



The Complete Chloroplast Genome of “Lasona” (*Allium cepa* var. *aggregatum* G.Don), an Important Indigenous Vegetable in the Northern Philippines

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ABSTRACT

Background: “Lasona” (*Allium cepa* var. *aggregatum* G.Don) is a multi-bulb cultivar commonly grown in the Northern Philippines as an annual crop. This, together with bulb onion, are the two main types of onions produced in the country, but the majority of onions exported from the Philippines are the latter. However, despite its importance to the human diet, no complete chloroplast genome sequence has been reported to date. Hence, the assembled plastome can be utilized as a resource for distinguishing the taxon and elucidating its evolutionary relationship with other *Allium* species, particularly within the *aggregatum* group.

Methods: Using Illumina sequencing data, we assembled and annotated the complete chloroplast genome of *A. cepa* var. *aggregatum* accession from the germplasm collection of the Institute of Crop Science, University of the Philippines, Los Baños, Laguna. Moreover, the morphology of the genotyped accession was characterized to ensure that the genome assembled is attributed to the correct taxon. The voucher specimen (ICROPS 1374) was deposited at the Philippine Herbarium of Cultivated Plants of the Institute of Crop Science.

Result: The complete chloroplast (cp) genome had a sequence length of 153,587-bp long, with 36.8% overall GC content. A total of 126 functional genes were found, comprising 80 mRNA genes, 38 tRNA genes and 8 rRNA genes. We annotated 43 genes that code for photosynthesis, 29 genes for self-replication and 6 other genes for other functions. This is the first complete chloroplast genome of *Allium cepa* var. *aggregatum*. The phylogenetic analysis of the assembled genome, together with 21 other *Allium* accessions and 12 other Amaryllidaceae species, revealed that the *A. cepa* accessions formed a distinct clade despite infraspecific plastome variations. Comparison among earlier published plastomes of *A. cepa* showed that the assembled *A. cepa* var. *aggregatum* chloroplast genome was distinct in sequence length and composition. The observed differences could be utilized to further elucidate differences needed for varietal identification.

Key words: Cv. Group *aggregatum*, Intraspecific diversity, Multiplier onion, Next generation sequencing, Plastome.

INTRODUCTION

Allium cepa var. *aggregatum* G.Don (1827) is locally referred to as “lasona,” an important culinary spice and vegetable, particularly in the Northern parts of the Philippines. It is a field-grown bulb belonging to the family of Amaryllidaceae. Otherwise known as shallots, potato onion, or multiplier onion, it is a distinct form in the *aggregatum* group placed under one of the horticultural classifications of *A. cepa* L. (Jones and Mann, 1963). It is characterized by having round, depressed, aggregated bulbs with brown outer coats and undeveloped scape (Don, 1827; Jiemei and Kamelin, 2000). The genus *Allium*, which includes economically significant bulbous vegetables such as onion, is considered native to southwestern Asia. However, it is now cultivated globally (Devi *et al.*, 2015; Rahaman *et al.*, 2021). In general, the center of origin of the genus *Allium* extensively spreads from the Mediterranean basin to central Asia (van der Meer *et al.*, 1997).

A. cepa var. *aggregatum* largely contributes to the human diet and is being utilized as an ornamental, natural alternative to synthetic food preservatives and for medicinal purposes (Saraswathi *et al.*, 2017; Shabir *et al.*, 2020; van der Meer *et al.*, 1997). In the Philippines, it is commonly grown for greens and cooked as a vegetable called “lasona gulay”. Along with yellow and red bulbs, “lasona” is one of

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the most popular varieties of onions, alongside garlic, traded in the country (DA-BAR, 2021). Despite the numerous and distinct variations in appearance, flavor and usage, the differentiation between *Allium* cultivars-particularly onions-remains challenging. This difficulty arises due to the complex nature of *Allium* species and their hybrids, which often exhibit overlapping characteristics (Shabir *et al.*, 2020). Nevertheless, considering these vegetables' widespread cultivation and economic significance, a comprehensive understanding of their genetic variability is indispensable (Rahaman *et al.*, 2021; Ananthan and Balakrishnamoorthy). Hence, we assembled, characterized and compared the chloroplast genome of “lasona” (*A. cepa* var. *aggregatum*) with other *A. cepa* accessions. The assembled plastome can be utilized as a resource for distinguishing the taxon and elucidating its evolutionary relationship with other *Allium* species, particularly within the *aggregatum* group.

MATERIALS AND METHODS

Characterization of plant material

A. cepa var. *aggregatum* were collected from the field gene bank of the Crop Breeding and Genetic Resources Division, Institute of Crop Science University of the Philippines Los Baños, Laguna. Its type locality is at San Nicolas, Ilocos Norte (18°7' 59.7105", 120°36' 10.71"). The prepared voucher specimen (ICROPS 1374) was deposited in the Philippine Herbarium of Cultivated Plants of the Institute of Crop Science, University of the Philippines Los Baños, Laguna, Philippines (<https://cafs.uplb.edu.ph/icrops/>, Dr. Renerio P. Gentallan Jr., rpgentallan@up.edu.ph). Subsequently, the morphology of the accession was characterized based on the prominent morphological markers in the *Allium cepa* group described by Don (1827) to verify the identity of the utilized accession. The whole plant, inflorescence and fruits were photographed *in situ* and *ex situ*. The quantitative traits measured are reported as the mean±standard deviation of the mean while qualitative traits were observed.

DNA extraction, chloroplast genome sequencing, assembly and annotation

Fresh *A. cepa* var. *aggregatum* leaves (0.5 g) were used to extract genomic DNA using the slightly modified CTAB method by Doyle and Doyle (1987). Sequencing was performed using the HiSeq-PE150 platform (Illumina Inc., San Diego, CA, USA) by NovogeneAIT Genomics Singapore PTE LTD (Singapore). The 150-bp paired-end raw reads (20278894) were produced which were subsequently filtered to generate 20248894 of cleaned reads. Using GetOrganelle v1.7.5+ software (Jin *et al.*, 2020), these were assembled into a circular genome. The circularized genome was annotated, then mapped using CPGAVAS2 (Shi *et al.*, 2019) and subsequently visualized using OGDRAW (Greiner *et al.*, 2019). The assembled cp genome sequence was submitted to GenBank with the accession number OP756522 of the National Center for Biotechnology Information (NCBI).

Phylogenetic analysis and genome comparison

The assembled chloroplast genome sequence of *Allium cepa* var. *aggregatum*, along with the complete chloroplast genome sequences of 33 Amaryllidaceae species available from the NCBI website, were used to elucidate phylogenetic relationships. The multiple sequence alignment was performed using MAFFT (Kato and Standley 2013) and a Maximum Likelihood (ML) phylogenetic tree was constructed using the Hasegawa-Kishino-Yano, Gamma distribution (HKY+G) model (Hasegawa *et al.*, 1985) with 1,000 bootstrap replicates in MEGA-XI (Tamura *et al.*, 2021). To determine and visualize the contraction and expansion of IR regions, the chloroplast genome of *Allium cepa* var. *aggregatum* was compared with other closely related *Allium* species using IRScope (Amiryousefi *et al.*, 2018).

RESULTS AND DISCUSSION

Morphological characterization of the plant material

The morphological characteristics of the accession (ICROPS 1374; Fig 1) align with the descriptions provided

Table 1: List of genes annotated in the chloroplast genome of *A. cepa* var. *aggregatum*.

Function	Gene names
Photosystem I	<i>psaA, psaB, psaC, psal, psaJ</i>
Photosystem II	<i>psbA, psbB, psbC, psbD, psbE, psbF, psbI, psbJ, psbK, psbL, psbM, psbN, psbT, psbZ, ycf3</i>
Cytochrome b/f complex	<i>petA, petB, petG, petN</i>
ATP synthase	<i>atpA, atpB, atpE, atpF, atpH, atpI</i>
NADH dehydrogenase	<i>ndhA, ndhB (× 2), ndhC, ndhD, ndhE, ndhF, ndhG, ndhH, ndhI, ndhJ, ndhK</i>
Rubisco large subunit	<i>rbcL</i>
DNA-dependent polymerase	<i>rpoA, rpoB, rpoC1, rpoC2</i>
Small subunit of ribosome	<i>rps11, rps12 (× 2), rps14, rps15, rps16, rps18, rps19 (× 2), rps3, rps4, rps7(× 2), rps8</i>
Large subunit of ribosome	<i>rpl14, rpl16, rpl2 (× 2), rpl20, rpl22, rpl23 (× 2), rpl32, rpl33, rpl36</i>
Proteins of unknown function	<i>ycf4</i>
Other genes	<i>accD, ccsA, cemA, clpP, matK</i>

(× 2): Indicates genes have two copies.

by Don (1827). *Allium cepa* var. *aggregatum* (ICROPS 1374) exhibits green foliage with a shaft that is 29.67 ± 1.34 mm long and 3.52 ± 0.23 mm in diameter. The bulb is broadly elliptic, aggregated, with thin deep purplish red skin and white flesh. The bulbs also have protective bulb-coat leaves, purplish, brown, or white (Geerinck, 1993). Each aggregate is composed of 5 to 8 bulbs, this “dividing” habit constantly appears in breeding lines of the common large-bulbed forms of *A. cepa* (Jones and Mann, 1963).

Complete genome assembly and annotation of *Allium cepa* var. *aggregatum*

The assembled complete cp genome of *Allium cepa* var. *aggregatum* (GenBank no. OP756522) had a typical quadripartite circular structure and a sequence length of 153,587 bp, which is longer than the chloroplast genomes of its closest related *Allium* species, *Allium cepa* (153,529 bp; KM088013.1), *Allium cepa* strain CMS-T (153,568 bp; KM088015.1) and *Allium cepa* genotype normal (N)



Fig 1: Morphological characteristics *Allium cepa* var. *aggregatum*; whole plant (a), aggregate bulbs (b) and dissected bulb (c).

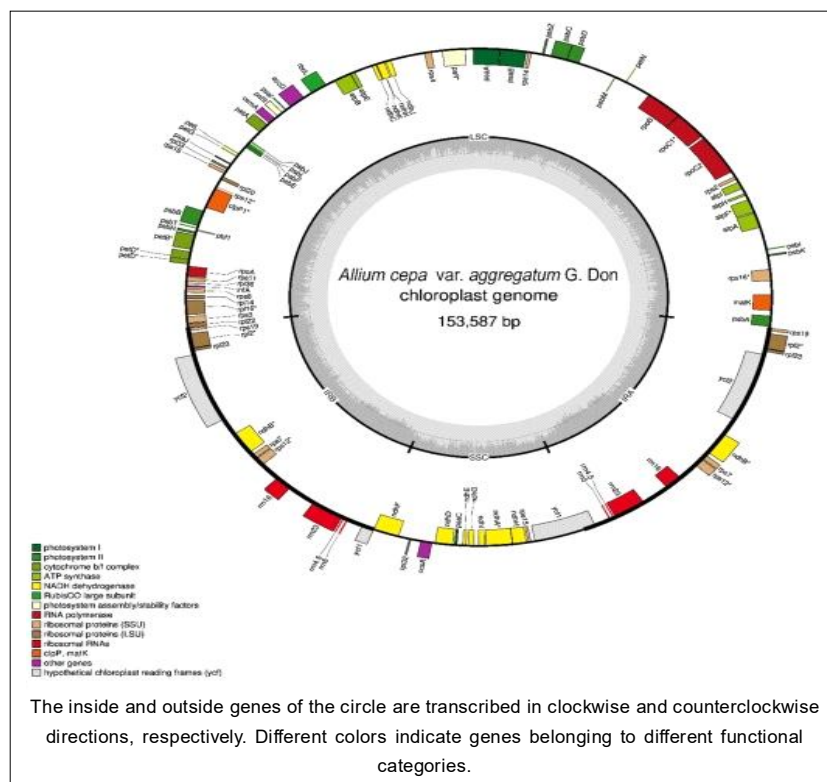


Fig 2: Chloroplast gene map of *A. cepa* var. *aggregatum* genome.

(153,538 bp; KF728080.1) available in NCBI. It includes a pair of inverted repeats (IRa and IRb) with 26,468 bp each that separates the rest of the genome sequences into two single-copy regions: LSC region (17,931 bp in length) and SSC region (17,031 bp in length) (Fig 2). The overall GC content of the cp genome is 36.8% with base compositions of 31.3% A, 31.9% T, 18.1% G and 18.7% C. A total of 126 coding genes were annotated in the cp genome of *A. cepa* var. *aggregatum* comprising 38 tRNA genes, 8 rRNA genes and 80 protein-coding genes. Based on the functions of these genes, there are 43 photosynthesis-related genes, 29 self-replication genes, 6 other genes with different functions (*matK*, *cemA*, *accD*, *clpP*, *cCsA* and *inFa*) and 1 unknown conserved open reading frame (Table 1). This is the first reported complete chloroplast genome of *A. cepa* var. *aggregatum*.

Phylogenetic analysis

Phylogenetic analysis showed that *A. cepa* var. *aggregatum* clustered with other *Allium cepa* accessions to form a distinct clade. However, despite their close relationship, *A. cepa* var. *aggregatum* was markedly separated from the six other *A. cepa* accessions with strong bootstrap support (Fig 3). The observed topology within the *A. cepa* clade may be attributed to infraspecific differences initially observed in this taxon with 19 recorded botanical varieties that are presently considered as heterotypic synonyms (POWO, 2022). This indicates that infraspecific plastome differences are observed between *A. cepa* individuals, unlike other taxa with conserved plastome sequences across accessions (Waters and Schaal, 1991; Agrawal *et al.*, 2014; Liu *et al.*, 2021). Moreover, we observed that *A. cepa*

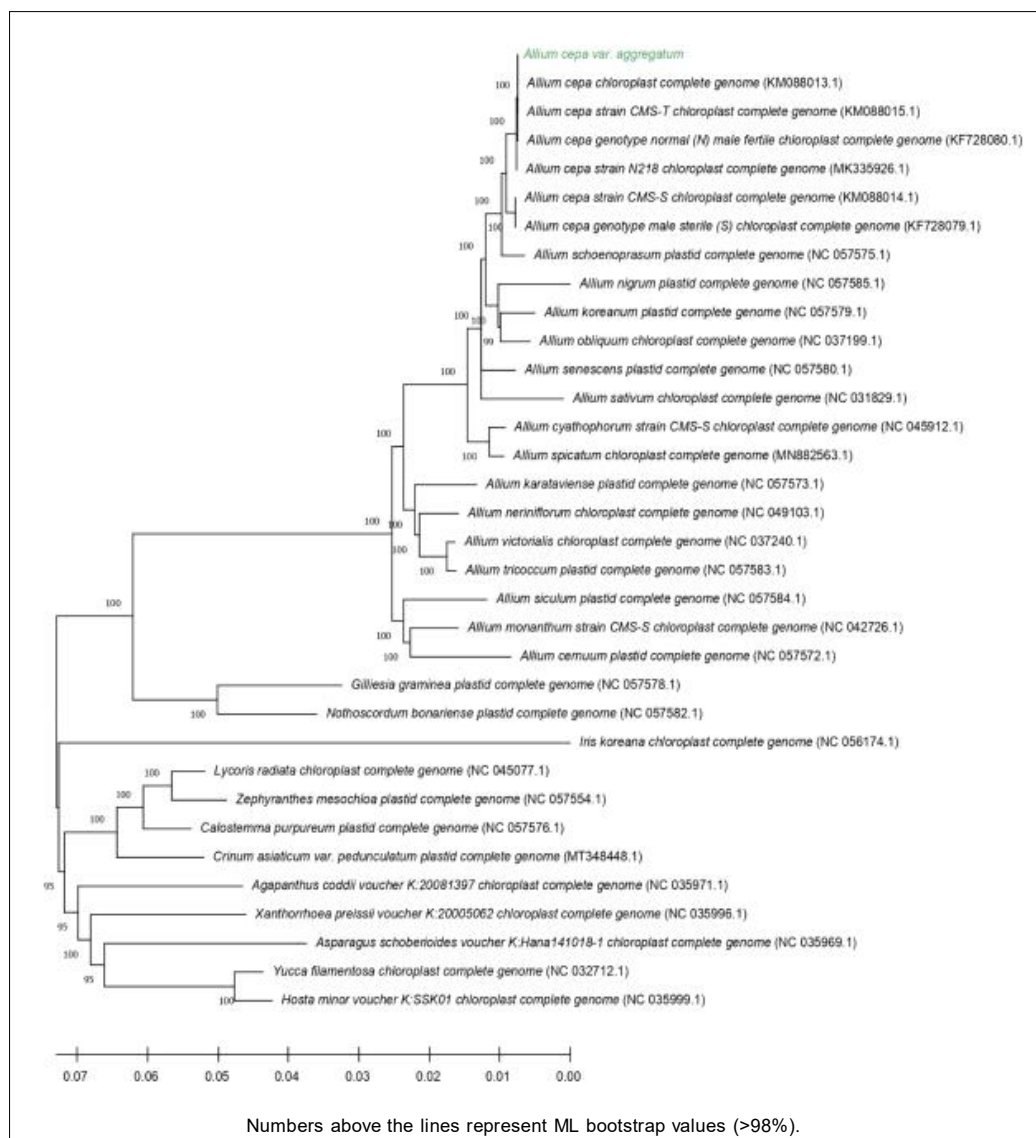


Fig 3: Phylogenetic tree reconstructed using maximum likelihood (ML) method based on complete chloroplast genome sequences of the 33 Amaryllidaceae species.

accessions were closely related to *A. schoenoprasum*. A similar relationship, within and between species, was resolved by Jimenez *et al.*, (2020) where they included plastomes of fertile and sterile strains of *A. cepa* in their phylogenetic analysis of *Allium* species in the Amaryllidaceae family.

Plastome comparison

Inverted Repeat (IR) lengths of *Allium* cp genomes ranged from 12,186 bp to 26,468 bp with *A. cepa* genotype male sterile (S) and *A. cepa* genotype normal (N) having the shortest and longest IR regions, respectively. Differences were observed across all junction sites among the seven *A. cepa* plastomes (Fig 4) *rps19* and *rpl22* were distributed

in LSC/IRb boundary across six *A. cepa* accessions with varying distances of two genes to the junction. Only the *rpl22* of *A. cepa* var. *aggregatum* was placed 30 bp away from the LSC/IRb boundary. For SSC/IRa boundaries, the *ycf1* gene located on the SSC region had a varying extension length to the IRa regions between the six *A. cepa* accessions while this gene was absent in *A. cepa* var. *aggregatum*. The *ndhF* genes, located primarily in the SSC region, were extended by 1 bp and overlapped the IRb region for five out of seven *A. cepa* genotypes at the IRb/SSC boundary. Moreover, there is also differential placement of *psbA* gene in reference to the IRa/LSC junction. The size variations, as observed in the seven plastomes sequenced

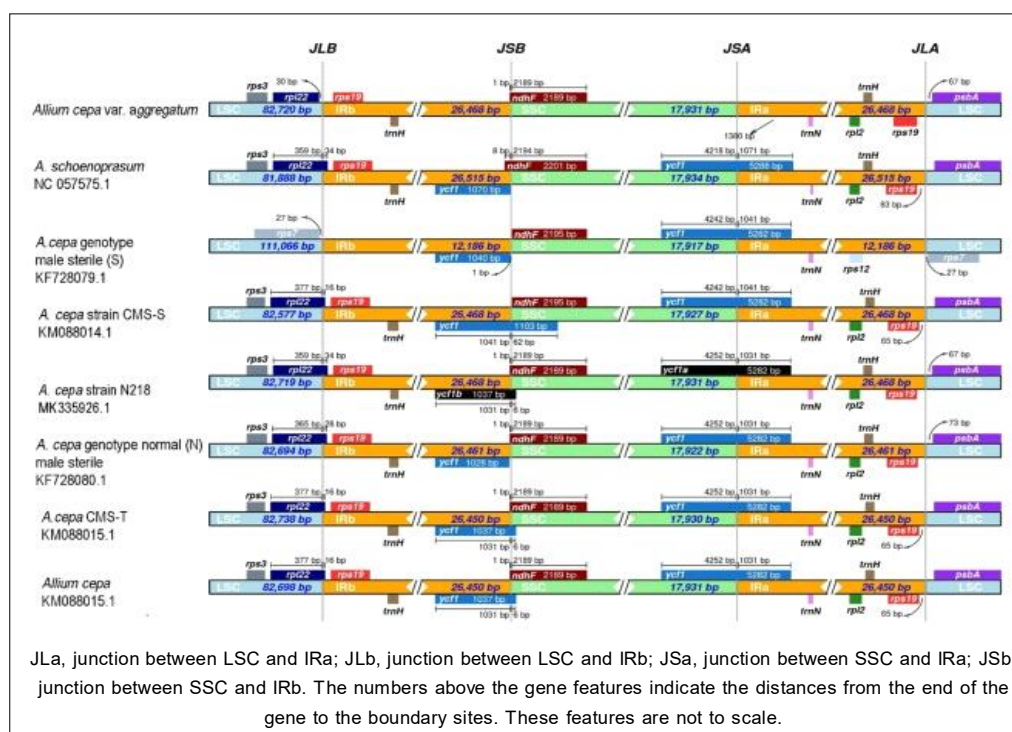


Fig 4: Comparison of the LSC, IR and SSC boundary regions among the 8 chloroplast genomes.

Table 2: Chloroplast genome features of *Allium cepa* genotypes.

Genome feature	<i>A. cepa</i> var. <i>aggregatum</i>	<i>A. cepa</i> genotype normal (N)	<i>A. cepa</i> CMS-S	<i>A. cepa</i> CMS-T
Genbank accession	OP756522	KF728080	KM088014	KM088015
Total length (bp)	153.587	153.538	153.440	153.568
LSC length (bp)	82.720	82.694	82.577	82.738
SSC length (bp)	17.931	17.922	17.927	17.930
IR length (bp)	26.468	26.461	26.468	26.450
mRNA genes	91	83	86	86
tRNA genes	38	38	38	38
rRNA genes	10	8	8	8
GC content (%)	36.8	36.8	36.8	36.8
A (bp)	31.3	31.3	31.3	31.3
T (bp)	31.9	31.9	31.9	31.9
G (bp)	18.1	18.1	18.1	18.1
C (bp)	18.7	18.7	18.7	18.7

from different *A. cepa*, may be attributed to the expansion and contraction of the IR and SSC junction sites (Amiryousefi *et al.*, 2018). The compositions of these genomes were also different in terms of the number of mRNA and rRNA genes (Table 2). These observed variations, along with the differences in the junction sites, provide additional resolution to the differences observed in the *A. cepa* plastomes. This further supports the distinctness of the *A. cepa* var. *aggregatum* plastome sequenced. These recorded chloroplast genome variations could further facilitate generation of DNA barcodes needed for germplasm identification at the variety level.

CONCLUSION

The complete chloroplast genome of an accession of *A. cepa* var. *aggregatum* was assembled and annotated and the morphology of the genotyped accession was also successfully characterized. The complete chloroplast (cp) genome is 153,587-bp long with 126 coding genes comprising 80 mRNA genes, 38 tRNA genes and 8 rRNA genes. Out of the mRNA genes, 43 genes code for photosynthesis, 29 genes for self-replication and 6 other genes for other functions. This is the first complete chloroplast genome of *Allium cepa* var. *aggregatum*. A phylogenetic analysis of the assembled genome, along with 21 other *Allium* accessions and 12 other Amaryllidaceae species, revealed that *A. cepa* var. *aggregatum* formed a distinct clade despite infraspecific plastome variations. The chloroplast genome was also distinct in sequence length and composition. This reported complete chloroplast genome of *A. cepa* var. *aggregatum* could be a valuable resource to further clarify differences needed for varietal identification of *Allium* species.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

There are no ethical issues associated with the plant material used in the study. The species is not endangered and not on the CITES and IUCN Red list and the sample was not collected from a protected area. The plant materials were collected from the field gene bank of the Crop Breeding and Genetic Resources Division, Institute of Crop Science, University of the Philippines Los Baños, Laguna, in compliance with the guidelines of the Institute of Crop Science.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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