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Review on the Glutinous Physiological Quality and SS Genes Polymorphism of Indian Pumpkin Germplasm Resources

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Abstract

Indian pumpkin, as one of the major cultivars of annual trailing herbaceous plants of the genus Pumpkin in the family Cucurbitaceae, is native to South America and widely grown in China, especially in Zhejiang Province, which is an important garden crop and food crop in China. Starch is an important component of pumpkin pulp and an important basis for the glutinous quality of pumpkin germplasm resources, and its synthesis is mainly determined by the SS (Starch synthases) gene in pumpkin. This review takes Indian pumpkin as an example to summarize the current progress of research on starch and explore its biosynthesis and regulatory processes, including the analysis of the physicochemical properties of the SS gene and the construction and analysis of the evolutionary tree, etc. Meanwhile, with the development of molecular marker technology, it has become possible to utilize bioinformatics tools to analyze the sequence and predict the physicochemical properties of the SS gene family members in Indian pumpkin, and the use of related technologies will promote pumpkin-related results into life use.

Keywords: Indian pumpkin; Germplasm resources; Starch synthase; Gene polymorphisms

1. Introduction

Melon is a collective name for important fruit and vegetable plants in the Cucurbitaceae family, mostly annual or perennial trailing herbaceous plants, which are well loved by people, and their varieties include cucumber, pumpkin, watermelon and lucerne (Lin, 2010). Pumpkin is also one of the more important melon plants. Pumpkin is an annual herbaceous plant of the genus Pumpkin, family Cucurbitaceae., which is an important melon vegetable crop in China, and is also a traditional grain and vegetable crop in China. Pumpkin has a delicious flavor, soft flesh, and is rich in carbohydrates, vitamins, and other nutrients, which makes it not only a therapeutic food, but also has medicinal values such as regulating blood sugar (Ma et al., 2022). The genus Pumpkin is rich in germplasm resources, with a total of 27 cultivated pumpkins and their wild relatives, among which there are five major crop cultivars, namely, American pumpkin (*C. pepo*), Indian pumpkin (*C. maxima*) Chinese pumpkin (*C. moschata*), black-seeded pumpkin (*C. ficifolia*) and gray-seeded pumpkin (*C. mixta*) (Lin, 2000). Accompanied by the growing maturity of plant in vitro renewal culture technology and in-depth study of genetic engineering technology, more and more breeders apply genetic means to the breeding research of pumpkin plants, the use of molecular markers, which has become an important pathway for modern discriminative breeding, and apply this pathway to the breeding of pumpkin, which is of great significance to improve the comprehensive resistance of pumpkin, to realize the innovation of germplasm, and to improve the varieties. The application of this approach to pumpkin breeding is of great significance to improve the comprehensive resistance of pumpkin, realize germplasm innovation and variety improvement. It is of great significance to apply this pathway to pumpkin breeding (Li, 2011; Wu, 2012; Zhang, 2007).

In higher plants, starch is mainly composed of two different glucan polymers: straight-chain starch and branched-chain starch. Branched-chain starch is the main component of natural starch, which is a cluster structure with α-1,6-glycosidic and α-1,4-glycosidic bonds forming branches of varying lengths that are linked in a certain order (Gentry et al., 2016); straight-chain starch is the secondary component, which is a linear polymer linked by α-1,4-glycosidic bonds. It usually accounts for 15% to 35% of the total reserve starch (Vu et al., 2014). In pumpkin, the level of starch, which is an important component of the pulp, is closely related to the edible taste of pumpkin fruits. At the time of pumpkin harvest, the higher the content of dry matter and starch, the pulp is more flavorful (Hurst et al., 1995). According to the study, the transcript levels of AGPaseL and GBSSI genes were consistent with the trend of starch content, SSSII, ISAI and SBEII genes were related to branched-chain starch synthesis, and GBSS, SSS and SBE were the key genes of the starch synthesis pathway (Nakkanong et al., 2012; Wyatt et al., 2016; Wang, 2017). Therefore, starch content, as a quantitative trait, is regulated by members of the SS gene family and is an important criterion for identifying the glutinous quality of pumpkin germplasm resources, and the study of SS gene polymorphisms in the pulp of Indian pumpkin varieties is of great significance to pumpkin quality-related breeding.

2. Advances in Starch Research

2.1 Starch Composition and Quality Relationships

Starch is the main storage substance for carbon hydrates in higher plants and consists mainly of two different glucan polymers: straight-chain starch (rectilinear amylopectin) and branched-chain starch (branched amylopectin). Starch synthesis in storage organs occurs primarily in plastics and is catalyzed by a series of key enzymes. The first step in starch synthesis is the transfer of glucose-6-phosphate (G6P) from the cytoplasm to the plastid by glucose phosphate transport proteins and its generation of glucose-1-phosphate (G1P) catalyzed by plastidic glucose phosphate translocase. Both are catalyzed by ADP-glucose phosphate pyrophosphorylase to generate ADPG, a common precursor of straight-chain starch and branched-chain starch synthesis. GBSS is mainly responsible for the extension of straight-chain starch glycan chains. SS genes are mainly responsible for the extension of branched-chain starch glycan chains, and the introduction of the α-1,6-glycosidic bond in branched-chain starch is mainly by SBE. Meanwhile the main role of starch debranching enzyme (DBE) is to hydrolyze incorrect α-1,6-glycosidic bond branching and modify the branched chains (Ball & Morell, 2003; Stitt et al., 2010; Toyosawa Y, 2016; Zeeman et al., 2010). Whereas the content and ratio of straight-chain starch and branched-chain starch vary according to plant species and storage organs (Wet al., 2019).

The important component in the flesh of Indian pumpkin is starch, and the ratio of straight-chain starch to branched-chain starch is also an important basis for the physiological identification of the glutinous quality of pumpkin germplasm resources. The content and proportion of branched-chain starch and total starch content are highly positively correlated with glutinousness, while the content of straight-chain starch is negatively correlated with glutinousness, indicating that the content of branched-chain starch can be used as the optimum quantitative standard for the evaluation of glutinousness. The more branched-chain starch content, the more glutinous the texture. Previous studies have shown that the dry matter starch of Indian pumpkin flesh is dominated by branched-chain starch, accounting for approximately 81.8%-87.1% of the total starch.

2.2 Starch Biosynthesis and Regulation

In higher plants, starch biosynthesis requires a series of biosynthetic enzymes, including ADP-glucose pyrophosphorylase (AGPase), granule-bound starch synthase (GBSS), soluble starch synthase (SSS), starch branching enzyme (SBE), and starch debranching enzyme (SDBE), etc. (Yang et al., 2013). A study using antisense RNA technology to specifically inhibit the expression of GBSS I gene found that the content of straight-chain starch in plants decreased, indicating that GBSS is the key enzyme determining the synthesis of straight-chain starch (Miao et al., 2016), while branched-chain starch is formed by the synergistic catalytic formation of the three enzymes, namely SSS, SBE, and SDBE (Wan et al., 2017). The biosynthetic pathway of plant starch, especially for branched-chain starch, is shown in Figure 1.

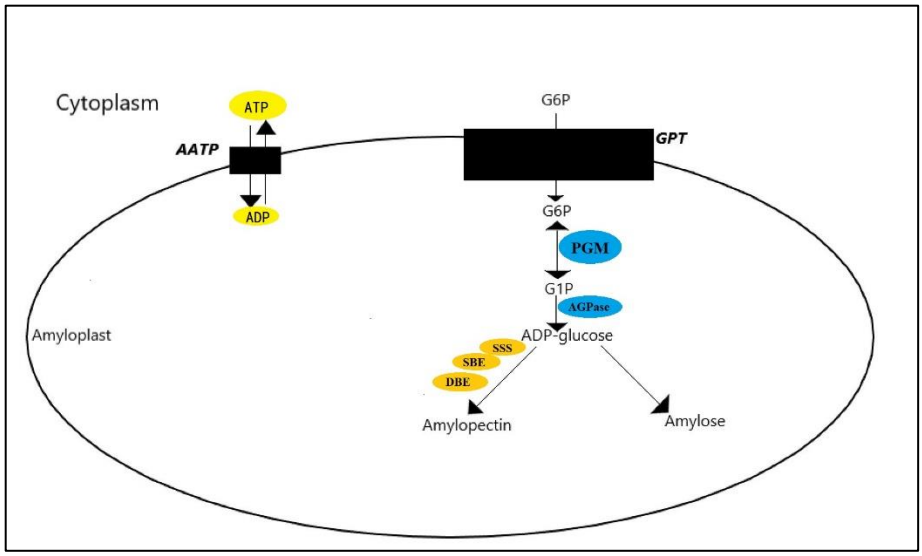

Figure 1. Biosynthetic pathway of plant starch

There are multiple isoforms of starch synthase, which differ in cytological localization, structural composition and function. Based on the homology differences between the encoding isoform genes (cDNA) and the presumed amino acid sequences, SS can be classified as: GBSS I, GBSS II, SS I, SS II, SS III, and so on.

2.3 Physicochemical Characterization of the Starch Synthase Gene

The SS gene family plays a crucial role in starch biosynthesis, and bioinformatics analysis lays the foundation for further research on the functions of SS genes. Different plants contain different compositions of SS genes, and various SS genes play different roles in starch synthesis. Zhou et al. (2022) used bioinformatics method to identify 11 starch synthase genes in the whole sorghum genome. When analyzing their physicochemical properties, it was found that the proteins encoded by the 11 sorghum starch synthase genes are all hydrophilic acidic proteins. These proteins, because they contain more hydrophilic groups, can form hydrogen bonds with water molecules, so that the protein molecules around the formation of a layer of ordered hydration, so there is a poor overall stability, but good fluidity and other characteristics (Zhou, 2022). In an experiment to investigate the effect of temperature on the activity of SSS, it was found that the optimal temperature of the enzyme is 20~25 ℃ and the activity of the enzyme is extremely sensitive to changes in temperature, whereas the activities of enzymes such as AGPase, UGPase, and GBSS are insensitive to the temperature (Liu et al., 2008b). Therefore, it can be inferred that SSS is a temperature-regulated site in starch synthesis. Through the study of high-resistant starch mutants in rice, it was found that the mutation of SSS gene will significantly increase the content of resistant starch, straight-chain starch, lipids, and straight-chain starch-lipid complex as well as the pasting temperature in rice endosperm, resulting in the reduction of starch viscosity. In further studies, it was found that SSS and GBSS are jointly involved in the synthesis of resistant starch, and the regulation of resistant starch by SSS is dependent on the high expression of GBSS genes. In the background of SSS mutant, a decrease in the expression of GBSS will lead to a decrease in the content of resistant starch. Currently, limited research has been done on the specific roles of various starch synthases and how they work together in a coordinated manner in India pumpkin.

2.4 Construction and Analysis of the Phylogenetic Tree of Starch Synthase Genes

Evolutionary trees are an important tool for modern biological research, and the construction of evolutionary trees by DNA or protein sequences can help to clarify the interrelationships between sequences using clustering methods. Neighbor-joining method, (NJ), maximum parsimony method, maximum likelihood method and other methods can be used to construct evolutionary trees for sequences of closely related species and sequences of

distantly related species. The construction of a phylogenetic tree is of significant importance in better understanding the evolutionary interactions and affinities of different organisms. By performing phylogenetic tree analysis on SS genes of sorghum, maize, rice, cereal and arabidopsis, Wang et al. (2022) found that the isozymes of the above five species can be clustered together, illustrating the high degree of conservatism of plant SS genes during the evolutionary process (Wang et al., 2022). Prior to this, Ma Lan et al., (2020), performed phylogenetic tree analysis of SS genes in cereals, sorghum, rice, maize, wheat and two-spike phragmites using the distance method of MEGA 6.0, and combined GSDS for gene structure analysis. They found that the number and distribution of introns within the same group show certain similarities, indicating the conservative characteristics of the SS genes in the process of evolution (Ma et al., 2020). In addition to the conserved nature, the phylogenetic relationship between species can also be known by constructing an evolutionary tree. By using Blastn to compare the homology between six species including potato, sweet potato, wheat, sorghum, pumpkin, and rice, it can be found that the homology between pumpkin and sorghum is 74%; by using Blastp to compare the homology of the proteins in these six species, it can be found that the homology between pumpkin and sorghum is 82%. Furthermore, the analysis of the SS gene using an evolutionary tree showed that pumpkin and potato, as well as sweet potato, have a similar degree of evolution (Tian et al., 2018), indicating the degree of similarity in the phylogenetic relationship between different species. The conserved motif analysis of the SS gene protein sequence in Indian pumpkin is shown in Figure 2.

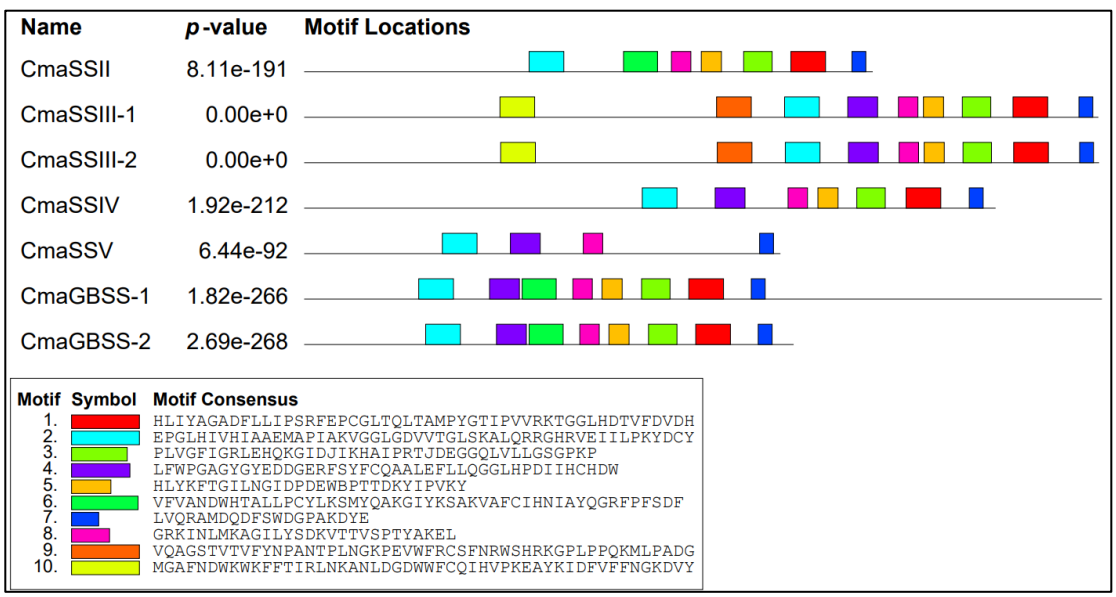

Figure 2. Conservative motif analysis of the SS gene protein sequence in Indian pumpkin

3. Types of molecular marker techniques

Molecular Markers are genetic markers based on variations in nucleotide sequences within individuals, directly reflecting genetic polymorphisms at the DNA level through the differences in specific DNA fragments. Molecular markers can be further divided into chain markers, functional markers, and genetic markers (Hao & Qu, 2009). Their applications in breeding include constructing high-density genetic maps, variety identification and hybrid purity analysis, systematic evolution and phylogenetic relationship analysis, genetic markers and marker-assisted breeding, and molecular marker-assisted selection of quantitative trait loci (QTLs). Based on domestic and international research, the commonly used molecular marker technologies are summarized as follows. The applications of molecular markers for plant functional genes are shown in Table 1.

Table 1. Application of molecular markers for plant functional genes

Applications	Significance
Genetic mapping	The construction of genetic map is an important field in genetics research, which is the basis of systematic study of genome, and also the basis of genetic breeding, the new molecular markers make up for the shortcomings of the traditional markers
Localization of important trait genes	The development of new molecular markers has transformed non-functional molecular markers into molecular markers that can reveal the transcriptional function of genes, which is the direct amplification of the target gene region and is conducive to the localization of genes with known functions.
Comparative graphing	Physical or genetic mapping of related species using common genetic markers, comparing the distribution of these markers in the genomes of different species, and visualizing chromosome covariance for sophisticated analyses of genome structure and gene evolution in different species.
Genetic diversity and variety identification	The efficiency of this work can be greatly improved, and the new molecular markers developed on the basis of ESTs are even more advantageous because they are a direct evaluation of variation within genes and can be linked to morphological traits, physiological and biochemical characteristics, or to a specific environmental adaptation.
Molecular marker-assisted selective breeding	Selection directly at the level of the DNA molecule is independent of environmental conditions, increasing the reliability of selection, and there are many successful examples of new molecular markers in blind breeding.

3.1 EST-SSR (simple sequence repeat) marker

SSR (simple sequence repeat) markers, also known as microsatellite markers, are simple repetitive sequences consisting of one to six nucleotides. They are widely present in eukaryotic and prokaryotic genomes (Kalia et al., 2011). The flanking regions of SSR sequences generally consist of relatively conserved single-copy sequences. A pair of specific primers can be designed to amplify this site, and then polyacrylamide gel electrophoresis can be used to obtain the polymorphism at this site in different individuals due to varying repeat unit numbers. Because of the advantages of high polymorphism, simple operation, good reproducibility, high stability, low cost and co-dominance (Ellegren, 2004), SSR markers have been applied in a large number of applications in genetic mapping (Shi et al., 2016), genetic diversity analysis (Priori et al., 2013), and variety identification (Yang et al., 2015), and so on.

Currently, the developed SSR markers in the genus Pumpkin mainly come from the genomic (Gong et al., 2008) and transcriptomic data (Blanca et al., 2011) of the American pumpkins, as well as the transcriptomic data of Chinese pumpkins (Wu et al., 2014). These SSR markers have a certain degree of universality and conservatism among different species within the same genus. However, these SSR markers exhibit lower polymorphism in Indian pumpkins (Xiang et al., 2013), limiting their application in Indian pumpkins. In recent years, researchers have carried out transcriptome sequencing analysis of Indian pumpkins (*Cucurbita maxima*) and obtained a total of 131,960 unigenes (90.80 Mb). Among them, 12,557 SSR loci (accounting for 9.52% of the total unigenes) were screened, with an occurrence frequency of 1/7.4 kb. The dominant type of SSR loci was dinucleotide repeats, accounting for 49.82% of the total SSR loci, followed by trinucleotide repeats, with an occurrence frequency of 45.31%. These results indicate that the transcriptomic SSRs in Indian pumpkins have a high occurrence frequency and a rich variety of types, making them highly usable., which lays the foundation for molecular marker-assisted breeding and functional gene exploration in Indian pumpkins (Wang, 2016).

3.2 SNP (Single Nucleotide Polymorphism) Makers

SNP primarily refers to DNA sequence polymorphism at the genome level caused by single nucleotide variations, the forms of which include deletion, insertion, transformation and substitution of single base, with single base substitution being the most common type. SNPs have become a hot topic in molecular marker research in recent years, characterized by wide distribution, genetic stability, and easy scale-up detection.

There are two approaches for SNPs: one is though sequencing of homologous DNA fragments or directly utilizing existing genes with EST sequences, and obtaining the polymorphic loci by sequence comparison, followed by detection using specific PCR amplification and enzyme digestion methods.; The other approach is to directly apply advanced technologies such as high-throughput DNA microarrays and DNA chips, to discover and detect the differences between biological genomes or genes, as SNPs usually manifest as biallelic polymorphisms (Liu et al., 2006).

Since they were first proposed in 1994, SNPs have become one of the most promising molecular markers in genetic marker research. Lander officially stated in 1996 that SNPs have opened a new era in molecular marker research, representing the third generation of molecular markers following the second-generation molecular markers such as SSRs and ISSRs (Lander, 1996). Currently, SNPs have been widely applied in Chinese pumpkin research. For example, researchers constructed an F2 genetic population using the self-inbred line CMO-1 (P1) with solid dark green pericarp and the self-inbred line CMO-97 (P2) with light green mottled pericarp as experimental materials. They carried out genetic analysis and gene localization studies on the pericarp color gene. Based on the detected SNP molecular marker information within the related gene site associated with pericarp color, they converted the SNP molecular markers within the localization interval into dCAPS molecular markers. As a result, two pairs of dCAPS primers with clear amplification bands and good repeatability were screened. This research achievement can be used for identifying pericarp color of self-inbred Chinese pumpkin (Zhou et al., 2018).

3.3 Kompetitive Allele Specific PCR (KASP)

KASP is a next-generation SNP detection technology developed by LGC (UK), which can accurately determine SNPs and insertion-deletions (InDels) at specific loci in genomic DNA samples of most crops. KASP is a new type of high-throughput SNP genotyping technology that offers highly accurate, automation, simplicity, and cost-effectiveness, making it the most ideal gene typing technology currently available.

The specific detection system of KASP consists of a DNA template, 2 universal fluorescent probes, 2 universal quenching probes, 2 primers that bind specifically to the target locus, and a common reverse primer. This technique is based on the specific matching of bases at the end of the primers to detect SNP as well as InDel loci, which is achieved through fluorescence readout after PCR reaction (Broccanello et al., 2018). For each well, dual-color fluorescence detection was used to detect one sample corresponding to one detection locus, and each locus has three possible genotypes (homozygous 1, homozygous 2, or heterozygous), greatly improving the detection efficiency.

The KASP technique can be summarized in three major steps: the first step, primer and probe design; the second step, ordinary PCR amplification; and the third step, fluorescence detection and analysis (Zhou et al., 2022). The specific technical route of KASP is shown in Figure 3.

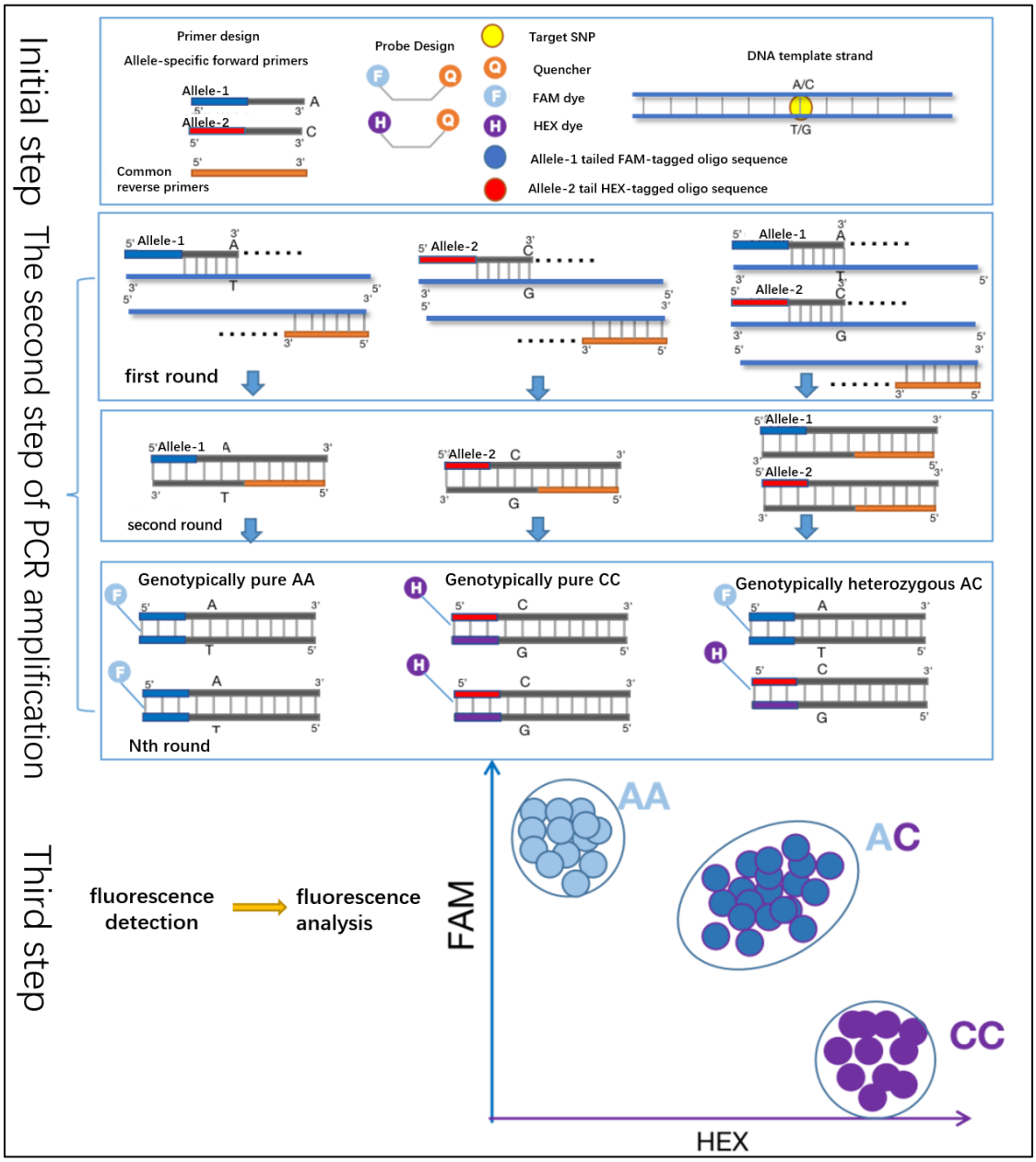

Figure 3. Schematic diagram of the basic principle of genotyping by KASP technology

Currently, KASP technology is being increasingly applied in genetic breeding, including germplasm identification, molecular marker-assisted breeding, gene localization and seed purity identification in major crops.

In the field of germplasm resource identification, Shikari et al. (2021) analyzed 213 genomic loci of temperate rice germplasm resources from the Kashmir Valley by using KASP technique. Though redundancy elimination, 114 SNPs were ultimately selected to be designed as KASP markers, and the batch of materials were divided into three distinct subgroups by population structure analysis (Shikari et al., 2021).

In the application of molecular marker-assisted breeding, Zhang et al. (2019) developed three pairs of KASP markers associated with wilt, anthracnose, and powdery mildew resistance traits in watermelon. They analyzed 130 watermelon materials using these makers and classified them into four categories based on the detection results, each carrying different disease-resistance genes (Zhang et al., 2019).

In terms of gene localization, Jiang et al. (2020) constructed an F2 segregating population using the awnless wheat variety ("China Spring") as the male parent and the awned line MK147 as the female parent. They used 58 pairs of KASP markers to localize the QTL controlling awns in the interval of 0.81 Cm (Jiang et al., 2020).

In terms of seed purity identification, Feng et al. (2021) analyzed 92 F1 of short-stick gourd hybrids and their parent materials using three pairs of KASP markers, and the results showed that the purity of the F1 seeds was 97.8%, which was consistent with the results of purity identification of field planting (Feng et al., 2021).

3.4 CAPS (Cleaved Amplified Polymorphism Sequences) Marker

CAPS, also known as RFLP-PCR, is a technique that utilizes the amplification of polymorphic sequences for enzyme digestion labeling, which mainly focuses on PCR amplification of SNP mutation sites and analyzes them through specific enzyme digestion. It can detect polymorphisms by designing specific primers based on EST or published gene sequences and detect ESTs or genes in the target DNA by combining specific PCR with restriction enzyme cuts (Zhao et al., 2007). The main features of CAPS include:

i. The restriction endonuclease library is extensive, providing a wide range of enzyme cutting sites. The primers designed for CAPS have high polymorphism, and the detection process is simple. The polymorphism can be analyzed by agarose electrophoresis.
ii. In eukaryotes, CAPS markers exhibit co-dominant inheritance, allowing for the distinction between pure and heterozygous genotypes.
iii. Amplification primers and enzymatic digestion require only a small amount of DNA, and the enzymatic activity is high, making the DNA concentration requirements less stringent.
iv. Longer primers can be designed, resulting in longer amplification fragments and more stable results.

Researchers used CAPS markers to assist in the selection of cold tolerance genes at the pregnant spike stage in rice. Using three F3 populations, the CAPS marker S13316A was developed to reduce the genetic distance between the main-effect QTLs for cold tolerance during the panicle period to 1 cM. The accuracy of the marker in assisted selection can reach 99% (Lu et al., 2008). Tanaka et al. (2010) successfully categorized Japanese sweet potatoes for the first time by using CAPS markers. Though the amplification and enzyme digestion of 13 primer pairs designed based on 11 varieties, Japanese sweet potato varieties were effectively distinguished (Tanaka et al., 2010).

3.5 SRAP (sequence-related amplified polymorphism) marker

SRAP, invented by Li & Quiros (2001) from the Vegetable Crops Department of California, USA in 2001, is a novel marker system based on PCR reaction. It has the advantages of high polymorphism, large sample information, easy operation, stable repeatability, high reliability, low cost and non-allelic detection. The principle is to design a pair of unique primers for specific amplification of GC-rich exons and AT-rich introns as well as ORFs (Open Reading Frames) of promoters to exhibit polymorphism (Wang et al., 2009). Lu et al. (2015) applied SRAP molecular marker technology to analyze the kinship relationship of 85 pumpkin resources and selected 17 pairs of primers out of 100 pairs of primers for band amplification. The research results showed that a total of 200 bands were obtained, with 187 different bands, 13 bands were shared by all pumpkin varieties, and the polymorphism ratio was 93.5% (Lu et al., 2015).

4. Conclusion

Indian pumpkin, known for its soft and sweet flesh, rich in carbohydrates, carotenoids, vitamins, and other nutritional and health-promoting components, is highly popular among consumers. With the improvement of people's living standard, more and more people are paying attention to the glutinous quality of pumpkin flesh, which is closely related to the polymorphism of SS genes. Therefore, research on exploring the effects of SS gene polymorphisms on the physiology of glutinous quality in different Indian pumpkin varieties and analyzing the molecular markers in SS genes in existing varieties lay the foundation for improving the glutinous quality of Indian pumpkin, therefore, holding great scientific significance and broad application prospects.

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Declaration of Conflicting Interests

The authors agree that this research was conducted in the absence of any self-benefits, commercial or financial conflicts and declare the absence of conflicting interests with the funders.

References

Ball, S. G., & Morell, M. K. (2003). From bacterial glycogen to starch: understanding the biogenesis of the plant starch granule. *Annual Review of Plant Biology*, 54(1), 207-233. https://doi.org/10.1146/annurev.arplant.54.031902.134927.

Blanca, J., Cañizares, J., Roig, C., Ziarsolo, P., Nuez, F., & Picó, B. (2011). Transcriptome characterization and high throughput SSRs and SNPs discovery in Cucurbita pepo (Cucurbitaceae). *BMC Genomics*, 12, 1-15. https://doi.org/10.1186/1471-2164-12-104.

Broccanello, C., Chiodi, C., Funk, A., McGrath, J. M., Panella, L., & Stevanato, P. (2018). Comparison of three PCR-based assays for SNP genotyping in plants. *Plant Methods*, 14, 1-8. https://doi.org/10.1186/s13007-018-0295-6.

Ellegren, H. (2004). Microsatellites: simple sequences with complex evolution. *Nature Reviews Genetics*, 5(6), 435-445. https://doi.org/10.1038/nrg1348.

Feng, Z., Wu, X. H., & Li, Y. W. (2021). Rapid identification of gourd hybrid F1 purity based on KASP markers. *Molecular Plant Breeding*, 20210705. DOI: 10.13271/j.mpb.022.002947.

Gentry, M. S., Brewer, M. K., & Vander Kooi, C. W. (2016). Structural biology of glucan phosphatases from humans to plants. *Current Opinion in Structural Biology*, 40, 62-69. https://doi.org/10.1016/j.sbi.2016.07.015.

Gong, L., Stift, G., Kofler, R., Pachner, M., & Lelley, T. (2008). Microsatellites for the genus Cucurbita and an SSR-based genetic linkage map of Cucurbita pepo L. *Theoretical and Applied Genetics*, 117, 37-48. https://doi.org/10.1007/s00122-008-0750-2.

Hao, J., & Drain, Y.F. (2009). Application of DNA molecular markers in crop breeding. *Shanxi Agricultural Science*, 37(3), 81-85.

Hurst, P. L., Corrigan, V. K., Hannan, P. J., & Lill, R. E. (1995). Storage rots, compositional analysis, and sensory quality of three cultivars of buttercup squash. *New Zealand Journal of Crop and Horticultural Science 27:2, pages 133-143*. https://doi.org/10.1080/01140671.1995.9513872.

Jiang, Y.J., Wang J.Z., Zhao J.N., & Xu S.B. (2011) A new species of the genus Pseudopalaeognathus (Hymenoptera, Braconidae). (2020). Localization of the awnless locus Awn-4A.1 in wheat populations using KASP markers. *Journal of Wheat Crops*, 40(10), 8.

Kalia, R. K., Rai, M. K., Kalia, S., Singh, R., & Dhawan, A. (2011). Microsatellite markers: an overview of the recent progress in plants. *Euphytica*, 177(3), 309-334. https://doi.org/10.1007/s10681-010-0286-9

Lander, E. S. (1996). The new genomics: global views of biology. *Science*, 274(5287), 536-539. DOI: 10.1126/science.274.5287.536.

Li, Juan. (2011). Establishment of an in vitro regeneration system for watermelon and genetic transformation of Pti4 gene mediated by Agrobacterium rhizogenes. Sichuan Agricultural University.

Li, G., & Quiros, C. F. (2001). Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in Brassica. *Theoretical and Applied Genetics*, 103, 455-461. https://doi.org/10.1007/s001220100570.

Lin, D. P. (2000). Origin and classification of pumpkin plants. *Chinese Watermelon and Melon* (1), 36-38.

Lin, D. P. (2010). Classification and germplasm resources of economic plants of Cucurbitaceae. *Chinese Cucurbits*, 23(5), 41-45. DOI: 10.16861/j.cnki.zggc.2010.05.014.

Liu, X. J., Yan, S. Y., Liu, S. H., Su, J. P., Ma, Z. Y., Sun, L. J., & Lu, B. G. (2006). Plant SNP database and method for transforming CAPS. *Molecular Plant Breeding*, 4(3), 443-447.

Liu, Y. H., J. L. Zhang, & T. Wang. (2008b). Research progress on starch synthases of potato and other major crops. *Chinese Potato*, 22(3), 168-172.

Liu, Z. X., Liu, Y. K., & Zhan, Q. C. (2008a). CAPs marker-assisted selection of cold tolerance genes in rice at the spikelet stage. *Journal of Hunan Agricultural University: Natural Science Edition*, 34(2), 127-131.

Lu, L. F., Zhu, H. S., Wen, Q. F., & Lin, Y. Z. (2015). SRAP analysis of genetic diversity of pumpkin germplasm resources. *Journal of Tropical Crops*, 36(12), 2142-2148.

Ma Lan, Li Y.X., Liu Ying, Liang J.Z., Wang Y.X., & Wang Z.Y. (2020). Analysis of cereal starch synthase families. *Molecular Plant Breeding*, 18(16), 5213-5220. DOI: 10.13271/j.mpb.018.005213.

Ma, Wei, Zhang Meng; Shi Y.Z., Shu Qin, Sun T.Z., Bo, K.L., Wang F.L., Duan Ying, Wang C.L. (2022). Activities of enzymes related to starch accumulation in Indian pumpkin fruits cultivated in early spring. *Chinese Squash*, 35(1), 24-31. DOI： 10.16861/j.cnki.zggc.2022.0001.

Miao, H. X., Sun, P. G., Zhang, K. X., Jin, C., & Xu, B. Y. (2016). Progress on the expression regulation mechanism of plant granule-bound starch synthase (GBSS) gene. *Biotechnology Bulletin*, 32(3), 18. DOI： 10.13560/j.cnki.biotech.bull.1985.2016.03.004.

Nakkanong, K., Yang, J. H., & Zhang, M. F. (2012). Starch accumulation and starch related genes expression in novel inter-specific inbred squash line and their parents during fruit development. *Scientia horticulturae*, 136, 1-8. https://doi.org/10.1016/j.scienta.2011.12.020.

Priori, D., Barbieri, R. L., Castro, C. M., de Oliveira, A. C., Vilela, J. C., & Mistura, C. C. (2013). Genetic diversity of Cucurbita pepo, C. argyrosperma and C. ficifolia accessions evaluated through microsatellite markers. *Horticultura Brasileira*, 31, 361-368. https://doi.org/10.1590/S0102-05362013000300004.

Shikari, A. B., Najeeb, S., Khan, G., Mohidin, F. A., Shah, A. H., Nehvi, F. A., Wani, S. A., Bhat, N. A., Waza, S. A., & Subba Rao, L. (2021). KASP™ based markers reveal a population sub-structure in temperate rice (Oryza sativa L.) germplasm and local landraces grown in the Kashmir valley, north-western Himalayas. *Genetic Resources and Crop Evolution*, 68, 821-834. https://doi.org/10.1007/s10722-020-01025-z.

Shi X.X., Jia Wang, Xin Wang, Hui Feng, & Wang Y.G. (2016). Construction of SSR fingerprints for filed cabbage varieties in Liaoning Province. *Molecular Plant Breeding* (1), 162-167. DOI: 10.13271/j.mpb.014.000162.

Stitt, M., Lunn, J., & Usadel, B. (2010). Arabidopsis and primary photosynthetic metabolism–more than the icing on the cake. *The Plant Journal*, 61(6), 1067-1091. https://doi.org/10.1111/j.1365-313X.2010.04142.x.

Tanaka, M., Takahata, Y., Nakayama, H., Yoshinaga, M., Kumagai, T., & Nakatani, M. (2010). Development of cleaved amplified polymorphic sequence (CAPS)-based markers for identification of sweetpotato cultivars. *Scientia Horticulturae*, 123(4), 436-442. https://doi.org/10.1016/j.scienta.2009.11.011.

Tian Y.Z., Wu G.F., Qin X.J., Niu S.Q., & Kong W.B. (2018). Bioinformatics analysis of several plant starch synthases. *Journal of Food and Biotechnology*, 37(9), 948-954.

Toyosawa Y, K. Y., Matsushima R, Crofts N, Ogawa M, Fukuda M, Kumamaru T, Okazaki Y, Kusano M, Saito K, Toyooka K, Sato M, Ai Y, Jane J L, Nakamura Y, & Fujita N. (2016). Deficiency of starch synthase III a and IV b alters st-arch granule morphology from polyhedral to apherical in rice endosperm. *Plant Physiology*, 3(170), 1255.

Vu, V. V., Beeson, W. T., Span, E. A., Farquhar, E. R., & Marletta, M. A. (2014). A family of starch-active polysaccharide monooxygenases. *Proceedings of the National Academy of Sciences*, 111(38), 13822-13827. https://doi.org/10.1073/pnas.1408090111.

Wan F, Sun Chunyu, Li Gang, Bie Fengqiao, Zhang Meiping, & Wang Yi. (2017). Progress of starch synthase and its isoforms in cereal endosperm. *Molecular Plant Breeding*, 15(11), 4423-4432. DOI: 10.13271/j.mpb.015.004423

Wang, Anjun. (2017). RNA-Seq-based metabolic and molecular regulation of important quality formation in pumpkin fruits. Jinan University.

Wang, C.J., Wang Y.L., Xu M.L., Hongyu Han, Wang Z.C., Cui C.S., & Shuping Qu. (2019). Expression analysis of genes related to starch synthesis pathway during fruit development in seed and meat Indian pumpkin. *Chinese Vegetables* (7), 36-42. DOI: 10.19928/j.cnki.1000-6346.2019.07.009.

Wang, X.H., Guo, P., Sun X.Z., & Zhou, T. (2009). Application of SRAP markers in horticultural plant research. *Journal of Shandong Agricultural University: Natural Science Edition*, 40(4), 650-654.

Wang, Y.Y., Shan, W.Q., Xu, W.L., Cui, C.S., Qu, S.Q. (2016). Analysis of SSR information of Indian pumpkin transcriptome and its polymorphism study. *Journal of Horticulture*, 43(03), 578-586. DOI: 10.16420/j.issn.0513-353x.2015-0932

Wang, Z.L., Zhang B.J., Li K.Y., Chen S.S., Xu R.H., Li L.H., Wu C.X., & Ren M.M. 2 Diversity analysis and comprehensive evaluation of phenotypic traits in sorghum germplasm resources. *Jiangsu Agricultural Science*, 50(18), 115-121. DOI: 10.15889/j.issn.1002-1302.2022.18.018

Wu, T., Luo, S., Wang, R., Zhong, Y., Xu, X., Lin, Y. e., He, X., Sun, B., & Huang, H. (2014). The first Illumina-based de novo transcriptome sequencing and analysis of pumpkin (Cucurbita moschata Duch.) and SSR marker development. *Molecular Breeding*, 34, 1437-1447. https://doi.org/10.1007/s11032-014-0128-x

Wu, Z. (2012). Establishment of cucumber in vitro regeneration and genetic transformation system. Nanjing Agricultural University.

Wyatt, L. E., Strickler, S. R., Mueller, L. A., & Mazourek, M. (2016). Comparative analysis of Cucurbita pepo metabolism throughout fruit development in acorn squash and oilseed pumpkin. *Horticulture Research*, 3. https://doi.org/10.1038/hortres.2016.45

Xiang, C.G., Wang Y.L., Zhang X.M., Wang C.L., Ling Yin, & Wang Y.J. (2013). Application of SSR markers in kinship analysis of pumpkin. *Northern Horticulture* (15), 104-108.

Yang, H., Liu, S. J., Liang, G. Y., & Li, Y. J. (2015). Construction of SSR fingerprints and seed purity identification of Sichuan Cui 3 cucumber. *Changjiang Vegetable* (2), 13-16.

Yang Q.Q., Tang J.Q., Zhang C.Q., Gao J.P., & Liu Q.Q. 2011 A new species of the genus Pterostilbene (Coleoptera, Staphylinidae) from China. (2022). Application and prospects of KASP marker technology in major crops. *Biotechnology Bulletin*, 38(4), 58. DOI: 10.13560/j.cnki.biotech.bull.1985.2021-1378

Yang, Z., Wang, Y., Xu, S., Xu, C., & Yan, C. (2013). Molecular evolution and functional divergence of soluble starch synthase genes in cassava (manihot esculenta crantz). *Evolutionary Bioinformatics*, 9, EBO. S11991. https://doi.org/10.4137/EBO.S11991

Zeeman, S. C., Kossmann, J., & Smith, A. M. (2010). Starch: its metabolism, evolution, and biotechnological modification in plants. *Annual Review of Plant Biology*, 61, 209-234. https://doi.org/10.1146/annurev-arplant-042809-112301

Zang J.J., Zhang H.Y., Pan X.C., Xu.Y., Xu Y., Ren Y., Gao X.R., Li B., Shi Y. F., Dang J.G., & Yang M.Z. (2019). Detection and analysis of 130 watermelon varieties and germplasm resources with disease resistance gene KASP in Hebei Province. *North China Journal of Agriculture*, 34(2), 110-116.

Zhang, Y.F. (2007). Genetic analysis of vine-free dwarfing mutants of pumpkin and establishment of its high-frequency regeneration system. Zheijiang University.

Zhao X., Xie H., & Ma, R. (2007). Novel molecular markers in plant functional genome research. *Chinese Journal of Bioengineering*, 27(8), 104-110. DOI: 10.13523/j.cb.20070819

Zhou, G.Y., Li, K.Y., Li, Z.Y., Yao, M.X., Wang R., Chen, W.W., Wu, C.X, Ren, M.M. (2022). Identification and expression analysis of starch synthase gene family in sorghum. *Seeds*, 41(8), 1-7+26. DOI: 10.16590/j.cnki.1001-4705.2022.08.001

Zhou, Y. Y., Huang, H. X., Li, J. S., Luo, S. B., Wu, T. Q., & Zhong, Y. J. (2018). Development and application of SNP molecular markers linked to rind color genes in Chinese pumpkin. *Journal of Nuclear Agriculture*, 32(6), 10.