The Comparative Analyses of Six Complete Chloroplast Genomes of Morphologically Diverse *Chenopodium album* L. (Amaranthaceae) Collected in Korea

**Jongsun Park**, **Juhyeon Min**, **Yongsung Kim**, and **Youngjae Chung**

1InfoBoss Inc., 301 Room, 670, Seolleung-ro, Gangnam-gu, Seoul, Republic of Korea
2InfoBoss Research Center, 301 Room, 670, Seolleung-ro, Gangnam-gu, Seoul, Republic of Korea
3Department of Biological Sciences, Sungkyunkwan University, Suwon, Republic of Korea
4Department of Biology, Shingyeong University, Hwaseong 18274, Republic of Korea

Correspondence should be addressed to Jongsun Park; starflr@infoboss.co.kr and Youngjae Chung; chenopodium@hanmail.net

Received 22 February 2021; Accepted 9 April 2021; Published 27 April 2021

1. Introduction

*Chenopodium album* sensu stricto belonging to *C. album* aggregate is an annual cosmopolitan weed native to Eurasia [6]. It is one of the notorious weeds which reduce crop yield by exploiting resources such as light and nutrients from soil [6]. In contrast, *C. album* sensu stricto has been recognized as a morphologically diverse species with difficulty in species identification [1, 11, 12]. This hexaploid species exhibited wide phenotypic plasticity covering morphological variations of other *Chenopodi-
**Results**

### 2.1. Six Complete Chloroplast Genomes and Their Morphological Features of *Chenopodium album* Collected in Korea

Six samples of *C. album* collected in Korea named CAGAP004, CAGOH01, CAJEJG05, CCANG01, CSJUK01, and CVHUP01 were selected based on their distinct morphological features and collected in different geographical positions in Korean Peninsula (Table 1 and Figure 1). The leaf shape of the six *C. album* samples presents a wide range which is from lanceolate to ovate. Their leaf margin shows the two types: (i) serrate (CAGAP004, CAGOH01, and CCANG01) and (ii) entire (CAJEJG05, CSJUK01, and CVHUP01; Figure 1 and Table 1). In addition, the thickness of leaves of the six samples is also divided into two types: (i) thick type (CAGAP004, CAJEJG05, and CVHUP01) and (ii) thin type (CAGOH01, CCANG01, and CSJUK01).

To understand the relationship between morphological features with geographical distribution and its genetic background of chloroplast genomes, we completed chloroplast genomes of the six *C. album* samples (Table 1). The six chloroplast genomes have a typical quadripartite structure which has one large single-copy (LSC), one small single-copy, and two inverted repeat (IR) regions (Figure 2). The length of the six chloroplast genomes ranges from 151,906 bp (CCANG01) to 152,199 bp (CAGAP004; Table 2), presenting 293 bp differences. They are similar to that of one of the two previously sequenced *C. album* chloroplast genomes, of which GenBank accession is NC_034950 (152,167 bp) [23]. Interestingly, the other chloroplast genome of *C. album*, MF418659, displays much shorter (150,272 bp) than those of the remaining *C. album* chloroplast genomes [24]. Their overall GC contents are conserved as between 37.2% and 37.3%, which are slightly higher than that of MF418659 (37.0%; Table 2). The GC contents of LSC and SSC regions are identical in the seven *C. album* chloroplast genomes including the firstly sequenced chloroplast of *C. album* (NC_034950), which are 35.3% and 31.0%, respectively. Similarly, GC contents in the IR region of the seven chloroplast genomes are from 42.7% to 42.8% (that of MF418659 is 43.4%).

All seven *C. album* chloroplast genomes including NC_034950 contain 129 genes including 84 protein-coding genes (PCGs), 8 ribosomal RNAs (rRNAs), and 37 transfer RNAs (tRNAs; Table 3). Seventeen genes are duplicated in IR regions including 6 PCGs (rpl2, rps12, ndhB, rps12, rps7, and ycf1), 4 rRNAs (rrn16, rrn23, rrn4.5, and rrn5), and 7 tRNAs (trnI-CAU, trnL-CAA, trnV-GAC, trnL-GAU, trnA-UGC, trnR-ACG, and trnN-GUU). The number of PCGs from the seven *C. album* chloroplast genomes is the same except MF418659 (Table 3), presenting that MF418659 has a quite different gene configuration. It has six additional PCGs, rpl23, ycf15, and ycf68 in the IR region, and loses psaJ PCG. In addition, it also misses one of the two rps12, displaying the same sequences of three exons of rps12 to the rest seven *C. album* chloroplast genomes; however, this missed rps12 should be added in the annotation of MF418659, resulting in 89 PCGs. After reannotation of two *Chenopodium quinoa* chloroplast genomes (KY635884 and MF805727), one ycf1 and four additional PCGs, ycf1, ycf2, and two rps12, are added, respectively, resulting in all chloroplast genomes of *Chenopodium* species having the same number of genes except MF418659 (Table 3).

In the seven *C. album* chloroplast genomes, there are six ATP synthase subunit genes, 11 NADH dehydrogenase genes, four RNA polymerase genes, six genes encoding subunits of cytochrome b/f complex, and 15 photosystem
subunit II genes. Five genes encoding photosystem subunit I are found in the seven *C. album* chloroplast genomes (Table 4), while MF418659 has only four genes with losing *psaJ*. There are 20 ribosomal proteins presented in the seven *C. album* chloroplast genomes consisting of 12 ribosomal proteins encoding small subunit and eight genes for large subunits (Table 4). Interestingly, an additional *rpl23* gene is found only in the MF418659 chloroplast genome. Other remaining genes encode acetyl-CoA-carboxylase (*accD*), translation initiation factor (*infA*), protease (*clpP*), chloroplast envelope membrane protein (*cemA*), maturation K gene (*matK*), and cytochrome c biogenesis protein (*ccsA*; Table 4). The number of hypothetical proteins is four among the seven *C. album* chloroplast genomes, except for MF418659 which has six genes (Table 4), presenting the different gene configuration of MF418659.

In the seven *C. album* chloroplast genomes, nine PCGs contain one intron (*rps16, atpF, rpoC1, petB, petD, rpl16, rps12, ndhB*, and *ndhA*) and only *clpP* and *ycf3* have two introns, which are conserved across chloroplast genomes of the other *Chenopodium* species. MF418659 chloroplast also has the same intron structure as the remaining *Chenopodium* chloroplast genomes. Taken together with the different properties of the MF418659 chloroplast genome including its length, GC ratio, and a number of genes, we suspected that MF418659 chloroplast genome might not be *C. album*; hence, we will exclude MF418659 for further analyses conducted in this study.

### 2.2. Nucleotide Diversity and Intraspecific Variations Identified from the Seven *Chenopodium album* Chloroplast Genomes

To investigate nucleotide diversity (*π*) and intraspecific variations of *C. album* chloroplast genomes, the six *C. album* complete chloroplast genomes sequenced in this study are aligned against the previously sequenced *C. album* chloroplast genome (NC_034950). The average value of nucleotide diversity is 0.0000625 (Figure 3), and a total of 56 single nucleotide polymorphisms (SNPs) and 26 insertion and deletion (INDEL) regions (308 bp in total) are identified. The LSC region, where the average nucleotide diversity is the highest (*π* = 0.00102), contains 35 SNPs (62.5%) and 16 INDEL regions (47 bp in length; 59.3%). Fifteen SNPs

---

### Table 1: List of six *Chenopodium album* samples used in this study.

| Sample name | Voucher number | Leaf shape | Leaf margin | Leaf thickness | GPS coordinates |
|-------------|----------------|------------|-------------|----------------|-----------------|
| CAGAP004    | KYS130730      | Lanceolate | Serrate     | Thick          | 32°43'25.76"N 126°92'45.26"E |
| CAGOH01     | sgu180626      | Ovate      | Serrate     | Thin           | 34°29'30.09"N 127°21'19.84"E |
| CAJEJG05    | sgu180521      | Lance-ovate| Entire      | Thick          | 33°10'16.00"N 126°15'57.73"E |
| CCANG01     | sgu180601      | Widely ovate| Serrate     | Thin           | 37°35'35.75"N 126°30'53.71"E |
| CSJUK01     | sgu180926      | Lanceolate | Entire      | Thin           | 37°58'19.00"N 128°45'51.00"E |
| CVHUP01     | sgu180918      | Lanceolate | Entire      | Thick          | 35°32'54.26"N 126°40'27.30"E |

* All vouchers were deposited in Sung Kyun Kwan University Herbarium (SKKU) in Korea.

---

*Figure 1: Geographical distribution of six *Chenopodium album*. The red arrows indicate the locations where six *C. album* samples were collected in South Korea. Pictures of voucher specimen were displayed on the left or right side of pictures habit in situ of the six samples.*
(26.8%) and 5 INDEL regions (193 bp in length; 8.93%) are found in the SSC region, displaying that the number of SNPs in the LSC region is larger more than twice that of the SSC region; however, the total length of INDEL regions in the SSC region is about 4 times greater than that of the LSC region. The main reason for this phenomenon is the presence of the 162 bp INDEL located between rpl32 and trnL-UAG genes. An IR region covers three SNPs and three INDEL regions (68 bp in length), which corresponds to the lowest nucleotide diversity in the IR region ($\pi = 0.0000146$). The low level of sequence variations in the IR region is known as a general phenomenon in the chloroplast genomes [25–27].

![Figure 2: A circular gene map of six Chenopodium album chloroplast genomes. Genes shown outside are transcribed clockwise, and inside the circle are transcribed counterclockwise. Genes are color-coded to distinguish different functional groups. The dark grey and the light grey plots in the inner circle correspond to the GC content and AT content, respectively.](image)
and rpl32 is found in the intron of Most of them are less than 5 bp long; however, 66 bp INDEL shift mutation. Seven INDEL regions (26.9%) are identified respectively: none of these INDEL regions cause any frame-

together. The PCG containing the largest SNPs is psbA (π = 0.00163) and six intergenic regions (trnH-psbA, petN-psbM, rpl11-rpl36, rpl36-infA, ycf1-ndhF, and rpl32-trnL; Figure 3). CcsA contains two nonsynonymous SNPs and one synonymous SNP, and matK has two nonsynonymous SNPs and one 6 bp INDEL, presenting that ccsA displays the highest SNP density among the PCGs. The highest π value of the intergenic region is observed between trnH and psbA (π = 0.00128; Figure 3).

### 2.3. Comparative Analysis of Simple Sequence Repeat (SSR) Polymorphisms on Chloroplast Genomes of C. album

In the seven C. album chloroplast genomes, 376 normal SSRs are identified (Table 5; Supplementary Tables 1–7). In addition, we also identified 280 extended SSRs and 3,039 potential SSRs on the seven chloroplast genomes (see Materials and Methods; Supplementary Tables 1–7). We analyzed only normal SSRs hereafter because normal SSRs can be commonly recognized as SSRs in various studies (see Materials and Methods). The unit length of normal SSRs varies from 1 bp (monoSSR) to 5 bp (pentaSSR), and the numbers of normal SSRs in each chloroplast genome are from 53 to 55, displaying an almost similar manner: CAGAP004 contains 55 normal SSRs, the largest, and CAJEJG05, CCANG01, and CSJUK01 contains 54 normal SSRs, while CAGOH01, NC_034950, and CVHUP01 have 53 normal SSRs (Table 5). Interestingly, no hexaSSR is identified on the seven C. album chloroplast genomes. The majority of normal SSRs is monoSSR (60.6%), and pentaSSR (3.70%) is the least (Figure 4(a)). In monoSSR, only an A/T motif was detected in all seven chloroplast genomes.

The overall distribution of normal SSRs on the seven C. album chloroplast genomes is similar to each other (Figure 4(b)). The intergenic region displays the largest number of normal SSRs, then coding, intron, and noncoding regions are in order (see Materials and Methods; Figure 4(b)). In all seven C. album chloroplast genomes, four normal SSRs are found in the noncoding region of rnr23, and 11 normal SSRs are identified in the coding regions of rpoC2, rpoB, atpB, rpoA, ycf1, and ndhB. In total, 30 to 33 normal SSRs were found in the intergenic regions on the seven chloroplast genomes. Most of the normal SSRs in the intergenic region are shared among the seven chloroplast genomes; however, CAJEJG05 and CAGAP004 chloroplast genomes have two distinct normal SSRs in their intergenic region of trnR-trnN and rpl32-trnL caused by one-bp INDEL and one SNP changing “T” to “A,” respectively. Also, CCANG01

| Strain name | GenBank accession | Whole (bp) | LSC (bp) | SSC (bp) | IR (bp) | Whole (bp) | LSC (bp) | SSC (bp) | IR (bp) | GC contents |
|-------------|------------------|------------|--------|--------|--------|-----------|--------|--------|--------|------------|
| CCANG01     | MW446241         | 151,906    | 83,681 | 17,969 | 25,128 | 37.3%     | 35.3%  | 31.0%  | 42.7%  |            |
| CSJUK01     | MW446242         | 152,197    | 83,679 | 18,132 | 25,193 | 37.2%     | 35.3%  | 31.0%  | 42.7%  |            |
| CAJEJG05    | MW446243         | 152,183    | 83,680 | 18,130 | 25,194 | 37.2%     | 35.3%  | 31.0%  | 42.7%  |            |
| CVHUP01     | MW446244         | 152,190    | 83,832 | 18,132 | 25,113 | 37.2%     | 35.3%  | 31.0%  | 42.8%  |            |
| CAGOH01     | MW446245         | 152,196    | 83,679 | 18,131 | 25,193 | 37.3%     | 35.3%  | 31.0%  | 42.7%  |            |
| CAGAP004    | MW446246         | 152,199    | 83,681 | 18,130 | 25,194 | 37.2%     | 35.3%  | 31.0%  | 42.7%  |            |
and CSJK01 chloroplast genomes have additional monoSSR between \textit{ndhC} and \textit{rbcL} by insertion of 1 bp nucleotide "T." In contrast, the deletion of a single nucleotide of "A" between \textit{ndhF} and \textit{rpl32} caused the removal of intergenic SSRs in CAGOH01, CCANG01, and CSJK01 chloroplast genomes. All seven \textit{C. album} chloroplast genomes have seven common normal SSRs located in the intronic regions of five PCGs, \textit{rps16}, \textit{atpF}, \textit{ycf3}, \textit{rpl16}, and \textit{ndhA}. In the case of CAGOH01 and CCANG01 chloroplast genomes, one additional monoSSR is identified in the intronic region of \textit{trnK} because one SNP changing "A" to "T" occurred in both chloroplast genomes. Besides, CSJK01 has an extra unique

Table 3: List of chloroplast genomes used for comparative analyses in this study.

| Family            | Species name                  | NCBI accession   | Total length (bp) | # of PGCs | # of tRNAs | # of rRNAs | GC ratio (%) | Reference          |
|-------------------|-------------------------------|------------------|-------------------|-----------|------------|------------|--------------|-------------------|
| Chenopodioideae   | \textit{Chenopodium album}    | MW446241         | 151,906           | 84        | 37         | 8          | 37.3%        | This study        |
|                   | \textit{Chenopodium album}    | MW446242         | 152,197           | 84        | 37         | 8          | 37.2%        | This study        |
|                   | \textit{Chenopodium album}    | MW446243         | 152,183           | 84        | 37         | 8          | 37.2%        | This study        |
|                   | \textit{Chenopodium album}    | MW446244         | 152,190           | 84        | 37         | 8          | 37.2%        | This study        |
|                   | \textit{Chenopodium album}    | MW446245         | 152,196           | 84        | 37         | 8          | 37.3%        | This study        |
|                   | \textit{Chenopodium album}    | NC_034950        | 152,167           | 84        | 37         | 8          | 37.2%        | [23]              |
|                   | \textit{Chenopodium album}    | MF418659         | 150,272           | 89        | 33         | 8          | 37.0%        | [24]              |
|                   | \textit{Chenopodium ficifolium} | NC_041200     | 151,923           | 84        | 37         | 8          | 37.3%        | [45]              |
|                   | \textit{Chenopodium quinoa}    | KY635884         | 152,075           | 84        | 36         | 8          | 37.2%        | [61]              |
|                   | \textit{Chenopodium quinoa}    | MF805727         | 151,069           | 84        | 36         | 8          | 37.2%        | [62]              |
| Chenopodioidae    | \textit{Chenopodium quinoa}    | NC_034949        | 152,099           | 84        | 36         | 8          | 37.2%        | [23]              |
|                   | \textit{Chenopodium quinoa}    | NSDK10013185.1   | 152,282           | N/A       | N/A        | N/A        | 37.2%        | [63]              |
|                   | \textit{Atriplex centralasiatica} | NC_045304    | 152,237           | 85        | 37         | 8          | 37.3%        | [64]              |
|                   | \textit{Dysphania ambrosioides} | NC_041201    | 151,689           | 84        | 36         | 8          | 36.9%        | [65]              |
|                   | \textit{Dysphania botrys}      | NC_042166       | 152,055           | 83        | 37         | 8          | 36.8%        | [66]              |
|                   | \textit{Dysphania pumilio}     | MK541016        | 151,960           | 84        | 36         | 8          | 36.9%        | [39]              |
|                   | \textit{Oxybasis glauca}       | NC_047226       | 151,655           | 84        | 37         | 8          | 36.9%        | [49]              |
|                   | \textit{Spinacia oleracea}     | NC_002202       | 150,725           | 96        | 37         | 8          | 36.8%        | [68]              |
|                   | \textit{Chenopodium quinoa}    | NC_027225       | 153,232           | 84        | 37         | 8          | 36.2%        | Unpublished       |
|                   | \textit{Chenopodium bigelovii}  | NC_027226       | 153,076           | 83        | 37         | 8          | 36.3%        | Unpublished       |
|                   | \textit{Chenopodium brachiata} | NC_027224       | 153,324           | 84        | 37         | 8          | 36.2%        | Unpublished       |
| Salicornioideae   | \textit{Salicornia europaea}   | NC_027225       | 153,232           | 84        | 37         | 8          | 36.2%        | Unpublished       |
|                   | \textit{Salicornia bigelovii}  | NC_027226       | 153,076           | 83        | 37         | 8          | 36.3%        | Unpublished       |
|                   | \textit{Salicornia brachiata}  | NC_027224       | 153,324           | 84        | 37         | 8          | 36.2%        | Unpublished       |
| Suaedoideae       | \textit{Suaeda japonica}       | NC_042675       | 152,109           | 83        | 37         | 8          | 36.4%        | [46]              |
|                   | \textit{Suaeda japonica}       | MK764271        | 152,112           | 80        | 37         | 8          | 36.4%        | [38]              |
|                   | \textit{Suaeda salsa}          | NC_045302       | 151,642           | 85        | 37         | 8          | 36.4%        | [69]              |
|                   | \textit{Suaeda glauca}          | NC_045303       | 149,807           | 85        | 37         | 8          | 36.5%        | [70]              |
|                   | \textit{Suaeda malacosperma}    | NC_039180       | 151,989           | 83        | 37         | 8          | 36.4%        | [71]              |
|                   | \textit{Bienertia sinuspersici} | KU726550       | 153,472           | 86        | 36         | 8          | 37.8%        | [72]              |
| Salsoloideae      | \textit{Haloxylon ammodendron} | NC_027668       | 151,570           | 85        | 37         | 8          | 36.6%        | [73]              |
|                   | \textit{Haloxylon persicum}    | NC_027669       | 151,586           | 85        | 37         | 8          | 36.6%        | [73]              |
| Betoideae         | \textit{Beta vulgaris}         | EF534108        | 149,696           | N/A       | N/A        | N/A        | 35.4%        | Unpublished       |
|                   | \textit{Beta vulgaris} subsp. vulgaris | KJ081864 | 149,635           | 85        | 37         | 8          | 37.0%        | [74]              |
|                   | \textit{Beta vulgaris} subsp. vulgaris | KR230391 | 149,722           | 81        | 29         | 8          | 37.0%        | [75]              |
| Paronychieae      | \textit{Gymnocarpos przewalskii} | NC_036812   | 150,636           | 81        | 37         | 8          | 36.5%        | [66]              |

* indicates that the species name should be reconsidered. ** Numbers in parenthesis are the original number of PCGs based on the annotation, and numbers outside of parenthesis indicate the number of PCGs based on our reannotation results.
tetraSSR “GTCT” in the intronic regions of *ycf3* by 4 bp insertion. These differences of normal SSRs among the seven chloroplasts of *C. album* can be utilized as molecular markers to distinguish their origins inside the Korean Peninsula once more chloroplast genomes of *C. album* in Korea are available.

Due to the different lengths of the LSC and SSC regions, the density of SSRs per Kbp was calculated. Interestingly, three chloroplast genomes, CAGOH01, CCANG01, and CSJUK01, display similar density in both the LSC and SSC regions, while the remaining four chloroplast genomes present that the density in the SSC region is larger than that of the LSC region. Since in the SSC region of CAGAP004, CAJEJG05, CVHUP01, and NC_034950 there is an additional intergenic normal SSR located in between *ndhF* and *rpl32* (Figure 4(c)). The densities of normal SSRs in IR regions of all seven chloroplast genomes are lowest (0.159 to 0.198 normal SSRs/Kbp; Figure 4(c)).

To understand conserved normal SSRs across the seven chloroplast genomes, we calculated SSR groups which contain normal SSRs of which left and right flanking sequences are similar to each other (see Materials and Methods). In total, 58 SSR groups and two singleton SSRs were identified, and 50 of 58 SSR groups (86.2%) contain seven normal SSRs originating from all seven chloroplast genomes, called the common SSR group. Eleven out of the 50 common SSR groups (22.0%) are located in the intronic region, and 36 common SSR groups (72.0%) are in the intergenic region (Figure 4(b)), which is congruent to the analysis result of normal SSRs mentioned in the previous section. Five intergenic loci contain two common SSR groups, *rpl32-trnL, atpH-atpI, ycf3-trnS, trnQ-psbK, and trnK-rps16* in each, and 31 intergenic loci contain one common SSR group. Two singleton SSRs were found in CSJUK01 and CCANG01 chloroplast genomes. These intraspecific variations of normal SSRs will provide insights into changes of SSRs inside the species, which can also be utilized to develop molecular markers of *C. album* efficiently.

2.4. Phylogenetic Analysis of Korean *C. album* Chloroplast Genome Sequence. Bootstrapped maximum likelihood (ML)

| Category                  | Group of gene          | Name of gene                      |
|---------------------------|------------------------|-----------------------------------|
| Ribosomal RNAs            |                         | *rrn*4.5, *rrn*5, *rrn*16, *rrn*23|
| Transfer RNA genes        |                         | *trnA-UGC, trnC-GCA, trnD-GUC, trnE-UUC, trnF-GAA, trnG-GCC, trnH-GUG, trnL-CAU, trnL-GAU, trnK-UUU, trnL-CAA, trnL-UAA, trnL-UAG, trnM-CAU, trnN-GUU, trnP-UGG, trnQ-UGU, trnR-AGG, trnR-UCU, trnS-GCU, trnS-GGA, trnS-UGA, trnT-GGU, trnT-UGU, trnV-GAC, trnV-UAC, trnW-CCA, trnY-GUA* |
| Self-replication          | Small subunit of ribosome | *rps2, rps3, rps4, rps7, rps8, rps11, rps12, rps14, rps15, rps16, rps18, rps19* |
|                           | Large subunit of ribosome | *rpl2, rpl14, rpl16, rpl20, rpl22, rpl32, rpl33, rpl36* |
|                           | RNA polymerase          | *rpoA, rpoB, rpoC1, rpoC2* |
|                           | Translation initiation factor | *infA* |
| Photosynthesis            | ATP synthase           | *atpA, atpB, atpE, atpF, atpH, atpI* |
|                           | NADH dehydrogenase subunit | *ndhA, ndhB, ndhC, ndhD, ndhE, ndhF, ndhG, ndhH, ndhI, ndhJ, ndhk* |
|                           | Cytochrome b/f complex subunit | *petA, petB, petD, petG, petL, petN* |
|                           | Photosystem subunit I subunit | *psaA, psaB, psaC, psaI, psaj* |
|                           | Photosystem subunit II subunit | *psbA, psbB, psbC, psbD, psbE, psbF, psbH, psbI, psbJ, psbK, psbL, psbM, psbN, psbT, psbZ* |
|                           | Rubisco large subunit   | *rbcL* |
|                           | Maturase                | *matK* |
|                           | Protease                | *clpP* |
|                           | Envelope membrane protein | *cemA* |
|                           | Subunit of acetyl-CoA- carboxylase | *accD* |
|                           | Cytochrome c-type biogenesis protein | *ccsA* |
| Other genes               |                         |                    |
| Genes of unknown function | Hypothetical reading frame | *ycf1, ycf2, ycf3, ycf4* |

Table 4: List of genes encoded by *Chenopodium album* chloroplast genomes.
and Bayesian inference (BI) phylogenetic trees of 34 Amaranthaceae chloroplast genomes including the six *C. album* chloroplasts sequenced in this study and one outgroup species, *Gymnocarpos przewalskii*, were constructed (see Materials and Methods). Phylogenetic trees present that six *C. album* chloroplast genomes are clustered with the previously sequenced *C. album* chloroplast genome (NC_034950) with high supportive values of ML and BI except the node containing NC_034950 in the ML tree (Figure 5(a)). The *C. album* s. str. clade is divided reciprocally into two clades in both trees. CAGAP004 and CAJEJG05 sharing the morphological feature of narrow leaves and collected in Jejudo island (Figure 1) only exhibit a correlation of geographical locations with high supportive values of ML and BI (Figure 5(b)). Leaf shape and margin of the six samples are not correlated to the two clades of *C. album* (Table 1 and Figure 5(b)); however, leaf thickness of the six *C. album* presents correlation to the clades: the clade containing CAGAP004, CAJEJG05, and CVHUP01 shows thick leaves, called as a thick-leaf clade, and the clade consisting of CAGOH01, CCANG01, and CSJUK01 displays thin leaves, called as a thin-leaf clade (Table 1 and Figure 5(b)). NC_034950 clustered in the thick-leaf clade was not possible to be confirmed whether its leaves are thick or not. Taken together, the phylogenetic relationship of the six *C. album* chloroplast genomes seems not to be highly correlated with their morphological features and geographical locations, supporting that their high plasticity of morphology links to other factors such as nuclear markers, polyploidy, or any regulatory factor of leaf morphologies. With the additional chloroplast genomes as well as nuclear marker sequences of *C. album* collected in Korea, these relationships of morphological features and geographical locations will be more explicit.

### 3. Discussions

#### 3.1. Species Incongruency of *C. album* Chloroplast Genomes

In this study, we sequenced six chloroplast genomes of *C. album* s. str. collected in Korea displaying various morphological features. One of the previously sequenced chloroplast genomes of *C. album*, MF418659, is quite different from the remaining *C. album* chloroplast genomes in the aspects of gene configuration of chloroplast genome (Table 3) as well as phylogenetic relationship (MF418659 chloroplast genome...
Figure 4: Number of normal SSRs identified on the seven *Chenopodium album* chloroplast genomes. (a) Displays the number of SSR types in each seven *C. album* chloroplast genome. The X-axis means the seven *C. album* samples, and the Y-axis indicates the number of normal SSRs. Five different colors indicate the five types of SSRs, monoSSRs, diSSRs, triSSRs, tetraSSRs, and pentaSSRs. The table below the X-axis presents numbers of SSRs along with samples and the five SSR types. (b) Shows the distribution of SSRs in noncoding, intron, intergenic, and coding regions along with the samples. The X-axis means the seven *C. album* samples, and the Y-axis indicates the number of normal SSRs. Four different colors correspond to the four different regions. The table below the X-axis shows the number of SSRs along with samples and the four regions. (c) Shows SSR density (# of SSRs/kb) of the LSC, SSC, and IRs regions along with the samples. The X-axis means the seven *C. album* samples, and the Y-axis indicates the number of normal SSRs along with samples and the three regions. Three different colors in the bar graphs mean the three regions. The table below the X-axis shows SSR density along with samples and the three regions.
Prior possibility of BI, respectively. Chloroplast genomes sequenced in this study were presented as bold characters.

The phylogenetic tree was drawn based on the ML tree. Numbers on branches in the phylogenetic tree indicate bootstrap values of ML and BI (Figure 5). These differences indicate that MF418659 may be located outside of the clade of Chenopodium and Atriplex in Figure 5). These differences indicate that MF418659 may neither be C. album nor genus Chenopodium.

It is partially supported by the fact that the collection site of MF418659 is the Himalayan area in India [24] where C. album has been mainly cultivated as crops [7–9]. Usually, species diversity of the Himalayan area is higher due to its wide variety of climates as well as various climatic perturbations that have been applied to different locations in the Himalayan area [29]. Considering the phylogenetic position of MF418659 (Figure 5), it is possible that MF418659 is misidentified or an unreported species which is very different from C. album.

This kind of incongruency problem of species has sometimes been found during comparative analyses in plant species. For instance, two Magnolia chloroplast genomes, Magnolia insignis (NC_035657) and Magnolia alba (NC_037005), were reported as examples of misidentification species based on the phylogenetic analysis based on complete chloroplast genomes [30]. This problem can occur due to not enough taxonomic coverage of whole chloroplast genomes or misidentification of the samples used in the studies because of difficulties in species identification based on morphologies. Therefore, the identification of MF418659 should be revised in some ways, such as species identification of the voucher used in the previous study or sequencing and analyzing more samples of C. album collected in the Himalayan area.

**3.2. Possible Causes of C. album s. str. Morphological Variations at the Molecular Level.** Based on cytogenic and nuclear molecular marker analysis of Chenopodium species, C. album is distinct to C. ficifolium (B genome diploid) and C. quinoa (A genome tetraploid) [14], and two major groups of C. album were identified based on the phylogenetic tree based on rrm5 and ITS sequences [14]. In comparison to the phylogenetic tree which displays that C. album and C. ficifolium were clustered in one clade with high supportive values (Figure 5), a maternal lineage of both species is nearer than that of biparental lineage. Several C. quinoa chloroplast genomes were clustered in the distinct clade to that of C. album and C. ficifolium (Figure 5), reflecting the different types of their genomes [14]. This phylogenetic tree based on complete chloroplast genomes (Figure 5) also indirectly supports that various intraspecific evolutionary events in several Chenopodium species, including C. album and C. quinoa, may have occurred, such as hybridization and polyploidization [2, 14].

Polyploidization and hybridization events can usually cause morphological plasticity and diversity; e.g., Nicotiana species display various flower colors based on events of polyploidization [31], and Centaurea stoebe, polyploidy species, shows that it causes various phenotypes to climate, resulting in boosting its invasion [32]. Similarly, morphological variations of C. album are not related to maternal lineage (Figures 1 and 5). It can be inferred that C. album presents various morphological differences because it is hexaploidy.
species. It can also be interpreted that these morphological variations are not fully genetically fixed but may be caused by nuclear genes related to leaf development, such as Class I KNOX genes, homeobox transcription factors which can regulate leaf shapes in *Arabidopsis thaliana* [33]. In this study, we found that the leaf morphology of six *C. album* has a weak correlation with their phylogenetic relation (Figures 1 and 5). If diverse leaf shapes of *C. album* are caused by these key regulators, we can deduce that the general trend of evolutionary process inferred from organelle genomes including chloroplast cannot explain this diversity because these regulators can display different evolutionary speeds and patterns from those of organelle genomes.

Several studies tried to delimitate species presenting different morphological features using whole chloroplast genome sequences. For instance, a phylogenetic tree constructed based on chloroplast genomes of *Anemopaegma acutifolium* supported that two leaf morphological trait types of *A. acutifolium* were caused by different maternal origins [34]. In the case of *Triplostegia glandulifera* and *T. grandiflora*, their chloroplast genomes were used for solving the boundary of the two species; however, they could not explain the high morphological plasticity of them [35]. Therefore, further analyses with more chloroplast genomes of *C. album* expressing various phenotypic characteristics will be necessary to understand the origin of its morphological plasticity.

### 3.3. Evaluation of Level of Intraspecific Variations on *C. album* Chloroplast Genomes

The intraspecific variations identified among the seven *Chenopodium album* chloroplast genomes (56 SNPs and 26 INDEL regions) are compared with the previous studies which investigated intraspecific variations on chloroplast genomes. Twenty cultivars and wild types of *Ricinus communis* (Castor bean) displayed 162 SNPs and 92 INDEL regions [28], which is three times more than those of *C. album*. Sixty-three chloroplast genomes of *Macadamia integrifolia* (Macadamia nut) are collected in eastern Australia, which is a smaller geographical range of *C. album*. Four hundred and seven SNPs [36] are detected from them, which is seven times more than the number of SNPs identified in this study. Comparing with that of our study, the numbers of intraspecific variations identified from *C. album* chloroplast genome are relatively lower. In the case of *Dioscorea polystachya* (Chinese yam), six chloroplast genomes collected in Northern and Southern China displayed 141 SNPs and 44 INDEL regions [37]. Its geographical coverage is larger than that of *C. album*, and climates of the six regions are quite different than those of *C. album*; the larger number of intraspecific variations in *D. polystachya* is reasonable.

To evaluate intraspecific variations on *C. album* chloroplast genomes considering its geographical distribution, various studies which identified intraspecific variations of organelle genomes from the plant species collected in Korea were surveyed. To compare intraspecific variations between two samples in the same species, we conducted a pairwise comparison of *C. album* chloroplast genomes, resulting in 0 to 33 SNPs and 7 to 36 INDEL regions being identified from the seven *C. album* chloroplast genomes. In the case of *Suaeda japonica* collected in Korea with different morphological features, only three SNPs and three INDEL regions were identified [38], which is mostly smaller than those identified in *C. album*. The number of intraspecific variations identified from *Dysphania pumilio*, another Amaranthaceae species, is 24 SNPs and one INDEL region [39], which is also in the range of those of *C. album*. Based on these previous results, intraspecific variations identified from the seven *C. album* chloroplast genomes are similar to those of Amaranthaceae species.

### 4. Materials and Methods

#### 4.1. DNA Extraction of Natural Collection of Korean *C. album*

Six samples of *C. album* were collected in various places in the Korean Peninsula (Table 1 and Figure 1). All vouchers of the six samples were deposited to the Sung Kyun Kwan University Herbarium (SKKU; Table 1). Their total DNA was extracted from fresh leaves of the six samples using a DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany).

#### 4.2. Genome Sequencing and De Novo Assembly of the Natural Collection of Six *C. album* Chloroplast Genomes

Genome sequencing was performed using HiSeqX at Macrogen Inc., Korea, from the extracted DNA of the six *C. album*. *De novo* assembly with confirmation was accomplished with Velvet v1.2.10 [40] after filtering raw reads using Trimmomatic v0.33 [41]. After obtaining the first draft of the chloroplast genome sequences, gaps were filled with GapCloser v1.1.2 [42], and all bases from the assembled sequences were confirmed by checking each base in the alignment (tvie mode in SAMtools v1.9 [43]) against the assembled chloroplast genome generated with BWA v0.7.17 [44]. All these processes were conducted under the environment of the Genome Information System (GeIS; http://geis.infoboss.co.kr; Park et al., in preparation) like other Amaranthaceae chloroplast genomes assembled [38, 39, 45–49].

#### 4.3. Chloroplast Genome Annotation

*Geneious Prime*® 2020.2.4 (Biomatters Ltd, Auckland, New Zealand) was used for chloroplast genome annotation based on the *C. album* chloroplast genome (NC_034950) [23] by transferring annotations while correcting exceptional cases, including missing start or stop codons. tRNA was predicted and confirmed based on the prediction of tRNAscan-SE v2.0.6 [50]. A circular map of *C. album* chloroplast was drawn by using the OGDRAW v1.3.1 [51].

#### 4.4. Identification of Sequence Variations on the Complete Chloroplast Genomes of *C. album*

Single nucleotide polymorphisms (SNPs) and insertions and deletions (INDELs) were identified from the pair-wise alignments of two selected chloroplast genomes conducted by MAFFT v7.450 [52]. When the number of INDELs was calculated, continuous INDEL bases were considered one INDEL. In addition, we denote the four regions: (i) coding region is exon that encodes a protein, (ii) intron regions indicate the region which does not translate inside protein-coding genes, (iii) intergenic regions are the sequence between two genes, and (iv) noncoding region means the sequence located in tRNAs or rRNAs.
4.5. Identification of Simple Sequence Repeats (SSRs). Simple sequence repeats (SSRs) were identified on the chloroplast genome sequence using the pipeline of the SSR database (SSRDB; http://ssrdb.infoboss.co.kr; Park et al., in preparation). Based on the conventional definition of an SSR on the chloroplast genome, monoSSR (1 bp) to hexaSSR (6 bp), the total length of SSRS on the chloroplast genome exceeds 10 bp. Owing to the different criteria of SSRS on chloroplast genomes, we adopted the criteria used in chloroplast genomes of *Dysphania* [47] and *Arabidopsis thaliana* [53] and mitochondrial genome of *Rosa rugosa* [54] as follows: the monoSSR (unit sequence length of 1 bp) to hexaSSR (6 bp) are used as normal SSRS, and heptaSSR (7 bp) to dec-aSSR (10 bp) are defined as extended SSRS. Among the normal SSRS, pentaSSRs and hexaSSRs for which the repeat number of unit sequences is 2 are classified as potential SSRS. Classification of regions on chloroplast genome was conducted in the same way described in the above section.

4.6. Comparison of SSRs Identified from Seven *C. album* Chloroplast Genomes. SSRs identified from seven *C. album* chloroplast genomes were compared on their flanking sequences under the environment of the SSRDB (http://ssrdb.infoboss.co.kr; Park et al., in preparation). The pipeline of the SSR comparison implemented in the SSRDB used in various organelle genome studies [53, 55] was used with the following conditions: a cut-off e value of 1e − 10 and a maximum flanking sequence for the comparison of 60 bp.

4.7. Nucleotide Diversity Analysis. Nucleotide diversity was calculated using the method proposed by Nei and Li [56] based on the multiple sequence alignment of *Chenopodium* chloroplast genomes using a Perl script used in previous studies [47, 53, 57]. The window size was set to 500 bp, and the step size was 200 bp when using the sliding-window method. Genomic coordination of each window was compared to the gene annotation of the chloroplast genome under the GenomeArchive® (http://www.genomearchive.net/) [58] environment for further analyses.

4.8. Construction of Phylogenetic Trees. The whole 34 Amaranthaceae chloroplast genomes and one outgroup of *Gymnocarpos przewalskii* chloroplast genome were aligned by MAFFT v7.450 [52], and alignment quality was checked manually. The maximum likelihood (ML) tree was reconstructed in IQ-TREE v1.6.6 [59]. In the ML analysis, a heuristic search was used with nearest-neighbor interchange (NNI) branch swapping, TVM+F+I model, and uniform rates among sites. All other options used the default settings. Boot-strap analyses with 1,000 pseudoreplicates were conducted with the same options. The posterior probability of each node was estimated by Bayesian inference (BI) using the MrBayes v3.2.7a [60] plug-in implemented in Geneious Prime® 2020.2.4 (Biomatters Ltd, Auckland, New Zealand). The HKY85 model with gamma rates was used as a molecular model. A Markov chain Monte Carlo (MCMC) algorithm was employed for 1,100,000 generations, sampling trees every 200 generations, with four chains running simulta-

neously. Trees from the first 100,000 generations were discarded as burn-in.

5. Conclusions

We completed the six chloroplast genomes of *Chenopodium album* showing various morphological features. The structure and gene order of chloroplast are conserved among seven *C. album* including the previously sequenced chloroplast genome (NC_034950). The average nucleotide diversity calculated from the seven *C. album* chloroplast genomes is 0.0000625, and a total of 56 SNPs and 26 INDEL regions are found. In comparison to the other cases of chloroplast intraspecific variations, *C. album* chloroplasts present a low level of sequence variation. The number of normal SSR identified from the seven *C. album* chloroplast genomes ranges from 33 to 35 displaying similar distribution and density of SSRS. Interestingly, specific SNPs and INDEL regions in intronic and intergenic regions make SSR variation among the seven chloroplasts. All seven *C. album* chloroplast genomes are clustered in high supportive values of ML and BI trees with a short length of branches. In addition, one of the morphological characters of *C. album* s. str., the thickness of leaves, presented correlation with the phylogenetic position. Taking together the results in this study, our six chloroplast genomes of *C. album* s. str. will provide the way to investigate intraspecific features of chloroplast genomes, also the insights of intraspecific variations to understand various characteristics of one species including morphological features.

Data Availability

Chloroplast genome sequences of *C. album* sequenced in this study can be accessed via accession numbers MW446241 to MW446246 in NCBI GenBank.

Conflicts of Interest

The authors declare that they have no competing interests.

Authors’ Contributions

Jongsun Park are Juhyeon Min are co-first authors.

Acknowledgments

This work was supported by the InfoBoss Research Grant (IBG-0001) and Grant from Rural Development Administration (PJ01385505). We also thank Dr. Suhyeon Park for discussing about the misidentification problem of chloroplast genomes and Mr. Woochan Kwon for giving constructive ideas for a better manuscript.

Supplementary Materials

Supplementary Table 1: list of SSRs identified in CAGAP004 of *C. album*. Supplementary Table 2: list of SSRs identified in CAGOH01 of *C. album*. Supplementary Table 3: list of SSRs identified in CAJEJG05 of *C. album*. Supplementary Table 4:
References

[1] F. Habibi, P. Vít, M. Rahiminejad, and B. Mandák, "Towards a better understanding of the Chenopodium aggregate (Amaranthaceae) in the Middle East: a karyological, cytometric and morphometric investigation," Journal of Systematics and Evolution, vol. 56, no. 3, pp. 231–242, 2018.

[2] B. Mandák, K. Krak, P. Vít et al., "Hybridization and polyploidization within the Chenopodium _Aggregate_ analyzed by means of cytological and molecular markers," Molecular Phylogenetics and Evolution, vol. 129, pp. 189–201, 2018.

[3] B. Mandák, P. Trávníček, L. Paštová, and D. Kořinková, "Is hybridization involved in the evolution of the Chenopodium _Aggregate_? An analysis based on chromosome counts and genome size estimation," Flora-Morphology, Distribution, Functional Ecology of Plants, vol. 207, no. 7, pp. 530–540, 2012.

[4] B. Mandák, K. Krak, P. Vít et al., "How genome size variation is linked with evolution within Chenopodium _Sensus lato_," Perspectives in Plant Ecology, Evolution and Systematics, vol. 23, pp. 18–32, 2016.

[5] P. Vít, K. Krak, P. Trávníček, J. Douda, M. N. Lomonosova, and B. Mandák, "Genome size stability across Eurasian Chenopodium _Species_ (Amaranthaceae)," Botanical Journal of the Linnean Society, vol. 182, no. 3, pp. 637–649, 2016.

[6] E. N. Jellen, B. A. Kolano, M. C. Sederberg, A. Bonifacio, and P. J. Maughan, "Chenopodium," in Wild Crop Relatives: Genomic and Breeding Resources, pp. 35–61, Springer, 2011.

[7] T. Partap and P. Kapoor, "The Himalayan grain chenopods. I. Distribution and ethnobotany," Agriculture, Ecosystems & Environment, vol. 14, no. 3–4, pp. 185–199, 1985.

[8] T. Partap and P. Kapoor, "The Himalayan grain chenopods. III. An under-exploited food plant with promising potential," Agriculture, Ecosystems & Environment, vol. 19, no. 1, pp. 71–79, 1987.

[9] L. Luczaj and W. M. Szymański, "Wild vascular plants gathered for consumption in the Polish countryside: a review," Journal of Ethnobiology and Ethnomedicine, vol. 3, no. 1, p. 17, 2007.

[10] T. Partap, B. D. Joshi, and N. Calwey, Chenopods: Chenopodiaceae, International Plant Genetic Resources Institute (IPGRI), 1998.

[11] H. A. Wahl, "A preliminary study of the genus Chenopodium in North America," Bartonia, vol. 27, pp. 1–46, 1952.

[12] Y. Chung, A taxonomic study of the Korean Chenopodiaceae, [Ph.D. thesis], Sungkyunkwan University (in Korean), Seoul (Korea), 1992.

[13] K. Krak, P. Vít, A. Belayayev, J. Douda, L. Hreussová, and B. Mandák, "Allopolyplid origin of Chenopodium _s. str._Chenopodiaceae: a molecular and cytogenetic insight," PLoS One, vol. 11, no. 8, article e0161063, 2016.

[14] B. Kolano, J. McCann, M. Oskroda et al., "Parental origin and genome evolution of several Eurasian hexaploid species of Chenopodium (Chenopodiaceae)," Phytotaxa, vol. 392, no. 3, pp. 163–185, 2019.
[32] M. A. Hahn, M. Van Kleunen, and H. Müller-Schärer, "Increased phenotypic plasticity to climate may have boosted the invasion success of polyploid Centaurea stoebe," *PloS One*, vol. 7, no. 11, article e50284, 2012.

[33] A. Hay and M. Tsiantis, "KNOX genes: versatile regulators of plant development and diversity," *Development*, vol. 137, no. 19, pp. 3153–3165, 2010.

[34] F. Firetti, A. R. Zuntini, J. W. Gaiarsa, R. S. Oliveira, L. G. Lohmann, and M. A. Van Sluys, "Complete chloroplast genome sequences contribute to plant species delimitation: a case study of the Anemopaegma species complex," *American Journal of Botany*, vol. 104, no. 10, pp. 1493–1509, 2017.

[35] Y.-T. Niu, F. Jabbour, R. L. Barrett et al., "Combining complete chloroplast genome sequences with target loci data and morphology to resolve species limits in _Triplostegia_ (Caprifoliaceae)," *Molecular Phylogenetics and Evolution*, vol. 129, pp. 15–26, 2018.

[36] C. J. Nock, C. M. Hardner, J. D. Montenegro et al., "Wild origins of macadamia domestication identified through intraspecific chloroplast genome sequencing," *Frontiers in Plant Science*, vol. 10, p. 334, 2019.

[37] J. Cao, D. Jiang, Z. Zhao et al., "Development of chloroplast genomic resources in Chinese Yam (Dioscorea polystachya)," *BioMed Research International*, vol. 2018, Article ID 6293847, 11 pages, 2018.

[38] Y. Kim, J. Park, and Y. Chung, "The comparison of the complete chloroplast genome of *Suaeda japonica* Makino presenting different external morphology (Amaranthaceae)," *Mitochondrial DNA Part B*, vol. 5, no. 2, pp. 1616–1618, 2020.

[39] J. Park and Y. Kim, "The second complete chloroplast genome of *Dysphania pumilio* (R.Br.) mosyakin & clemants (Amaranthaceae): intraspecies variation of invasive weeds," *Mitochondrial DNA Part B*, vol. 4, no. 1, pp. 1428–1429, 2019.

[40] D. R. Zerbino and E. Birney, "Velvet: algorithms for de novo short read assembly using de Bruijn graphs," *Genome Research*, vol. 18, no. 5, pp. 821–829, 2008.

[41] A. M. Bolger, M. Lohse, and B. Usadel, "Trimmomatic: a flexible trimmer for Illumina sequence data," *Bioinformatics*, vol. 30, no. 15, pp. 2114–2120, 2014.

[42] Q.-Y. Zhao, Y. Wang, Y.-M. Kong, D. Luo, X. Li, and P. Hao, "Optimizing de novo transcriptome assembly from short-read RNA-Seq data: a comparative study," *BMC Bioinformatics*, vol. 12, Suppl. 14, p. S2, 2011.

[43] H. Li, B. Handsaker, A. Wysoker et al., "The sequence alignment/map format and SAMtools," *Bioinformatics*, vol. 25, no. 16, pp. 2078–2079, 2009.

[44] H. Li, "Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM," 2013, https://arxiv.org/abs/1303.3997.

[45] Y. Kim, Y. Chung, and J. Park, "The complete chloroplast genome of *Chenopodium ficifolium* Sm. (Amaranthaceae)," *Mitochondrial DNA Part B*, vol. 4, no. 1, pp. 872–873, 2019.

[46] Y. Kim, J. Park, and Y. Chung, "The complete chloroplast genome of *Suaeda japonica* Makino (Amaranthaceae)," *Mitochondrial DNA Part B*, vol. 4, no. 1, pp. 1505–1507, 2019.

[47] Y. Kim, J. Park, Y. Chung et al., "Comparative analysis of chloroplast genome of *Dysphania ambrosioides* (L.) Mosyakin & Clemants understanding phylogenetic relationship in genus *Dysphania* R. Br.," *Korean Journal of Plant Resources.*, vol. 32, pp. 644–668, 2019.

[48] Y. Kim, Y. Chung, and J. Park, "The complete chloroplast genome sequence of *Dysphania pumilio* (R.Br.) Mosyakin & Clemants (Amaranthaceae)," *Mitochondrial DNA Part B*, vol. 4, no. 1, pp. 403–404, 2019.

[49] Y. Kim, Y. Chung, and J. Park, "The complete chloroplast genome of *Oxybasis glauca*(L.) S. Fuentes, Uotila & Borsch (Amaranthaceae) as the first chloroplast genome in genus *Oxybasis*," *Mitochondrial DNA Part B*, vol. 5, no. 2, pp. 1410–1412, 2020.

[50] T. M. Lowe and P. P. Chan, "TRNAscan-SE on-line: integrating search and context for analysis of transfer RNA genes," *Nucleic Acids Research*, vol. 44, no. W1, pp. W54–W57, 2016.

[51] S. Greiner, P. Lehwalk, and R. Bock, "OrganelleGenomeDRAW (OGDRAW) version 1.3. 1: expanded toolkit for the graphical visualization of organelar genomes," *Nucleic Acids Research*, vol. 47, no. W1, pp. W59–W64, 2019.

[52] K. Katoh and D. M. Standley, "MAFFT multiple sequence alignment software version 7: improvements in performance and usability," *Molecular Biology and Evolution*, vol. 30, no. 4, pp. 772–780, 2013.

[53] J. Park, H. Xi, and Y. Kim, "The complete chloroplast genome of *Arabidopsis thaliana* isolated in Korea (Brassicaceae): an investigation of intraspecific variations of the chloroplast genome of *Korean A. thaliana*," *International Journal of Genomics*, vol. 2020, Article ID 3236461, 18 pages, 2020.

[54] J. Park, H. Xi, Y. Kim, S. Nam, and K.-I. Heo, "The complete mitochondrial genome of new species candidate of *Rosa rugosa* (Rosaceae)," *Mitochondrial DNA Part B*, vol. 5, no. 3, pp. 3433–3437, 2020.

[55] J. Lee, J. Park, H. Xi, and J. Park, "Comprehensive analyses of the complete mitochondrial genome of *Ficulus binodulus* (Coleoptera: Lucanidae)," *Journal of Insect Science*, vol. 20, no. 5, p. 10, 2020.

[56] M. Nei and W.-H. Li, "Mathematical model for studying genetic variation in terms of restriction endonucleases," *Proceedings of the National Academy of Sciences*, vol. 76, no. 10, pp. 5269–5273, 1979.

[57] J. Park, H. Xi, and S.-H. Oh, "Comparative chloroplast genomics and phylogenetic analysis of the *Viburnum dilatatum* complex (Adoxaceae) in Korea," *Korean Journal of Plant Taxonomy*, vol. 50, no. 1, pp. 8–16, 2020.

[58] J. Park and H. Xi, "Genome Archive (R): standardized genome repository for supporting large-scale genome analyses," in *Proceedings of Plant and Animal Genome XXVI Conference*, January 13-17, 2018.

[59] L.-T. Nguyen, H. A. Schmidt, A. Von Haeseler, and B. Q. Minh, "IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies," *Molecular Biology and Evolution*, vol. 32, no. 1, pp. 268–274, 2015.

[60] J. P. Huelsenbeck and F. Ronquist, "MRBAYES: Bayesian inference of phylogenetic trees," *Bioinformatics*, vol. 17, no. 8, pp. 754–755, 2001.

[61] S. O. Rabah, C. Lee, N. H. Hajrah et al., "Plastome sequencing of ten nonmodel crop species uncovers a large insertion of mitochondrial DNA in cashew," *Plant Genome*, vol. 10, no. 3, 2017.

[62] K. Wang, L. Li, S. Li et al., "Characterization of the complete chloroplast genome of *Chenopodium quinoa*Wild,*" *Mitochondrial DNA Part B*, vol. 2, no. 2, pp. 812–813, 2017.

[63] C. Zou, A. Chen, L. Xiao et al., "A high-quality genome assembly of quinoa provides insights into the molecular basis of salt bladder-based salinity tolerance and the exceptional nutritional value," *Cell Research*, vol. 27, no. 11, pp. 1327–1340, 2017.
X.-J. Zhang, N. Wang, L.-Y. Zhang, S.-J. Fan, and X.-J. Qu, "Characterization of the complete plastome of Atriplex centra-lasiatica (Chenopodiaceae), an annual halophytic herb," *Mitochondrial DNA Part B*, vol. 4, no. 2, pp. 2475-2476, 2019.

Y. Kim, Y. Chung, and J. Park, "The complete chloroplast genome sequence of Dysphania ambrosioides (L.) Mosyakin & Clemants (Chenopodiaceae/Amaranthaceae sensu APG), a medicinal plant and invasive species in Korea," *The Korean Journal Of Weed Science*, vol. 39, pp. 42–42, 2019.

Z. Yang, Y. Zhang, L. Pan, and C. Fu, "Characterization of the complete chloroplast genome of Gymnocarpos przewalskii, an endangered species in China and Mongolia," *Conservation Genetics Resources*, vol. 10, no. 4, pp. 717–721, 2018.

Y. Kim, K.-I. Heo, S. Lee, and J. Park, "Complete chloroplast genome sequence of the Pseudostellaria longipedicellata S. Lee, K. Heo & SC Kim (Caryophyllaceae)," *Mitochondrial DNA Part B*, vol. 3, no. 2, pp. 1296-1297, 2018.

C. Schmitz-Linneweber, R. M. Maier, J.-P. Alcaraz, A. Cottet, R. G. Herrmann, and R. Mache, "The plastid chromosome of spinach (Spinacia oleracea): complete nucleotide sequence and gene organization," *Plant Molecular Biology*, vol. 45, no. 3, pp. 307–315, 2001.

X.-J. Qu, X.-T. Li, L.-Y. Zhang, X.-J. Zhang, and S.-J. Fan, "Characterization of the complete chloroplast genome of Suaeda salsa (Amaranthaceae/Chenopodiaceae), an annual succulent halophyte," *Mitochondrial DNA Part B*, vol. 4, no. 2, pp. 2133-2134, 2019.

X.-J. Qu, L.-K. Liu, L.-Y. Zhang, X.-J. Zhang, and S.-J. Fan, "The complete chloroplast genome of an annual halophyte herb, Suaeda glauca (Amaranthaceae)," *Mitochondrial DNA Part B*, vol. 4, no. 2, pp. 2780-2781, 2019.

J.-S. Park, I.-S. Choi, D.-H. Lee, and B.-H. Choi, "The complete plastid genome of Bienertia sinuspersici (Amaranthaceae/Chenopodiaceae), a vulnerable halophyte in coastal regions of Korea and Japan," *Mitochondrial DNA Part B*, vol. 3, no. 1, pp. 382-383, 2018.

B. Kim, J. Kim, H. Park, and J. Park, "The complete chloroplast genome sequence of Bienertia sinuspersici," *Mitochondrial DNA Part B*, vol. 1, no. 1, pp. 388-389, 2016.

W. Dong, C. Xu, D. Li et al., "Comparative analysis of the complete chloroplast genome sequences in psammophytic Haloxylon species (Amaranthaceae)," *PeerJ*, vol. 4, article e2699, 2016.

H. Li, H. Cao, Y.-F. Cai, J.-H. Wang, S.-P. Qu, and X.-Q. Huang, *The complete chloroplast genome sequence of sugar beet (Beta vulgaris ssp. vulgaris)*, Taylor & Francis, 2014.

K. B. Stadermann, B. Weisshaar, and D. Holtgräwe, "SMRT sequencing only de novo assembly of the sugar beet (Beta vulgaris) chloroplast genome," *BMC Bioinformatics*, vol. 16, no. 1, pp. 1–10, 2015.