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Genetic Diversity and DNA Fingerprinting in Broccoli Carrying Multiple Clubroot Resistance Genes Based on SSR Markers

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Abstract: To identify cultivars quickly and accurately, DNA fingerprinting of 10 broccoli varieties was performed by using simple sequence repeat (SSR) marker technology. Highly informative and polymorphic SSR markers were screened using broccoli and rapeseed. Out of the 93 SSR marker pairs, 21 pairs were selected and found to have good polymorphism. Each marker pair generated 1 to 10 polymorphic bands with an average of 4.29. The average polymorphism information content (PIC) was 0.41 with a range from 0.16 to 0.95. Six selected marker pairs established the fingerprinting of the 10 accessions and their unique fingerprints. Cluster analysis of 10 accessions showed that the genetic similarity coefficient was between 0.57 and 0.91. They can be divided into 3 groups at the genetic similarity coefficient (GSC) of 0.73. The above results indicated that DNA fingerprinting could provide a scientific basis for the identification of broccoli polymerized multiple clubroot resistance genes. Research shows that SSR marker-based DNA fingerprinting further ensures plant seed purity.

Keywords: broccoli; genetic diversity; SSR markers; fingerprinting; clustering analysis

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1. Introduction

Broccoli (*Brassica oleracea* L. var. *italica*), rich in vitamin C, protein, minerals and anticancer ingredients of sulforaphane, is widely planted in the world, especially in China and America, covering more than 50% of the worldwide area [1]. In the past 20 years, the total product amount of broccoli has increased one-fold in the world (https://www.fao.org/statistics/databases/en/, accessed on 6 February 2022). Recently, broccoli has been widely planted in China [2].

In the past 10 years, male sterility has been widely used in plant breeding of *Brassica oleracea* [3–5]. Recently, some elite parents have been regularly used in cross-breeding programs of broccoli, which results the genetic variation between new varieties and homogenization cultivars relatively start to narrow. Moreover, due to the exchange of germplasm across in different areas, broccoli resources from the same genetic background may have different names in different places [1,6]. As a result, it is difficult to distinguish broccoli germplasm from morphological characteristics alone. In fact, we usually using visual measurement to observe varieties' identity and purity on the spot, which is a conventional method of establishing these characteristics. However, it is susceptible to environmental changes resulting in a lack of stability, and it is time-consuming [7].

To date, biochemical markers such as random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), intersimple sequence repeat (ISSR) and simple sequence repeat (SSR) have been widely used in crop identification, which have polymorphic and spatiotemporal features and are not influenced by environmental factors [8]. Among these marker types, SSRs are widely used in seed purity detection and genetic diversity analysis, they have good polymorphism, simple operation, and are not

affected by external and internal environments, including growth and development time. Therefore, SSR markers have been used to characterize genetic diversity in crops, such as rice [9], corn [10], wheat [11], and tomato [12]. In addition, SSR markers are also a common method to map domains and multiple alleles and genes in *Brassica* crops, including Chinese cabbage, cabbage, broccoli and cauliflower [1,13–16].

Clubroot is a soil-infective disease caused by *Plasmodiophora brassicae* Woronin, which is now spreading all over the world and becoming the most serious disease of Cruciferae crops [17]. This disease inflicts much damage on the production of Chinese cabbage, turnip ($2n$ = 20, AA) and broccoli ($2n$ = 18, CC). Phytopathological studies on the differentiation of clubroot fungi races in representative countries were inspected, and ten types (races) were identified in this pathogen [18]. Among them, race 4 is predominantly identified in China [19]. To date, most studies have focused on the location and mining of resistance loci and the verification of their function. Four clubroot resistance (CR) genes have been cloned thus far: *Crr1*, *CRa*, *CRd* and CRb^{kato} [20,21]. *Crr1* and *CRa* belong to the TIR-NB-LRR gene family and are mainly found in turnip and Chinese cabbage [21].

However, there is still no commercial broccoli cultivar resistant to clubroot in China. Through embryo bioengineering technology, we have created a few materials of broccoli crosses with rapeseed named 'Huashuang 5R', a resistant cultivar to clubroot. Since the International Plant Variety Protection Act (http://en.wikipedia.org/wiki/Plant_Variety_Protection_Act_of_1970, accessed on 7 February 2022) was signed, the intellectual property rights of new varieties have attracted more attention worldwide. Thus, it is necessary to analyze genetic diversity and construct DNA fingerprints to support the protection of these resources. The purpose of the study was to analyze the genetic diversity and construct DNA fingerprints of 10 broccoli accessions materials collected from the Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences (IVF-CAAS), which are based on 21 pairs of SSR primer pairs.

2. Materials and Methods

2.1. Plant Materials and Genomic DNA Isolation

A total of 10 broccoli accessions, including one negative control B1024 ($2n$ = 20, AA) and the other two parental varieties (broccoli inbred line 'B71' and rapeseed 'Huangshuang 5R'), were all developed and enhanced by IVF-CAAS. 'Huangshuang 5R' was initially bred by Huazhong Agricultural University and then improved by self-pollination. According to distant hybridization with embryo bioengineering technology, broccoli accessions polymerized multiple CR genes in 2020, as shown in Table 1. All of the resources were planted on the experimental farm of IVF-CAAS, on 10 July 2021. When the plant seedling age was 25 days, plant genomi DNA was extracted by the modified cetyltrimethyl ammonium bromide (CTAB) method [1]. DNA samples were stored at −20 °C for later use.

Table 1. Information on 10 broccoli genotypes resistant to clubroot.

Accessions	Species	Generations	CR Genes
B368	Broccoli (*Brassica oleracea* L. var. *italica*) × Wild cabbage (*Brassica macrocarpa* Guss.) × Rape (*Brassica napus* L.)	BC1	*CRa*, *CRb*, *CRc*, *Crr2*, *Crr3*
B578	Broccoli (*Brassica oleracea* L. var. *italica*) × Rape (*Brassica napus* L.)	F_1	*CRa*, *CRb*, *CRc*, *CRk*, *Crr1*, *Crr2*, *Crr3*
B606	Broccoli (*Brassica oleracea* L. var. *italica*) × Red cabbage (*Brassica oleracea L.* var. *capitata*) × Turnip (*Brassica rapa* L. ssp. *rapa*)	$BC1F_3$	*CRa*, *CRb*, *CRc*, *Crr2*, *Crr3*
B611	Broccoli (*Brassica oleracea* L. var. *italica*) × Red cabbage (*Brassica oleracea L.* var. *capitata*) × Turnip (*Brassica rapa* L. ssp. *rapa*)	$BC1F_3$	*CRa*, *CRb*, *CRc*, *Crr2*, *Crr3*
B831	Broccoli (*Brassica oleracea* L. var. *italica*) × Wild cabbage (*Brassica macrocarpa* Guss.) × Rape (*Brassica napus* L.)	BC1	*CRa*, *CRb*, *Crr1*, *Crr2*, *Crr3*

Table 1. *Cont.*

Accessions	Species	Generations	CR Genes
B910	Broccoli (*Brassica oleracea* L. var. *italica*) × Turnip (*Brassica rapa* L. ssp. *rapa*)	F_1	*CRa*, *CRb*, *Crr1*, *Crr2*, *Crr3*
B936	Broccoli (*Brassica oleracea* L. var. *italica*) × Turnip (*Brassica rapa* L. ssp. *rapa*)	BC1	*CRa*, *CRb*, *Crr1*, *Crr2*, *Crr3*
B1019	Broccoli (*Brassica oleracea* L. var. *italica*) × Cabbage (*Brassica oleracea* L. var. *capitata*) × Turnip (*Brassica rapa* L. ssp. *rapa*)	F_1	*CRa*, *CRb*, *Crr1*, *Crr2*, *Crr3*
B1024	Choi Sum (*Brassica campestris* L. ssp. *chinensis* var. *utilis Tsen et Lee*) × Chinese cabbage (*Brassica pekinensis Rupr.*)	F_1	*CRa*, *CRb*, *CRc*, *CRk*, *Crr1*, *Crr2*, *Crr3*
B1027	Broccoli (*Brassica oleracea* L. var. *italica*) × Rape (*Brassica napus* L.)	BC1	*CRa*, *CRb*, *Crr1*, *Crr2*, *Crr3*

Note: Identification and details of CR loci in each cultivar are unpublished.

2.2. Analysis and Amplifications of SSRs

Broccoli and rapeseed were amplified using 93 pairs of SSR markers synthesized by the Beijing Genomics Institute (BGI, Beijing, China), with select broccoli inbred line 'B71' and rapeseed 'Huangshuang 5R' as the representative of diploid (CC) and tetrapolid (AACC), respectively, in the sprout period. Finally, 21 pairs of SSR markers which have clear bands and strong polymorphisms were selected to identify the collected broccoli germplasm materials containing more than 5 disease resistance loci.

Ultimately, 21 SSRs were selected as shown in Table 2. The PCR amplification system is as follows: 10.0 µL of Taq Master Polymerase (TaKaRa Bio, Dalian, China), 2.0 µL of markers (1.0 µL of P1 and 1.0 µL of P2), 3.0 µL of DNA template and 5.0 µL of double-distilled water, total volume is 20.0 µL. PCR amplification (BioRad, Hercules, CA, USA) was conducted with denaturing at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 45 s, and final synthesis at 72 °C for 7 min. The PCR products were separated on 8% nondenaturing polyacrylamide gels. After electrophoresis, the gels were stained as previously described [1,16].

Table 2. Sequences of SSR markers used in this study.

Marker Names	Forward Marker (5′ to 3′)	Reverse Marker (5′ to 3′)
LTSSR13	TCATCCTCCATACCTCTCCG	CCGGTTCAGGTTGAAGAAAA
LTSSR81	GTCTCGCCATGTCTCCTCTC	TGGCATCACACTAGCTACGC
LTSSR94	GCGAAACCAAGAGAGGAGAG	CAGAGGAAGGGGAGGAAGAG
LTSSR132	CCAGAAGGAACCAGCAAGAG	AAATGTCAATGCCACTGCAA
LTSSR180	TGAATATGATAGCGGAGGGG	AACGGGGGAACGTTTAGATT
LTSSR188	AGACAGCGGAGACGGTAATG	CGATAGAGAGATGGGCAACG
LTSSR304	AAACGAACCACTTTGTTGCC	CTTCTGTTTCTTCTTCGCCG
LTSSR340	GCGACTTCCACTCCTCACTC	GAAGACAGCAAAGGACCAGC
LTSSR349	TTCAACGGCAAACATGTGAT	ACCCACCATTGAACAAGAGC
LTSSR484	AAACCAGAGCCAAAAGCAAA	GTGGTTATGGAAGTGGTGGG
LTSSR544	CGGGGGTTTATTAGGGAAAA	AGACTGTGGCGCTTTTTGTT
LTSSR584	GCCACCGTTGTAAAAGTGCT	TCATCATCATCGTCCCTTGA
LTSSR688	ATTTTCATGCGTGTGTTCCA	ACCACCCCAATCTCGTTACA
LTSSR712	GGATTGGTAATGGTTGGTCTG	AAGCATCCGTGGTCGTTAAG
LTSSR785	TCAAGGCAACTGTGAACCAA	GTGATCGCCTTATCCTTTCG
BoSF2421	CACTCAGAGGAGGAGGTTGC	GCCACGTGTAGGCATGTAGA
BoSF2435	CCCACAATTCGGTATTCACC	GTCTTGCACCACCGAAAGAT
BoSF2587	AGCTTCTCGCCCTGACAATA	ACCGGCATCAAATCTCAATC
BoSF2733	CTCCGGTCATTGATATTGGC	GCCTTTTTGGTGCATGTTTT
BoSF2749	GCCAAGACTTCACGGTCATT	TGTCTGTCTGGTCCCTCTTT
BoSF2860	CATGCTTGCCTGAAAAGACA	CCTTGTACTGCTCCTCTGCC

2.3. Genetic Diversity Analysis

As in our previous report, a band of the same fragment size was recorded as a marker allele and scored as 1, 0 and 2 for a band, no band and a deletion, respectively [1,16]. The genetic relationships between genotypes of different materials were analyzed by using the NTSYSpc package version 2.1 (New York, NY, USA), and the original phylogenetic tree was constructed based on the neighbor-joining method, The SSR markers could distinguish all cultivars and be identified as core markers with the indicated polymorphisms. The similarity and cluster analysis were performed according to our previous reports [16].

2.4. DNA Fingerprinting

The characteristic band of a resource is the common characteristic band of most sample individuals. The SSRs were identified as core markers with indicated polymorphisms that could distinguish all resources. A set of binary data was obtained from the '0' and '1' data produced by core SSR markers. Quick response (QR) codes, including information on species carrying CR genes and DNA fingerprinting together with core SSR markers, were carried out online (https://cli.im/, accessed on 8 February 2022).

2.5. Data Analysis

Polymorphism information content (PIC) was estimated with PowerMarker 3.51 (Raleigh, NC, USA) [7]. The SSR markers were named by the marker pairs and the estimated molecular weight of the fragment, which was used in DNA fingerprinting. Count the electrophoresis results and calculate their PIC values (Table 3). $PIC_i = 1 - \Sigma_{j=1} P_{ij}^2$, P_{ij} represents the frequency of occurrence of the j-th band pattern of marker i, and the total band pattern of marker i is from 1 to n.

Table 3. SSR markers used in this study and their amplification results.

Marker Names	Number of Amplified Bands	Number of Polymorphic Bands	The Percentage of Polymorphic Bands (%)	PIC
LTSSR13	8	8	100	0.33
LTSSR81	4	4	100	0.25
LTSSR94	10	10	100	0.17
LTSSR132	1	1	100	0.36
LTSSR180	4	4	100	0.16
LTSSR188	4	4	100	0.21
LTSSR304	3	3	100	0.28
LTSSR340	4	4	100	0.30
LTSSR349	5	5	100	0.36
LTSSR484	4	4	100	0.40
LTSSR544	1	1	100	0.36
LTSSR584	3	3	100	0.50
LTSSR688	7	7	100	0.43
LTSSR712	2	2	100	0.95
LTSSR785	5	5	100	0.71
BoSF2421	4	4	100	0.82
BoSF2435	4	4	100	0.72
BoSF2587	6	6	100	0.19
BoSF2733	4	4	100	0.35
BoSF2749	2	2	100	0.18
BoSF2860	5	5	100	0.61
Average	4.29	4.29	100	0.41

2.6. Ploidy Detection by Flow Cytometry

Leaves were sampled from plants with 5 or more disease resistance loci, and the broccoli (CC) and oilseed rape (AACC) used as control to establish the population for measuring the DNA contents. The corresponding peak value of the G0/G1 phase was adjusted to 200. We took 200 mg of the sample to be tested and added 2 mL Galbraith's buffer, a disposable blade

was used to chop the tissue. After filtering a 400-mesh filter, the liquid in a 2 mL centrifuge tube was centrifuged at 500 r/min for 3 min, and then the supernatant was discarded. Finally, the PI dye solution was added to dye the nuclei, placed on ice condition for 30 min, and the nuclei were measured by the machine (Dolezel et al. 2007).

3. Results

3.1. SSR Marker Statistics

A total of 21 pairs of SSR markers were selected for screening the polymorphisms among 10 broccoli resources with multiple CR genes. In total, 90 polymorphic bands were obtained (Table 3). The percentage of polymorphic bands (PPB) of each marker pair was 100%. High polymorphism proved that the tested materials belonged to two cultivated resources: diploid ($2n$ = 18, CC) and allotetraploid ($4n$ = 48, AACC). The number of fragments simplified by each marker varied from 1 to 10, with an average of 4.29. The average PIC, which is a measure of heterozygosity, was as low as 0.16 (LTSSR180) and as high as 0.95 (LTSSR712), with an average of 0.41. There were 6 SSRs, LTSSR584, LTSSR712, LTSSR785, BoSF2421, BoSF2435, and BoSF2860, with high PIC values greater than 0.50, sharing 28.57% of the total polymorphic SSR markers. Six pairs of markers (LTSSR13, LTSSR81, LTSSR94, LTSSR180, LTSSR340, LTSSR688) with more amplification bands were selected, and the amplification results are shown in Figure 1.

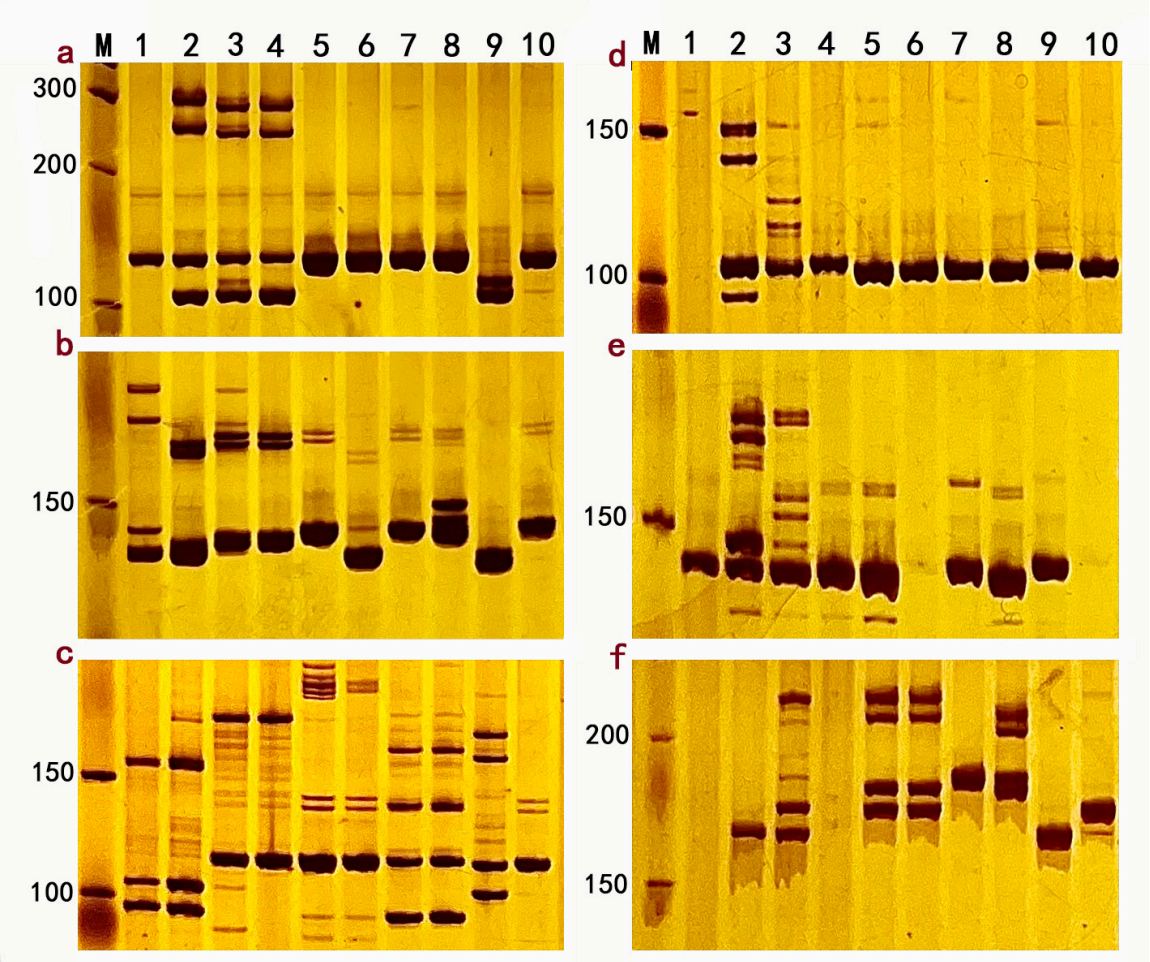

Figure 1. The PCR results of some SSR markers in 10 broccoli genotypes. M, D500 Marker, letters (**a**–**f**) represent markers LTSSR13, LTSSR81, LTSSR94, LTSSR180, LTSSR340, and LTSSR688, and numbers 1 to 10 represent B368, B578, B606, B611, B831, B910, B936, B1019, B1024, and B1027, respectively.

3.2. DNA Fingerprinting of 10 Broccoli Accessions

Using 21 pairs of SSR markers to amplify 10 broccoli germplasm materials to construct DNA fingerprints, a total of 90 polymorphic bands were obtained, and the SSR amplification results were encoded as a string of zeroes and ones. These strings were arranged in a digital fingerprint of the 10 tested broccoli resources. Table 4 shows the digital DNA fingerprint was constructed by five core SSR markers (LTSSR94, LTSSR688, LTSSR13, BoSF2587, BoSF2860) and that all 10 broccoli resources could be uniquely identified. The SSR marker LTSSR13 appeared as heterozygous bands in only one material (B1024), LTSSR94 appeared as heterozygous bands in seven materials (B606, B611, B831, B910, B936, B1019, and B1027), LTSSR688 appeared as heterozygous bands in seven materials (B606, B831, B910, B936, B1019, B1024, B1027), no bands were amplified in two materials (B368 and B611), BoSF2587 appeared as heterozygous bands in eight materials (B606, B611, B831, B910, B936, B1019, B1024 and B1027), and BoSF2860 appeared as hybrid bands in all materials. The QR codes for the 10 accessions are shown in Figure 2 with corresponding genetic information: species carrying five to seven CR genes and DNA fingerprinting based on five core SSR markers. For example, by scanning the QR code of B368, you could obtain the following information: the variety was B368, whichever was broccoli (*Brassica oleracea* L. var. *italica*) hybrid with wild cabbage (*Brassica macrocarpa* Guss.) and then with Rape (*Brassica napus* L.), and it contained clubroot resistance genes *CRa*, *CRb*, *CRc*, *Crr2* and *Crr3*. The binary molecular identity card was 11000100000000000001000000100000100, and DNA fingerprinting was based on 5 core SSR markers: LTSSR94, LTSSR688, LTSSR13, BoSF2587 and BoSF2860.

Table 4. DNA fingerprinting of 10 broccoli resources by SSR markers.

Rank	Name	Binary Molecular Identity Card
1	B368	11000100000000000001000000100000100
2	B578	11000100001000000101001010010000 1010
3	B606	00100000011100001101010100011010 1001
4	B611	00100000010000000101010100011010 1001
5	B831	00100000000110011000100000001000 0101
6	B910	00100000000110011000100001000001000 0
7	B936	10011001000001000000100001000000100
8	B1019	10011001000011110000100001000001000 0
9	B1024	01100010101000000110100000010000 1000
10	B1027	00100000000100000000100000110101000 0

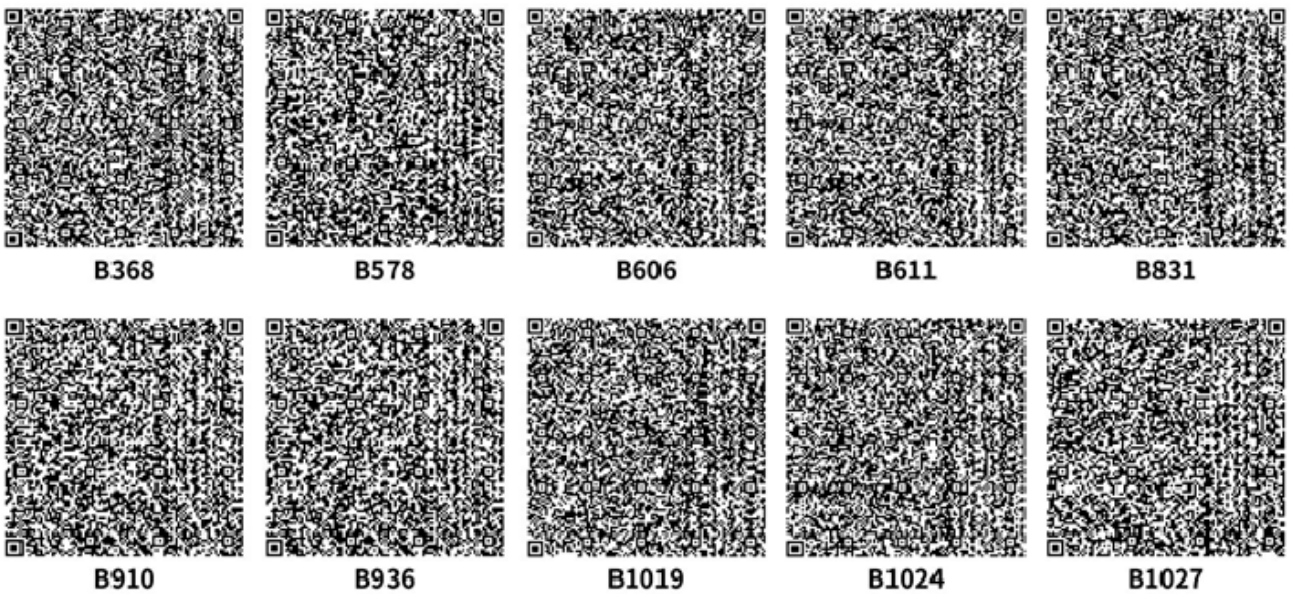

Figure 2. QR codes of the tested materials.

Accessions of B611 and B606 were identified uniquely by as many as 4 pairs of SSR markers, followed by accessions of B831 and B910 by 2 SSR markers, whereas each of the other 6 accessions was identified by one specific pair of SSR markers. The amplified fragments of marker LTSSR94 are shown in Figure 1. Among these 21 pairs of SSR markers, 5 identified either one or two accessions, and LTSSR94 identified 7 resources, followed by BoSF2860, BoSF2587 and LTSSR688, with 6 resources each. LTSSR13 and LTSSR349 identified 5 resources, 7 SSRs identified 4 accessions, and 3 SSRs identified 3 accessions.

3.3. Genetic Diversity

Cluster analysis was performed on 10 materials based on SSR markers to analyze the genetic diversity of these materials. The GSC ranged from 0.57 to 0.91, with an average of 0.77. When the heritability coefficient is 0.73, the broccoli accessions can be divided into three main groups (I, II, and III), as shown in Figure 3. We also found that broccoli accessions of B368 and B1024 formed subgroups I and III, respectively, which proved and verified the differences in genetic background (Table 1). There was a wide genetic background of B368 containing broccoli, wide cabbage, and rapeseed, while B1024 was the F_1 generation of Choi Sum cross with Chinese cabbage. Subgroup II contained 8 resources; among these accessions, B578, B606, B611, B936, and B1019 clustered together, indicating a close interrelationship between them, while the other cluster, including B831, B910, and B1027, showed a closer interrelationship. From the generation information shown in Table 1, we found that these resources all initially derived from the allotetraploid, although segregation did exist. According to the comparisons of clubroot genes, all of these broccoli resources in subgroup II were consistent with their pedigrees, so our study increased the genetic variation in broccoli cross-breeding programs.

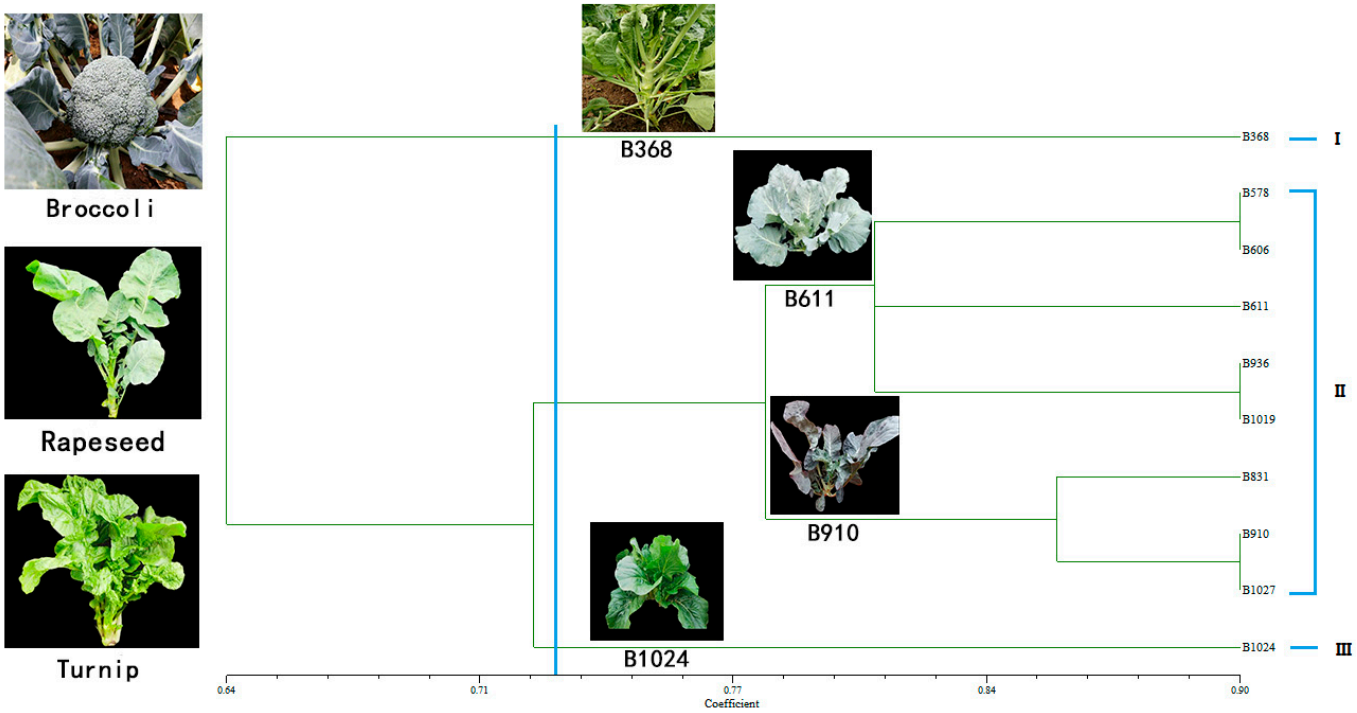

Figure 3. Genetic diversity of 10 broccoli accessions. Dendrogram constructed from cluster analysis by UPGMA. The 10 broccoli accessions were divided into three major groups (I, II, and III).

3.4. Ploidy Identification

From Figure 4, we could find that there were morphological diversity and clubroot resistance loci in these improved cruciferous germplasm. At the same time, detection of various ploidy levels via DNA content measurement also verified their genetic information. In Figure 4, B606 and B611 are diploid materials, B578 is a hybrid offspring of broccoli and

rapeseed, and the ploidy identification is close to tetraploid, and B831 was BC1 generation of broccoli (*Brassica oleracea* L. var. *italica*) × wild cabbage (*Brassica macrocarpa* Guss.) × rapeseed (*Brassica napus* L.), B936 was BC1 generation of broccoli (*Brassica oleracea* L. var. *italica*) × turnip (*Brassica rapa* L. ssp. *rapa*), B1024 was F_1 generation of choi sum (*Brassica campestris* L. ssp. *chinensis* var. *utilis Tsen et Lee*) × Chinese cabbage (*Brassica pekinensis* Rupr.), and B1027 was BC1 generation of broccoli (*Brassica oleracea* L. var. *italica*) × rape (*Brassica napus* L.). Other than that, B910 and B936 were detected as two heteroploids because their fluorescence peaks were both between 200 and 400, which were both F_1 generation of broccoli (*Brassica oleracea* L. var. *italica*) × turnip (*Brassica rapa* L. ssp. *rapa*).

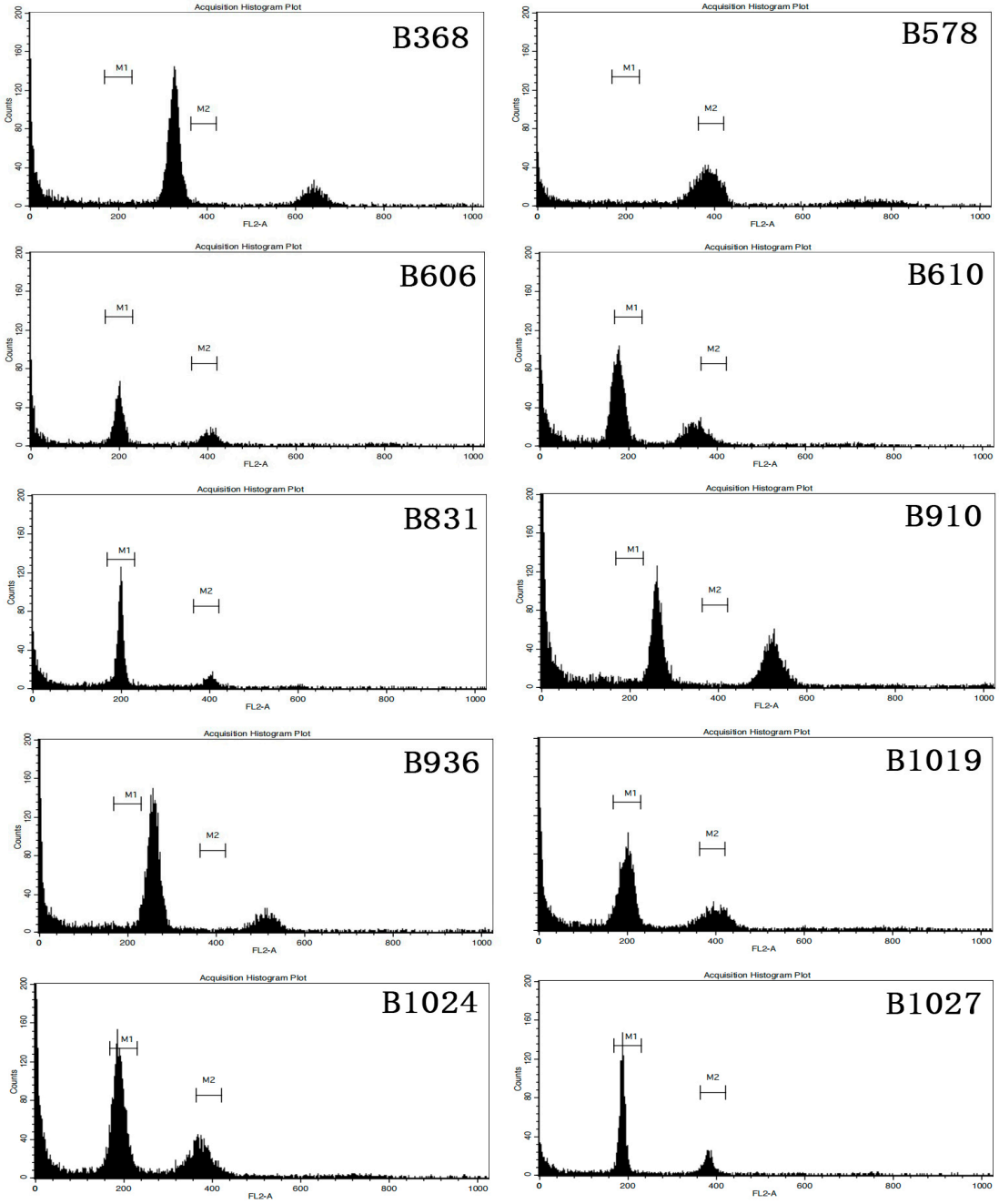

Figure 4. The detection of ploidy by flow cytometry.

4. Discussion

To date, a variety of DNA markers have been widely used in crops for germplasm evaluation, DNA fingerprinting, genetic diversity analysis, genetic mapping, and QTL mapping. Single-nucleotide polymorphisms (SNPs) based on the genomic sequence of varieties, together with SSRs, are suitable molecular markers for DNA fingerprinting and improving the precision of variety differentiation, as proven by previous reports. However, compared with SNPs, SSRs are the better choice for DNA fingerprinting and genetic diversity studies due to their low cost, reliability, and allelic diversity [22]. In recent decades, SSR markers have been widely used in field and horticultural crops [6,23]. In this study, 21 pairs of SSR markers differentiated 10 broccoli genotypes with 100% polymorphic loci, indicating that SSR markers are an effective molecular marker type for DNA fingerprinting and genetic diversity.

In previous reports, most studies of DNA fingerprinting based on SSRs or SNPs focus mainly on cultivar crops; as a result, these polymorphic markers are usually only used in genetic diversity or DNA fingerprinting among intraspecific cultivars or interspecific plants. In this study, a few core SSRs (LTSSR13) could be used to identify interspecific and intergeneric hybrids in homozygotes or resources in genetic diversity, as well as *Brassica oleracea* and *Brassica napus* crops, which is a new approach for distinguishing *Brassica* plants in identifying homozygotes.

Quick response (QR) codes have been widely used in mobile applications due to their convenience and the pervasive built-in cameras on smartphones. In this study, we proposed ScreenID to enhance QR code security by identifying its authenticity, which embeds a QR code with information from a unique screen DNA fingerprint of broccoli with CR genes. This study provided a model for the interaction between the camera and screen in the temporal and spatial domains. More extensive experiments have demonstrated that ScreenID can efficiently differentiate screens of different models, species and manufacturers and thus improve the security of QR codes [24].

The clubroot disease resistance genes of cruciferous vegetables are mainly from turnip, radish, rapeseed, and Chinese cabbage [25,26], and most of these resistance materials usually contain a single CR gene, which may cause serious weakening and increasing the planting risk. In the future breeding, the varieties with resistance to multiple physiological races could fundamentally solve the problem of clubroot on crucifers. In this study, 10 disease-resistant *Brassica* plants aggregating five or more clubroot resistance genes were finally obtained, and these important materials might help us to accelerate the development of breeding for broccoli and the other *Brassica* crops resistant to multiple physiological races.

From this study, another achievement is assessing the genetic diversity of 10 broccoli genotypes. These 10 broccoli resources were divided into three distinct groups, indicating that high genetic variation was present in broccoli carrying multiple CR genes, which was first announced in previous studies [17,27]. In particular, our study provided more information on the genetic diversity of *Brassica oleracea* crosses with *Brassica napus* and turnip than cultivars [28]. This study also showed that the genetic diversity of 10 broccoli resources was relatively large, with genetic similarity coefficients ranging from 0.57 to 0.91, which has broadened the genetic variation in broccoli, *Brassica napus*, and turnip generations in cross-breeding programs [1]. Moreover, more generations should be improved and purified, as well as further research on the molecular mechanism of domain genes, serving for broccoli breeding and other *Brassica* crops, in disease resistance, stress resistance, and high quality [17].

5. Conclusions

These novel results described 21 high-efficiency SSR markers used for genetic diversity and DNA fingerprinting of broccoli carrying multiple CR genes. A DNA fingerprint based on generations of *Brassica oleracea* crossed with *Brassica napus* and *Brassica oleracea* crossed with turnip further than cultivars was initially constructed. Additionally, our study

provided 10 new germplasms in clubroot resistance, which could be beneficial to broccoli breeding in disease resistance, as well as the other *Brassica oleracea* crops.

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