the efficiency of the latter. The operation steps of AFLP technique include using two kinds of

restriction endonucleases, namely rare cleaving enzyme and common cleaving enzyme, to cleave genomic DNA. Next, according to the nucleotide sequences of used linker and restriction site, primers are set, and the double-stranded linker is connected to the end of DNA fragment, followed by specific PCR amplification, and finally the amplified fragment is separated. AFLP technique combines the advantages of RFLP and RAPD, and has the advantages of high polymorphism level, fast molecular recognition, fast reaction speed, more detection sites and less DNA consumption. However, this technique also has some challenges and limitations. Firstly, AFLP requires high DNA purity and endonuclease quality. Secondly, the operating cost is high and the technical requirements are relatively high. Therefore, AFLP technique faces certain difficulties in popularization and application. In specific research and application, it is necessary to weigh and choose its unique advantages and limitations, in order to achieve the best experimental results.

### Inter-simple sequence repeat (ISSR)

Inter-simple sequence repeat (ISSR), also known as ISSR technique, makes use of the characteristics of simple sequence repeat (SSR) widely existing in genomic DNA. This technique carries out PCR amplification on a sequence with reverse SSR by setting a pair of specific primers at two ends, thus resulting in polymorphism of amplified fragments caused by different numbers of simple sequence repeat units<sup>[19]</sup>. Compared with other molecular marker techniques, ISSR technique has more polymorphism. It can cover the whole genome and has the characteristics of extremely rich quantity and high information content. The experimental process is relatively simple and the results are reproducible, and the requirements for the quantity and quality of DNA are low, so the operation is convenient. Because of its advantages, ISSR technique is currently one of the most widely used molecular markers.

## Application of Molecular Markers in *C. oleifera*

### Diversity analysis of *C. oleifera*

With the attention paid to woody oil tree species of *C. oleifera*, it is a key work to study its genetic diversity. It has been difficult for traditional breeding methods to meet the demand for high-yield and high-quality *C. oleifera* varieties, so molecular breeding techniques came into being, which provides the possibility for breeding better varieties. Under this background, the analysis of genetic diversity of *C. oleifera* not only helps to make better use of germplasm resources, but also provides theoretical support for variety breeding and improvement.

The research on genetic diversity of *C. oleifera* in various regions shows its rich characteristics. For example, Zou *et al.*<sup>[20]</sup> studied excellent clones of *C. oleifera* in Youxian County, and found the correlation between biological characteristics and molecular markers, showing a certain genetic diversity. Xie *et al.*<sup>[21]</sup> analyzed the population of *C. oleifera* in Guangning, Guangdong Province, and showed that it had high Nei's gene diversity and Shannon diversity index. In addition, Zuo<sup>[22]</sup> analyzed the genetic

diversity of *C. oleifera* in Hubei Province by SRAP markers, and the results showed the rich genetic diversity of *C. oleifera* in many areas. Meanwhile, the study on genetic diversity of four wild *C. oleifera* species<sup>[23]</sup> also showed high observed heterozygosity and expected heterozygosity. Yan *et al.*<sup>[24]</sup> analyzed 25 *C. oleifera* materials by SRAP technique and found that these resources were rich in genetic diversity. In addition, the study on *C. oleifera* by Huang<sup>[25]</sup> showed that it also had rich genetic diversity characteristics.

Generally speaking, from the research of many regions and different species, there is rich genetic diversity in *C. oleifera* germplasm resources. It provides an important scientific basis for further breeding, rational utilization and protection of *C. oleifera* germplasm resources. In future research, we can further explore the genetic diversity of *C. oleifera* by combining various molecular marker techniques, and provide a more solid foundation for the development of *C. oleifera* industry.

### Construction of DNA fingerprint of *C. oleifera* and identification of true and false hybrids

With the rapid increase of planting area of *C. oleifera*, the problem of mixed varieties and uneven quality has become more and more prominent, and the situation of "homonym and synonym" is common. Therefore, it is very important to construct the DNA fingerprint of *C. oleifera* variety resources for variety identification and true-false hybrid identification.

Zhou *et al.*<sup>[26]</sup> used seven pairs of SSR markers to construct DNA fingerprints of 43 cultivars of *C. oleifera*, and 35 polymorphic loci were obtained, showing the polymorphism level of 97.22%. Four specific marker combinations (COg SSR16, COgssr37, COe SSR4 and COe SSR44) completely distinguished these 43 camellia cultivars. Lin *et al.*<sup>[27]</sup> screened 31 core SNP loci with high polymorphism from 221 common *C. oleifera* germplasm resources, and the accuracy reached over 91.36%. Li<sup>[28]</sup> constructed the DNA fingerprint of wild *C. oleifera* by ISSR molecular marker technique, and obtained through amplification, 166 bands, of which 159 bands were polymorphic, accounting for 95.78%. Dai *et al.*<sup>[29]</sup> identified 32 varieties of *C. oleifera*, and obtained 86 bands through amplification, including 51 polymorphic bands, and the percentage of polymorphic loci reached 60.28%. Luo *et al.*<sup>[30]</sup> studied *Camellia huana* T. L. Ming et W. J. Zhang and *C. oleifera*, and obtained through amplification, 440 bands, among which 385 bands were polymorphic, accounting for 87.5%. Liu *et al.*<sup>[31]</sup> selected 10 primers from 10 excellent clones of *C. oleifera* in Cenxi, Guangxi, and obtained through amplification, 108 bands, of which 81 bands were polymorphic, showing a polymorphism rate of 75%. Li used eight pairs of primers to amplify main *C. oleifera* varieties in Fujian Province, and got 313 loci, among which 151 loci were polymorphic, accounting for 48.24% of the total loci. These fingerprints can be used to identify *C. oleifera* materials.

### Association analysis

Association analysis, also known as linkage disequilibrium

mapping (LD mapping) or association mapping, usually takes natural population as the research object, and performs analysis by associating the phenotypic diversity of target traits with the polymorphism of genetic markers or candidate genes on the basis of linkage disequilibrium (LD), so as to determine the relationship between target traits and genetic markers or candidate genes in a certain population<sup>[32]</sup>. Although association analysis has been widely used in maize<sup>[33]</sup>, wheat<sup>[34]</sup>, rice<sup>[35]</sup>, soybean<sup>[36]</sup> and other crops, its application in *C. oleifera* research is relatively rare.

Dong *et al.*<sup>[37]</sup> explored SSR molecular markers closely related to important economic traits by taking *Camellia chekiangoleosa* Hu in Zhejiang as the research object. A total of 199 alleles were detected, among which 49 pairs of SSR primers were significantly correlated with nine traits, such as peel thickness, 1 000-seed weight, fresh seed yield, oleic acid and stearic acid ( $P < 0.01$ ), and the explanatory rate of phenotypic variation was between 13.51% and 56.55%. Extremely significant correlation between 21 SSR markers and above traits was detected by the GLM method. Eleven SSR markers were detected to have significant correlation with nine traits such as peel thickness, fresh seed yield, linolenic acid, oleic acid, stearic acid and total fatty acid ( $P < 0.05$ ) by the MLM method, and the contribution rates of phenotypic variation were in the range of 20.39% – 57.36%. Six pairs of markers related to oil quality were excavated. The application of correlation analysis can more accurately map quantitative traits such as yield, high quality, physiological characteristics, nutritional quality, beauty, storage resistance and stress resistance, thus improving research efficiency and accuracy. Lin *et al.*<sup>[38]</sup> conducted correlation analysis on oil content and fatty acid composition of *C. oleifera*, and used 279 hybrid individuals from six sibling families to conduct single marker-trait association tests, and detected 90 single marker-trait associations and 1 haplotype-trait association, and the explanatory rates of phenotypic variation were in the range of 1.87% – 17.93%. Moreover, they also verified the association between six SNP markers from Cofad2-A, CoSAD1 and CoSAD2 and traits ( $Q < 0.10$ ). These SNP markers identified are expected to be used in marker-assisted selection in the future to improve the oil content and quality of *C. oleifera*.

### Prospects

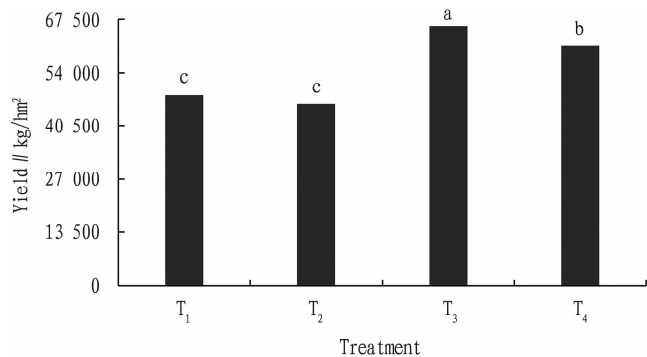
With the rapid development of economy and the improvement of living standards, the traditional breeding model of *C. oleifera* has been difficult to meet people's needs. Traditional breeding methods mainly rely on phenotypic selection, which lacks scientificity. As a woody plant, *C. oleifera* has a long growth cycle, and it takes as long as 10 years from selecting excellent varieties to cultivating mature varieties. Such traditional method, which takes a long time and lacks scientific basis, not only has low efficiency and low yield, but also faces many challenges in cultivation techniques. The characteristics of woody plants cause many difficulties in the breeding process. Due to the lack of clear correspondence between phenotypes and genotypes of traits, it is impossible to

accurately map target genes at the molecular level. As a result, the accuracy of selected offspring is low, which restricts the further development of *C. oleifera* industry. In the early stage of variety breeding, finding loci related to yield traits and adopting methods such as variety screening, high-yield location and genetic improvement can greatly improve breeding efficiency. At present, molecular markers have made remarkable progress in soybean, maize, rice and other crops, but due to the relatively short research on *C. oleifera*, the application of molecular markers in *C. oleifera* is relatively rare, lacking sufficient data support. Therefore, molecular marker technique will be an important direction of *C. oleifera* breeding in the future. Molecular markers lay a foundation for molecular-assisted breeding, while target linkage mapping and association analysis can effectively support the exploration of beneficial genes, thus shortening the breeding cycle and accelerating the breeding process. Through these technical means, new varieties of *C. oleifera* with high yield, high quality and stress resistance can be bred more accurately, and the *C. oleifera* industry can be promoted to develop in a more scientific, efficient and sustainable direction.

## References

- [1] PAN YR. Cultivation techniques and pest control of *Camellia oleifera* [J]. Forest By-Product and Speciality in China, 2022(6): 48–49. (in Chinese).
- [2] GUO XY. Analysis on cultivation techniques of high-yield *Camellia oleifera* [J]. Shanxi Agricultural Economics, 2020: 115–116. (in Chinese).
- [3] RONG YL. Study on key techniques of high-yield oil tea cultivation [J]. Low carbon world, 2020(10): 206–207. (in Chinese).
- [4] TEIXEIRA AM, SOUSA CA. Review on the biological activity of *Camellia* Species [J]. Molecules, 2021(26): 2178.
- [5] YANG L, GU Y, ZHOU J, et al. Whole-genome identification and analysis of multiple gene families reveal candidate genes for theasaponin biosynthesis in *Camellia oleifera* [J]. Int J Mol Sci [J]. 2022(23): 6393.
- [6] QUAN W, WANG A, GAO C, et al. Applications of Chinese *Camellia oleifera* and its by-products: A review [J]. Front Chem., 2022(10): 921246.
- [7] MENG J, FANG XP, SHI XM, et al. Our country walnut industry development current situation, problems and suggestions [J]. Chinese Oils and Fats, 2023(48): 84–86, 103. (in Chinese).
- [8] DENG SL. Research on the development status of *Camellia oleifera* and afforestation technology [J] 2023(2): 106–108. (in Chinese)
- [9] XIAO KY, LIU J. Progress in breeding of common *Camellia oleifera* [J]. Science of Biological Hazard, 2021(44): 114–118. (in Chinese)
- [10] DING XF, CHEN QB, WANG JY, et al. Problems and countermeasures in the development of oil tea industry in Hubei Province [J]. Hubei Forestry Science and Technology, 2022(51): 70–72. (in Chinese)
- [11] TAN XF. Advances in molecular breeding of *Camellia oleifolia* [J]. Journal of Central South University of Forestry and Technology, 2023(43): 1–24. (in Chinese)
- [12] LI HB, WANG S, DING HM, et al. Development of EST-SSR markers in the transcriptome of *Camellia camellia* [J]. Journal of Plant Physiology, 2017(53): 1267–1278. (in Chinese)
- [13] XIN JS, GUO JL, ZHANG RB. Comparison and analysis of several common molecular marker techniques for identification of seed purity and variety authenticity [J]. Seed, 2005(6): 58–60. (in Chinese).
- [14] TIAN QQ, HUANG JJ, WEN Q, et al. Current situation and trends in molecular breeding of *Camellia camellia* [J]. Forest Science in Southern China, 2021(49): 53–59, 73. (in Chinese)
- [15] ZHANG ZZ, GUO ML, ZHANG JD. Molecular marker technology and its application in medicinal plants [J]. Journal of Pharmaceutical Practice, 2007(3): 137–140. (in Chinese)
- [16] XING XQ. Application of RAPD technology [J]. Food Science and Technology, 2010(35): 314–316. (in Chinese)
- [17] WANG HY, CHEN M, LIAO ZH, et al. Molecular markers of RFLP, RAPD and AFLP and their applications in plant biotechnology [J]. Journal of Biology, 1999(4): 24–25, 19. (in Chinese)
- [18] VOS P, HOGERS R, BLEEKER M, et al. AFLP: A new technique for DNA fingerprinting [J]. Nucleic Acids Res, 1995(23): 4407–4414.
- [19] ZHAO X, XIE H, MA RC. Emerging molecular markers in plant functional genomes [J]. Chinese Journal of Bioengineering, 2007(8): 104–110. (in Chinese)
- [20] ZOU YL. Evaluation of superior clones and studies on genetic diversity of Youxian oil tea [M]. Beijing: Chinese Academy of Forestry, 2021. (in Chinese).
- [21] XIE H, ZHU WJ, ZHANG YZ, et al. Genetic diversity of *Camellia camellia* germplasm based on SRAP markers [J]. Journal of Central South University of Forestry and Technology, 2017(37): 93–97, 113. (in Chinese).
- [22] ZUO XZ. Optimization of SRAP markers and genetic diversity of *Camellia camellia* in Hubei Province [M]. Wuhan: Huazhong Agricultural University, 2012. (in Chinese).
- [23] XIANG TT. Investigation and evaluation of germplasm resources and population genetic structure of wild oil tea [M]. Ya'an: Sichuan Agricultural University, 2022. (in Chinese).
- [24] YAN HQ, ZHENG W, DAI JN, et al. Genetic diversity analysis of *Camellia hainanensis* cultivars based on SRAP markers [J]. Molecular plant breeding, 2022(20): 1901–1908. (in Chinese).
- [25] HUANG Y. Genetic diversity analysis of *Camellia oleifolia* based on SRAP molecular markers [J]. Science of Forestry, 2013(49): 43–50. (in Chinese).
- [26] ZHOU WC, WEN Q, YANG J, et al. SSR fingerprint construction and cluster analysis of *Camellia camellia* cultivar [J]. Molecular plant breeding, 2017(15): 238–249. (in Chinese).
- [27] LIN P, WANG KL, YAO XH, et al. Molecular identification of major *Camellia* species resources based on transcriptome SNP [J]. Agricultural Sciences in China, 2023(56): 217–235. (in Chinese).
- [28] LI GS. ISSR genetic relationship and fingerprint construction of 5 wild *Camellia* species [M]. Changsha: Central South University of Forestry and Technology, 2014. (in Chinese).
- [29] DAI HP, ZHAO H, JIA GL, et al. Establishment of ISSR fingerprint database of *Camellia camellia* varieties [J]. Journal of Agricultural Sciences, 2013(22): 101–107. (in Chinese).
- [30] LUO ZQ, LU J, LI RJ, et al. ISSR analysis of germplasm resources of *Camellia chinensis* in Guizhou and construction of fingerprint database [J]. Rural Science and Technology, 2020(11): 118–120. (in Chinese).
- [31] LIU K, ZHANG NY, WANG DX, et al. ISSR identification and genetic analysis of superior clones of Youzha Cen Soft series [J]. Journal of Central South University of Forestry and Technology, 2016(36): 22–26. (in Chinese).
- [32] YUE QC, FU GD, ZHANG CF, et al. Progress in application of plant association analysis [J]. Jiangsu Agricultural Sciences, 2019(47): 24–30. (in Chinese).
- [33] DING YF, WANG JL, CHEN FQ, et al. Correlation between SSR diversity and ear traits in maize inbred lines [J]. Acta Prataculturae Sinica, 2020(29): 143–153. (in Chinese).

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**Fig. 1** Effects of functional organic materials on watermelon yield and quality

## Conclusions and Discussion

Stem diameter, vine length and leaf number are important reference indexes for measuring the growth speed and plant health of watermelon. The results of this study showed that compared with the simple application of garlic straw or onion straw, the functional organic material composed of garlic straw and chicken manure and the functional organic material composed of onion straw and sheep manure could significantly increase the stem diameter, vine length and leaf number of watermelon, and the functional organic material composed of garlic straw and chicken manure had the greatest promotion effect on watermelon morphogenesis. It is because chicken manure, compared with sheep manure, garlic straw and onion straw, contains higher organic matter, nitrogen, phosphorus, potassium and medium and trace elements, which can increase the content of soil active organic carbon, promote the propagation and growth of rhizosphere soil microorganisms, and not only improve soil physical and chemical properties and soil structure, but also provide more nutrients for plant growth<sup>[3]</sup>.

Excellent nutritional quality is one of the important production goals of watermelon cultivation, and it is also an important prerequisite for watermelon to obtain high economic benefits. The central sugar, side sugar and center-to-side difference are important reference indexes for evaluating the quality of watermelon fruit. High yield is another important reference index for watermelon to pursue high economic benefits. Rational application of organic fertilizer and chemical fertilizer is more conducive to the improvement of crop quality and yield than single application of

organic fertilizer or chemical fertilizer<sup>[4]</sup>. The results of this study showed that the functional organic material composed of garlic straw and chicken manure and the functional organic material composed of onion straw and sheep manure were more conducive to the increase of central sugar content and marginal sugar content of watermelon, the decrease of center-to-side difference and the improvement of watermelon yield and quality than the simple application of garlic straw or onion straw, because chicken manure and sheep manure could improve soil aggregate structure and soil enzyme activity and provide a suitable soil environment for plant growth<sup>[5-6]</sup>. The functional organic material T<sub>3</sub>, composed of garlic straw and chicken manure, performed relatively well, which might be because chicken manure has a comprehensive and balanced nutrition and long-lasting fertilizer effect, contains macroelements of nitrogen, phosphorus and potassium, medium elements such as calcium and magnesium and trace elements such as iron, magnesium and zinc, which are necessary for crop growth, and is also rich in organic sugars such as humic acid, various enzymes and beneficial microorganisms, thus providing a comprehensive and lasting nutrient supply for plant growth<sup>[7]</sup>.

## References

- [1] NIU XS, JU XT. Organic fertilizer resources and utilization in China[J]. Journal of Plant Nutrition and Fertilizer, 2017, 23(6): 1462–1479. (in Chinese).
- [2] XIE GH, BAO WQ, LIU JJ, *et al.* An overview of researches on livestock and poultry excreta resource in China[J]. Journal of China Agricultural University, 2018, 23(4): 75–87. (in Chinese).
- [3] MA YL, WU GH, SHEN HT, *et al.* Effects of combined application of sheep manure-derived organic fertilizer and chemical fertilizer on tobacco growth and soil fertility[J]. Acta Agriculturae Nucleatae Sinica, 2021, 35(10): 2423–2430. (in Chinese).
- [4] LIU W, SI RT, FAN JH, *et al.* Effects of different fertilization patterns on yield and quality of mango[J]. Chinese Journal of Tropical Crops, 2021, 42(3): 761–768. (in Chinese).
- [5] LI TC, HUANG J, MA CB, *et al.* Soil organic matter content and its relationship with pH and bulk density in agricultural areas of China[J]. Journal of Soil and Water Conservation, 2020, 34(6): 252–258. (in Chinese).
- [6] BOWLES TM, ACOSTA MV, CALDERÓN F, *et al.* Soil enzyme activities, microbial communities, and carbon and nitrogen availability in organic agroecosystems across an intensively-managed agricultural landscape[J]. Soil Biology and Biochemistry, 2014(68): 252–262.
- [7] GUAN TX, MA GT, MA ZL, *et al.* Effects of continuous application of chicken manure on field-grown cucumber yield, quality, and soil properties[J]. Journal of Plant Nutrition and Fertilizer, 2021, 27(8): 1351–1360. (in Chinese).

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- [34] XU X, ZHANG DH, ZHAO JS, *et al.* Genome-wide association analysis of spikelets number traits in common wheat[J]. Journal of Plant Genetic Resources, 2022(23): 1098–1110. (in Chinese).
- [35] WANG FB, SHI J, ZHENG Z, *et al.* Genetic diversity and correlation between agronomic traits and SSR markers in rice[J]. Journal of Sichuan University (Natural Science Edition), 2019(56): 976–982. (in Chinese).
- [36] WANG ZY, NIAN H, SONG EL, *et al.* SSR correlation analysis of

important agronomic traits in South China soybean[J]. Journal of Agricultural Sciences, 2015(43): 6–8, 11. (in Chinese).

- [37] DONG L, TIAN QQ, HUANG B, *et al.* Association between economic traits and SSR markers in *Camellia carthae*, Zhejiang[J]. Molecular plant breeding, 2022(20): 4710–4722. (in Chinese).
- [38] LIN P, YIN H, YAN C, *et al.* Association genetics identifies single nucleotide polymorphisms related to kernel oil content and quality in *Camellia oleifera*[J]. J Agric Food Chem, 2019(67): 2547–2562.

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