Chloroplast genome of white wild chrysanthemum, Dendranthema sp. K247003, as genetic barcode

Sang Kun Park¹, Soo-Jin Kwon², Jihye Park³, Minjee Lee³, So Youn Won², Young Chul Kim⁴,⁵, Yoon-Jung Hwang⁶, Seong-Han Sohn² and Jungho Lee³,⁶*

¹Floriculture Research Division, National Institute of Horticultural and Herbal Science (NIHHS), RDA, Jeonju 565-852, Korea
²Genomics Division, National Academy of Agricultural Science (NAAS), RDA, Jeonju 560-500, Korea
³Green Plant Institute, B-301, Heungdeok IT Valley, Giheung-gu, Yongin 446-908, Korea
⁴Korea Botanic Garden, Pyeongchang 232-953, Korea
⁵Department of Biology, Gangneung-Wonju National University, Gangneung 210-702, Korea
⁶Department of Life Science, Sahmyook University, Seoul 139-742, Korea

*Correspondent: jlee@greenplant.re.kr

Dendranthema boreale and D. indicum are easily distinguished from other Korean Dendranthema spp. by having yellow flowers. We have found a putative new taxon of Dendranthema having white flowers, except for sharing most characters with Dendranthema boreale. The chloroplast (cp) genome of the putative new taxon of Dendranthema, Dendranthema sp. K247003, registered in National Agro-Biodiversity Center (ABC), was completely characterized as a genetic barcode. The cp-genome of Dendranthema sp. K247003 was 151,175-bp in size: LSC was 82,886-bp, IR 24,971-bp, SSC 18,347-bp. The cp-genome of Dendranthema sp. K247003 contains 113 genes and 21 introns consisted of 79 protein coding genes, 4 RNA genes, and 30 tRNA genes, with 20 group II introns and one group I intron. Some of the genes and the introns were duplicated in IR. The cp-DNA of Dendranthema sp. K247003 is distinguished from that of D. boreale IT121002 by 67 SNPs in genic regions of 24 protein coding genes and by a 9-bp INDEL in ycf1. Further cp-DNA study will give us better information on genetic markers of Dendranthema species.

Keywords: Asteraceae, chloroplast genome, Compositae, Dendranthema, INDEL, SNP

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more than six Asteraceae genera were reported (Dempewolf et al., 2010; Doorduin et al., 2011; Nie et al., 2012; Liu et al., 2013; Walker et al., 2014). Among them, 47 cp-genomes were reported only from agriculturally important Helianthus (Shaw et al., 2007; Timme et al., 2007; Bock et al., 2014), Asteraceae. For Dendrantha, while published as Chrysanthemum, some IGS regions of chloroplast were used for genetic diversity study (Liu et al., 2012). For MAS, genetic information on generic regions is valued than that of IGS regions. Thanks to the dramatic development of Next Generation Sequencing method (NGS) in recent years, it has become possible to complete chloroplast genome sequencing at low cost. Complete cp-genomic sequences have become more useful as genetic barcode of plants (Nock et al., 2011; Li et al., 2015). Here, we report the complete genome of Dendrantha sp. K247003 as genetic barcode.

MATERIALS AND METHODS

Chloroplast DNA extraction, genome sequencing, assembly, and PCR-based validation

White flowered chrysanthemum, Dendrantha sp. K247003, was collected at the population of D. boreale in Geounri, Yeongwol of Gangwon province (N: 37°15’ 18.9” E: 128°31’39.3”). The plant was registered at Agro-Biodiversity Center (ABC), Rural Agricultural Administration (RDA), as genetic resources for vegetative clones (IT number: K247003). The plant was propagated in Floriculture Research Division, National Institute of Horticultural and Herbal Science (NIHHS), RDA. Fresh leaves of Dendrantha sp. K247003 were collected from the Floriculture Research Institute, NIHHS in Rural Development Administration (RDA), Jeonju, and stored in liquid nitrogen until usage. Total DNA was extracted using the Qiagen DNaseasy Plant Mini Kit (Qiagen, Hilden, Germany), and DNA concentration and quality were determined using a Scandrop nano-volume spectrophotometer (Analytik Jena, Germany). High quality DNA (concentration = 300 ng/μL, A260/280 ratio = 1.8-2.0, and A260/230 ratio = 1.7) was used for PCR and sequencing.

For NGS data production, purified DNA was fragmented and used to construct short-insert libraries (insert size, 200-bp), according to the manufacturer’s instructions (Illumina, USA). The short fragments were paired-end sequenced using an Illumina Hi-Seq 2500 sequencing system at NICEM of Seoul National University. NGS data (7.63 Gb of 82.97 M reads) were analyzed using CLC Genomic Workbench ver. 7.5.1 (Qiagen, Hilden, Germany), as described by Jeong et al. (2014). For Sanger sequencing, the whole cp-genome of Dendrantha sp. K247003 was PCR-amplified in ~1-2 kb fragments, and cp-genome structure was verified using Long PCR, with ~5-28 kb fragments, as described by Lee and Manhart (2002a; 2002b). Only PCR products ranging from ~1-2 kb were sequenced using Bigdye (ver. 3.1) and ABI3730 at NICEM of Seoul National University. Assembled cp-sequences were verified using Sequencher ver. 5.0 (Gencode, USA) by combining Sanger data and the assembled NGS sequence.

Genome annotation, genome comparison and sequence analysis

Protein coding and ribosomal RNA genes were annotated using DOGMA (http://dogma.ccbb.utexas.edu/; Wyman et al., 2004). The boundaries of each annotated gene were manually determined by comparison with orthologous genes from other known cp-genomes. Genes encoding tRNAs were first predicted using trNAscan (http://lowelab.ucsc.edu/ tRNAscan-SE; Lowe and Eddy, 1997) and ARAGORN, version 1.2 (http://130.235.46.10/ ARAGORN/; Laslett and Canback, 2004), and were manually verified by predicting the tRNA secondary structure. Circular genome maps were drawn using GenomeVx (Conant and Wolfe, 2008), followed by manual modification. The sequencing data and gene annotation were submitted to National Agricultural Biotechnology Information Center (NABIC), Jeonju, with accession number NG-0482-000001. The mVISTA program in ShuffLAGAN mode (Fraser et al., 2004) was used to compare the cp-genome of Dendrantha sp. K247003 with that of D. boreale IT121002 (NABIC: NG-0478-000001; unpublished).

RESULTS AND DISCUSSION

The cp-genome of Dendrantha sp. K247003, was determined (Fig. 1) and found to be 151,175 bp in length. It includes small and large single copy (SSC, LSC) regions of 18,347 bp and 82,886 bp, respectively, separated by a pair of 24,971 bp Inverted Repeats (IRs). A total of 113 genes were detected, including 79 protein coding genes, 30 tRNA genes, and four rRNA genes (Table 1). This cp-genome was also found to contain 20 different introns, including 19 group II introns and a group I intron with a cyanobacterial origin (Besendahl et al., 2000) found within the trnL_uuu gene. Three protein coding genes, including clpP, rps12, and ycf3, contain two group II introns (clpP_i1, clpP_i2, rps12_i1, rps12_i2, ycf3_i1 and ycf3_i2), and 14 genes contain a single group II intron: rpoC1_i1, rpl2_i, rpl16_i, rps16_i, atpF_i, petB_i, petD_i, ndhA_i, ndhB_i, trnA_ugc_i, trnG_ugg_i, trnL_gau_i, trnK_uuu_i, and trnV_uac_i. Among the 20 group II introns, the intron in rps12, between exons 1 and 2, is trans-splicing, while the other 19 group II introns are cis-splicing.
Eighteen genes, five introns, and parts of two genes and an intron are found within the IR, which has two copies. These 18 genes include seven protein-coding genes (ndhB, rpl2, rpl22, rpl23, rps7, rps19, ycf2), all four rRNA genes (16S, 23S, 4.5S, 5S), and seven tRNA genes (trnA_ugc, trnL_cau, trnL_gau, trnL_caa, trnN_guu, trnR_aceg, trnV_gau). The five introns are ndhB.i, rpl2.i, trnA_ugc.i, trnL_gau.i, and rps12.i2. The IR also contains the 5' end of ycf1 at the border with the SSC, resulting in one intact ycf1 and a 612-bp ψ-ycf1 in the cp-genome. In addition, the IR contains parts of the rps12 gene. This rps12 gene consists of three exons, rps12.e1, rps12.e2, and rps12.e3 (Lee, 1997); rps12.e1 is in the LSC, but rps12.e2 and rps12.e3 are in the IR. Thus, the genome contains a single copy of rps12.e1 but has two copies of rps12.e2 and rps12.e3. A cis-splicing group II intron, rps12.i1t, intervenes between rps12.e1 and rps12.e2. The rps12.i1t is split into two pieces, rps12.i1t1 and rps12.i1t2, because the rps12 gene is transcribed in two separate operons, the clpP operon (clpP-rps12.e1-rps12.i1t1-rpl20) and the 3' rps12 operon (rps12.i1t2-rps12.e2-rps12.i2-rps12.e3-rps7-ndhB).

Direct comparison of chloroplast genomes of Dendranthema sp. K247003 and D. boreale TT121002 using mVISTA program is shown in Figure 2. In the analysis, 97 IGS regions showed variation. In genes, 24 genes have variations. There were no variation in 30 tRNA and 4 rRNA genes. Among 79 protein coding genes, 24 genes had variation. There were two kinds of variations (Table 2). One was Single Nucleotide Polymorphism (SNP) and the other was INDEL. SNPs were found at 67 sites in 24 protein coding genes. The protein coding genes include 6 genetic system genes and 18 photosynthesis
Table 1. Gene list in chloroplast genome of *Dendranthema* sp. K247003.

| Gene                        | Genetic system genes | Conserved orf                          | ycf1 | ycf2 × 2 | ycf3** | ycf4 |
|-----------------------------|----------------------|----------------------------------------|------|---------|--------|------|
| matK                        | RNA polymerase       | matK                                   |      |         |        |      |
| rpoA                        | Ribosomal protein    | rpoA                                   |      |         |        |      |
| rpoB                        | Large subunits       | rpl14                                  |      |         |        |      |
| rpoC1*                      |                      | rpl16*                                 |      |         |        |      |
| rpoC2                       |                      | rpl20                                  |      |         |        |      |
| rps2                        |                      | rpl22                                  |      |         |        |      |
| rps3                        |                      | rpl23 × 2                              |      |         |        |      |
| rps4                        |                      | rpl32                                  |      |         |        |      |
| rps7 × 2                    |                      | rpl33                                  |      |         |        |      |
| rps8                        |                      | rpl36                                  |      |         |        |      |
| rps11                       |                      |                                        |      |         |        |      |
| rps12**α × 2                |                      |                                        |      |         |        |      |
| rps14                       |                      |                                        |      |         |        |      |
| rps15                       |                      |                                        |      |         |        |      |
| Photosynthesis genes        |                      |                                        |      |         |        |      |
| Acetyl-CoA carboxylase      |                      | accD                                   |      |         |        |      |
| ATP-dependent Clp protease  |                      | clpP**                                 |      |         |        |      |
| ATP synthase                |                      | atpA                                   |      |         | atpE   | atpF* |
| Cytochrome b                |                      | atpB                                   |      |         |        |      |
| Cytochrome b/f              |                      | atpE                                   |      |         |        |      |
| Cytochrome f                |                      | petA                                   |      |         |        |      |
| Cytochrome C biogenesis     |                      | petB*                                  |      |         |        |      |
| Membrane protein            |                      | petD*                                  |      |         |        |      |
| NADH dehydrogenase          |                      | petG                                   |      |         |        |      |
| Photosystem I               |                      | petL                                   |      |         |        |      |
| Photosystem II              |                      | petN                                   |      |         |        |      |
| Rubisco                     |                      |                                        |      |         |        |      |
| Translation initiation factor 1 |                      |                                        |      |         |        |      |
| Ribosomal RNA               |                      |                                        |      |         |        |      |
| Transfer RNA                |                      |                                        |      |         |        |      |
| rm16S × 2                   |                      |                                        |      |         |        |      |
| rm23S × 2                   |                      |                                        |      |         |        |      |
| rm4.5S × 2                  |                      |                                        |      |         |        |      |
| rm5S × 2                    |                      |                                        |      |         |        |      |
| rmE_UUC                     |                      |                                        |      |         |        |      |
| tmC_GCA                     |                      |                                        |      |         |        |      |
| tmD_GUC                     |                      |                                        |      |         |        |      |
| tmL_GAU* × 2                |                      |                                        |      |         |        |      |
| tmL_UAU*                    |                      |                                        |      |         |        |      |
| tmL_GUU* × 2                |                      |                                        |      |         |        |      |
| tmL_UAA*                    |                      |                                        |      |         |        |      |
| tmL_CAU × 2                 |                      |                                        |      |         |        |      |
| tmL_UAG                     |                      |                                        |      |         |        |      |
| tmL_CAUA × 2                |                      |                                        |      |         |        |      |
| tmL_GCA                     |                      |                                        |      |         |        |      |
| tmL_GUA                     |                      |                                        |      |         |        |      |
| tmM_CAU                     |                      |                                        |      |         |        |      |
| tmM_CAUA                    |                      |                                        |      |         |        |      |
| tmM_GCA                     |                      |                                        |      |         |        |      |
| tmM_GUA                     |                      |                                        |      |         |        |      |
| tmN_GAU* × 2                |                      |                                        |      |         |        |      |
| tmN_GUA                     |                      |                                        |      |         |        |      |
| tmN_UAU*                    |                      |                                        |      |         |        |      |
| tmN_UAA*                    |                      |                                        |      |         |        |      |
| tmN_GUU* × 2                |                      |                                        |      |         |        |      |
| tmN_UUA*                    |                      |                                        |      |         |        |      |
| tmN_UUA                     |                      |                                        |      |         |        |      |
| tmN_GUA* × 2                |                      |                                        |      |         |        |      |
| tmN_GUA*                    |                      |                                        |      |         |        |      |
| tmP_UUA*                    |                      |                                        |      |         |        |      |
| tmP_UUU                     |                      |                                        |      |         |        |      |
| tmP_UUA                     |                      |                                        |      |         |        |      |
| tmP_UUU* × 2                |                      |                                        |      |         |        |      |
| tmP_UUA*                    |                      |                                        |      |         |        |      |
| tmP_UUG                     |                      |                                        |      |         |        |      |
| tmP_UUG                     |                      |                                        |      |         |        |      |
| Pseudo gene                 |                      |                                        |      |         |        |      |
| ψ-ycf1                      |                      |                                        |      |         |        |      |
| ψ-rps19                     |                      |                                        |      |         |        |      |

*αintrong-copy genome contains a copy of rps12 exon 1 in LSC and two copies of rps12 exon 2 and 3 in IR.
Fig. 2. Comparison of chloroplast genomes of *Dendranthema* sp. K247003 and *D. boreale* IT121002 using mVISTA program. Grey arrows and thick black lines above the alignment indicate genes with their orientation and the position of the IRs, respectively. The Y-scale represents the percent identity between 50-100%. Genome regions are color-coded: Coding regions in blue; noncoding sequences (CNS) in red.
Table 2. Variation in cp-genic regions between Dendranthema sp. K247003 and D. boreale IT121002. SNP: Single Nucleotide Polymorphism.

| Gene name | Size (D. sp / D. boreale) | No of SNP | INDEL | Gene name | Size (D. sp / D. boreale) | No of SNP | INDEL |
|-----------|---------------------------|-----------|-------|-----------|---------------------------|-----------|-------|
| 1 psbA    | 1062                      | 1         | –     | 13 accD   | 1503                      | 4         | –     |
| 2 matK    | 1518                      | 2         | –     | 14 cemA   | 690                       | 1         | –     |
| 3 rpoB    | 3183                      | 1         | –     | 15 rpoA   | 1008                      | 2         | –     |
| 4 rpoC2   | 4152                      | 10        | –     | 16 rpl22  | 468                       | 2         | –     |
| 5 atpF    | 744                       | 1         | –     | 17 ycf2   | 6849                      | 1         | –     |
| 6 atpF    | 555                       | 2         | –     | 18 ndhF   | 2226                      | 1         | –     |
| 7 psbD    | 1062                      | 1         | –     | 19 ccsA   | 975                       | 3         | –     |
| 8 psaA    | 2205                      | 1         | –     | 20 ndhD   | 1503                      | 3         | –     |
| 9 psaA    | 2253                      | 2         | –     | 21 ndhI   | 561                       | 1         | –     |
| 10 ndhI   | 477                       | 1         | –     | 22 ndhA   | 1092                      | 3         | –     |
| 11 atpB   | 1479                      | 1         | –     | 23 ndhH   | 1236                      | 2         | –     |
| 12 rbcL   | 1479                      | 1         | –     | 24 ycfI   | 5016 / 5007               | 19        | 9-bp INDEL |

genes. As the genetic system genes, two conserved open reading frames (ycf1 and ycf2), maturase K gene (matK), 3 RNA polymerase genes (rpoA, rpoB and rpoC2) and ribosomal protein large subunit gene (rpl22) are included. Among photosynthesis genes, six genes of NADH dehydrogenase (ndhA, ndhD, ndhF, ndhF, ndhH, ndhI and ndhJ), three genes of ATP synthase (atpB, atpF and atpI), two photosystem I genes (psaA and psaB), two photosystem II genes (psbA and psbD), a rubisco gene (rbcL), a membrane protein gene (cemA), cytochrome C biogenesis gene (ccsA), and a Acetyl-CoA carboxylase gene (accD) were variable. In addition to SNP, 9-bp INDEL was found in ycf1 gene containing 19 SNPs.

**CONCLUSION**

This is the first report of chloroplast genome in Dendranthema, Asteraceae. As genetic barcode of Dendranthema sp. K247003, a possible new species, 151,175-bp of chloroplast genomic sequence was registered to NABIC (NG-0482-000001). The chloroplast genome is distinguished from that of D. boreale IT121002, by 67 SNPs and an INDEL in coding regions, in addition to 97 variable IGS sites. As suggested by Dong et al. (2015) in land plants, ycf1 would be useful for plant identification as having 19 SNPs and an INDEL in the comparison of Dendranthema sp. K247003 and D. boreale IT121002. In addition, as suggested by Li et al. (2015), we show that chloroplast genomic information is useful for genetic barcode in Dendranthema. Further characterization of organellar genomes using NGS data would facilitate our phylogenomic study and molecular marker developments in Dendranthema at low cost. Finally, further morphological and cytological studies on Dendranthema sp. K247003 are remained for the taxonomic treatment of this taxon.

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