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# Development of Kompetitive Allele Specific PCR markers for identification of persimmon varieties using genotyping-by-sequencing

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*Background:* Persimmon (*Diospyros kaki* Thunb.) is the most widely cultivated species of the genus *Diospyros*. In this study, genetic diversity and variations in persimmon genotypes were investigated using single nucleotide polymorphism (SNP) markers identified by genotyping-by-sequencing (GBS) analysis.
*Results:* Ninety-five persimmon accessions grown in the Pear Research Institute, National Institute Horticultural and Herbal Science, were sequenced using the Illumina Hiseq2500 platform and polymorphic SNPs were detected to develop molecular markers. These reliable SNPs were analyzed using the Kompetitive Allele Specific PCR (KASP) assay to discriminate among persimmon genotypes. GBS generated a total of 447,495,724 trimmed reads, of which 89.7% were raw reads. After demultiplexing and sequence quality trimming, 108,876,644 clean reads were mapped to the reference transcriptome. An average of 1,146,070 genotype reads were mapped. Filtering of raw SNPs in each sample led to selection of a total of 1,725,401 high-quality SNPs. The number of homozygous and heterozygous SNPs ranged from 1,933 to 6,834 and from 846 to 5,927, respectively.
*Conclusions:* Of the 49 SNPs selected for development of an identification system for persimmons, 15 SNPs were used in the KASP assay to analyze 32 persimmon accessions. These KASP markers discriminated among all accessions.
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## 1. Introduction

More than 190 species of deciduous trees, evergreen trees, and shrubs belong to the genus Diospyros. They include the food species persimmon trees (*D. kaki*), date-plum trees (*D. lotus*), American persimmon trees (*D. virginiana*), and moringa trees (*D. oleifera*) [1]. Persimmons, in particular, exhibit outstanding fruit qualities and have been cultivated at industrial scale in the United States, Australia, Europe, as well as their native habitat of East Asia [2]. Persimmon cultivation in Korea is presumed to have begun before the time of Three Hans. The first record of persimmon cultivation was found in 1236, in the Hyangyak Method of Emergency, a book of medications written during the Goryeo Dynasty.

The persimmon fruit is sweet, tasty, and very nutritious. This prompted the traditional practice of planting a persimmon tree in the garden. Sweet persimmon has only been relatively recently introduced to Japan in the 1960s, followed by its more widespread cultivation on farms. Many varieties, including 'Fuyu,' were selected from the bud mutation. Many such varieties have been generated in persimmon growing farms and have become commonly utilized as a material for plant breeding. In addition, seedlings from natural crossbreeding may by chance produce varieties with beneficial traits. For these accessions to be selected and used in promoting and registering new cultivars, accurate analyzed genetic data from collection to selection is necessary [3].

Persimmon trees are a perennial, woody plant. The accurate characterization of any given variety requires a long time. In the case of mixing of species during the growth of the resource, an accurate variety identification is difficult until the fruits can be examined. In particular, it is difficult to clearly discriminate between the resource discovered from bud mutation and the known main varieties. To overcome this difficulty, DNA molecular markers have been proactively identified based on genetic differences [3,4,5,6,7,8,9,10,11,12,13,14,15,16]. For these DNA

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marker-based approaches, various methods have been developed and used. These included including randomly amplified polymorphic DNA, restriction fragment length polymorphism, amplified fragment length polymorphism, and simple sequence repeats. Some require improved reproducibility or rely on complex analyses, which impedes their use for accurate variety identification, although they could be used to analyze the homology among different varieties. The genotyping-by-sequencing (GBS) technique was first developed using corn. GBS decodes and analyzes base sequences in the regions surrounding the sequence cut using the *Ape* KI restriction enzyme. The technique is based on next generation sequencing (NGS) but is more rapid and less expensive. The use of a barcoding system allows the analysis of many samples in one run [17,18]. GBS analysis has subsequently been applied to barley, wheat, and soybeans for rapid propagation [19,20]. In Korea, GBS has been applied to the genome studies regarding new cultivar promotion, pure line selection, and phylogenetic analysis in a variety of major crops [21,22,23].

Kompetitive Allele Specific PCR (KASP) is a novel method of single nucleotide polymorphism (SNP) genotyping that requires only a few SNP markers to genotype various samples. The KASP assay, developed by the LGC Genomics Ltd., is based on fluorescent signals, and is an efficient and low-cost genotyping method [24,25,26,27,28]. The accuracy and convenience of the KASP assay has made it popular in the analysis of corn (*Zea mays*) [29], wheat (*Triticum aestivum*) [30], and other plants.

Presently, the KASP assay was used to genotype persimmon accessions through the use of SNP markers obtained from the GBS analysis of the accessions. The aim was to verify the potential application of the technique in the identification of persimmon varieties.

## 2. Materials and methods

### 2.1. DNA extraction and GBS library construction

Young leaves were collected from 95 varieties of persimmon accessions and hybrid seedlings cultivated by the Pear Research Institute, National Institute Horticultural and Herbal Science. The DNeasy plant mini kit (QIAGEN, Valencia, CA, USA) was used to extract genomic DNA (gDNA) of high purity (Table 1). The extracted DNA was purified using the NanoDrop 8000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) to generate the GBS library. GBS analysis was conducted by Seeders Inc. (Daejeon, Republic of Korea). The GBS library was generated following the standard analysis method [17]. The barcode adapter consisted of two strands: F-5′-ACACTCTTTCCCTACACGACGCTCTTCC GATCTxxxx-3′ and R-5′-CWGyyyyAGATCGGAAG AGCGTCGTG-TAGGGAAAGAG TGT-3′, and the CWG as the *Ape* KI recognition site harbored common adapter-F and the complementary sequence [17]. The common adapter consisted of F-5′-CWGAGATCGGAA GAGCGGTTCAGCAGGAATGCCGAG-3′ and R-5′-CTCGGCATTCCTGCT GAACCGCTCTTCCGATCT-3′. For the GBS library, the 95 gDNA samples were treated with 3.6 U *Ape* KI (New England BioLabs, Ipswich, MA, USA) for 2.5 h (20 μL reaction volume) at 75°C. For pooling, the barcode and common adapters were attached to the treated fragments using 200 U T4 DNA ligase. The samples were purified using the QIAquick PCR purification kit (QIAGEN). The primers used in the PCR amplification were F-5′-AATGATACGGCGACCACCGAGATC TACACTCTTTCCCTACACGACGCTCTTCGATCT-3′ (58mer) and R-5′-C AAGCAGAAGACGGCATACGAGATCGGTCTCGGCATTCCTGCTGAACCG CTCTTCCGATCT-3′ (61 mer) [17]. The 3′ end of primer-F and primer-R contained 33 base sequences for complementary binding with the barcode adapter and common adapter, respectively. PCR was done at 95°C for 2 min, followed by 16 cycles of 95°C for 30 s, 62°C for 30 s, 68°C for 30 s, and finally 68°C for 5 min. The suitability of the PCR products for GBS analysis was determined through gel electrophoresis and quality control (QC) analysis.

### 2.2. Sequence pre-processing

Demultiplexing was carried out using the barcode sequence, followed by the removal of the adapter sequence using Cutadapt (version 1.8.3) [31] and sequence quality trimming using the DynamicTrim and LengthSort programs of the SolexaQA (v.1.13) package [32]. DynamicTrim removed the low-quality base at either ends of the short read based on the phred score to carry out a purification process for high-quality clean reads. LengthSort removed the reads from which excess bases have been cut by the DynamicTrim. For DynamicTrim, a phred score ≥20 was applied. For LengthSort, a short read length of ≥25 pb was applied.

### 2.3. Raw SNP detection and consensus sequence extraction

The mapping of cleaned reads after the pre-processing to the reference genome was carried out using the BWA (0.6.1-r104) program [33,34]. Mapping generates the BAM format file, which precedes the detection of raw SNP (In/Del) among the representative transcript and sequencing samples. The BAM format file generated through the mapping of cleaned reads to the reference genome was used to detect raw SNPs (In/Del). The consensus sequence was extracted using the SAMtools (0.1.16) program [33]. Prior to raw SNP (In/Del) detection, SNP validation was carried out using the SEEDERS in-house script [35]. To compare SNPs among the analytic targets, an integrated SNP matrix for the samples was produced. Based on the respective coordinates, the SNP type was classified as homozygous (SNP read depth ≥90%), heterozygous (40% ≤ SNP read depth ≤60%), and etc. (discernible types).

### 2.4. Extensive SNP search and preparation of KASP probe and primer

To develop molecular markers to identify persimmon varieties, 49 candidate SNP markers were selected, for which the homozygous and heterozygous types could be discriminated. From the selected SNP markers, the final 49 coordinates clearly discriminated homozygous and heterozygous types based on the read depth result in raw read mapping were selected. For these selected SNP marker coordinates, the mapping data used in the GBS analysis were applied to determine the mapping pattern of short reads as an image using the IGV program. Through IGV imaging, the final 15 SNP markers among the sequences surrounding SNPs, from the conserved regions whenever possible, were used to successfully design the KASP primer and probe by LGC Biosearch Technologies (KOD analysis). From each candidate SNP coordinate, Primer_Allele X, Primer_Allele Y, and Primer_Common were designed.

### 2.5. DNA-based marker development and genotyping

The genotyping of the newly developed KASP primer was performed for 32 persimmon accessions (Table 2). The KASP assay was performed using a real-time PCR device, where the end-point PCR method allowed the genotyping data to be checked for the SNP coordinates using the fluorescent signal of the KASP probe following the PCR reaction. The KASP PCR condition was slightly differentiated for each assay marker according to annealing temperature to optimize the reaction. The PCR mixture for the KASP assay comprised 5 μL of 10 ng/μL gDNA, 5 μL of 2× PCR MasterMix, 8.6 μL of distilled water, and 0.14 μL of AssayMix. The PCR reaction was carried out in a total volume of 10 μL. For the KASP PCR temperature condition, the touchdown PCR condition was

**Table 1**
Cultivars and breeding lines for GBS analysis.

| No. | Cultivars and accessions | Type[z] | No. | Cultivars and accessions | Type |
|---|---|---|---|---|---|
| 1 | Jowan | PCNA | 49 | Jinyangmulbansi | non-PCNA |
| 2 | Romang | PCNA | 50 | Hamanbansi | non-PCNA |
| 3 | Yeonsu | PCNA | 51 | Habcheonbansi | non-PCNA |
| 4 | Gampung | PCNA | 52 | Daegutungturi | non-PCNA |
| 5 | Wonmi | PCNA | 53 | Andongsusigam | non-PCNA |
| 6 | Wonchu | PCNA | 54 | Goseongchambansi | non-PCNA |
| 7 | Fuyu | PCNA | 55 | Gimhaedanseongsi | non-PCNA |
| 8 | Noansubunsu | PCNA | 56 | Gimhaechalgam | non-PCNA |
| 9 | FJ117 | PCNA | 57 | Sacheonchalgam | non-PCNA |
| 10 | Taishu | PCNA | 58 | Sancheonggojongsi | non-PCNA |
| 11 | Changwon | PCNA | 59 | Sancheongkurigam | non-PCNA |
| 12 | Wakagijiro | PCNA | 60 | Bonghwagolgam | non-PCNA |
| 13 | Daeandangam | PCNA | 61 | Sangjuhagdongsi | non-PCNA |
| 14 | Ro-19 | PCNA | 62 | Uljinwonsi | non-PCNA |
| 15 | Gosho | PCNA | 63 | Gwangjubaesi | non-PCNA |
| 16 | Shinsyuu | PCNA | 64 | Guryekurigam | non-PCNA |
| 17 | Jiro | PCNA | 65 | Mujudaesi | non-PCNA |
| 18 | 05-9-26 | PCNA | 66 | Saburouza | non-PCNA |
| 19 | 05-11-10 | PCNA | 67 | Saizyou | non-PCNA |
| 20 | 05-10-16 | PCNA | 68 | Aitsmisiraji | non-PCNA |
| 21 | 05-11-49 | PCNA | 69 | Daimaban | non-PCNA |
| 22 | 05-14-64 | PCNA | 70 | Goryengsusi | non-PCNA |
| 23 | 05-16-65 | PCNA | 71 | Sangjudungsi | non-PCNA |
| 24 | 05-17-68 | PCNA | 72 | Sangjuwonsi | non-PCNA |
| 25 | 08-7-62 | PCNA | 73 | Mujudaesi | non-PCNA |
| 26 | 05-8-62 | PCNA | 74 | Hamanmulgam | non-PCNA |
| 27 | 08-9-58 | PCNA | 75 | Myongjudolgam | non-PCNA |
| 28 | 08-7-87 | PCNA | 76 | Yecheonsusi | non-PCNA |
| 29 | Mino | non-PCNA | 77 | Uiseongsagogsi | non-PCNA |
| 30 | Emon | non-PCNA | 78 | Daidanemasi | non-PCNA |
| 31 | Zenzimaru | non-PCNA | 79 | Gwangjupasi | non-PCNA |
| 32 | Nishimurwase | non-PCNA | 80 | Damyangbaegjeongsi | non-PCNA |
| 33 | Inayama | non-PCNA | 81 | Jangseongsetogari | non-PCNA |
| 34 | Monbei | non-PCNA | 82 | Jangseongsusi | non-PCNA |
| 35 | Parter | non-PCNA | 83 | Hwasunpasi | non-PCNA |
| 36 | Honeymon | non-PCNA | 84 | Okcheonbansi | non-PCNA |
| 37 | Wangchu | non-PCNA | 85 | Okcheonbyonggam | non-PCNA |
| 38 | Chuyeon | non-PCNA | 86 | Yeonginbansi | non-PCNA |
| 39 | Guryejangdungi | non-PCNA | 87 | Yeonginjangjunsi | non-PCNA |
| 40 | Najupasi | non-PCNA | 88 | Gangneungjangsi | non-PCNA |
| 41 | Damyangkurigam | non-PCNA | 89 | Goseongdongcheolsi | non-PCNA |
| 42 | Jangseongsangchugam | non-PCNA | 90 | Siheungsangsi | non-PCNA |
| 43 | Changpyeongpasi | non-PCNA | 91 | 05-8-29 | non-PCNA |
| 44 | Hwasunbuduki | non-PCNA | 92 | 05-9-42 | non-PCNA |
| 45 | Jeongupbansi | non-PCNA | 93 | 05-10-63 | non-PCNA |
| 46 | Yesanwolhasi | non-PCNA | 94 | 05-11-61 | non-PCNA |
| 47 | Goesangolgam | non-PCNA | 95 | 05-10-18 | non-PCNA |
| 48 | Uiryeongbansi | non-PCNA | | | |

[z] PCNA: pollination constant non-astringent.

entered so that the assay probe accurately located the target site for the reaction. The basic PCR condition was 10 cycles of 94°C for 15 min, 94°C for 20 s, 61°C for 1 min (a decrease in temperature by 0.6°C per cycle), followed by 25 cycles of 94°C for 20 s and 55°C for 1 min, and finally 30°C for 1 min and subsequent fluorescence scan. The final genotyping was carried out through the specific fluorescent detection of two alleles using the CFX96 device (Bio-Rad, Hercules, CA, USA).

## 3. Results

### 3.1. GBS library construction

The GBS technique is very useful in searching for high-quality SNP markers, as it is an NGS-based technique that applies a restriction enzyme in analyzing the base sequence of a specific region. The barcode allows rapid analysis and single analysis of many samples at lower cost than NGS analysis. In addition, the identified SNP markers can be used in the mapping to the chromosomes after comparison with the reference genome [17,19,36]. Presently, a total of 95 persimmon accessions (17 sweet persimmon cultivars and 62 astringent persimmon cultivars) and hybrid seedlings (11 lines of sweet persimmon and five lines of astringent persimmon) were investigated. Following the extraction of gDNA from each sample, 2 μL of DNA was used for 1.5% agarose gel electrophoresis. The gDNA quality was determined to be suitable for the subsequent construction of the GBS library (Table 1).

The DNA isolated for the GBS library construction was treated with a restriction enzyme as previously detailed [17]. A high-purity GBS library is suitable for GBS analysis when it is free of the DNA fragment (128 bp dimer band) containing the adapter sequence and when the amplified DNA fragment is 170–350 bp [17,18]. In addition, the Illumina HiSeq2000 platform that was used is commonly used in the analysis of genomes and is most suitable to analyze the library products 100–400 bp size. In the case of paired-end reads, an ideal size of 250–500 bp is recommended [18,37,38]. Electrophoresis for the GBS library revealed that the library formed well through smear at 200–500 bp and high concentrations were detected at 200–300 bp. To determine

**Table 2**
The list of persimmon accessions for genotyping of the newly developed KASP primers.

| Cultivars | Type of fruits* | Cultivar | Type of fruits |
|---|---|---|---|
| Goseongchambansi | PCA | Gwangjupasi | PCA |
| Changpyeongpasi | PCA | Wangchu | PVA |
| Noansubunsu | PCNA | 05-14-64 | PCNA |
| Chuyeon | PVNA | Gampung | PCNA |
| Zenzimaru | PVNA | Jangseongsusi | PCA |
| Gimhaedanseongsi | PCA | Sancheongkurigam | PCA |
| Daimaban | PCA | Parter | PVA |
| Jangseongsetogari | PCA | Uljinwonsi | PCA |
| Jeongupbansi | PCA | Wonmi | PCNA |
| Gwangjubaesi | PCA | Yeonginjangjunsi | PCA |
| Jowan | PCNA | Bonghwagolgam | PCA |
| Gimhaechalgam | PCA | Jinyangmulbansi | PCA |
| Gangneungjangsi | PCA | Emon | PCA |
| Goseongdongcheolsi | PCA | Sangjuhgdongsi | PCA |
| Hamyangbansi | PCA | Taishu | PCNA |
| Goesangolgam | PCA | Sancheongdanseongsi | PCA |

* PCA: pollination constant astringent, PCNA: pollination constant non-astringent, PVNA: pollination variant non-astringent, PVA: pollination variant astringent.

whether the final library product was suitable for the GBS analysis, QC analysis was carried out (Fig. 1).

### 3.2. GBS sequencing data

The barcode and adapter sequences were removed from each sample file, and sequence quality trimming was performed (Table 3, Table 4). The Illumina HiSeq 2500 platform, was then used for paired-end read GBS sequencing. The total number of reads was 529.4 million and the total read length was 53.5 Gbp, with a mean of 101 bp. The sum of the raw reads from each sample was 499.2 million, with a mean of 5.3 million. The total length of the raw reads was 50.4 Gbp, with a mean of 0.5 Gbp. The sum of trimmed read numbers was 447.5 million with a mean of 4.7 million. The total length of trimmed reads was 37.3 Gbp, with a mean of 0.4 Gbp. The average length of trimmed reads was 83.2 bp. The number of trimmed reads was 89.7% of the total number of raw reads (Table 4).

### 3.3. Mapping

The clean reads of each sample obtained through demultiplexing and sequence quality trimming were mapped to the reference genome, and statistical values were generated. The mapping of clean reads used the BWA (0.6.1-r014). By downloading the RNA sequence from the NCBI and using the de novo assembly, the representative transcript was established as the reference (Table 5). From the overall 447.5 million trimmed reads, the mapped reads number was 108.9 million and the average mapped reads number was 1.1 million, accounting for 24.2% (data not shown).

### 3.4. SNP detection per sample

The raw SNP of each sample was used to produce an integrated SNP matrix for the 95 GBS persimmon samples. SNPs that satisfied the filter criteria were classified as homozygous, heterozygous, and neither homozygous and heterozygous. The total number of SNPs identified across all samples was 1.7 million. Among them, there were 437,000 homozygous SNPs and 385,000 heterozygous SNPs. The variety with the greatest and fewest identified SNPs was 'Yeongjinbansi' (total of 6,834) and 'Taishu' (total of 846), respectively. The number of homozygous and heterozygous SNPs ranged from 1,933 to 6,834 (mean 4,608), and 846 to 5,297 (mean 4,047), respectively (data not shown).

### 3.5. GBS read depth analysis to develop molecular markers for differentiating persimmon varieties

During the evolutionary process of persimmon, the chromosome number multiplied so that the species now comprises a hexaploid genome ($2n = 6\times$, $n = 15$). In persimmon, the genetic characteristics are those of heterozygotes. As it is a hexaploid plant, selecting the SNP coordinates that clearly distinguishes each variety is very difficult. Even when the SNP coordinates of heterozygous type have been selected, discrimination from the homozygous type becomes almost impossible in practice due to the small read depth or a biased read depth according to the combination (1:5, 2:4, 3:3, 4:2, 5:1) of the heterozygous type. Thus, in this study, polymorphic SNPs were selected by first selecting common SNPs for each group among the 133,819 coordinates of the

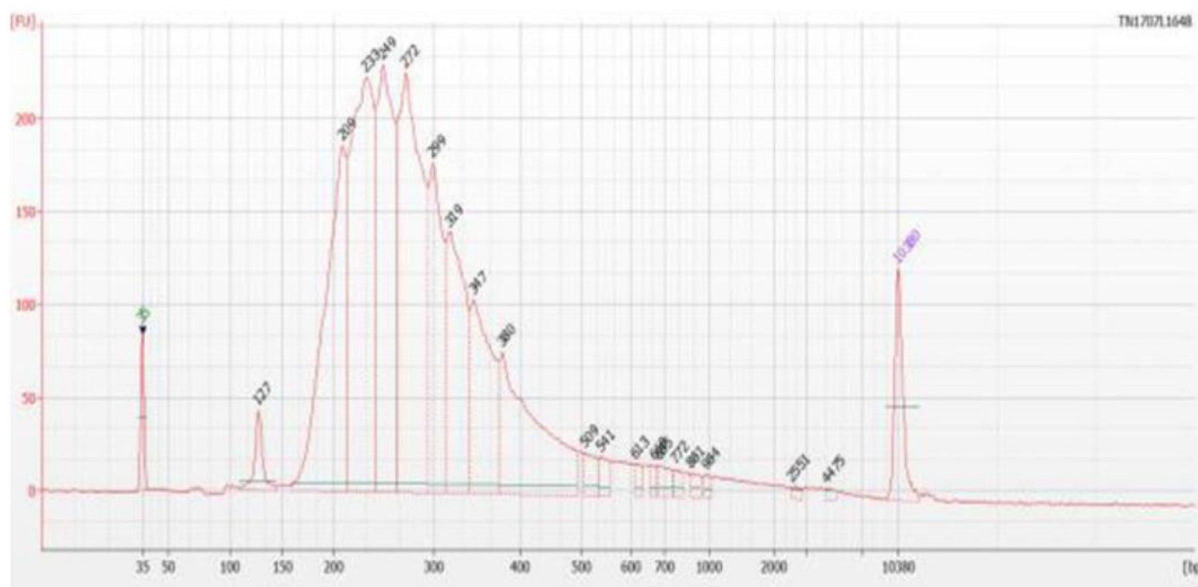


**Fig. 1.** Representative bioanalyzer image of GBS library-QC report.

**Table 3**
Summary of sequencing raw data.

| No. of barcode | No. of samples | No. of reads | Avg. length (bp) | Total length (bp) |
|---|---|---|---|---|
| 95 | 95 | 264,713,012 | 101 | 26,736,014,212 |
| | | 264,713,012 | 101 | 26,736,014,212 |
| | Total | 529,426,024 | Total | 53,472,028,424 |

**Table 4**
Summary of the GBS sequence data after alignment to the reference sequence.

| | Total | Average/plant |
|---|---|---|
| Total raw reads | 499,171,496 | 5,254,437 |
| Trimmed reads | 447,495,724 | 4,710,481 |
| Total length of raw reads (bp) | 50,416,321,096 | 530,698,117 |
| Total length of trimmed reads (bp) | 37,284,300,999 | 392,466,326 |
| No. of mapped reads | 108,876,644 | 1,146,070 |
| Total length of mapped regions (bp) | 285,777,869 | 3,008,188 |

integrated SNP matrix for the 95 persimmon samples, then comparing those common SNPs per group. Among the selected polymorphic SNP coordinates, those that discriminated between homozygous and heterozygous types were primarily selected. Among these, by examining the ratio of read depth for the heterozygous type, 49 SNP coordinates that clearly discriminated between homozygous and heterozygous types were finally selected. Fifteen SNP coordinates, among the 49, were then used as the molecular markers in the KASP assay for the identification of persimmon varieties.

### *3.6. SNP marker validation through KASP*

The selected SNP coordinates were used in developing 15 new KASP markers (Table 6). To verify the potential use of these markers, validation was performed using the persimmon accessions and hybrid seedlings. KASP genotyping comprises the KASP assay mix, KASP master mix, and samples. The KASP assay mix consists of three allele specific primers (two forward primers and one reverse primer), each with a linker (allele specific tail) for the attachment of a fluorescent probe (Table 7). The KASP master mix contains the fluorescent probes (FAM, HEX) that can attach to the primers in the KASP assay mix. The fluorescent probes attach to the allele specific tail in the primer. Genotyping is based on the location of scatter spots expressed by the fluorescent probes. For homozygous SNPs, the fluorescent probe binds to only one allele specific primer to form a scatter spot on the top left (*Y*-axis) or bottom right (*X*-axis). For heterozygous SNPs, the simultaneous binding of the fluorescent probe to two allele specific primers forms a scatter spot in the middle between the *Y*- and *X*-axes. This allows discrimination between the homozygous and heterozygous types. For the simultaneous genotyping of multiple samples, the scatter spots form a cluster based on each SNP type so that the SNP type of each sample can be quickly checked (Fig. 2). The analysis of the 15 KASP primers for 32 varieties of persimmon accessions and hybrid seedlings revealed that the scatter spots formed a cluster for all varieties, which clearly discriminated between the homozygous and heterozygous types (Table 8 and Fig. 2).

## 4. Discussion

Persimmon trees are a traditional fruit tree in Korea. Persimmon fruit has long been closely associated with life in Korea. Our ancestors consumed the fruit as a dessert or a medication. Most indigenous persimmon species in Korea are astringent persimmons. These are distributed mainly in the south and north Gyeongsang provinces, south and north Jeolla provinces, and the Yeongdong region in Gangwon province. Classification of astringent persimmons in Korea has focused on horticultural characteristics. Cho and Cho [39] classified 186 species as being nationally distributed.

Recent studies have utilized DNA molecular markers to discriminate cultivars in several crops. The use of DNA molecular markers is based on the differences in DNA base sequences. The various methods that have been developed and used include including randomly amplified polymorphic DNA, restriction fragment length polymorphism, amplified fragment length polymorphism, and simple sequence repeats. The GBS analysis used in this study is based on the NGS technique, in which only the restriction enzyme sites are selectively analyzed in sequencing to allow a rapid and inexpensive analysis of many samples.

In this study, numerous SNP markers were identified by GBS analysis. Forty-nine SNP markers that allowed the identification of persimmon varieties were selected using the GBS analysis for persimmon accessions and hybrid seedlings. Of these markers, 15 led to a successful primer and probe designs for KASP assay. Real-time PCR demonstrated that these markers could clearly differentiate the homozygous and heterozygous types. The remaining SNP markers were thought to require a suitable PCR condition or to have duplicated loci or unsuccessful design for the primer in regions surrounding the SNP [25,40,41]. These identified molecular markers are the first KASP markers to be developed for the identification of persimmon varieties. By genotyping, these 15 KASP markers accurately identified different regions in 32 varieties of persimmon accessions and hybrid seedlings. Recently, disputes have frequently arisen among persimmon farms concerning the introduction of new varieties. Since persimmon is a perennial plant, a long time (3–5 years) is required before the growth of fruits, which are needed to determine whether a new variety has been introduced. Even when fruit growth occurs, they can be similar in shape, which complicates the visual identification of varieties. In such cases, the use of markers that can specifically identify varieties could enable an easy and rapid identification of different varieties of persimmon. Recent studies in Korea are proactively focusing on the development and distribution of new cultivars, with the goal of provide a substitute for the sweet persimmon varieties introduced from Japan. In cases where mixing

**Table 5**
Published representative *D. kaki* transcripts.

| Representative transcript No. | Total length (bp) | Min. length (bp) | Max. length (bp) | Avg. length (bp) | N50 length (bp) |
|---|---|---|---|---|---|
| 31,258 | 30,524,525 | 200 | 10,510 | 976 | 1,695 |

*The data used in *De novo* assembly (NCBI accession number: SRX2212171, SRX2212170) [34].

**Table 6**
The sequences information of 15 SNPs finally selected for KASP assay.

| SNP markers ID | Flanking sequences |
| --- | --- |
| Persimmon C | ATCATATCTCGCCCAAAGCTGAGGCTCCACCC<br>TCCAAAAACACACCGCATCGAACCCCGTAACCCCCCGAAAGCCTCTCTCTCGCTCTCGGCCATG<br>GGGGCTCACCGCCGCTTCGACCCGGTTTCGAA<br>ATGCAGCAGCGAGGGAAGGTCCAATCAAACAGTGGCCTCCGACCTCGAC<br>GGCACGCTCCTCGTCTCGACAAG[A/C]GCATTTCCTTATTTCCTTCTCGTCGCCCTCGAAGCCGGCAGCCTAGTACGCGCTCTGCTCCTTCTGGCCT<br>CGGTTCCGTTCGTCTACTTCACCTACCTCTTCGTCTCTGAAGCC<br>TTCGCCATCCAGACTCTGATCTTTATCGCCTTCGCCGGAATCAAGATCCGAGACATTGAGCTCGCGGCGAGGTCGGTGCTGCCC<br>AAGTTCTACTCCGAGGACGTCCATCCGGAGACTTGGAGGGTTTTCAATTCCTTCGGAAAGAGATATATAATCACTGCGAATCCGAGGATTATGGTCGAGCAT |
| Persimmon D | CTCAGCTCCATTGGAGTACGAGAAGAAATACATGGACAATCACCAACCAGCTCGTACATTGTTGCGTGCAAT<br>AAGCAAGGAATTGTTGAGTTTAAATGAATACCATGCTAAGACCATAGCAAC<br>AGTCTTGGGGAAGCCAATGCTGATGGCTATTTTAGAAGAAAATGTAGATTTTGTC<br>GCAGTAATTATTAAAGATTGCCCAGATATGCTGCGGATTGAGATCGATGGATTAACACTTTTTTCATA<br>CGCAATTTCAAAAGGCCTTCGGAAAATCT<br>ACAAGTTTGCATGTACAAGGCCTGA[G/A]ACGGGACTTTTAGCAACCGTACTTCCCGATCGCTATGGTAACAC<br>TATCCTTCATCAAGCTGCAAAGTTATCACTGTCCCAATCTCTCGGTCCAGCTATAAAGATGCAGATAGAGCTAC<br>AACGATTTAAGGAGGTGGAGAAGATGGCACCTATGTGCAGAACAGTAGTGAACAATATGAACCAAACTCCAAAAGATTT<br>ATTTATAATGGAACACAAGGAACTTAAGAAAAACGCACGAGACTGGTGGAAGGA |
| Persimmon E | TTTGATGCTTTTATAGGTTCTACGGTTGACCTGTATGCACACACGGATTCAGTTTTTGGACCTGGAAAAAACTTGGA<br>AGATGAAAGACTGAAGGATACTTCAACACTTTTGGCTTCAGCTACTAATGACTGGAG<br>TGAAGATGGCCTTTGGAATAACTCAAACTTCTCGGCATCTAGCCTGACTGGACATGTTGAGGCAACCATCAAGAGTAAGG<br>ATGGGATAGCAGCAGATAAATTAGACACTCCTTCTTCAAGTGTTGATTGGTTTTTGGATGATCAA<br>TGGCAAACCAACAGCATGACT[A/C]CTCTTGACAACAAGACAAGCAAAGAAGATGATTCGTTTAATGTGTGGAATGATTTTGCAAGTTCTACTAGGAC<br>CCAAGGTGCTTCAACAAATTCGTTGACACAAAGTACTTATCAGAATGCTGCTGTTGACGAACAAACATCAAATACAATTCTTCCGGGCTCAACCAACA<br>ACTTTCAGGAAATGGATTTTGGTAGTTTTTCACAATCAGATCTCTTTTCTGGATTATCTAACAATCAGCATGATTCTGTAGAAGTGAAC<br>AGCATGCAATCAGAAGTCCATGTCTCACGGAGGATGGATG |
| Persimmon I | TGAGGAGCTTCTTCCTCTGATGATGTGTGCAATTGAGCGCCATCCAAACAGCGGCACACGGGATTCCTTGACCCACACATTG<br>TTTAACTTAATCAAACGCCCAGATGAACAGCAGAGACGAATTATAA<br>TGGAGGCCTGTGTAACGCTTGCGAAGAATGTAGGAGAAATGAGAACAGAGACAGAACTACTTCCCCAGTGTTGGGAACA<br>AATTAATCACATGTACGAGGAGCGCAGGCTGCTTGTTGCTCAATCATGTG<br>GAGAGCTTGCAGAATTTGTTCGCCCTGAGATTCGTGATTCCCT[T/C]ATCTTGTCTATTGTGCAACAATTGATAGAGGATTCCG<br>CAACTGTTGTCCGTGAAGCTGCTGCTCATAATCTGGCATTGCTCCTTCCGCTCTTCCCAAACACGGACAA<br>ATATTTCAAGGTTGAGGAGATGATGTTT<br>CAGTTGGTTTGCGATCCCTCTTGGGTGGTTGTGGAAACGACAATCAAAGAATTGGTCCCTGCTTTAATAAAATGGGGAA<br>ACAAGTTGGATCATATATTACGAGTTCTACTCTCTCACATCTTAAGCTCTGCTCAACGTTGT<br>CCGCCACTTTCGGGGGTTGAAGGT |
| Persimmon J/Y | TTCCAGCATGTATGCTCCCAAACGTTGAATCGGTTCACCGGACACCGACACCATCTGGCGCAGATCAGTTATCAGCCCTTGTGTGGCATACAG<br>GTCATTTTCTGATACTGCTTTTGCGCACGCAACCAGAGCCTGTCTCAAGTCCCGT<br>CTCGGGATTTCTTCCATCGTTTGTCTCCAGCTCTCCATCTCCATTGAGGCTACACCTCCCTGCACGGTACTATCATAACTGTCAAATAAATCAG<br>AATCAGGTCCCAGCATTACAGTTTCCAGTTCCCGCAGCTTATGCTTAAGTTCATTGAC[G/A]TCTTCAGTTATGCAGGAACCGCTTCTTGGGGAGC<br>CGTAGTTGTTGTCGGGAGAGTAGTGCCCTAGTTCTGGAGGATATGACTGACACTCTTGCTG<br>CGACATGGGGCTTTCACCGGCTGAGAAACTGAAAGTCGAGGGTGAATTGTGGACAGTG<br>TAACAGCCAGTTGCTGAAGATGACTCCAGGGTCCGATACTTTTCCCGGGAATTCTGCAAGAAGTTACTCCCCTGGCTGCTGTTACCATGGCTGAA<br>CTGGGGGCCCAAGTCTTGAAAATAGGGATCAACCTCTTGCACCAGCTTATAA |
| Persimmon K | CTTTTTTTTTCCTTCCTTTTCCAGAAAACAATTAGCAACTACTTGTTAACTCCCCAAAAGAAGGGTATTTACACAAACTAGGTCAACTCTCAAAGTCG<br>GGGCTGGGTAAAGCATCTGTGCAAGATGAGCCATCACATCTTCAATGACCCCTATCTA<br>CCGGCATTGCTGCAGAATTTCCTTTGAAGCTAGCAGCAGCCACCAAACAACTGTACCATCGCCTCAGGAATCATGATCGCGACCTCCTCAAAGGATCC<br>CTTGTGAAGAAGGGATGCTTCAAGGCCTCCCGTGCCGTTAGCCTC[G/A]CCGAAGGATCGTATTTTAACAGCCCTTGCAGTAGATGTATTAGATCCCCGG<br>CCGAATGGTCTACCTGCTGCATGATTAGGTTCTGTAGCCGTGGGAG<br>CTTTAGAACGGCCTTAATACTTTCTCTTGATGTTGCGCCTTCCGGCCAGTCTAATCTCCCCTT<br>CCTGATGTATTTCTCGGCATGACGGTCTGCTCTCTTTAGCATGTGCTGGGGGAGTGGACCAAGTACCCTTTCCATCATGGCAAGGTGCTCCAAGTTCTCATGG<br>GTTTGAAACAATGCTTCACCCGTACACAATTCCACTA |
| Persimmon M | CACACGGATTCAGTTTTTGGACCTGGAAAAAACTTGGAAGATGAAAGACTGAAGGATACTTCAACACTTTTGGCTTCAGCTACTAATGACTGGAGTGAAGAT<br>GGCCTTTGGAATAACTCAAACTTCTCGGCATCTAGCCTGACTGGACATGTTGAGGCAA<br>CCATCAAGAGTAAGGATGGGATAGCAGCAGATAAATTAGACACTCCTTCTTCAAGTGTTGATTGGTTTTTGGATGATCAATGGCAAACCAACAGCATGACTACTCT<br>TGACAACAAGACAAGCAAAGAAGATGATTCGTTT[A/G]ATGTGTGGAATGATTTTGCAAGTTCTACTAGGACCCAAGGTGCTTCAACAAATTCGT<br>TGACACAAAGTACTTATCAGAATGCTGCTGTTGACGAACAAACATCAAATA<br>CAATTCTTCCGGGCTCAACCAACAACTTTCAGGAAATGGATTTTGGTAGTTTTTCACAATCA<br>GATCTCTTTTCTGGATTATCTAACAATCAGCATGATTCTGTAGAAGTGAACAGCATGCAATCAGAAGTCCATGTCTCACGGAGGATGGATGA<br>TGCAAAAGTTAAAGTTGACCTGGAAGGGGCTTATGGAG |
| Persimmon O | ACCTGAATCATCATCTCTATTTCCCGGATGTTGTAGTTATTATTTAAGTTGCAATCTACGATAGCATCCAGTCTTTTCTCCCTTTGAAGTTTCTTGACA<br>TGGTCAAGCAATAACACATCATCTTCTTCTTCCAGTCGTGAGAAGTCAATCGCCCTCTGACCTGTCACAAGCTCTAGCA<br>GCATTATCCCATAACCAAAAACATCAGTCCGCTCGGATGACTTTCCAGTCGACAAGTATTCAGGAGCTATGTGACCCATCGTCCCACGAACTT<br>GAGTTGTCACATTTGTCTTTCTCACATCC[G/A]<br>CTAACTTTGCCAGGCCAAAGTCACCAACAACTGCTTCAAAATCTTCATCCAGTAAAACATTAGCAGCCTTAACATCCCGATGAATAATTTTAGG<br>ATTACAGTGTTCATGAAGGTACTCCAGCCCACGTGCTGTGCCTAAAGCCACTCGTTTTCTTGTAGGCCAATCTAAAACAGGATCACCAG<br>GTTTAAGCTCTCGTAAACAATAAGCAACGCTTAGGTTCTGCATGAAGGGATACACCAAAAGGCGTTCTGTTGGTGTTGTGCAGAACCCGATCAACCG<br>TAATAGATTCCTGTGAACAG |

(*continued on next page*)

**Table 6** (*continued*)

| SNP markers ID | Flanking sequences |
|---|---|
| Persimmon P | TGGCAGTGGCCGAGGCACCAAAGCCAGCAACAGGAGCTGGCTCCATCCGGCTAGGATACCAGCTTGGAACACCTCCCATAACTCGAGGATCTTCATGAGATGGGGCAA<br>CAGGCTTGCCAAGTTCCCACTCACCATGCGCAAAATGGCATTTGTCACCAAATTTGCAGCCCTCAGCAGTGTTATATTTG<br>TTGCACATTTTGGTTTTAACTACTGGCGCAGAAGAACCATTGGGAACAGAAGCCGCCGCAGTCACATTCCTTGATACAGGAGCTGGCCCCAAGTTCATCATCTGTGCA<br>ACCA[T/C]ATTATATCCGCCAGGAACATAATGAAGGAAGTGGCAATTCTCTCCAAAAGGGCAGCCAGAAGTACTGAAAAACTTCG<br>TGCAAGGCTTCAATTTGCTTCCTAAACCAGTTGATGAGTCCATTTCTGTCCTGAATTTCTTAGCA |
| Persimmon Q | CTTCAGTCTCATCAGGCAATGACTCAGTGTAGCCTCCACAAGAACCGGTGTTGGTGCAATTGGAGCAAAGATCAGTGGTGGTCGTCGTGGT<br>GGCAAAAGAAGACCGGAAGGACAGCCGCACGGCGCTCGGCC<br>GAGTCTCTGGCAGCTCCATGAGAGGTTCATCAGGGTTCATTAAGATGTGAACCACCTTCCTCATCCTAG<br>GCCTCAACATGAAATCTGGATGCAAACAAGCCAGCCCCACAAGCAAAGCCCTTTTCACTTGTTCCTCGTCATATTTTTGGCCCTGCAGCTGCTTG<br>TCCA[T/C]ACAGTTCAGCAATGCCCCTGTTTCCTGCAAATCCCACACACTGTCCACCAGGC<br>TGTTTTCGTCCATGATCCCTCTTGATCTTTTCCCACACACCAGTTCCAGCACCACCATTCCAAAGCTGTACACATCCGATTCCGGTG<br>TGGCCCTCCCGGTGAAGCTGACTTCCGGGGCCAAGTAGCCGGGAGTCCCGGCTATAATGGTTGTGACCG<br>AGGCTGCGTCTTGGAGTAGTAATCTTGCCAGCCCGAAATCGCCTAGATGCGCATTGTAGTCTCCATCCAGCATCACATTGTTTGGCTTCAC |
| Persimmon S | GCGTCCCTCGTCCGTTGTCGCTGACACATATCGAGAAGGTGTTGCTGCTGCTGTTTCCCGTGTTCCTTGTCCCGTTGTCCGTCGTCGCTGCTTC<br>ATCTTCCTCTCTCCCTTCGCCCT[A/G]TCATCTCCTGCGTCACAGCGTTCCCGATTCGGCTTCACATCTCCGGCGTCACAGTGCCCCATCATCTCCATCTTCTTTGCTGCT<br>GCCACTGCCGCTGCTCGCCGGCGTCAGAGGAATTCGGATATGAGAGACTTGTTCAGGTG<br>CTCCTGTGTGTCTGCGTGGATGCATAAATAGGACATTCTCCAATTCTTTGCCAATGGGCTGAAACTTGGAAG<br>TTGCATCCTTACAACTACAGACATCGAACATCCTGTTTTGTATGGTGACGGAATTATCTTTTTGGATCACAGATCATTGATATGG |
| Persimmon T | GTTTTACCCACAAAGATCAGCCAACACTGTTGTAGTTGACTTCTGTTTTTCCCCCCCCCTTTTTTTCATCCCAGAGGACTAGCTGTTACTAGGTTGTTGCTCACAATAT<br>TGCCCTTAGGGCGTCCTCTGCATCGATGTTGGGAGCTAA<br>AGATGGAATAGACCCCGCCAATATGCTCAATGTATCGGCAGCAATATGATGAGAAGCTATATTCTTCCTGGAA<br>CAAAAGGGTCAAAACCTTCCACAACCCATGCTGGATTCATAGGCTTACTTTTACCATCAGTGTAATGATAAAGCACCGA[C/T]ACTCCAGGACCATGTAAGCCTGT<br>TTAGCGGCACCAGTTGCTGCGACAGGACAAATTAGTAGTTATACCAATGAAC<br>AAAGGCCAATTGCTAGTCCCTAACAGTGGCATCTGCAATTTTGAACTATGGATGGTGCAGATGATCCCCTC<br>TCAGCCCCTCTTCTTGTTGAGCCCCAACTGCTTGCTTTGTCTCTGTAGTGGAGACCTTTTTTGATGGAAAGTTCTGTA<br>AATACAGAATTGAATGGAATGCTTTATAATTTAGC |
| Persimmon V | AGGAAATGGATACGTCAAGATCACTTTCATGTACAAAACTACTTGAAACTGATGGTTCAGAGGCAAAGCCCTTGTCTACCTTTGACTCTTCGAATAGTAAGCT<br>TGAAGATGACTGCTCTGAAATTGGAGCAAAGCAGCACTTTGGTTCT<br>CTTACTGCAGATTGTAGCGACTCATTTCATACCATGGGATTTTCATCTCCATTGCCTAGCATGCCTCTTTG<br>GAAGACAATGGAGGATATCTCAGCACCTTGTATGCCGATCAATGATAGTCCTGAAGATAAAGGGCAGGCTGCAGAATATG[T/C]ATCTGAACTTCCTGATA<br>AAGATAATGCTAAAAGTAAAAGTTCATTAGAGCATGCTGTTAGGCATTCA<br>GGAGCCACATTGGCTGACAAACATAATAGTGATGGCTTCTTTAGTTCAGCTGCTATATCAGACAGTGAATTTGTAGACCTCCCAG<br>ACTCACTTTTGGACTTCTCAAATGAGGATGGGGTTCTCTTCGTAGATGTAGATCGGAAAAGCACATTGGA<br>TAAGTCTGGTTGTGATACCACACTGGAGTCTGGCTACCATAATAAACTGCTTTTGAGTTCTTCCAAGGATGTTGATGA |
| Persimmon W | TGAGTCGGACTGTTTACATGGACAACCTATCGCCACAGGTCACCGAAGCTGTCTTGAAGACTGCACTCAGTCAGTTTGTGAATGTGAACAAAGTTCATATCA<br>TTCCAAACTACACGCACCCAAGCGATGCAGCGGGTCGTGCCTTGGTTGAG<br>ATGGAGAATCCAAAGCAGGCTGAGCAAATTGTGCGTGAGATGGGTAACTTACCATTCATGATGTCAGGGAT<br>GCCAAGACCCGTCCGGGCACGTGCTGCTGATCCGGAGATGTTTGATGATCGTCCAAGGAAACCTGGGAAAAGGATTA[C/G]TTTCCGGTGGTTGGACCAGCAG<br>GACCCTGATTTCCAGGTTGCGAAGAAGCTCAAGGATCTGGCAA<br>GGAAAAATGCTGCGGAAGCGTCATTCATTCTGGAGCGGCAAGTGGATGAAGAAGAGAAGCTTGCAAACCAACAGTGGAAAACCCTGAAGGC<br>GAATTACAAGAAATATGAGCTGATAGATGGGGTGCTGGGCGATGGGACTGCTTTGCGATTAG<br>CAGAACGTTATAACACCCGAATCTTTGATGATAAATAATCCTTACATAACCCACCACCCTCCCCTGTTTCTACTTGCCCCAG |
| Persimmon AA | AGAATCAAGTGCAACAACATGCTCATCGCCAGCAGAGACAAGAATAATGTGGATACACTTTCTTCTAAATGAGTGGATTGCACGTGGCTGTAGCTCATTGTGCT<br>GCTCTCCATGGCCAAGGCAGAAATTGG<br>TACCAGACCCAAATGAGTGGACTGTCCCATCATTCATGACAGCCAAGGCATAACTTGGACCAGCAGCAATCTGAACTACAGATCCCATGCTCTCA<br>AGAAATTCTACAATTGTGGGTGTTGGCCTATCAAGGGTGTCACCATGGCCAAGCTGTCCCTGTGCATTCATTCC[G/A]CATGTGTAGACATGGCCTTGTCTTGTGA<br>GGAACATGGTAAAATTAAGCCCTGCAGCA<br>ACCTGCTTGCAAGCGATTCCATTTAATGCTTCCACAAGTCTGGGCCTGAATATTGGACGACCTGTATCTCTATGTCCACAACAAAATGA<br>TGAATTACCTCCACACGTAAAAACCTCTCCAGATTGCATGACAAAAGCAGCATGATTGTGGGAGGCCGAGACA<br>TGGGCCACATGGGCAGTAGATGGGAAACTAATCCTGGTAAATGCCACACATTGGGTTGTTTCAGACCCATGACCAAGAACA |

**Table 7**
Primer sequences of KASP markers.

| SNP markers ID | Primer_AlleleX (5′–3′) | Primer_AlleleY (5′–3′) | Primer_Common (5′–3′) |
|---|---|---|---|
| Persimmon_C | GACGAGAAGGAAATAAGGAAATGCG | CGACGAGAAGGAAATAAGGAAATGCT | GGCACGCTCCTCGTCTCRACAA |
| Persimmon_D | AAGTACGGTTGCTAAAAGTCCCGTT | GTACGGTTGCTAAAAGTCCCGTC | CCTTCGGAAAATCTACAAGTTTGCATGTA |
| Persimmon_E | CAATGGCAAACYAACAGCATGACTA | CAATGGCAAACYAACAGCATGACTC | AAAYGAATCATCTTCTTTGCTTGTCTTGTT |
| Persimmon_I | GCCCTGAGATTCGTGATTCCCTT | CCCTGAGATTCGTGATTCCCTC | CTCTATCAATTGTTGCACAATAGACAAGAT |
| Persimmon_J/Y | CAGCTTATGCTTAAGTTCATTGACA | CAGCTTATGCTTAAGTTCATTGACG | CGACAACAACTACRGCTCCCCAA |
| Persimmon_K | GGCTGTTAAAATACGATCCTTCGGT | GCTGTTAAAATACGATCCTTCGGC | CTTCAAGGCCTCMCGTGCCGTT |
| Persimmon_M | AAGACAAGCAAAGAAGATGATTCRTTTG | CAAGACAAGCAAAGAAGATGATTCRTTTA | TAGTAGAACTTGCAAAATCATTCCACACAT |
| Persimmon_O | GTCACRTTTGTCTTTCTCACATCCG | GTCACRTTTGTCTTTCTCACATCCA | AGCAGTTGTTGGYGACTTTGGCCT |
| Persimmon_P | CCAAGTTCATCATCTGTGCAACCAT | CAAGTTCATCATCTGTGCAACCAC | GAGAGAATTGCCACTTCCTTCATTATGTT |
| Persimmon_Q | GCCCTGCAGCTGCTTGTCCAT | CCCTGCAGCTGCTTGTCCAC | GACAGTGTGTGGGATTTGCAGGAAA |
| Persimmon_S | CGCTGTGACGCAGGAGATGAC | ACGCTGTGACGCAGGAGATGAT | CTGCTTCATCTTCCTCTCTCCCTT |
| Persimmon_T | CAGGCTTACATGGTCCTGGAGTA | AGGCTTACATGGTCCTGGAGTG | TTACCATCAGTGTAATGATAAAGCACCGA |
| Persimmon_V | GCATTATCTTTATCAGGAAGTTCAGATG | AGCATTATCTTTATCAGGAAGTTCAGATA | GCCGATCAATGAYAGTCCTGAAGATAA |
| Persimmon_W | CAAGGAAACCTGGGAAAAGGATTAC | CAAGGAAACCTGGGAAAAGGATTAG | CTGCTGGTCCAACCACCGGAAA |
| Persimmon_AA | AAGACAAGGCCATGTCTACACATGT | GACAAGGCCATGTCTACACATGC | TCACCATGGCCAAGCTGTCCCT |

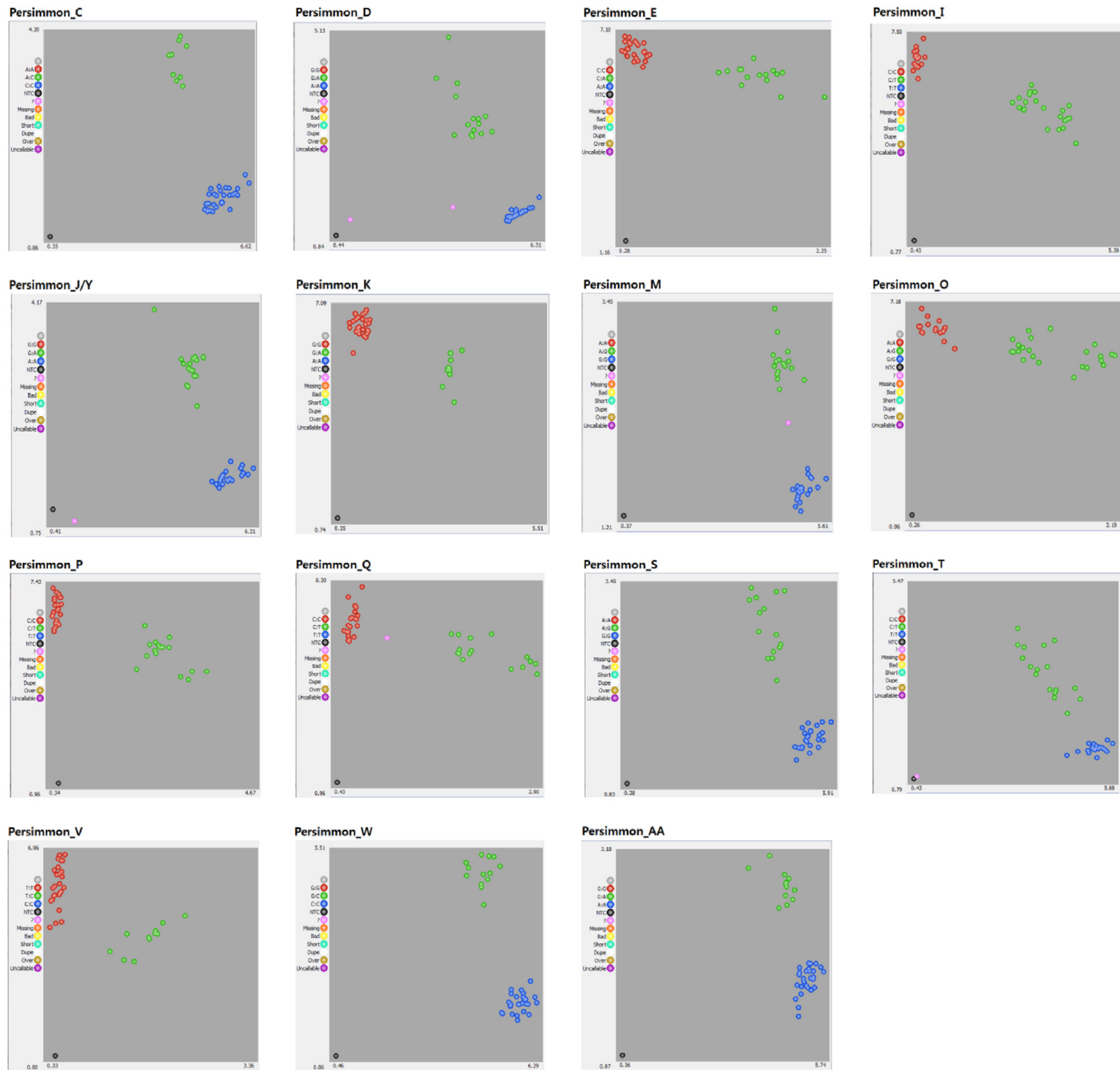


**Fig. 2.** Genotyping results of persimmon cultivars using the KASP assay technique. The scatter plots with the *X*- and *Y*-axes represent the allele discrimination of the genotypes. The red and blue dots denote the homozygous alleles and the green dots denote the heterozygous alleles.

**Table 8**
Validation of KASP markers and the list of genotyped persimmon cultivars.

| Cultivars | SNP markers for KASP assay | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1* | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
| Goseongchambansi | C** | R | C | C | A | G | G | A | C | C | G | T | T | S | A |
| Changpyeongpasi | C | A | M | Y | A | G | R | A | C | C | G | T | T | C | R |
| Noansubunsu | M | A | C | C | R | G | G | A | C | Y | G | Y | T | C | A |
| Chuyeon | C | A | M | C | A | R | R | A | C | Y | R | Y | Y | C | R |
| Zenzimaru | C | A | M | Y | R | G | R | R | C | C | G | T | T | S | A |
| Gimhaedanseongsi | C | A | C | C | A | G | G | A | C | C | G | Y | T | C | A |
| Daimaban | C | A | C | C | A | G | G | A | C | Y | G | T | T | C | A |
| Jangseongsetogari | C | A | C | Y | A | G | G | A | C | C | G | T | T | C | A |
| Jeongupbansi | C | A | C | Y | A | R | G | A | C | C | G | Y | T | G | A |
| Gwangjubaesi | C | A | M | C | A | G | R | R | C | C | R | T | Y | G | A |
| Jowan | M | A | C | C | R | G | G | R | C | Y | G | T | T | C | A |
| Gimhaechalgam | C | A | C | Y | A | G | G | A | Y | C | G | T | T | C | A |
| Gangneungjangsi | C | R | C | Y | A | G | R | A | Y | C | G | T | T | C | A |
| Goseongdongcheolsi | C | R | C | Y | A | G | R | A | Y | C | G | T | T | C | A |
| Hamyangbansi | C | A | M | Y | A | G | R | A | Y | C | G | T | T | C | A |
| Goesangolgam | C | A | M | C | A | R | R | A | C | C | G | T | T | S | A |
| Gwangjupasi | C | A | M | Y | A | G | R | A | Y | C | G | T | T | C | R |
| Wangchu | M | A | C | Y | A | G | G | R | C | C | G | T | Y | C | A |
| 05-14-64 | C | A | M | Y | A | G | R | R | Y | C | R | Y | Y | C | R |
| Gampung | C | R | M | Y | R | G | R | R | C | Y | G | T | Y | C | A |
| Jangseongsusi | C | A | C | Y | R | G | G | A | C | C | G | T | T | C | A |
| Sancheongkurigam | M | A | C | Y | A | G | G | R | C | C | G | T | T | S | A |
| Parter | M | R | M | Y | R | R | R | A | Y | C | R | Y | Y | C | A |
| Uljinwonsi | C | R | C | C | A | R | G | A | Y | C | G | T | T | C | A |
| Wonmi | C | R | C | C | R | G | G | R | C | Y | R | Y | Y | C | A |
| Yeonginjangjunsi | C | A | M | C | A | G | G | A | C | C | R | T | Y | S | A |
| Bonghwagolgam | C | A | C | C | R | G | G | A | C | C | G | T | T | S | A |
| Jinyangmulbansi | C | R | C | Y | R | G | G | A | C | C | G | T | T | C | R |
| Emon | C | A | C | Y | R | G | G | A | C | C | R | T | T | C | A |
| Sangjuhgdongsi | C | A | C | Y | A | R | G | A | C | C | G | Y | T | C | A |
| Taishu | C | A | C | C | R | G | G | R | C | Y | G | T | T | C | A |
| Sancheongdanseongsi | C | A | M | C | A | G | R | A | C | Y | G | T | T | S | A |

* SNP marker ID, 1: Persimmon_C, 2: Persimmon_D, 3: Persimmon_E, 4: Persimmon_I, 5: Persimmon_J/Y, 6: Persimmon_K, 7: Persimmon_M, 8: Persimmon_O, 9: Persimmon_P, 10: Persimmon_Q, 11: Persimmon_S, 12: Persimmon_T, 13: Persimmon_V, 14: Persimmon_W, 15: Persimmon_AA.
** Nucleotide base, M: A or C, R: A or G, Y: C or T, S: G or C.

of a foreign variety is suspected during the seedling growth stage, the variety identification markers could be used to remove the foreign variety. This could block the mixing of varieties from the stage of seedling growth.

## Conflict of interests

The authors report no relationships that could be construed as a conflict of interest.

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