1. Introduction

The family Plantaginaceae (formerly Veronicaceae) is divided into 10 tribes viz., Plantaginiae, Angelonieae, Gratioleae, Antirrhiniae, Caliliricheae, Sibthorpieae, Chelonieae, Veroniceae, and Russelieae (Stevens, 2001) and considered to be a monophyletic group closely allied to the Scrophulariaceae (Rahn, 1996; Olmstead et al., 2001; Stevens, 2001; Albach et al., 2005a). Earlier studies on Plantaginaceae (Rahn, 1996) found it difficult to find a sister group for the monophyletic Plantaginaceae which often appeared as sister to Buddlejaceae, Oleaceae, Globulariaceae, and Lentibulariaceae. Plantaginaceae was further separated from its allied families based on advanced morphological characters like phyllodial and parallel-veined leaves, presence of hairs in the leaf axil, protogynous flowers, scarious corolla, absence of a disc, etc. and it was considered to be a monogenic family with the only genus Plantago divided into six subgenera. Later, The Angiosperm Phylogeny Group (1998) and Olmstead et al. (2001) revealed that the family is not monogenic and segregated various genera under the family Plantaginaceae. Olmstead et al. (2001) also revealed the presence of a total of nine tribes in family Plantaginaceae (Veronicaceae group), however, the tribal placement was not confirmed due to poor support, polytomy and paraphyletic placement of few tribes. Albach et al. (2005a) further classified Plantaginaceae into 12 different tribes quoting the family highly heterogeneous which includes plants ranging from aquatic to alpine habitats. Lately, APG-IV (Chase et al., 2016), reclassified Plantaginaceae with 10 tribes. None of the earlier studies, however, made efforts to understand the phylogenetic placement of several tribes of Plantaginaceae. Sparse attempts have been made to understand the plastome structure, evolution, and phylogenomics. In the present study, we have made an effort to understand the gene content and plastome evolution in the family Plantaginaceae using the newly generated plastome sequence of Veronica ovata subsp. kiusiana, a taxon native to SE Asia. In the first-ever attempt, plastomes of seven out of 10 tribes of Plantaginaceae have been compared to understand the evolution across the tribes of Plantaginaceae. The size of the plastome of V. ovata subsp. kiusiana is 152,249 bp, showing a typical quadripartite structure containing LSC, SSC, and two IRs with the sizes of 83,187, 17,704, and 25,679 respectively. The plastome comparison revealed the unique deletions in ycf2 and ndhF genes of members of different tribes, and also revealed high nucleotide variable hotspots. The study also revealed six highly variable genes and intergenic spacer viz. rps16, rpl15-ycf1, cssA-ndhD, ndhC-trnV, petN-psbM, and ycf1-trnN as potential DNA barcodes for the genus Veronica. The phylogenomic study revealed the sister relationship between V. ovata subsp. kiusiana and V. persica and also suggested the tentative placement of seven tribes in the family Plantaginaceae.
genera which remained unresolved. Very few studies used the molecular markers to resolve the complexity within the family Plantaginaceae (Olmstead et al., 2001; Albach et al., 2005a & b; Estes & Small, 2008; Refulio-Rodriguez & Olmstead, 2014; Yousefi et al., 2016). Olmstead et al. (2001) carried out molecular systematic study of the family Scrophulariaceae based on three chloroplast markers viz. rbcL, ndhF, and rps2. They also considered Plantaginaceae (formerly Veronicaceae) in the study. Albach et al. (2005a) analyzed the relationships within Plantaginaceae. They circumscribed the family into 12 tribes based on four nuclear and plastid markers. Albach et al. (2005b) attempted to unravel the diversification of tribe Veronicae in New Guinea using the markers ITS and trnL-F. Yousefi et al. (2016) tried to resolve the relationships within the tribe Antirrhinace for the Flora Iranica region based on markers ITS and rpl32-trnl. Moreover, the efficacy of the markers was never tested in any of the phylogenetic studies. Recently, Choi et al. (2016) reported plastome sequences of two species of Veronica viz. V. nakaiana Ohwi and V. persica Poir. The study also reported plastome sequence of Veronicastrum sibiricum (L.) Pennell, an allied genus of Veronica.

In the present study, we have sequenced the plastome of Veronica ovata subsp. kiusiana (Furumii) Albach a southeast Asian endemic species and compared it with the V. nakaiana Ohwi and V. persica Poir. plastomes to understand their nucleotide diversity and codon usage. Further, the data was used to compare the plastome structure and evolution across the tribes of Plantaginaceae.

2. Material and methods

2.1. Sampling, DNA extraction, and sequencing

The voucher specimens of Veronica ovata subsp. kiusiana were collected from Seorak Mountain, Yangyang-gun, Gangwon Province of the Republic of Korea in July 2014. The specimens were deposited at the Herbarium of Korea Research Institute of Bioscience and Biotechnology (Voucher number KRIB 0004193). Seeds from the same specimen were germinated and DNA was extracted from fresh young leaves. DNA extraction was carried out using the DNeasy Plant Mini Kit (QIAGEN, Cat. No. 69104) according to the manufacturer’s protocol.

TruSeq DNA PCR-Free Library Prep Kit (Illumina) was used for library preparation. Total genomic DNA was sheared and short inserts (550 bp) paired-end libraries prepared by adding unique barcodes. After the library preparation, the DNA sample was run in a single lane of an Illumina HiSeq 10X with a read length of 151 bp. The final raw data output was 13.72 GB including both forward and reverse reads.

2.2. Plastome assembly and annotation

The raw reads obtained after Illumina sequencing were analyzed using FastQC V0.11.7 (Andrews, 2010) software to ensure the quality of reads and Phred score. The adaptors and low-quality bases were trimmed by using Trimmomatic V0.38 (Bolger et al., 2014). The high-quality reads without adaptors were extracted and were used for the final assembly of the plastome. Both forward and reverse reads with a read length of 150 bp and insert size of 300 bp, with seed sequence were imported in NOVOPlasty V3.8.2 (Dierckxsens et al., 2017). The De-novo as well as the reference-based assembly was performed to confirm the accuracy of the assembled plastome. The closest allied reference of Veronica nakaiana (KT633216) was selected for reference-based assembly. The assembly and orientation of Inverted Repeats (IRs), Large Single Copy (LSC), and Small Single Copy (SSC) regions were confirmed by NCBI blast and graphic view using Geneious prime 2020.1.2.

2.3. Genome comparison

Representative plastomes of seven tribes of Plantaginaceae were considered and aligned using a Geneious prime 2020.1.2 plugin MAFFT v7.450 (Katoh and Standley, 2013). The representative plastomes of seven tribes were considered and presented in Table 1. All the sequences were available on the NCBI database except V. ovata subsp. kiusiana (generated during this study) and Antirrhinum majus L. which was received from the Chinese Academy of Sciences, Beijing, China (Courtesy: Snapdragon Genome Database©Yongbiao, 2020). The compared plastomes were analyzed for conserved and variable regions using MultiPIPMaker (http://pipmaker.bx.psu.edu/pipmaker/) (Schwartz et al., 2000).

2.4. Simple sequence repeats (SSRs) and repeat analysis

The assembled plastome of Veronica ovata subsp. kiusiana was used to find out the Simple Sequence Repeats (SSRs) by using the online server MISA (Beier et al., 2017) (http://webblast.ipk-gatersleben.de/misa/index.php?action=1). The threshold for mono, di, tri, tetra, penta, and hexa nucleotides was kept as 10, 5, 4, 3, 3, and 3 respectively. The number, position, and size of SSRs were compared with other Plantaginaceae members. While REPuter (Kurtz et al., 2001) (https://bibiserv2.cebitec.uni-bielefeld.de/reputer), an online server was used to detect the position of forward, reverse, complement, and palindromic repeat sequences of V. ovata subsp. kiusiana in plastome. The threshold for repeat length was set to 30 with more than 90% similarity and hamming distance was set to 3. The repeat sequences of V. ovata subsp. kiusiana was compared with the other Plantaginaceae members.

2.5. Nucleotide diversity and interspecific variation in Veronica

The Single Nucleotide Polymorphisms (SNPs), and codon usages were analyzed separately using DnaSP v6.12.01 software (Rozas et al., 2017) for three plastomes of Veronica. Three plastomes of Veronica spp. were aligned using MAFFT v7.450 (Katoh and Standley, 2013). The aligned sequences were imported in DnaSP v6.12.01 software to perform Nucleotide diversity (Pi) and sliding window analysis.

2.6. Codon usage

The Protein-coding genes of three species of Veronica viz. V. ovata subsp. kiusiana, V. nakaiana, and V. persica were imported in DnaSP v6.12.01 software (Rozas et al., 2017) and were analyzed separately. The percent codon usage was further confirmed using Geneious Prime 2020.1.2 (https://www.geneious.com). To examine the frequency and uniformity of Synonymous codon and codon biases, the Relative Synonymous Codon Usage (RSCU) was also determined in DnaSP v6.12.01 software (Rozas et al., 2017).

2.7. Phylogenetic analysis

To understand the phylogenetic placement of Veronica ovata subsp. kiusiana in Plantaginaceae, samples were selected based on its allied genera and tribes from the family. The ingroup and
The outgroup consisted of 14 and three taxa respectively from the closely allied Scrophulariaceae family. A total of 17 plastomes were aligned using a Geneious prime 2020.1.2 plugin MAFFT v7.450 (Katoh and Standley, 2013). The aligned sequences were further used for phylogenetic tree construction using the Maximum Likelihood (ML) method in IQtree 1.6.12-MacOSX (Nguyen et al., 2015). The best fit model was tested using the IQtree Model test pipeline, and GTR + F + R4 was selected as the best fit model using the Bayesian Information Criterion (BIC). To construct the phylogenetic tree, 1000 Bootstrap replications were used of which the optimal

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**Table 1**

Representative species and tribes included in this study.

| No. | Genus     | Species                        | Tribe      | GenBank Accession No. |
|-----|-----------|--------------------------------|------------|-----------------------|
| 1   | Veronica  | Veronica ovata subsp. kiusiana | Veroniceae | MT671999              |
|     |           | Veronica nakaiana              |            | NC031153              |
|     |           | Veronica persica               |            | NC031344              |
| 2   | Veronicastrum | Veronicastrum sibiricum (L.) Pennell | Veroniceae | NC031345              |
| 3   | Hemiphragma | Hemiphragma heterophyllum Wall. | Sibthorpieae | NC045398             |
| 4   | Antirrhinum | Antirrhinum majus L.           | Antirrhineae | http://bioinfo.sibs.ac.cn/Amm/ |
| 5   | Penstemon | Penstemon fruticosus (Pursh) Greene | Cheloneae | MG201976             |
| 6   | Bacopa    | Bacopa monnieri (L.) Wettst.    | Gratioleae | NC047469             |
| 7   | Digitalis | Digitalis lanata Ehrh.         | Digitalideae | NC034688            |
| 8   | Plantago  | Plantago ovata Forssk.         | Plantagineae | MH165324            |

Fig. 1. Plastome map of *Veronica ovata* subsp. *kiusiana*. Genes drawn inside the circle are transcribed clockwise and those outside are counter-clockwise. Genes belonging to different functional groups are shown in different colors. The innermost circle denotes GC content across the plastome.
The Illumina sequencing generated 36,725,388 reads. The average organellar coverage was 1,527X. The final trimmed reads obtained from Trimmomatic V0.38 were assembled in NOVOPlasty which resulted in two circular contigs. Both were manually checked and mapped with the reference sequence in Geneious Prime 2020.1.2. The final assembled plastome length was 152,249 bp.

3. Results

3.1. Plastome assembly

The Illumina sequencing generated 36,725,388 reads. The average organelle coverage was 1,527X. The final trimmed reads obtained from Trimmomatic V0.38 were assembled in NOVOPlasty which resulted in two circular contigs. Both were manually checked and mapped with the reference sequence in Geneious Prime 2020.1.2. The final assembled plastome length was 152,249 bp.

3.2. Plastome characteristics

The plastome of Veronica ovata subsp. kiusiana exhibits a typical plastome structure, having LSC (Large Single Copy), SSC (Small Single Copy), and a pair of IRs (Inverted Repeats). The total length of the plastome is 152,249 bp while that of LSC, SSC, and IR regions is 83,187, 17,704, and 25,679 bp respectively (Fig. 1, Table 2). The GC content of the whole plastome is 38%. The plastome sequence generated has been deposited to NCBI (Accession no. 460554.508 representing the consensus tree was selected. To visualize the output of IQtree, the tree file was imported in Figtree V1.4.4 (https://github.com/rambaut/figtree/releases) (Rambaut, 2018).

| Table 2 |

Comparison of major features of Veronica ovata subsp. kiusiana plastome with allied Plantaginaceae members.

| Plastome feature | Veronica ovata subsp. kiusiana | Veronica nakaiana | Veronica persica | Veronicastrum majus | Hemiphagrum heterophyllum | Antirrhinum majus | Penstemon fruticosus | Bacopa monnieri | Digitalis lanata | Plantago ovata |
|------------------|-------------------------------|------------------|-----------------|---------------------|--------------------------|----------------|---------------------|---------------|---------------|--------------|
| Genome length    | 152,249                       | 152,319          | 150,198         | 152,930             | 152,700                  | 152,690        | 152,704             | 152,495       | 153,108        | 162,116      |
| % GC content     | 38                            | 37.9             | 37.9            | 38.3                | 38.1                     | 37.9           | 37.9                | 37.6          | 38.6          | 38.2         |
| LSC              | 83,187                        | 83,195           | 81,850          | 83,615              | 83,243                   | 85,045         | 83,784              | 83,764        | 83,934        | 82,084       |
| SSC              | 17,704                        | 17,702           | 17,418          | 17,801              | 17,831                   | 17,929         | 17,823              | 17,395        | 17,688        | 5272         |
| IRs              | 25,679                        | 25,711           | 25,465          | 25,727              | 25,808                   | 24,858         | 25,249              | 25,668        | 25,743        | 37,380       |

| Genes number of genes | 130 | 133 | 130 | 131 | 133 | 132 | 133 | 132 | 130 | 149 |
| CDS               | 85  | 88  | 86  | 86  | 86  | 87  | 87  | 88  | 85  | 98  |
| tRNA              | 37  | 37  | 36  | 37  | 39  | 37  | 37  | 38  | 36  | 43  |
| rRNA              | 8   | 8   | 8   | 8   | 8   | 8   | 8   | 8   | 8   | 8   |
| % GC LSC          | 36.1| 36.0| 36.0| 36.5| 36.2| 36.0| 36.0| 35.5| 36.8| 36.9|
| % GC SSC          | 32.0| 31.7| 31.6| 32.3| 32.1| 32.0| 31.7| 31.4| 32.7| 31.7|
| % GC IR           | 43.2| 42.2| 43.2| 43.3| 43.2| 42.9| 43.2| 42.8| 43.4| 40.0|
| No. of genes      | 112 | 112 | 112 | 112 | 112 | 112 | 112 | 112 | 112 | 112 |
| duplicated in IR  | 19  | 19  | 19  | 19  | 19  | 21  | 19  | 19  | 19  | 31  |
| No. of genes      | 24  | 24  | 24  | 24  | 24  | 24  | 24  | 24  | 24  | 28  |

| Table 3 |

Gene composition of the chloroplast genome of Veronica ovata subsp. kiusiana.

| Category | Group | Name |
|----------|-------|------|
| Photosynthesis-related genes | Rubisco | rbcL |
| Photosystem 1 | psaA, psaB, psaC, psaL, psaJ |
| Photosystem 2 | psaA, psaB, psaC, psaD, psaE, psaF, psbH, psbI, psbJ, psbK, psbL, psbM, psbN, psbT, psbZ |
| APT synthase | atpA, atpB, atpE, atpF, atpH, atpl |
| NADPH | petA, petB, petD, petG, petL, petN |
| Cytochrome b/f complex | nadA, nadB, nadC, nadD, nadH, nadE, nadF, nadG, nadH, nadL, nadN, nadK |
| Transcription and translation related genes | Transcription | rpoA, rpoB, rpoC1, rpoC2 |
| Translation initiation factor | rps2, rps3, rps4, rps7*, rps8, rps11, rps12*, rps14, rps15, rps16*, rps18, rps19 |
| Ribosomal proteins | rpl2*, rpl14, rpl16, rpl20, rpl22, rpl23*, rpl33, rpl36 |
| RNA genes | Transfer RNA | tmnA-UGC*, tmnA-ACA*, tmnA-GCA, tmnB-UGC, tmnB-ACA, tmnB-GCA, tmnC-UGC, tmnC-ACA, tmnC-GCA, tmnD-UCG, tmnD-ACA, tmnD-GCA, tmnE-UCG, tmnE-ACA, tmnE-GCA, tmnF-UCG, tmnF-ACA, tmnF-GCA, tmnG-UCG, tmnG-ACA, tmnG-GCA, tmnH-UCG, tmnH-ACA, tmnH-GCA, tmnI-UCG, tmnI-ACA, tmnI-GCA, tmnJ-UCG, tmnJ-ACA, tmnJ-GCA, tmnK-UCG, tmnK-ACA, tmnK-GCA, tmnL-UCG, tmnL-ACA, tmnL-GCA, tmnM-UCG, tmnM-ACA, tmnM-GCA, tmnN-UCG, tmnN-ACA, tmnN-GCA, tmnO-UCG, tmnO-ACA, tmnO-GCA, tmnP-UCG, tmnP-ACA, tmnP-GCA, tmnQ-UCG, tmnQ-ACA, tmnQ-GCA, tmnR-UCG, tmnR-ACA, tmnR-GCA, tmnS-UCG, tmnS-ACA, tmnS-GCA, tmnT-UCG, tmnT-ACA, tmnT-GCA, tmnU-UCG, tmnU-ACA, tmnU-GCA, tmnV-UCG, tmnV-ACA, tmnV-GCA, tmnW-UCG, tmnW-ACA, tmnW-GCA, tmnY-UCG, tmnY-ACA, tmnY-GCA, tmnZ-UCG, tmnZ-ACA, tmnZ-GCA, ycf1, ycf2, ycf3, ycf4, ycf15 |
| Other genes | RNA processing | cemA |
| Carbon metabolism | gltA, gltB |
| Fatty acid synthesis | accD |
| Proteolysis | clpP |
| Conserved reading frame | ycf1, ycf2, ycf3, ycf4, ycf15 |

1. Genes with Intron.
2. duplicated genes.
MT671999). The plastome of Veronica ovata subsp. kiusiana is characterized by the presence of a total of 130 genes, comprising 85 CDS, 37 tRNAs, and 8 rRNAs (Table 2). The list of genes is presented in Table 3.

The genome alignment using MultiPip resulted in the highly conserved plastomes among the seven tribes of Plantaginaceae except for few variable regions in Veronica persica, Antirrhinum majus, Bacopa monnieri, and Plantago ovata (Table 4, Fig. 2). Veronica persica, Antirrhinum majus, and Bacopa monnieri exhibited deletions in intergenic spacer of various genes, whereas Plantago ovata revealed deletions in both intergenic spacers as well as in three genes (Table 4).

3.3. Repeat and SSR analysis

In the present study, we determined SSRs of Veronica ovata subsp. kiusiana as well as nine other Plantaginaceae members. The type, distribution, and size of SSRs were examined. Based on the SSR analysis, a total of 47 microsatellites were detected. Sixteen forward, 17 reverse, and 12 forward-reverse repeats were detected in the plastome of V. ovata subsp. kiusiana (Fig. 3a). 43, 1, and 2 SSRs were found in the LSC, IR, and SSC regions respectively (Fig. 3b). All the SSRs found in V. ovata subsp. kiusiana were AT-rich. The size of the SSRs ranged from 10 to 121 bp (Fig. 4).

The number of SSRs in the compared plastomes varied from 14 (Antirrhinum majus) to 57 (Hemiphragma heterophyllum). Also, the highest numbers of SSRs were located in the LSC region followed by SSC and IRs in all the compared plastomes (Fig. 3a & b).

The reputer analysis yielded a total of 34 repeat sequences in the plastome of Veronica ovata subsp. kiusiana. 16 forward, 17 palindromic, and one reverse repeat were detected, however, no complement type of repeats was observed. Other compared plastomes also exhibited a high number of forward and palindromic repeats and a low number of reverse and complement repeats (Fig. 5a). Most of the repeats ranged in the size of 30–49 in all compared plastomes except in Plantago and Antirrhinum where the repeat sizes varied from 30 to 8000 (Fig. 5b).

3.4. Nucleotide diversity and interspecific variation in Veronica

The alignment of the plastomes of Veronica ovata subsp. kiusiana, V. nakaiana, and V. persica was also studied. 4979 SNPs and 5255 indel mutations were observed. Sliding window analysis between the three plastomes exhibited some hotspot regions. High nucleotide diversity and Pi distance was observed in two genes and four intergenic spacer regions viz., rps16, rps15-ycf1, ccsA-ndhD, ndhC-trnV, petN-psbM, and ycf1-trnN (Fig. 6).

3.5. Codon usage

The three Veronica plastomes were compared for their codon usage. The plastomes of Veronica ovata subsp. kiusiana, V. nakaiana, and V. persica are characterized by 26050, 26791, and 26,522 codons and 78150, 80373, and 79,584 nucleotides respectively. The most abundant amino acid is Leucine while the least abundant is Cysteine in all the three compared plastomes (Fig. 7). Codon usage is biased towards A and T in all the three plastomes. The high codon preference (RSCU) is 1.78, 1.82, and 1.90 while the low codon preference is 0.46, 0.31, and 0.31 in the plastomes of V. ovata subsp. kiusiana, V. nakaiana, and V. persica respectively (Supplementary Table 1).

3.6. Phylogenomic analysis

This is the first-ever study to understand relationships between tribes of Plantaginaceae using plastome data. The phylogenomic analysis included representation of seven tribes of Plantaginaceae viz., Plantagineae, Digitalideae, Veroniceae, Sibthorpieae, Antirrhineae, Cheloneae, and Gratioleae. Veronica ovata subsp. kiusiana appeared sister to V. nakaiana (BP = 100/100) (Fig. 8). V. persica appeared sister to the clade of V. ovata subsp. kiusiana and V. nakaiana. Tribes Plantagineae and Digitalideae appeared sister (BP = 99.6/98) while tribes Veroniceae and Sibthorpieae appeared sister (BP = 100/100). Single representatives are available for the tribes Antirrhineae, Cheloneae, and Gratioleae which appeared sister to the clade of Plantagineae/Digitalideae and Veroniceae/Sibthorpieae (BP = 100/100).

4. Discussion

This is the first-ever study on the characterization and comparison of plastomes of seven tribes of Plantaginaceae. The plastome alignment revealed significant deletions in genes and intergenic spacers of the seven tribes of Plantaginaceae (Table 4, Fig. 2). The genus Veronica showed highly conserved plastome and depicted
few deletions in the intergenic spacers of *Veronica persica*. While *Plantago ovata* possesses high variability among the seven tribes of Plantaginaceae due to the expansion of IRs and genome size (Fig. 2). *Plantago ovata* exhibited huge variations in terms of gene composition, genome size, and rearrangements (Asaf et al., 2020). While we found that there was a 1,124 bp long deletion in *ycf2* gene of *Plantago ovata* and this could be due to the rearrangement and expansion of IRs.

Simple Sequence Repeats (SSRs) are the short stretches of 1–6 nucleotides found in coding as well as non-coding regions of the genome (Schlötterer, 2000; Ellegren, 2004). In this study, we compared the type and size of SSRs across all the seven tribes. The least number of SSRs were reported in *Antirrhinum majus* of tribe Antirrhinae. The compared plastomes exhibited the highest number of mononucleotide repeats while most of the repeats were AT-rich which was in accordance with the earlier available plastomes for the genus (Choi et al., 2016).

The family Plantaginaceae has been studied extensively by various researchers but phylogenetic positions of several genera remained unresolved. Very few studies used the molecular mark-

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**Fig. 3.** a. Comparison of types of SSRs across 10 plastomes. b. Comparison of the position of SSRs across 10 plastomes.
ers (nuclear - ITS, plastid - atpB, matK, ndhF, psbB, rbcl, rpl32-trnL, rps3, rps4, rps16, trnF, trnK, and trnV-atpE) to resolve the complexity within family Plantaginaceae (Olmstead et al., 2001; Albach et al., 2005ab; Estes & Small, 2008; Refulio-Rodriguez & Olmstead, 2014; Yousefi et al., 2016). Later, Choi et al. (2016) studied the plastome structure and nucleotide diversity in Veronica nakaiana and V. persica and proposed five probable markers (trnG-trnM, trnT-trnL, ycf4-cemA, petD-rpoA, and rpl2-trnL). Though, the efficacy of the markers was never tested in any of the phylogenetic study. The makers proposed by Choi et al. (2016) were selected from the 114 highly variable genes (Coding, noncoding regions, and Introns). However, our study used the complete plastome of three species of Veronica viz. V. ovata subsp. kiusiana, V. nakaiana, and V. persica, and revealed few more high variable regions and mutational sites. Here we propose six highly variable genes and intergenic spacer viz. rps16, rps15-ycf1, ccsA-ndhD, ndhC-trnV, petN-psbM, and ycf1-trnN (Fig. 6). These markers possess high nucleotide diversity and will be helpful in the future to resolve the complexes within genera of family Plantaginaceae. The plastome possesses high variability in LSC, SSC, and followed by IRs (Li & Zheng, 2018), similar was observed in Veronica. The study of Olmstead et al. (2001) highlighted the deletion/insertion of a few bases in ndhF gene of Digitalis, Hemiphragma, Plantago, and Veronica, which was supported by our study. We observed the deletion of three bases (TTC) at 713 bp to the upstream of 3’ of ndhF gene in the tribe Veronicaeae, Sibthorpieae, DigitaliIdeae, and Plantagineae. To confirm this deletion in the future, we need robust sampling from all the tribes of Plantaginaceae.

The Leucine and Cysteine were the most and least common amino acids in the plastome of the genus Veronica, respectively (Fig. 7). Our study was also supported by the previous findings on the plastomes of Plantago ovata (Asaf et al., 2020). The study also supports the presence of AT codon biases towards the third position of codons, similar to most of the Angiosperms (Jiang et al., 2017; Asaf et al., 2020).

Our phylogenetic analysis confirmed the position of Veronica ovata subsp. kiusiana in the genus Veronica, which appeared sister to V. nakaiana (Fig. 8). The phylogenetic placement of genus Veronica was found to be in congruence with the previous studies (Choi et al., 2016; Asaf et al., 2020). The phylogenetic tree also revealed the placement of seven tribes within the family Plantaginaceae (Fig. 8) but due to the sampling limitation, we could not confirm the phylogenetic position. In the future, the inclusion of robust sampling will confirm the phylogenetic position of all ten tribes in the family Plantaginaceae.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2020.09.040.
Fig. 5. Comparison of a. types of repeats and b. the number of repeats across 10 compared plastomes.
Fig. 6. Nucleotide diversity and hotspot regions between Veronica plastomes. The X-axis represents the nucleotide position and Y-axis represents nucleotide diversity (Pi).

Fig. 7. Codon usage of three Veronica taxa. X-axis: Amino acid, Y-axis: codon usage in percentage. * indicates the stop codon.

Fig. 8. Phylogenomic tree showing the placement of seven tribes of family Plantaginaceae. All the nodes in the tree have 100% bootstrap support.
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