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Comparative Analysis of Cichorieae Tribe (Asteraceae) Chloroplast Genomes: Insight to Structure, Repetitive DNA, and Phylogeny.

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Abstract

The whole chloroplast genome has been increasingly used over the past three decades for systematic and ordered analysis of evolutionary relationships, variation within species, and developmental studies. There were several other studies on the Asteraceae family, but the studies were on the family level. This study investigates the plastomes of an important Asteraceae tribe, Cichorieae, that are widely distributed, morphologically complex, include several species reproduce apomictically. Plastomes belonging to 36 different species in 12 different genera have been studied in detail and compared. The comparisons were based on plastome structure, number of coding and noncoding genes, and number of rRNA and tRNA genes. Further, the repetitive DNA types, frequency, and their position in the chloroplast genomes have been analyzed. Moreover, SSRs types and frequency were compared and analyzed. In addition, a number of coding and noncoding regions were studied and used to construct a phylogenetic tree.

The investigation of this study indicated the distinctiveness of sequence divergence among the plastomes of Cichorieae tribe species in various aspects. The results provided excellent phylogenetic linkages that can be used for advanced comparative studies at both generic and species levels. Most noncoding and coding regions showed the least variation; however, major variations were shown by those regions that have rarely been used in chloroplast phylogenetics. Regions of coding (*clpP*, *matK*, *ndhA*, *rpoC1*, *rpoC2*, and *ycf1*) and non-coding (*ndhF-rpl32*, *petN-psbM*, *psbE-petL*, *rps4-ndhJ*, *rps15-ycf1*, *trnL-trnF*, *trnT-psbD*, and *ycf3-trnS*) are recommended to use in studying Cichorieae tribe members to achieve better phylogenetic construction, population genetic analysis, and use as a marker for accurate and automated species identification. The tandem repeat of 21-30 period size is the most variable among the Cichorieae tribe plastomes. Ttra-, penta-, and hexanucleotide SSR types were found in *Cichorium intybus*, *Crepidiastrum*, *Sonchus*, and *Taraxacum* plastomes, while, they were not found in other plastomes under study. These SSRs may be useful for identifying species, detecting hybridization, determining phylogeny, and differentiating populations.

Keywords: Whole Chloroplast genome, Plastomes, Cichorieae tribe, Phylogenetic, Repetitive DNA, SSRs.

1. Introduction

Species belonging to the tribe Cichorieae LAM. & DC. (also called Lactuceae), distributed mainly in the temperate regions of the northern hemisphere. This tribe includes ca. 93 genera, excluding mix population of apomictic and sexual reproductions of *Hieracium* L., *Pilosella* Hill., and *Taraxacum* Wigg. These three genera alone include nearly 1,400 species. The Cichorieae tribe includes many economic, cultivated, and locally important species, such as lettuce (*Lactuca* L.), chicory (*Cichorium* L.), and some invasive worldwide distributed weeds such as dandelion (*Taraxacum*) (Jeffrey, 2001; Salih *et al.*, 2017).

According to Carlquist (1976), morphological, physiological, cytological (chromosome counts), and biochemical variables were used to evaluate the variation in the Cichorieae tribe taxa at the level of intra- and inter-

specific levels. Thus, species diversity, phylogenetic relationships, and evolution of the tribe members can be studied using both phenotypic and genotypic features (Lundberg and Bremer, 2003; Kilian *et al.*, 2009).

In eukaryotic cells of autotrophic organisms, chloroplast (plastid) genomes are essential organelles. Plant chloroplast genomes (plastome, ctDNA, cpDNA), in terms of their genomes and genetic systems, are independent and are uniparentally inherited (Raubeson and Jansen, 2005; Kilian *et al.*, 2009; Gholipour and Kohnehrouz, 2017). Plastome comprises a large genomic sequence; its size in plants ranges from 85 - 218 kbp (Palmer, 1991; Daniell *et al.*, 2016), with a very low rate of recombination. Moreover, gene contents and order are highly conserved among plant chloroplast genomes. The above features all together make the chloroplast genome a valuable source for comparative analysis within the genera, tribe, and family members, and a good marker for

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plant phylogenetic analysis and DNA barcoding. Also, chloroplast genome provides an opportunity for analyzing the relationships between distant and closely related species, as well as the analysis of variation at the intraspecific and interspecific levels by analyzing the repetitive sequences, thereby producing a valuable investigation into plant diversity, molecular evolution, and cytogenetic relationships (Salih *et al.*, 2017; Yaradua *et al.*, 2020).

Here in this study, 36 chloroplast genomes belonging to the tribe Cichorieae were selected to compare and analyze: the characterization and structure patterns of chloroplast genomes, gene contents, phylogenetic relationships, selecting polymorphic regions for developing chloroplast marker, and intermolecular combination and microstructural variation of repetitive DNA domains in Cichorieae tribe. The result of this investigation will provide valuable information on chloroplast genome evolution among these tribe members.

2. Materials and Methods

2.1. Genome Content and Organization of plastomes

Sequences of 36 Cichorieae tribe species plastomes (complete chloroplast genome) belonging to 12 genera were obtained from National Center for Biotechnology Information (NCBI). The plastome selection was according to the plastome genome availability in the NCBI gene bank. All plastomes belonging to the Cichorieae tribe were downloaded first, and then all plastomes belonging to each genus within the Cichorieae tribe were separately analyzed by aligning them to ensure diverse plastome genomes and similar genomes were omitted, as there were several duplicated plastomes for the same species in NCBI with a different accession number. Further, only up to 8 plastomes were randomly selected for a single genus (*Taraxacum* and *Sonchus* L.).

The size and GC% of all 36 Cichorieae tribe species plastomes were compared using the GENEIOUS prime 2021 (Kearse *et al.*, 2012). Annotations of the plastome sequence were used to examine gene contents and their organization in the plastomes. Then, the transfer RNA gene prediction program tRNAscan-SE was used to confirm the number and location of tRNA genes (Table 1). Later, dot-plot analyses were used to compare each plastome with that of *Nicotiana tabacum* L. (Solanaceae - NC_001879) (Shinozaki *et al.*, 1986), to examine the gene order conservation and identify the position of the Long Single Copy (LSC), Short Single Copy (SSC), two identical Inverted Repeat (IR), and the two inversion events nested in LSC region (Inv-a, Inv-b), respectively (Kearse *et al.*, 2012).

Using the IR-plot visualization tool, which is available at (https://irscope.shinyapps.io/irapp/), Genome plotting was used to represent the location of the sites connecting the IRs to the SSC and LSC regions in order to show the genetic organization of 17 representative chloroplast genomes (Amiryousefi et al., 2018).

2.2. Sequence variation analysis of chloroplast genomes

For comparing DNA sequences, the online program mVISTA (Frazer et al., 2004) was used to identify

sequence similarities and differences. *Cichorium intybus* chloroplast genome and its annotation were used as the reference chloroplast genome with the rest of the 35 Cichorieae plastomes. The program uses Shuffle-LAGAN mode to align the sequences. The high sequence polymorphism region of the genes and spacers regions were selected to use later in phylogenetic constructions.

2.3. Phylogenetic analysis

Phylogenetic relationships were constructed using high sequence diversity of the protein-coding genes and most polymorphic intergenic regions; these regions were analyzed and selected using the mVISTA alignment. The most polymorphic regions were extracted from each of the 36 plastomes. A multi-sequence alignment tool with default parameters was used to align sequences of each regions by using GENEIOUS prime 2021 software (Kearse *et al.*, 2012). Subsequent to sequence alignments, each alignment was double-checked manually in the GENEIOUS program alignment viewer to avoid any faults or mismatches by the alignment software.

Maximum likelihood (ML) trees were constructed, using whole chloroplast genomes, LSC, SSC, and IR regions, also the most polymorphic protein-coding genes and most polymorphic intergenic regions by MEGA11 (Tamura *et al.*, 2021). To infer the best-fit substitution model for these polymorphic datasets, the best-fit evolutionary model was scored according to the Bayesian Information Criterion (BIC) by using MEGA11 with 1000 replicates bootstrap consensus tree (Felsenstein, 1985). Bootstrap replicates of the tree branches less than 50% were collapsed. For the out-group, the *N.tabacum* plastome was used (Shinozaki *et al.*, 1986).

2.4. Repeat sequence identification

Elements of repetitive DNA in chloroplast genomes of the Cichorieae tribe were investigated using three different programs. Size and location of forward, invert/palindrome, and complement repeats were analyzed using the REPuter v1.0 program (Kurtz et al., 2001), using the program parameters 30 bp as a minimum repeat size, 3 kb for the hamming distance, and a 90% sequence identity threshold. Further, TRF v4.09 (Benson, 1999) was used to identify tandem repeat sequences (> 10 bp in length); program parameters were 2, 7, and 7 for matches, mismatches, and indels, respectively, and the minimum alignment score and maximum period size were set to 50 and 500, respectively. Likewise, microsatellite identification tool MISA v1.0 was used to analyze the number and type of Single Sequence Repeats -SSRs (≥ 10 bp), with thresholds of 10, 5, 4, 3, 3, and 3 repeat units for mono-, di-, tri-, tetra-, Penta-, and hexanucleotides, respectively (Thiel et al., 2003).

3. Results and Discussions

3.1. Structural and organization comparison of Cichorieae tribe plastomes

The Cichorieae tribe of Asteraceae is the most taxonomically researched tribe; its members display easy identification by overall morphological characteristics. However, an extensive range of evolutionary features made it difficult to identify natural groups. Consequently, many taxonomists classified this tribe member at both generic and suprageneric levels differently. This study provided genetic comparisons of Cichorieae tribe members by utilizing the whole chloroplast genome in order to assess genetic diversity at low taxonomic levels and systematic analyses.

Cichorieae tribe plastomes show a typical quadripartite circular structure consisting of one LSC and SSC and two IRs (IRa and IRb) as indicated by using dot blot analysis in the GENEIOUS program (Figure 1). The average genome size of the 36 Cichorieae tribe plastomes was 152 kbp, ranging from 151,173 bp (*Taraxacum erythrospermum*) up to 153,017 bp (*Ixeris repens*). Each IRs region size ranged from 24,420 (*T.erythrospermun*) to 25,174 bp (*Ixeris polycephala*). The LSC region size ranged from 82,924 bp (*Hypochaeris radicata*) to 84,386 bp (*Crepidiastrum sonchifolium*), and the SSC region with the size of about 18,150 bp (*H. radicata*) to 18,596 bp (*Lactuca sativa*) (Table 1).

The plastomes of tribe Cichorieae were with an overall average of 37.7% GC%; there was slight variation among the species with values ranging from 37.5 to 37.8 %. The L.sativa plastomes were the only plastomes found to have the lowest GC% content (37.5%) for their whole genomic chloroplast sequence, while each of (Crepidiastrum denticulatum, Crepidiastrum lanceolatum, H. radicata, Lapsanastrum humile, Reichardia ligulata) had the highest GC content (37.8%) in their plastomes (Table 1). However, the GC% contents of IR regions (43.0-43.2%) were much higher than the LSC region (35.7-36.0%) and SSC region (31.0-31.6%). This variation is certainly related to the high GC% content of ribosomal RNA (rRNA) genes (54.2-55.4%) located and distributed only within IR regions. These results show that the GC contents comparison among Cichorieae tribe plastomes showed high similarities among genera and at the species levels (Table 1).

Previous studies have shown that expansions and contractions of the IRs caused variations in the chloroplast genome size and LSC and SSC length across contrasting genera. Accordingly, the IRs and their gene contents are the most conserved regions of the plastome, while the LSC region is the least conserved (Wang *et al.*, 2008; Zhu *et al.*,2016).

3.2. Gene contents and organization

The chloroplast genomes of the tribe Cichorieae encoded a range of functional genes (Table 1). The lowest number of plastome functional genes is 126 genes recorded in Sonchus boulosii, and the highest number is 136 genes recorded in each of H.radicata, Stebbinsia umbrella, Taraxacum brevicorniculatum, and Taraxacum kok-saghyz. The recorded functional genes include 90 protein-coding genes in the plastome of L.sativa, and S.umbrella. Likewise, 91 protein-coding genes were recorded in T.brevicorniculatum, and T.kok-saghyz plastomes, and the rest of the plastomes of Cichorieae tribe generally comprised of 89 protein-coding genes (Table 1). These differences in coding genes number took place with the presence of the ycf9 gene in L.sativa, pbf1 in S.umbrella, and ycf68 with ihbA genes in each of T.brevicorniculatum, and T.kok-saghyz plastomes. The above genes all were absent in the remaining Cichorieae tribe plastomes. However, according to Wicke et al. (2011), the angiosperm plastomes include 70 up to 88 protein-coding genes. In angiosperms, plastid genome gene number and order is conserved (Wolfe et al., 1987) in comparison to nuclear genome. This is because chloroplast sequences evolve at approximately half the speed of nuclear regions (Jansen et al., 2005; Walker et al., 2014).

Number of genes duplicated in IRs (from rps19 to rps15) were different among plastomes, 17-21 duplicated genes have been recorded in Cichorieae tribe plastomes (Table 1). There were 17 genes duplicated in IRs of *C.intybus, Dendroseris* plastomes, *Sonchus* plastomes, in addition to recording 18 genes duplicated in each of *Crepidiastrum* plastomes, *Ixeris* plastomes, *R.ligulata, S.umbrella, Taraxacum* plastomes, and *Youngia japonica,* whereas each of *H.radicata, L.humile* plastomes included 19 genes duplicated in IRs.

rRNA genes were 3-4 different genes, all duplicated in the IRs, of which plastomes of *Dendroseris, Lactuca, Sonchus, and R.ligulata* contain only 3 different rRNA genes (5S-rRNA, 16S-rRNA, and 23s-rRNA), and the rest of the Cichorieae tribe in this study contains 4 different rRNA genes (4.5S- rRNA, 5S- rRNA, 16S- rRNA, and 23S-rRNA) (Table 1). However, six tRNA genes were duplicated in IRs in Cichorieae plastomes under study.

Table 1. Size, gene contents, and gene orders of complete chloroplast genome sequences of 36 Cichorieae tribe species downloaded fro	m
the National Center for Biotechnology Information (NCBI).	

								% GC				No. of			No. of	
# C	Genome	Genome	LSC	SSC	IK	33.71 1	1.00		DNU	000	No. of	protein-	NO. O	I NO. OI	gene	Accession
# Genus	characteristics	Size	Length	Length	Lengtr	whole	LSC	IR	rkna	SSC	Functional	coding	tKINA	TKNA	duplicated	No.
		(bp)	(bp)	(bp)	(bp)	Genome	region		in IR	region	genes	genes	genes	genes	by IRs	
1 Cichorium L.	C. intybus L.	152,975	84,232	18,561	25,091	37.7	35.8	43.2	54.2	31.4	129	89	32	8	20	NC 043842.1
	C. denticulatum (Houtt.)		- , -	- ,	- ,						-		-	-	-	
	Pak & K.	152,689	84,154	18,519	24,942	37.8	35.9	43.2	55.2	31.4	135	89	38	8	21	NC_042149.1
2 Crepidiastrum	C lanceolatum Nakai	152 748	84 022	18 568	25.079	37.8	35.9	43 3	55.2	31.3	135	89	38	8	21	NC 0465121
Nakai.		102,710	01,022	10,000	20,077	57.0	5517	10.0	00.2	51.5	100	0,7	50	0	21	110_010012.1
	C. sonchifolium (Bunge)	152,603	84,386	18,547	24,835	37.7	35.8	43.2	55.2	31.5	134	89	37	8	21	NC_046513.1
	Pak & K.															
	& Arn	152,199	84,187	18,552	24,730	37.6	35.8	43.2	55.2	31.3	127	89	32	6	18	NC_051923.1
	D litoralis Skottsh	152 263	8/ 213	18 576	24 737	37.6	35.8	13.1	55 3	31.2	128	80	33	6	18	NC 051922.1
	D. macrantha Skottsh	152,205	84 213	18,576	24,737	37.6	35.8	43.1	55.3	31.2	128	80	33	6	18	NC 051924.1
	D. marginata Hook	152,205	04,215	10,570	24,757	57.0	55.0	45.1	55.5	51.2	120	07	55	0	10	NC_051724.1
3 Dendroseris D Don	& Arn	152,261	84,236	18,551	24,737	37.6	35.8	43.1	55.3	31.2	128	89	33	6	18	NC_051925.1
5 Denaroseris Bibon	D micrantha Hook															
	& Arn	152,327	84,287	18,564	24,738	37.6	35.8	43.1	55.3	31.2	128	89	33	6	18	NC_051921.1
	D. ninnata Hook. &															
	Arn.	152,290	84,258	18,568	24,732	37.6	35.8	43.1	55.4	31.2	128	89	33	6	18	NC_051926.1
	D. pruinata Skottsb.	152,348	84,291	18,583	24,737	37.6	35.8	43.1	55.4	31.2	128	89	33	6	18	NC 051920.1
4 Hypochaeris L.	H. radicata L.	151,330	82,924	18,150	25,128	37.8	36	43.1	54.9	31.6	136	90	38	8	21	NC 044795.1
	I. polycephala Cass.													_		
5 Ixeris Cass.	II. Ex DC.	152,776	84,084	18,344	25,174	37.7	35.9	43.0	55.2	31.2	135	89	38	8	21	NC_046514.1
	I. repens A. Grey.	153,017	84,242	18,495	25,140	37.6	35.8	43.1	55.1	31.2	135	89	38	8	21	MW092111.1
<pre></pre>	L. raddeana Maxim.	152,339	83,976	18,521	24,921	37.7	35.9	43.1	54.4	31.2	132	89	35	8	21	NC_056380.1
6 Lactuca L.	L. sativa L.	152,765	84,103	18,596	25,033	37.5	35.7	43.1	55.1	31	135	90	37	8	21	AP007232.1
7.1	L.humile (Thunb)Pak	152 592	94.044	10.462	25.025	27.0	25.0	42.0	55.0	21.4	125	80	20	0	21	NO 046515-1
/ Lapsanastrum P&K	&K.Bremer	152,582	84,000	18,402	25,027	37.8	35.9	45.0	55.2	51.4	155	89	38	8	21	NC_040515.1
8 Reichardia Roth.	R.ligulata (Vent.) K. & Sun.	152,620	84,205	18,525	24,945	37.8	35.7	43.2	55.3	31.1	127	89	32	б	18	NC_051919.1
	S. acaulis Dum. Cours.	152,071	84,334	18,244	24,746	37.6	35.8	43.1	55.2	31.5	127	89	32	б	18	NC_042382.1
	S. arvensis L.	151,967	84,251	18,184	24,766	37.6	35.8	43.1	55.2	31.5	127	89	32	6	18	NC_054161.1
	S. asper (L.) Hill.	151,849	84,156	18,217	24,738	37.6	35.8	43.1	55.3	31.4	127	89	32	6	18	NC_048510.1
	S. boulosii Chamboul.	152,016	83,988	18,566	24,731	37.6	35.8	43.0	55.2	31.2	126	89	31	6	18	NC_042244.1
9 Sonchus L.	S. canariensis (Sch.Bip.)	152.075	84.337	18.245	24.746	37.6	35.8	43.1	55.3	31.5	127	89	32	6	18	NC 042381.1
	Boulos.	152 105		10,502		07.6	25.0	10.1	55.0	21.2	120	00	25	,	10	-
	S. leptocephalus Cass.	152,406	84,331	18,583	24,740	37.6	35.8	43.1	55.5	31.2	130	89	35	6	19	MN334533.1
	S. oleraceus L.	151,849	84,156	18,217	24,738	37.6	35.8	43.1	55.5	31.5	127	89	32	6	18	NC_048452.1
	S. webbu Scn. Bip.	152,194	84,209	18,427	24,749	37.0	33.8	45.1	55.5	51.5	127	89	32	0	18	NC_042585.1
10 Stebbinsia Lipsch.	S. umbreua (Franch.) Lipsch.	152,462	84,125	18,561	24,888	37.7	35.9	43.1	55.2	31.2	136	90	38	8	21	NC_051973.1
	T.brevicorniculatum Korol.	151.282	83.862	18.578	24.421	37.7	35.9	43.3	55.1	31.2	136	91	37	8	21	NC 032056.1
	T. coreanum Nakai.	151,451	84,019	18,500	24,466	37.7	35.8	43.3	55.1	31.2	135	89	38	8	21	MN689808.1
	T. erythrospermum Resser.	151,173	83,812	18,521	24,420	37.7	35.8	43.2	55.1	31.2	135	89	38	8	21	MN689810.1
	T. hallaisanense Nakai.	151,554	84,066	18,524	24,482	37.7	35.8	43.3	55.1	31.2	135	89	38	8	21	MW067130.1
11 Taraxacum Wigg.	T. kok-saghyz Rodin.	151,338	83,986	18,490	24,431	37.7	35.9	43.2	55.1	31.3	136	91	37	8	21	NC_032057.1
	T. mongolicum Hand. Mazz	151,451	84,052	18,541	24,429	37.7	35.8	43.3	55.1	31.2	135	89	38	8	21	NC_031396.1
	T. officinale Wigg.	151,324	83,895	18,567	24,431	37.7	35.9	43.3	55.1	31.2	135	89	38	8	21	NC_030772.1
	T. platycarpum Dahlst.	151,307	83,922	18,507	24,439	37.7	35.9	43.3	55.1	31.3	135	89	38	8	21	NC_031395.1
12 Youngia Cass.	Y. japonica (L.) DC.	152,540	84,047	18,445	25,024	37.7	35.9	43.2	55.2	31.4	135	89	38	8	21	NC_046516.1



Figure 1. Dot-plot analyses show the position of the LSC, SSC, IRs, and the two inversions (Inv-a, Inv-b) in the LSC region.

The tRNA gene numbers ranged from 31 tRNA in S.boulosii up to 38 tRNA in plastomes of Taraxacum, Ixeris, Crepidiastrum, H.radicata, S.umbrella, and Y.japonica. The tRNA genes that were present in some plastomes and absent in others include trnfM-CAU, trnG-GCC, trnG-UCC, trnI-CAU, trnI-GAU. They were absent in each of the Dendroseris, Lactuca, and Sonchus plastomes. Furthermore, there were no trnfM-CAU and trnG-UCC genes in each of the plastomes of C.intybus, and R.ligulata. And trnI-GAU were not recorded in each of C.intybus and R.ligulata. There is no trnG-GCC in T.brevicorniculatum or T.kok-saghyz. Contrarily, the plastomes of C.intybus, Dendroseris berteroana, Lactuca raddeana, and S.boulosii lack any trnV-UAC annotation. Only the plastomes of R.ligulata, C.intybus, and C.sonchifolium lack the trnI-CAU gene, trnL-UAA, and *trn*Y-GUA, respectively.

In contrast to the absence of a particular gene in some plastomes, examination of the LSC region of Cichorieae plastomes showed duplication of some genes in certain lineages. The results showed variation in numbers of trnF-GAA repeated in Taraxacum plastomes compared to all other Cichorieae tribe plastomes. The trnF-GAA gene is doubled in (Taraxacum coreanum, Taraxacum Taraxacum mongolicum, hallaisanense, Taraxacum officinale, and Taraxacum platycarpum) plastomes and tripled in (T.brevicorniculatum, T.erythrospermum, and T.kok-saghyz) plastomes. As the results show, this duplication and triplication could be restricted to Taraxacum chloroplast genomes only and previously encountered by Salih et al. (2017) in comparing three Taraxacum plastomes with several different Asteraceae plastomes. Furthermore, Wittzell (1999) studied different Taraxacum taxa based on trnL-trnF region sequence diversity. The diversification in repeating trnF-GAA gene provides support for Taraxacum diversification on evolutionarily old and younger/derived taxa.

3.3. Inverted repeat regions contraction and expansion

It has been found that all land plants exhibit expansion and contraction of IR regions and junction formation (LSC/IR and IR/SSC), which is an indication of plastome evolution (Zhang et al., 2013; Walker et al., 2014). The border between LSC and IRs along with the border of the IR and SSC regions of the 36 Cichorieae plastomes were compared with that of the N. tabacum as a reference genome to study the impact of the junction formation and expansion of the IR regions. The N.tabacum was chosen for this comparison because none of the inversion events (the two inversions in the LSC region and SSC inversion event) occurred in its plastome (Timme et at., 2007; Liu et al., 2013; Zhang et al., 2013), Additionally, the plastome of N.tabacum is regarded as an early angiosperm genome structure. Only plastomes that show variation at their junctions are represented in IR-plot in Figure 2, (D.berteroana, for example, stands in for the other two species in the genus. Each of S.boulosii, Sonchus canariensis, Sonchus leptocephalus, Sonchus oleraceus, and Sonchus webbii are represented by the Sonchus acaulis, while Sonchus arvensis shares connections with Sonchus asper. Each of T.kok-saghyz and T.platycarpum is represented by T.brevicorniculatum, whilst T.coreanum T.erythrospermum, represents T.hallaisanense, T.mongolicum, and T.officinale).

In all the 36 plastomes, the border between LSC and IRb starts at the location of the *rps*19 gene, resulted to produce a pseudogene at the end of IRb, the length of this pseudogene is equal to the expansion of the IRb into the *rps*19 gene. The IRb of *Crepidiastrum* spp., *L.humile*, and *Y.japonica* were extended lesser (about 32 bp) into the *rps*19 gene, whereas *I.polycephala* and *I.repens* had extended into the *rps*19 pseudogene at the end of IRb by 93 and 97 bp, respectively.

In addition, there were different events of IRb region extension into the *ycf1* gene in the 36 Cichorieae tribe plastomes. The *Dendroseris, I.repens, R.ligulata, S.arvensis, S.asper, T.brevicorniculatum,* and *T.kok-saghyz* there IRb region extended into the *ycf1* gene, while in the rest of the plastomes IRb region extended into the *ycf1* gene because of the re-inversion event. This extension created a pseudogene of about 462-472 bp length of the *ycf1* gene at the beginning of the IRa region.

This conserved border among Cichorieae tribe plastomes is similar to the results found among angiosperm plastomes, which are mostly located within *rps*19 or *ycf*1 genes (Walker *et al.*, 2014; Downie and Jansen, 2015) (Figure 2).

Moreover, the gene *ndh*F is expanded and overlapped by 46 bp to the gene ycf1 in the downstream of IRb/SSC region in the plastomes of T.coreanum, T.erythrospermum, T.hallaisanense, T.mongolicum, T.officinale, and T.platycarpum, while, the ndhF gene of the rest of Cichorieae plastomes (Cichorium intibus, Crepidiastrum plastomes, H.radicata, I.polycephala, Lactuca plastomes, S.boulosii, L.humile, S.acaulis, S.canariensis, S.leptocephalusm, S.oleraceus, S.webbii, S.umbrella, and Y.japonica) was located 4 to 51 bp away from the downstream of the IRb/SSC border and did not overlap with ndhF gene. By contrast, Dendroseris plastomes, I.repens, R.ligulata, S.arvensis, S.asper, T.brevicorniculatum, and T.kok-saghyz had the ndhF gene located up to 16 bp from the upstream of the SSC/IRa junction without overlapping (Figure 2).

Furthermore, another junction is IRa/LSC junction, starting with the *trn*H gene located entirely within the LSC region, with various distances from the IRa/LSC junction. The plastome of *H.radicata* with 26 bp was the longest intergenic space of *trn*H genes from the border of SSC/IRa among the 36 plastomes species, while the plastomes of *Sonchus, R.ligulata,* and *Dendroseris* have only 2 bp spacer from the border of SSC/IRa, which is the shortest intergenic spacer of *trn*H genes from the border of SSC/IRa among the 36 plastomes species (Figure 2).

3.4. SSC and LSC inversion events

Despite the two large IRs events in the chloroplast genome, there has been another inversion event in the SSC region of the plastomes when comparing the Cichorieae tribe plastomes with that of N.tabacum. The inversion events in the SSC region and LSC region were investigated by dot-plot analysis using GENEIOUS program (Figure 1) and double-checked manually by visualizing the alignment of the 36 complete chloroplast genomes of the Cichorieae tribe. The gene orders in the SSC region began with the ycf1 gene and ended with the ndhF gene in a completely reversed SSC. These gene orders (SSC inversion) are represented by some of the Cichorieae tribe plastomes including Dendroseris plastomes, I.repens, R.ligulata, S.arvensis, S.asper, T.brevicorniculatum, and T.kok-saghyz (Figure 2). The rest of the Cichorieae tribe plastomes in this study do not represent these gene order; thus, their SSC was not inverted (Figure 2). The results also showed that there were differences in occurring SSC region inversion at the species level, as indicated in plastomes of Taraxacum, Ixeris, and Sonchus. The inversion occurred in some species plastomes and was absent in other plastomes belonging to these three genera (Figure 2). These structural variations and sequence rearrangements of the Cichorieae chloroplast genomes are very important for providing a vital resource for molecular evolution and phylogenetic studies (Ogihara et al., 1988). IR/SSC junction expansion and constriction along with the inversion of the SSC region were of interest to several researchers to study earlier in detail (Palmer, 1991; Wang et al., 2008; Downie and Jansen, 2015; Walker et al., 2015; Zhu et al., 2016). In addition, it has been demonstrated that chloroplast DNA within individual plants exhibits a form of heteroplasmy in

which the plastome exists in two equimolar states (inversion isomers) that differ in the relative orientation of the SSC region (Palmer, 1983; Walker et al., 2015). This re-inversion is considered an ordinary phenomenon among chloroplast genomes of higher plants, and it is not a product of any evolutionary event.

Another and third inversion events in chloroplast genomes are the two inversions in the LSC region, as they are a specific feature of the Asteraceae chloroplast genomes (Salih *et al.*, 2017). These two inversion events in the LSC region were shared among the plastome of all the 36 Cichorieae tribe plastomes under study when compared to *N.tabacum* (Figure 1). The two inversion events consist

of the large inversion of about 22 kbp inversion (Inv-a) and a smaller inversion (Inv-b) sized 2.6 kb nested within the large inversion region (Inv-a). The only differences between these two inversions in Cichorieae tribe plastomes studied compared to the same inversion present in other Asteraceae plastomes are from the size of these inversions, as it is recorded in other Asteraceae chloroplast genomes the inv-a size is about 22.8 kb and the inv-b is about 3.3 kb (Kim *et al.*, 2005). Consequently, the results from the current study showed that the Inva and Invb size are small in comparison to same inversion in other Asteraceae plastomes.



Figure 2. IR-plot of 17 plastomes. Each species and their corresponding chloroplast genome sequence length are depicted to the left of each track. Genes transcribed in positive and negative strands are presented above and below of their corresponding tracks with from right-to-left and left-to-right directions, respectively. The arrows are showing the bp distance of the start or end coordinate of a given gene from the corresponding junction site. For the genes extending from a region to another, the T bar on the top or below shows the extent of their parts with their corresponding values. The genes in the vicinity of the junctions are the realistically scaled projections of the bp distances for each

site. JLB= junction between LSC and IRb, JSB= junction between SSC and IRb, JSA= junction between SSC and IRa and JLA= junction between IRa and LSC.

3.5. Sequence divergence

Overall sequence identity and divergent regions within the Cichorieae tribe species at the plastome level were performed using mVISTA (Frazer et al., 2004) programs by calculating and visualizing regions with high sequence variations among the 36 plastomes (Appendix 1). The results showed that the structure and gene order conservation in Cichorieae tribe plastomes indicate evolutionary conservation at the plastome level (Appendix 1). The non-coding regions (intergenic spacers) were more divergent than the coding regions. However, there was an exception of some coding region diversity among Cichorieae plastomes, including ccsA, clpP, matK, ndhA, ndhF, rbcL, rpoC1, rpoC2, ycf1, ycf2, and ycf3 (Appendix 1). One of these coding genes was located in IRs, 4 of them were located in the SSC region, one (ycf1) in the junction of the IR/SSC region, and the rest of the 5 coding genes were located in the LSC region. There was a high level of similarity recorded in IRs sequences and a low level of similarity recorded in the LSC sequences. The differences in the level of similarity between IRs and LSC sequences possibly regarded as gene conservation resulted from the copy number variations in IR sequences (Khakhlova and Bock, 2006; Bock, 2014).

3.6. Phylogenetic tree construction and marker Identification

However, accurate phylogenetic construction can be achieved using the whole chloroplast genomes. Scientists constantly attempt to discover new and valuable plastid markers, through finding new plastome regions for investigating intra-genomic and interspecific variations in rates of molecular evolution. In the current study, the phylogenetic inference was built for the whole plastomes, each of the LSC, SSC, and IR regions separately, using N.tabacum as the outgroup. Furthermore, from the mVISTA analysis, a set of 37 regions with a high sequence diversity was selected (one intron is included among the 27 most polymorphic intergenic regions and the 10 most polymorphic coding genes). In order to perform a phylogenetic analysis, these regions were extracted from each plastome under study. The ML method was used based on the best-fit substitution model for such polymorphic datasets, scored according to the Bayesian information criterion (BIC). Mostly (for the coding, whole chloroplast genomes, LSC, SSC, and IR regions), the best substitution model was GTR+G., whereas T92+G was generally the best substitution model for intergenic spacers (Appendix 2). Phylogenetic analyses were conducted in MEGA11 (Tamura et al., 2021). The bootstrap consensus tree was inferred from 1000 replicates. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates were collapsed.

The whole chloroplast genome and LSC region have provided the phylogeny of the Cichorieae tribe with great success, and they result in the same ML tree topology (Figure 3 and Appendix 3). These data support the monophyly of the tribe Cichorieae with two clades.

Further, from the coding and noncoding regions phylogenetic inference, different genes and intergenic spacers have been identified as evolutionarily significant markers (Appendix 3); these markers have been used for phylogenetic analysis at the species level and below (Shaw et al., 2005; Kress et al., 2005; Takahashi et al., 2005; Daniell et al., 2006; Timme et al., 2007; Shaw et al., 2007). In the field of evolutionary phylogeny, chloroplast genes and genomes have been major research areas (Tremetsberger et al., 2013; Mandel et al., 2017; 2019). The resolution of the phylogenetic tree of the tribe Cichorieae is in progress with some results from the remaining topology in the current study (Appendix 3) and in already published works (Tremetsberger et al., 2013; Mandel et al., 2017; 2019). Incongruence exists in gene and intergenic spacer trees from current work and that of others (Tremetsberger et al., 2013; Mandel et al., 2017). However, among the 10 selected coding regions, only six of them (clpP, matK, ndhA, rpoC1, rpoC2, and ycf1) were detected as hypervariable regions in the present study, and they have been shown to be useful for inferring phylogenetic relationships within Cichorieae tribe plastomes. However, they have not been widely used possibly because of a lack of universal primers and the large number of primer pairs needed to sequence the entire region. The other four coding genes (rbcL, ndhF, ycf2, and ycf3) did not show satisfactory variability to use for inferring the phylogenetic tree (Kumar et al., 2009). Nevertheless, the rbcL coding region has been usually used in many previous studies as a phylogenetic marker for Asteraceae and many other plant families. However, using the rbcL coding gene in this study makes it impossible to derive a solid phylogenetic topology (Appendix 3).

In general, analysis of the sequences of non-coding regions generated a congruent phylogenetic topology with high support for most internal nodes. A high level of divergence was observed along intergenic spacers, including ndhF-rpl32, petN-psbM, psbE-petL, rps4-ndhJ, rps15-ycf1, trnL-trnF, trnT-psbD, and ycf3-trnS, and trees were fully congruent with the phylogenetic topology of the chloroplast genome. Although ycf4-cemA, trnH-psbA, and petB-petD spacers were frequently used to construct phylogenetic relationships among plant families (Doorduin et al., 2011), the results from this study did not show a congruent topology with high support among the Cichorieae tribe plastomes (Appendix 3). For example, the intergenic region between trnH and psbA genes has been the most preferred region for their primer amplification success and phylogenetic topology construction (Hebert et al. 2003; Hebert and Gregory 2005; Taberlet et al. 2007). In this investigation, this non-coding region does not succeed to generate a congruent phylogenetic topology for the Cichorieae tribe, possibly because these regions are short, and therefore may not yield enough variation to distinguish among closely related species (Shaw and Small, 2005). However, studies showed that some of these regions worked well when combining these short regions with other coding or noncoding region to construct a phylogenetic tree (Kumar et al., 2009).



Figure 3. Molecular Phylogenetic analysis of whole chloroplast genomes of 36 plastomes of the Cichorieae tribe by Maximum Likelihood (ML) method. The percentage of trees in which the associated taxa clustered together is shown next to the branches. *N.tabacum* is used as an outgroup. Evolutionary analyses were conducted in MEGA11.

The rest of the coding regions and intergenic spacers in Appendix 3 are possibly better to use to analyze the divergence that occurs in taxa at family and generic levels as mutational hotspots but not for the interspecific level. Thus, the above investigated coding (clpP, matK, ndhA, rpoC1, rpoC2, and ycf1) and non-coding (ndhF-rpl32, petN-psbM, psbE-petL, rps4-ndhJ, rps15-ycf1, trnL-trnF, trnT-psbD, and ycf3-trnS) regions have a higher level of variability, and they are with good congruent topology and high bootstrap support. These regions, however, having rarely been used, may represent a novel combination of markers for analyzing phylogenetic relationships among the Cichorieae tribe. Thus, universal primers for these coding and intergenic regions may be valuable for the investigation of phylogenetic inference and population genetic analysis of the Cichorieae tribe plastomes at various taxonomic levels. These regions can also be used as a marker for accurate and automated species identification, providing plant taxonomists with a speciesspecific DNA barcode catalogue that is simple to use (Shaw et al., 2005; Kress et al., 2005; Takahashi et al., 2005; Hughes et al., 2006; Daniell et al., 2006; Timme et al., 2007; Shaw et al., 2007).

Regarding the *Lactuca* plastome, as shown in Figure 3 and other phylogenetic topography in Appendix 3, *L.raddeana* nested in a clade including *Taraxacum* and *Ixeries* as it is the wrong place for the *Lactuca* genus, and *L.sativa* placed in the related genera of *Sonchus* and *Dendroseris* clade. So, no consistent conclusions have been reached based on the analysis of different coding and noncoding regions from this genus. However, the classification of *Lactuca* was also controversial in the previous studies (Wang *et al.*, 2013; Mandel *et al.*, 2019; Jiang *et al.*, (2021).

3.7. Repeat sequence analysis

The recombination events and genome rearrangements consequently have affected on occurring different repetitive DNA sequences and regions in a genome (Ogihara et al., 1988, Milligan et al., 1989). Different repeat types, sizes, and locations in chloroplast genomes of the Cichorieae tribe were analyzed through using three different programs. The size and location of forwarding (F), inverted (R)/ palindromic (P), and complement (C) repeats were determined using REPuter (Kurtz et al., 2001) (Figure 4). The repetitive calculations excluded the two largest palindromic repetitive DNA of IR regions for the chloroplast genome of 36 Cichorieae tribe plastomes. The Cichorieae tribe plastomes were conserved in their total number of repeats; however, there were some exceptions, as the majority of plastomes under study included 49 total number of repeats, except 45 repeats in S.boulosii, 48 repeats in S.umbrella and T.brevicorniculatum, 50 in S.arvensis and S.canariensis, and the highest number of repeats was 58 repeats recorded in I. polycephala plastome. The most abundant repeat type was forwarded repeats (11-130 bp of length) among the 36 Cichorieae tribe plastomes, ranging from 20 repeats in Y.japonica and 32 repeats in I.polycephala. Reverse repeats were the least abundant repeats, ranging from only one reversed repeat in S.arvensis, S.boulosii, T_{\cdot} hallaisanense, T. kok-saghyz, and 7 repeats in I.repens, L.humile, and Y.japonica (Figure 4).

Meanwhile, tandem repeat sequences (>10 bp in length) were identified with tandem repeat finder (TRF) v4.09 (Benson, 1999). The most abundant tandem repeats in Cichorieae plastomes were 46 tandem repeats in *S.boulosii*, and the lowest numbers were found in *R.ligulata* and *S.umbrella* which was only 28 tandem repeats (Table 2). The tandem repeat distributed abundantly and widely in the LSC region and were around 70% of total tandem repeats recorded in the LSC region, 7% in SSC, and 23% in IR regions. The majority of tandem repeats were located in intergenic spacers and were around 74% of total repeats found in plastomes, and 26% located in coding genes (Table 2). The tandem repeats of 21-30 period size are most variable among the Cichorieae tribe plastomes.

The simple sequence repeats (SSRs) are the small unit of repetitive DNA sequence occurring in both coding and non-coding regions. SSRs are useful DNA markers to study population genetics because of their co-dominant expression, polymorphic nature (Yamane *et al.*, 2009; Redwan *et al.*, 2015), and genetic variation among plant genotypes in chloroplast genomes (Vendramin *et al.*, 1999, Deguilloux *et al.*, 2004, Piya and Nepal, 2013). The Perlscript MIcroSAtellite identification tool MISA v1.0 (Beier *et al.*, 2017) was used to analyse the number and type of simple sequence repeats (SSRs) (Figure 5 and 6). The results showed that the total number of SSRs ranged from the lowest of 26 SSRs in C. sonchifolium, Y.japonica, and the most abundant is 42 SSRs in C. denticulatum (Figure 5). Number of each SSRs type identified in plastomes under study is 14-37 mononucleotide SSRs, 2-9 dinucleotide SSRs, 1-6 trinucleotide SSRs, 1-2 tetranucleotide SSRs in some plastomes, and in some cases 1-2 penta- and hexanucleotide chloroplast SSRs. The distribution of SSRs in the LSC region is more abundant than their distribution in SSC and IRs regions (Huotari and Korpelainen, 2012). The mononucleotide SSRs are the most abundant (Figure 5) among the SSRs repeats. In general, the intergenic SSRs are more abundant than the genic SSRs in Cichorieae tribe plastomes; this is the common result for occurring SSRs in Asteraceae plastomes as previously analyzed by Liu et al. (2012) and chloroplast genomes of some other plant families (Sherman-Broyles et al., 2014; Dong et al., 2018; Liu et al., 2020), which explains the lower polymorphism of coding region in comparison to non-coding region.

The majority of mononucleotide SSRs (14-29 bp) are composed of A/T type which comprises 46 (79%) of the total SSRs in the plastomes (Figure 6). The di-nucleotide SSRs type was AC/GT and AT/AT; however, the most abundant was AT/AT type of di-nucleotide SSRs comprises the 10 (28%) of the total SSRs, yet AC/GT was recorded only one time in C. sonchifolium plastome among the Cichorieae plastomes (Figure 6). The tri-nucleotide SSRs types of AAG/CTT, AAT/ATT, ATC/ATG comprised 3 (15%) of the total SSRs, although ATC/ATG tri-nucleotide SSR was recorded only one time in each of T.coreanum, T.hallaisanense, T. mongolicum plastomes among the other plastomes (Figure 6). The tetra-nucleotide (AAAG/CTTT, AAAT/ATTT, AATC/ATTG, AGGG/CCCT, and AGAT/ATCT) chloroplast SSRs was more abundant than tri-nucleotide SSRs, comprising 4 (20%) of the total SSRs; However, the tetra-nucleotide SSRs of the AGGG/CCCT type recorded twice in the plastomes of C.intybus and Crepidiastrum, while AGAT/ATCT only recorded once and was present in the plastomes of *Taraxacum* (Figure 6). Contrarily, pentanucleotide (AATCT/AGATT) chloroplast SSRs have only been detected in the plastome of L.raddeana, (AATAT/ATATT) chloroplast SSRs have only been detected in the plastome of T.mongolicum, and (AACCC/GGGTT) chloroplast SSRs have only been detected in the plastome of T.platycarpum. In addition, hexanucleotide (AATCTC/ AGATTG, AATCCT/ AGGATT, AAGGAT/ ATCCTT, AAAATT/ AATTTT, AGATAT/ATATCT) chloroplast SSRs were identified in each of Crepidiastrum, Sonchus, and Taraxacum plastomes only, recorded one time in each plastomes, and absent in other Cichorieae chloroplast genomes under study.

The above-mentioned SSRs types are valuable markers for species identification, hybridization, and introgression analyses, the study of plant phylogeny, and population differentiation (Yamane *et al.*, 2009; Haasl and Payseur, 2011; Sonah *et al.*, 2011; Laiadi *et al.*, 2019). The results of this study showed PCR-based analysis of tetra-, penta-, and hexanucleotide repeats is better to be used to detect diversity in the chloroplast genomes of Cichorieae tribe plastomes at the level of genera and species. Thus, the analysis of polymorphic microsatellites provides an important and not expensive experimental tool to examine several plant genetic issues such as genetic diversity, genetic structure, evolutionary history, and hybridization in native and agricultural species (Wheeler *et al.*, 2014).







Table 2. Shows the total number, size, location, and region of Tandem repeats (TRs) in Cichorieae tribe plastomes, identified with TRF v4.09 (Benson, 1999), with parameters of 2, 7, and 7 for matches, mismatches and indels, respectively. The minimum alignment score and maximum period size were set to 50 and 500, respectively.

			TRs J	period s	size		Location		Region		
#	Species	Total	11-20	21-30	31-40	>40	No. of tandem repeat located in spacer	No. of repeat located in gene	No. of TR in LSC	No. of TR in SSC	No. of TR in IRs
	C. intybus L.	40	32	4	4		31	9	27	7	6
	C. denticulatum (Houtt.) Pak & K.	40	26	10	4		30	10	29	3	8
	C. lanceolatum Nakai.	43	29	10	4		32	11	28	8	7
	C. sonchifolium (Bunge) Pak & K.	39	30	7	2		28	11	25	6	8
	D. berteroana Hook. & Arn.	32	22	6	4		24	8	22	2	8
	D. litoralis Skottsb.	33	22	7	4		25	8	23	2	8
	D. macrantha Skottsb.	33	22	7	4		25	8	23	2	8
	D. marginata Hook. & Arn.	33	22	7	4		25	8	24	1	8
	D. micrantha Hook. & Arn.	33	21	8	4		26	7	24	1	8
	D. pinnata Hook. & Arn.	34	22	8	4		26	8	24	2	8
	D. pruinata Skottsb.	34	22	8	4		26	8	24	2	8
	H. radicata L.	36	27	4	5		29	7	22	2	12
	I. polycephala Cass. Ex DC.	38	27	7	4		32	6	29	1	8
	I. repens A. Grey.	32	21	9	2		25	7	23	3	6
	L. raddeana Maxim.	37	22	12	3		27	10	23	4	10
	L. sativa L.	36	21	12	3		26	10	17	5	14
	L. humile (Thunb) Pak & K.Bremer	33	27	4	2		23	10	22	5	6
	R. ligulata (Vent.) K. & Sun.	28	18	4	6		15	13	18	2	8
	S. acaulis Dum. Cours.	36	23	9	4		24	12	24	4	8
	S. arvensis L.	38	23	11	4		28	10	25	1	12
	S. asper (L.) Hill.	38	26	8	4		19	19	29	1	8
	S. boulosii Chamboul.	46	31	11	4		33	13	35	3	8
	S. canariensis (Sch.Bip.) Boulos.	37	24	9	4		25	12	25	4	8
	S. leptocephalus Cass.	36	24	8	4		24	12	27	4	5
	S. oleraceus L.	39	26	9	4		25	14	29	2	8
	S. webbii Sch. Bip.	40	25	11	4		30	10	29	3	8
	S. umbrella (Franch.) Lipsch.	28	21	5	2		22	6	16	3	9
	T.brevicorniculatum Korol.	33	19	11	2	1	26	7	22	3	8
	T. coreanum Nakai.	30	17	11	2		24	6	20	2	8
	T. erythrospermum Resser.	36	21	12	2	1	27	9	28	0	8
	T. hallaisanense Nakai.	36	17	17	2		30	6	24	2	10
	T. kok-saghyz Rodin.	37	21	13	2	1	31	6	26	3	8
	T. mongolicum Hand. Mazz	29	19	8	2		23	6	21	2	6
	T. officinale Wigg.	35	19	14	2		29	6	26	1	8
	T. platycarpum Dahlst.	35	19	14	2		27	8	25	0	10
	Y. japonica (L.) DC.	31	25	4	2		21	10	22	2	7



Figure 5. The number of SSRs, each repeat sequence SSRs length was screened to be ≥ 10 bp. SSRs were identified with thresholds of 10, 5, 4, 3, 3, and 3 repeat units for mono-, di-, tri-, tetra-, Penta-, and hexanucleotides, respectively using the microsatellite identification tool MISA v1.0 (Thiel *et al.*, 2003).



Figure 6. The type of SSRs, each repeat sequence SSRs length was screened to be ≥ 10 bp. SSRs were identified with thresholds of 10, 5, 4, 3, 3, and 3 repeat units for mono-, di-, tri-, tetra-, Penta-, and hexanucleotides, respectively using the microsatellite identification tool MISA v1.0 (Thiel *et al.*, 2003).

4. Conclusion

Characteristic comparison among 36 Cichorieae tribe plastomes showed overall similarity of its plastomes structure. There were no significant differences among representatives plastomes of the tribe, these similarity among the tribe plastomes results of the remarkable stability of chloroplast characteristics at lower taxonomic level. However, there were some exceptions: number of rRNA genes was different among the plastomes, and there were little differences in the total number of coding, tRNA, and rRNA genes among the tribe plastomes. In addition, the large LSC region is the least conserved when all parts of the plastome are compared, including group of inversions (Inv a, Inv b), and insertions/ deletions region (*trn*F-GAA gene). The results also showed that there are differences in SSC region inversion that is occurring at the

species level, as indicated in plastomes of *Taraxacum*, *Ixeris*, and *Sonchus* plastomes, as the inversion occurred in some species plastomes and was absent in other plastomes belonging to these three genera. Furthermore, the LSC inversion (Inv-a and Inv-b) is smaller in Cichorieae plastomes in comparison with other Asteraceae plastomes; there is a 0.8 kb difference between the size of an Inv-a in Cichorieae chloroplast genomes and a 0.7 kb difference between the size of an Inv-b in Cichorieae.

The sequence alignments and phylogenetic trees highlighted six potentially concatenated coding regions include *clpP*, *matK*, *ndhA*, *rpoC1*, *rpoC2* and nine potentially concatenated non-coding regions *ycf1*, *ndhF-rpl32*, *petN-psbM*, *psbE-petL*, *rps4-ndhJ*, *rps15-ycf1*, *trnL-trnF*, *trnT-psbD*, and *ycf3-trnS*. These regions possibly are more variable than the regions used so far, at least at the level of a tribe in the Asteraceae family such as the Cichorieae tribe. These regions offer levels of variation better than the regions identified in previous studies and are therefore likely to be good choices for molecular studies at low taxonomic levels for the Cichorieae tribe members. The results also showed that most likely the sample sequence of *Lactuca sativa* in NCBI was incorrectly identified.

The most abundant repeat type was forwarded repeats among the 36 Cichorieae tribe plastomes and reverse repeats were the least abundant repeats, ranging from only one reversed repeat to 7 repeats. When it comes to tandem repetitive DNA, up to 46 repeats were documented, with the LSC area recording 70% of all tandem repeats, the SSC region 7%, and the IR regions 23%. The majority of tandem repeats were located in intergenic spacers and 26% located in coding genes. The tandem repeat of 21-30 period size is most variable among the Cichorieae tribe plastomes.

The distribution of SSRs in the LSC region is more abundant than their distribution in SSC and IRs regions. The mononucleotide SSRs are the most abundant among the SSRs repeats. In general, the intergenic SSRs are more abundant than the genic SSRs in Cichorieae tribe plastomes. The majority of mononucleotide SSRs are composed of A/T type which comprises 79% of the total SSRs in the plastomes. However, the tetra-, penta-, and hexa-nucleotide SSRs were recorded in some plastomes and absent in other plastomes of Cichorieae tribe, so these SSRs repeat could be valuable markers for species identification, hybridization, and introgression analyses, the study of plant phylogeny, and population differentiation.

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Appendix 1. Sequence comparison of 36 Cichorieae tribe plastomes. The mVISTA based similarity graphical information portrays sequence identity to *Cichorium intybus* as a reference plastome. Grey arrows above the alignment denote the gene orientation. A cut-off of 50% identity is used for the plots. In each plot, the Y-scale axis represents percent identity (50 to 100%). Dashed rectangles indicate highly divergent regions compared with the reference plastome.

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#### Cichorium

Alignment 9 Dendroseris-pinnata Dendroseris_pinnata 10 alignments Criteria: 70%, 100 bp Regions: 234

**Appendix 2**. The best-fitted model of evolution for the whole chloroplast genomes, LSC, SSC, IR and all polymorphic regions analyzed in this investigation. Each analysis involved 37 nucleotide sequences. The table shows the total positions in the final dataset for each model, and BIC scores (Bayesian Information Criterion) which describe the best substitution pattern for each models. Evolutionary analyses were conducted in MEGA11 (Tamura et al., 2021).

#	Region for phylogenetic	Model	total number of	BIC			
	analysis	WIGGET	positions in a dataset	DIC			
1.	Whole genome	GTR+G	136267	1038336.106			
2.	LSC	GTR+G	88267	938336.209			
3.	SSC	GTR+G+I	21241	149228.126			
4.	IR	GTR+G+I	26615	99146.24677			
5.	clpP	T92+G	2375	16915.02854			
6.	matK	GTR+G	1563	10589.44004			
7.	ndhA	GTR+I	2356	14965.56373			
8.	ndhF	GTR+G	2285	15021.52762			
9.	rbcL	T92+G+I	1459	8143.670817			
10.	rpoC1	GTR+G	2886	17048.15912			
11.	rpoC2	GTR+G	4209	23413.47337			
12.	ycf1	GTR+G+I	6433	53978.64294			
13.	ycf2	GTR+G	7094	27720.25902			
14.	ycf3	T92+G	2081	11034.21292			
15.	atpI-atpH	GTR	1314	8510.050189			
16.	ndhC-atpE	T92+G	153466	428169.7554			
17.	ndhF-rpl32	GTR+G	1427	11827.82275			
18.	petA-psbJ	T92+G	1092	8035.746059			
19.	petB-petD	T92+G	2081	11034.21292			
20.	petD-rps11	T92+G	1363	7936.536827			
21.	petG-rpl20	T92+G	2648	14800.50839			
22.	petN-psbM	GTR+G	5446	28823.03943			
23.	psaA-ycf3	T92+G	895	6287.743009			
24.	psaI-ycf4	T92	474	3577.660599			
25.	psbA-trnK	T92+G	266	2272.706353			
26.	psbE-petL	GTR+G	1514	10670.35651			
27.	rpl32-trnL UAG	GTR+G	1186	10568.76413			
28.	rps4-ndhJ	T92+G	3366	22221.07111			
29.	rps12-clpP	T92	179	1741.680704			
30.	rps15-ycf1	T92+G	574	5152.612542			
31.	rps16 intron	T92+G	1268	8537.406947			
32.	rps16-trnQ UUG	GTR+G	1377	11020.19351			
33.	trnH GUG-psbA	HKY+G	649	6108.51606			
34.	trnK-rps16	GTR+G	1102	8192.155682			
35.	trnL-trnFGAA	GTR+G	761	5598.25329			
36.	trnS-trnC GCA	T92+G	981	8039.29115			
37.	trnT GGU-psbD	T92+G	1513	10824.81313			
38.	trnT UGU-trnL	T92+G	971	7371.637396			
39.	ycf3-trnS GAA	GTR+G	1042	7956.211414			
40.	ycf4-cemA	T92+G	936	6352.32406			
41.	ycf15-rps7	T92+G	2060	8562.738488			

**Appendix 3**. Molecular Phylogenetic analysis by ML method. Each analysis involved 37 nucleotide sequences. Numbers on the nodes are bootstrap values based on 1000 replicates. *Nicotiana tabacum* is used as an out-group. Evolutionary analyses were conducted in MEGA11 (Tamura *et al.*, 2021).













Nicotiana tabacum Hypochearis radicata Cichorium intybus Laduce sadiva beris polycephala beris repens Laguas stum humile Youngia japonica Crepiciastrum sonchifoium Crepiciastrum denticulatum Crepiciastrum lanceolatum Laduce raddeana Stebbinsia umbrelia Taraxacum umbrelia Taraxacum umbrelia Taraxacum mongolicum Taraxacum kolk-saginy Sonchus super Sonchus super Sonchus super Sonchus super Sonchus super Sonchus super Sonchus acadis Sonchus acadis Sonchus acadis Sonchus acadis Sonchus acadis Sonchus acadis Dendrosers prinata Dendrosers prinata Dendrosers prinata Dendrosers prinata Dendrosers prinata Dendrosers prinata

rpoC1



Nicotiana tabacum Hypochaeris radicata Lactuce sativa Cichorium infyuis Reichardia ligulata Sonchus webbi Sonchus caulis Sonchus caulis Sonchus caulis Sonchus caulis Sonchus caulis Sonchus aculis Sonchus aculis Sonchus aculis Sonchus asper Sonchus asper Sonchus asper Sonchus asper Bendroseris prinata Dendroseris prinata Dendroseris micrantha Dendroseris machantha Dendrose







Reichardia ligulata Sonchus boulosii Sonchus arvensis Sonchus asper Sonchus vebbii Sonchus canariensis Sonchus acaulis Sonchus leptocephal endrosens massa eris polycephala Ixens polycephala Ixens polycephala Ixens repens Lapsanastrum humile Youngia Isponica Crepidiastrum sonchifol Crepidiastrum lanceola Lactuca raddeana Stebbinsia umbrella Taraxacum mongolicun Taraxacum nallaisanen Taraxacum hallaisanen Taraxacum nailaisanens Taraxacum erythrosperm Taraxacum platycarpum Taraxacum kok-saghyz Taraxacum brevicornicul Taraxacum officinale







liastrum lance





rpl12-clpP

rps15-ycf1



saghyz



90

90

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68 55

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Dichorium intybus leichardia ligulata

68

60

ycf15-rps7

