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The Application Status of Molecular Marker Assisted Selection Technology in the Breeding of Disease Resistant Tomatoes

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【**Abstract**】Tomato is rich in nutrient, tasty in flavor, and various in shape. It is a delicious kind of vegetable on the table and indispensable source of nutrition for the human body. However, at present, the tomato still faces problems like serious diseases, low disease resistance,long period to cultivate disease-resistant species,quality and yield decrease when many kinds of diseases occur, etc. It is of high importance to study and discuss how to improve the identification efficiency and the polymerization efficiency of resistance gene, and resolve tomato's problem of shortage in resistance genes, low disease resistance, and long period to cultivate disease-resistant species, etc. The paper uses PAMS (Penta-primer amplification refractory mutation system) analysis technology to test 8 resistance genes of 6 kinds of disease from 435 separate tomato plants. We have found that the PAMS technique can greatly improve the testing speed and accuracy in molecular assisted breeding compared with the traditional PCR technique. It can also shorten the breeding period to 2-3 years. We have already cultivated disease-resistant tomato "Jifan 143".

Keyword: tomatoes, molecular assisted breeding, PARMS

1.Background:

Tomato is rich in nutrient, and is beloved by the general consumer. It is the number one vegetable crop in the world. Until 2021, the production of tomatoes in Asia has accounted for 60 percent of the production of the world. China is the country with the largest tomato production. In the past 10 years, China's tomato production has been in a steady and rising trend in terms of cultivation area and output. According to the data of the National Bureau of Statistics, in 2020, China's tomato planting area will be 951.4 thousand hectares, and the output will be 48.749 million tons. China is the largest tomato seed market in the world, with a seed market size of more than 1.5 billion yuan. However, the daily production process of tomato protection cultivation is vulnerable to a variety of pathogens. There are more than 200 known tomato diseases, more than 90 kinds of tomato diseases in China, more than 10 kinds of main diseases, when a variety of diseases occur at the same time, not only will cause the decline in

yield and quality, but even lead to total crop failure. To solve the existing problems such as poor taste and flavor, few disease-resistance genes and weak comprehensive resistance ability, it is urgent to breed new varieties of high-quality multi-resistance tomatoes quickly and efficiently to adapt to the changing market demand.

In recent 10 years, the fastest developing technology of tomato breeding in China is molecular marker-assisted selection technology. Molecular Marker breeding (also known as marker assisted selection, Marker assisted selection, MAS) uses molecular markers closely linked to the target gene (or functional markers based on the target gene itself) as selection markers to complete the selection of target traits in the process of breeding.

Commonly used molecular markers are RLFP, RAPD, SSR, AFLP, ISSR, SCAR, CAPS, STS, SNP. With the discovery of genes for disease resistance and quality and their application in assisted selection, molecular marker technology has largely replaced artificial inoculation identification technology, and has become an effective tool for tomato breeding, which has greatly improved the accuracy and efficiency of selection, shortened the breeding years, and accelerated the breeding process.

Molecular assisted breeding technology can quickly and accurately select genotypes at the molecular level, and has obvious advantages. First, molecular markers can directly and accurately detect the genotype of the target gene, avoiding the blindness in traditional selection. Secondly, germplasm resources can be evaluated comprehensively and efficiently and resource utilization can be improved. Thirdly, it can improve the selection efficiency, avoid the screening difficulty of traditional breeding, and accelerate the breeding process. Fourth, it can be selected at seedling stage or early generations, eliminate undesirable plants, reduce the size of the population, and reduce the workload. Because of its high efficiency and accuracy, molecular assisted breeding technology has been widely used in disease resistance breeding and quality breeding.

Taking PARMS technology as an example, this paper uses SNP sites of resistance genes previously studied to quickly and simply detect SNP allele genotypes to improve detection efficiency, and analyzes and prospects the development prospects of molecular marker-assisted selection breeding technology through analysis of the results, with a view to making great progress in tomato disease resistance breeding.

2. Experimental Method:

2.1*Experimental material*:

In this study, 64 cherry tomato varieties, 48 large-fruit tomato varieties and 33 medium-fruit tomato varieties were selected. Two to three young leaves were collected from each variety, and a total of 435 tomato plants were isolated.

2.2 *DNA extraction:*

The fresh young leaves of the Plant were taken into the Ep test tube, and the grinding beads were added. After cooling with liquid nitrogen, the leaves were ground in a high-throughput tissue grinder, and then DNA was extracted using the NuClean Plant Genomic DNA Kit of Konweizi. The Nano Drop was used to adjust the DNA concentration to 10-100 ng.

2.3 *PARMS Amplfication:*

PARMS amplification: PARMS (Penta-primer amplification refractory mutation system) is an SNP PCR analysis technique that combines a pair of universal fluorescent primers, a pair of SNP allele-specific primers, and a reverse common primer.

The principle is shown in Figure 1: A is connected to FAM (the blue fragment in #3 is the blue fluorescent joint sequence of FAM, and #1 is connected to the blue fluorophore, part of which can be complementary with #3, so that the base and

fluorescence can be combined). If homozygous is blue on the typing diagram, the output result is FAM;

C is connected with HEX (the green fragment in #4 is HEX green fluorescent joint sequence, #2 is green fluorescent group, part of the sequence can be complementary with #4, so that the base and fluorescence combination), if the homozygous is green on the typing diagram, the output result is HEX;

The hybrid signal, red on the pattern, produces a FAMHEX output.

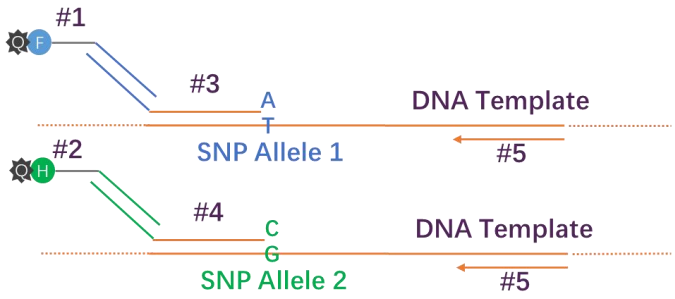

Figure 1 PARMS reaction principle

Note: #1, Allele 1 FAM fluorescent universal primer; #2, Allele 2 HEX fluorescent universal primer; #3, Allele 1 specific amplification primer (labeled primer); #4, Allele 2 specific amplification primer (labeled primer); #5, Universal reverse primers (labeling primers)

PCR reaction system was prepared according to PARMS master mix instructions and PCR amplification was performed. The PCR reaction system and cycle parameters were shown in Table 1 and Table 2.

Table1:PCR reaction system

Template DNA	1 μL

2 ×PARMS master mix	5 μL
10 mM labeled primer	0.15 μL
10 mM labeled primer	0.15 μL
10 mM universal reverse primers	0.4 μL
ddH2O	3.3 μL

Table 2:PCR reaction cycle parameters

Procedure	Temperature	Time
Step 1	94°C	15 min
Step 2	94°C	20 s
Step3	65°C (Each cycle drops 10 ℃)	1 min
	Step 2,step 3 have 10 cycles in total	
Step 4	94°C	20 s
Step 5	57°C	1 min
	Step 4,step 5 have 30 cycles in total	

2.4*Allele Genotype Identification:*

PARMS uses FAM and HEX as report fluorescence, ROX as reference fluorescence, uses Tecan F200 to scan FEM, HEX and ROX signals, and reads the end signal of the amplified product, as shown in the figure 2.

Label: FAM							
Mode			Fluorescence Top Reading				
Excitation Wavelength			492 nm				
Emission Wavelength			518 nm				
Excitation Bandwidth			10 nm				
Emission Bandwidth			5 nm				
Gain			100 Manual				
Number of Flashes			5				
Flash Frequency			400 Hz				
Integration Time			20 µs				
Lag Time			0 µs				
Settle Time			0 ms				
Z-Position (Manual)			20000 µm				
Start Ti	2023/11/23 10:43:16						
	Temperature: 24.7 ° C						
<>	1	2	3	4	5	6	7
A	792	689	645	662	731	707	601
B	758	653	657	670	640	600	612
C	898	691	1517	632	649	609	630
D	711	636	823	657	646	623	603
E	717	677	2133	1536	2016	624	1358
F	651	616	1390	814	1515	554	796
G	630	620	2030	619	1980	600	1973
H	618	609	1127	571	1234	557	1327

Figure 2:detection signal value

Online software SNP decoder(www.snpway.com/snpdecoder) is used to parse and convert fluorescent signals and get a clear and intuitive genotype map. Alleles connected to FAM fluorescent tag sequences converged near the X axis, alleles connected to HEX fluorescent tag sequences converged near the Y axis, and heterozygous alleles of the two alleles were displayed in the middle.

Figure 3 SNP decoder classifying operation

3. Application of molecular assisted breeding technique in tomato disease resistance breeding

3.1Tomato yellow leaf curl virus disease

Tomato yellow leaf curl virus disease is a devastating disease on tomatoes, causing huge economic losses in the world. It is an epidemic disease in most areas of China, mainly mediated by whitefly. It spreads rapidly and is difficult to control. So far, the researchers have identified six anti-Ty virus genes, of which Ty-1 and Ty-3 encoding the DFDGD family have been identified as alleles. Figure 4 and 5 showed the detection results of Ty-1 and Ty-2 resistance genes of yellow leaf curve virus disease. The results showed that 328 of 435 tomato isolates contained Ty-1 material, accounting for 75.40%, of which 283 were homozygous and 45 were heterozygous.

There were 43 materials containing Ty-2, accounting for 9.89%, of which 32 were homozygous and 11 were heterozygous.

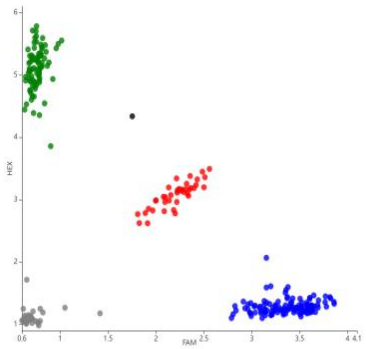

Figure 4 :Detection result of Ty-1 resistance gene of yellow leaf curl virus disease

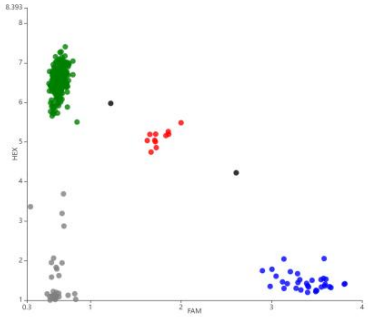

Figure 5 :Detection result of Ty-2 resistance gene of yellow leaf curl virus disease

Note: In the figure, blue dots indicate homozygous resistance genes, red dots indicate heterozygous resistance genes, green dots indicate no resistance genes, gray dots indicate no judgment, and black dots indicate negative controls. The following pictures are the same.

3.2 *Root knot nematode disease*

Root knot nematode disease is a soil-borne disease that is difficult to control and can be harmful from seedling stage to adult stage. Nine resistance genes have been found, and the Mi family plays a very important role in tomato breeding for resistance to root-knot nematodes. Figure 6 shows the detection results of the root knot nematode disease resistance gene Mi-1.2. The results show that 315 of the 435 tomato isolates contained Mi-1.2, accounting for 72.41%, among which 267 were homozygous and 48 were heterozygous.

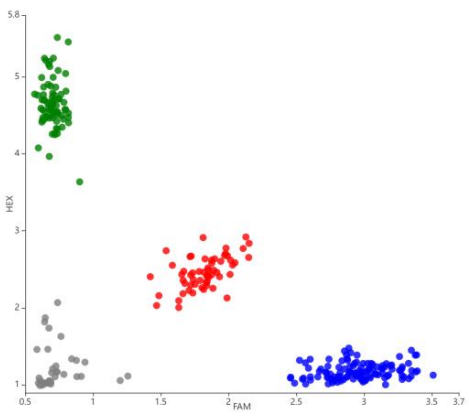

Figure 6 Detection results of root knot nematode resistance gene

3.3 T*omato leaf mold disease*

Tomato leaf mold disease is caused by Cladosporium flavicolor in the subphylum. This disease is more serious in the north of China, which leads to the decline of tomato quality, yield reduction and huge loss. The most effective way to control tomato leaf mold disease is to breed disease-resistant varieties. There are at least 24 resistance genes for leaf mold disease, among which Cf-5 and Cf-9 are the most widely used. FIG. 7 and 8 showed the detection results of leaf mold resistance genes Cf-5 and Cf-9. The results showed that there were 232 Cf-5 materials in 435 tomato isolates, accounting for 53.33%, of which 105 were homozygous and 127 were heterozygous. There were 186 materials containing Cf-9, accounting for 42.76%, of which 90 were homozygous and 96 were hybrid.

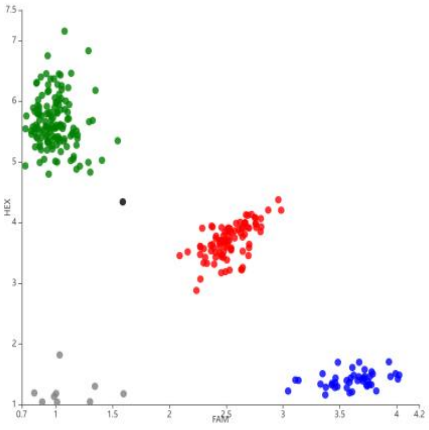

Figure 7 Detection results of leaf mold gene (Cf-5)

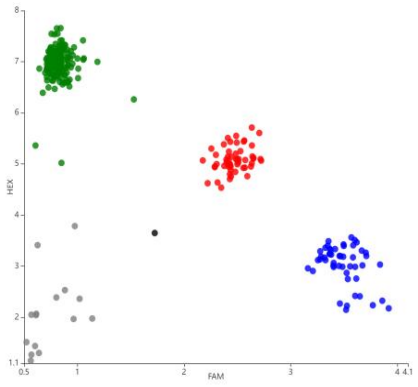

Figure 8 Detection results of leaf resistance mold resistance gene (Cf-9)

3.4 *Fusarium Crown and root rot disease*

Fusarium Crown and root rot disease occurs in most areas of tomato growth, and has

become one of the main diseases that harm tomato production. Previous studies have shown that tomato crown root rot resistance is controlled by dominant single gene Frl, which is crucial in tomato crown root rot resistance breeding. FIG. 9 shows the Frl detection results of the resistance gene of Fusarium Crown and root rot disease. The results show that among the 435 tomato isolates, 286 materials contains Frl, accounting for 65.75%, of which 154 were homozygous and 132 were heterozygous.

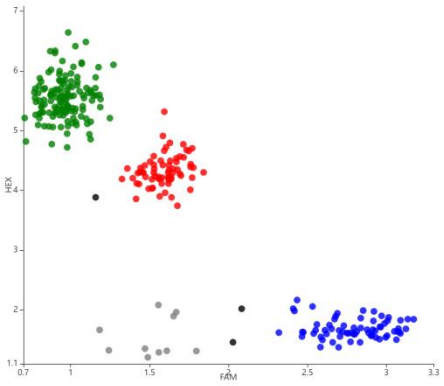

Figure 9 Detection results of Fusarium Crown and root rot resistance gene

3.5 Gray leaf spot disease

Gray leaf spot disease, also known as brown spot disease, is caused by Stemphylium solani, Stemphylium floridanum Stemphylium lycopersici, a small species of Stemphylium, which mainly harms leaves and fruits. It is considered to be a major disease of cultivated tomatoes and has threatened tomato-growing regions around the world. So far, only the resistance gene Sm has been identified as dominant and localized on chromosome 11 in tomatoes. It was isolated from the wild strain S. pimpinellifolium and is currently used to breed tomato resistant varieties. FIG. 10 shows the detection results of Sm resistance gene for grey leaf spot disease. The results show that there were 398 Sm materials in 435 tomato isolates, accounting for 91.49%, of which 336 were homozygous and 62 were heterozygous.

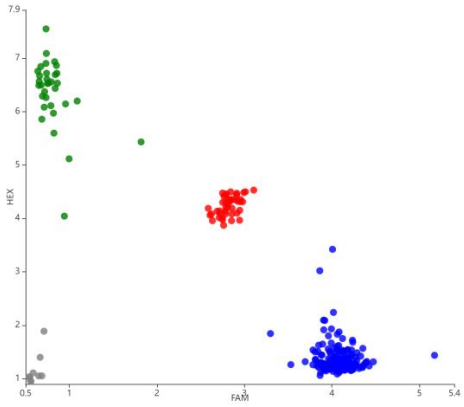

Figure 10 Detection results of gray leaf spot resistance gene

3.6 Late blight

Late blight occurred widely in the north of China, especially in the winter in the north of the facility cultivation and the south of the open field cultivation. At present, researchers have found a total of 6 late blight resistance genes Ph-1, Ph-2, Ph-3, Ph-4, Ph-5, PH-1 and PH-2, among which Ph-3 is the most widely used late blight resistance gene. FIG. 11 shows the detection results of late blight resistance gene Ph-3. The results show that among the 435 tomato isolates, there were 22 Ph-3 containing materials, accounting for 5.06%, of which 6 were homozygous and 16 were heterozygous.

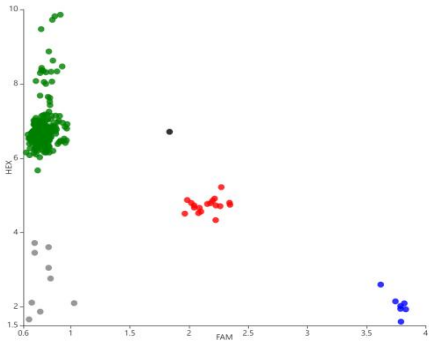

Figure 11 Detection results of late blight resistance gene

4. Conclusion:

In this study, PARMS technique was used to detect 8 resistance genes of 6 diseases in 435 tomato isolates. Jifan 143, a virus-resistant hard powder fruit, has been bred by this technique. The variety has the following characteristics: infinite growth type, strong growth potential and high fruit setting rate. Deep pink, high hardness, single fruit weight about 250 grams. Good disease resistance (resistance to tomato yellow leaf bending virus disease, tobacco Mosaic virus disease, leaf mold disease, late blight, wilt disease, including Ty-1, ty-5, Tm2a, Cf-9, Ph3, I2 resistance genes), the yield of about 14300 kg per mu. Suitable for winter and spring protected areas and solar greenhouse after autumn cultivation.

Figure 12 photo of ‘Jifan 143’variety of tomato

The application of molecular assisted breeding technology can detect the resistance genes contained in isolated tomato plants and varieties with high throughput, which can improve the detection speed and accuracy compared with the traditional PCR method.

5. Application prospect of molecular assisted breeding technology:

5.1*Actively carry out quantitative character marker research*

At present, researchers have detected allelic variation of 69 tomato traits, such as quality, disease resistance, stress resistance and yield, but most of the markers

developed so far are focused on quality traits, and there are few markers related to quantitative traits. Further research is needed to provide sites for tomato quality breeding and shorten quality breeding years.

5.2 *Develop molecular assisted breeding kits*

At present, the detection cost of molecular marker-assisted breeding is high and the machine is cumbersome. It is expected to develop relevant kits to reduce the cost and make the selection technology of molecular marker-assisted breeding a more convenient detection method.

5.3 *Upgrade breeding technique*

Molecular marker-assisted selection breeding is a combination of modern molecular biology and traditional breeding methods, and its application in tomato breeding has greatly promoted the progress of tomato breeding. However, with the development of The Times, it needs to be updated constantly. For example, gene editing technology can carry out directional and accurate modification of traits; the whole genome selection technology greatly reduces the field selection cost; intelligent design technology can build intelligent prediction model of design breeding and promote the overall upgrade of breeding methods.

referencing：

[1] ZHENG Jinliang. Comparison and Comprehensive Evaluation of Tomato in Early Spring Greenhouse Cultivation[D].Henan Institute of Science and Technology.2020.

[2] TAN Haiwen.WU Yongqiong.QIN Li .PAN Linghua.QIN Xiaoyan.XIONG Qiao.Variation and occurrence of tomato infection and venereal disease in China.China Vegetables.[J].2019(1):80-84.

[3] YANG Chaosha;WANG Shanshan;YIN Weiping;WANG Guohua;ZHANG Liyong;YIN Qingzhen;Detection of Six Disease Resistance Genes in Eighty-one

Tomato Germplasm Resources[J].Northern Horticulture.2022(20):1-9.

[4] SONG Jianjun,WANG Linshan,TIAN Peng,WANG Ruifei.Research progress on molecular marker for disease resistance genes in tomato[J].Journal of Northeast Agricultural University.2012, 43(04): 1-10.

[5] GUO Ying;HUA Qingchun;HU Mengxia;WANG Yong;YUAN Junxiu;YANG Fangping.Application and Prospect of Marker-assisted Selection in Crop Breeding[J].Journal of Cold-Arid Agricultural Sciences.2023,2(9):758-790.

[6] WANG Shanshan;YANG Chaosha;YIN Weiping;WANG Guohua;ZHANG Liyong;YIN Qingzhen;Identification and screening of multiple resistance and high quality cherry tomato resources[J].Journal of China Agricultural University.2022, 27(8): 121-130.

[7] WEI Cai;SHI Qian-qian;MAO Zhen-chuan;YANG Yu-hong;XIE Bing-yan;Resistance Identification and Evaluation against Meloidogyne incognita of Tomato with Mi Gene Family[J].China Vegetables.2016(6): 29-33.

[8] YU Shuan-cang, CHAI Min, JIANG Li-gang.Molecular markers of tomato leaf mould resistant genes Cf-9 、 Cf-11 and Cf-19[J].Acta Phytopathologica Sinica.2005, 35(3): 286-288.

[9]G F, R S M, W S J. Identification of RAPD markers linked to fusarium crown and root rot resistance (*Frl*) in tomato[J]. Euphytica. 1999, 105(3): 205-210.

[10]Yang H, Zhao T, Jiang J, et al. Mapping and screening of the tomato Stemphylium lycopersici resistance gene, *Sm*, based on bulked segregant analysis in combination with genome resequencing[J]. BMC Plant Biology. 2017, 17(1): 1-10.

[11]Jung J, Kim H J, Lee J M, et al. Gene-based molecular marker system for multiple disease resistances in tomato against Tomato yellow leaf curl virus, late blight, and verticillium wilt[J]. Euphytica. 2015, 205(2): 599-613.

[12]Ye Qingjing Yang Yuejian Wang Rongqing Ruan Meiying Zhou Guozhi Yao Zhuping.Research Progress of Tomato Leaf Mould Resistant Gene and Molecular Breeding[J].Molecular Plant Breeding.2004(03):313-320.

[13]WANG Tonglin;YE Hongxia;ZHENG Jirong;LI Ming;Research progress of main flavor compounds in tomato fruits[J].Acta Agriculturae Zhejiangensis.2020,32(8)：1514-1524.