

MITOGENOME ANNOUNCEMENT



The complete mitochondrial genome of *Allium fistulosum* L. (Amaryllidaceae)

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ABSTRACT

Allium fistulosum L. (Linnaeus, Carolus, 1753) is an aromatic vegetable with health benefits and medicinal value. In this study, the complete mitochondrial genome of *A. fistulosum* was determined. Circular mitochondrial DNA (mtDNA) was 382,053 bp in size, encoded 44 genes, and contained 26 protein-coding genes (PCGs), 14 tRNAs, and four rRNAs. Phylogenetic analysis of amino acid sequences of the 26 PCGs revealed that the closest relationship was between *A. fistulosum* and *Allium cepa*. The high-quality mitochondrial genomic sequences of *A. fistulosum* presented in this study will serve as a useful resource for a range of genetic, functional, evolutionary, and comparative genomic studies on this species of the Amaryllidaceae family.

ARTICLE HISTORY

Received 2 June 2023
Accepted 9 August 2023

KEYWORDS

Allium fistulosum L.;
mitochondrial genome;
phylogenetic analysis

Introduction

Allium fistulosum L. (Linnaeus, Carolus, 1753) is a perennial herb belonging to the family Amaryllidaceae that is widely distributed in Asia (Singh and Ramakrishna 2017). *A. fistulosum* is a typical spicy vegetable, is an excellent flavoring agent in dietary cooking and plays an important role in preventing human diseases (Gao et al. 2022; Tang et al. 2022).

The mitochondrial genome contains genetic and evolutionary information about the species, and its high conservation in eukaryotes makes it a useful marker for evolutionary research (Zheng et al. 2018). To date, only the complete mitochondrial genome of *Allium cepa* has been published in the Amaryllidaceae family (Kim et al. 2016), and there is no information on the mitochondrial genome of *A. fistulosum*. To our knowledge, this is the first study to report the complete mitochondrial genome of *A. fistulosum*.

Materials and methods

In the present study, fresh leaf sample of *A. fistulosum* were collected from the Beijing Vegetable Research Center of the Beijing Academy of Agricultural and Forestry Sciences (BVRC), Beijing, China (11°54'55" E, 40°21'58" 36 N) (Figure 1). The voucher specimen was deposited in the Germplasm Resource Bank of the BVRC under voucher number 31310 (contact person: Yongqin Wang; wangyongqin@nercv.org). Leaf mitochondrial DNA was extracted as previously described by

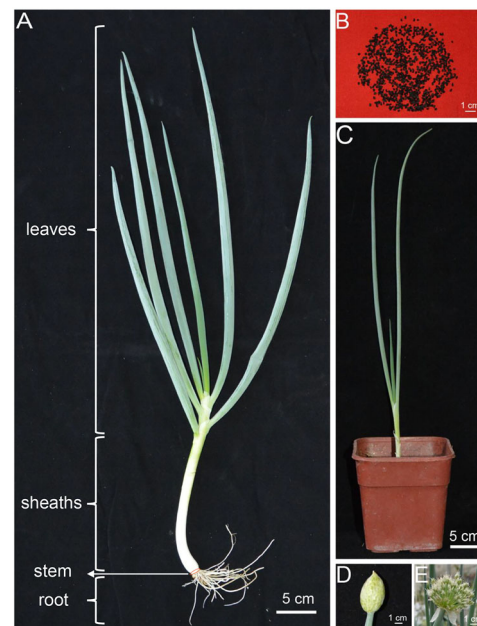







Figure 1. Species reference map and pictures of the growth and development process of *Allium fistulosum*. (A) Whole plant of *A. fistulosum*. *A. fistulosum* is a monocotyledonous plant with green tubular leaves. Pseudostems are formed by metamorphic development of leaf sheaths. The stem is extremely shortening. The root system is fibrous roots with strong regenerative ability. (B) Seeds. The seeds are black, shield-shaped, with a raised, angular back and a semicircular abdomen; (C) Seedling. The seedling is of elongated tubular leaf young leaves that are light green. (D) Unopened flower bud. The unopened buds are covered with a membrane; E: Blooming bud. The apical umbellate florets are spherically clustered and arranged radially. These photos were taken by the author of Jiayi Xing.

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 Supplemental data for this article can be accessed online at <https://doi.org/10.1080/23802359.2023.2248684>.

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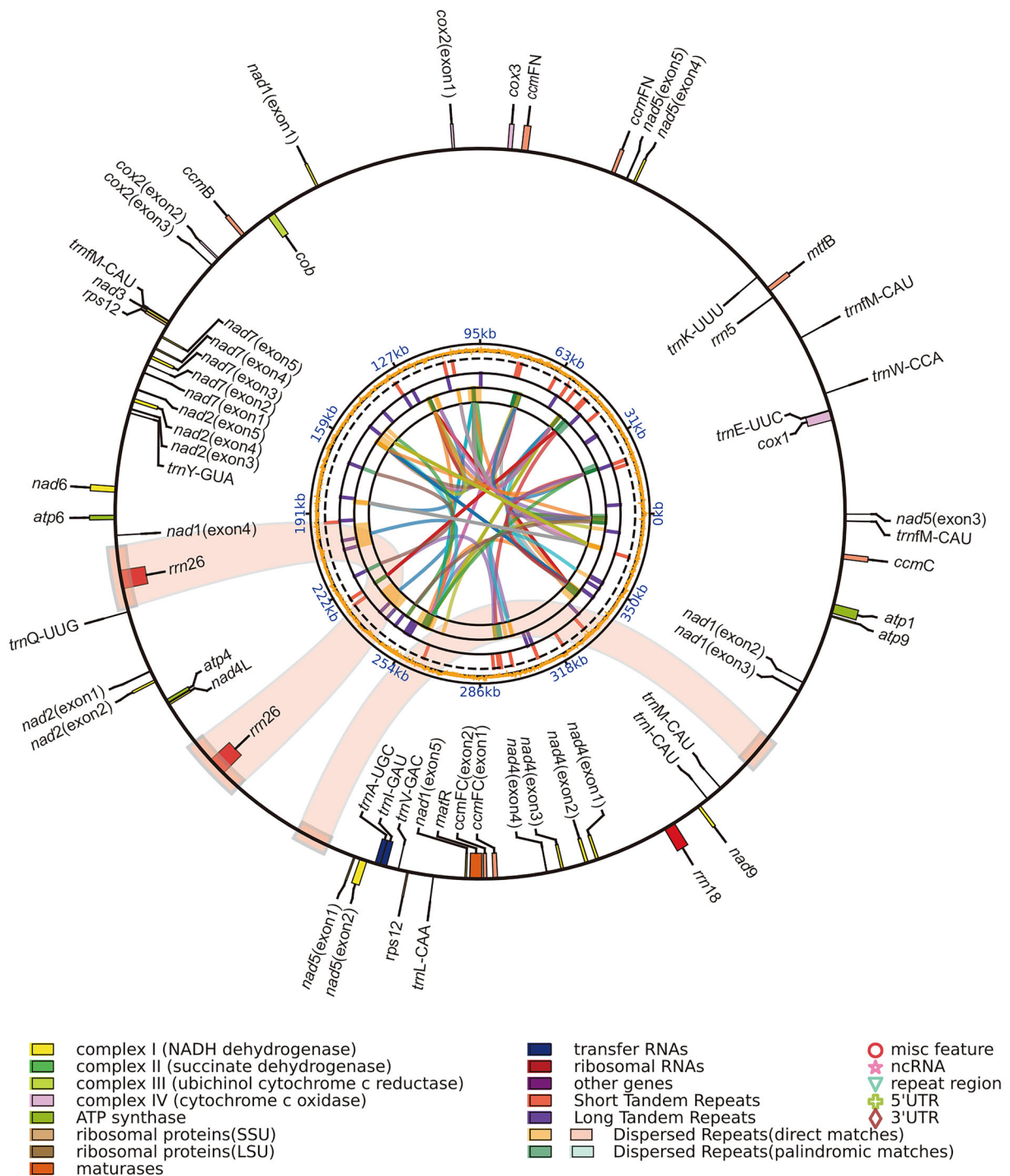


Figure 2. Mitochondrial genome map of *Allium fistulosum*, with 26 protein-coding genes (PCGs), 14 tRNAs, and four rRNAs. The rRNAs genes were filled in red, and genes with related functions are shown in the same color. Genes that are found in the inner and outer circle are transcribed clockwise and counterclockwise, respectively. The colored parabola in the center circle represents the dispersed repeats.

Chen et al. (2011). Purified DNA was sequenced on an Illumina HiSeq 4000 and PacBio, assembled in SPAdes v3.10.1 (Borgström et al. 2011). Geseq (<https://chlorobox.mpimp-golm.mpg.de/geseq.html>) was used to annotate the mitochondrial genome. OrganelleGenomeDRAW software (OGDRAW) was used to map the mitochondrial genome (Greiner et al. 2019). The complete mitogenome sequences of the nine species were downloaded from GenBank and aligned using the MAFFT website (Kato and Standley 2013). Phylogenetic analyses were performed using MEGA 6 with 1000 bootstrap replicates, and the model-finding tool in MEGA 6 was used to acquire the best

replacement model. During this process, maximum likelihood analysis was performed, and the cpREV model was chosen as the appropriate model for sequencing, with *Indigofera tinctoria* (MW448462) and *Nicotiana tabacum* (NC_006581) considered as outgroups. Primers for the identification of the *cox2* gene were referred to Kim et al. (2013).

Results

The mitochondrial genome of *A. fistulosum* was sequenced to an average depth of 1207 X (supplemental Figure S1),

Table 1. Gene information of the mitochondrial genome of *A. fistulosum*.

gene	Position		Length (bp)	Nucleic acid		Cod-ing strand	Codon		Anti-codons	Amino acid size
	Start	End		AT(%)	GC(%)		Start	Stop		
<i>atp1</i>	365329	366852	1524	54.92%	45.08%	+	ATG	TGA		507aa
<i>atp4</i>	223541	224104	564	58.33%	41.67%	-	ATG	TAA		187aa
<i>atp6</i>	191285	191998	714	61.62%	38.38%	+	ATG	CAA		237aa
<i>atp9</i>	364693	364917	225	55.11%	44.89%	+	ATG	CAA		74aa
<i>ccmB</i>	138077	138697	621	58.94%	41.06%	+	ATG	TGA		206aa
<i>ccmC</i>	374501	375316	816	55.76%	44.24%	+	ATG	TAG		271aa
<i>ccmFC</i>	288701	289455	1314	54.41%	45.59%	-	ATG	CGA		437aa
	287109	287667								
<i>ccmFN1</i>	87565	88611	1047	54.25%	45.75%	+	ATG	TAA		348aa
<i>ccmFN2</i>	72574	73182	609	50.90%	49.10%	+	ATG	TGA		202aa
<i>cob</i>	132065	133255	1191	57.85%	42.15%	-	ATG	TGA		396aa
<i>cox1</i>	15812	17389	1578	56.97%	43.03%	-	GTG	TAA		525aa
<i>cox2</i>	99750	100143	789	58.05%	41.95%	+	ATG	TAA		262aa
	143966	144283								
	145567	145643								
<i>cox3</i>	90211	90909	699	56.51%	43.49%	+	ATG	TGA		232aa
<i>matR</i>	284822	286822	2001	49.03%	50.97%	-	ATG	TAG		666aa
<i>mttB</i>	39797	40546	750	55.33%	44.67%	+	ATG	TGA		249aa
<i>nad1</i>	123405	123789	978	57.06%	42.94%	+	ACG	TAA		325aa
	352743	352825								
	351067	351258								
	194608	194666								
	283998	284256								
<i>nad2</i>	218026	218178	1467	59.24%	40.76%	+	ATG	TAA		488aa
	219697	220088								
	173248	173408								
	171486	172058								
	169816	170003								
<i>nad3</i>	157436	157792	357	57.14%	42.86%	+	ATG	TAA		118aa
<i>nad4</i>	306216	306676	1488	58.20%	41.80%	-	ATG	TGA		495aa
	304319	304833								
	300173	300595								
	297678	297766								
<i>nad4L</i>	224251	224553	303	64.03%	35.97%	-	ACG	TAA		100aa
<i>nad5</i>	265084	265313	2013	58.32%	41.68%	+	ATG	TAA		670aa
	266162	267377								
	381969	381990								
	68784	69178								
	70318	70467								
<i>nad6</i>	186485	187453	969	55.31%	44.69%	+	ATG	TAG		322aa
<i>nad7</i>	166802	166944	1185	55.53%	44.47%	-	ATG	TAG		394aa
	165414	165482								
	163797	164263								
	162309	162552								
	160072	160333								
<i>nad9</i>	325365	325862	498	57.23%	42.77%	+	ATG	TGA		165aa
<i>rps12</i>	157838	158215	378	55.03%	44.97%	+	ATG	TGA		125aa
<i>rps12</i>	274278	274520	243	58.02%	41.98%	+	ACT	TAG		80aa
<i>trnM</i>	157290	157356	67	46.27%	53.73%	+			CAU	
<i>trnE</i>	17466	17537	72	50.00%	50.00%	-			UUC	
<i>trnK</i>	42923	42995	73	50.68%	49.32%	-			UUU	
<i>trnL</i>	278698	278778	81	50.62%	49.38%	+			CAA	
<i>trnfM</i>	30450	30523	74	45.95%	54.05%	+			CAU	
<i>trnI</i>	327445	327518	74	55.41%	44.59%	-			CAU	
<i>trnM</i>	330298	330370	73	60.27%	39.73%	-			CAU	
<i>trnfM</i>	380793	380866	74	43.24%	56.76%	+			CAU	
<i>trnQ</i>	207503	207574	72	51.39%	48.61%	+			UUG	
<i>trnV</i>	272758	272829	72	51.39%	48.61%	-			GAC	
<i>trnW</i>	20333	20406	74	48.65%	51.35%	+			CCA	
<i>trnY</i>	173964	174046	83	49.40%	50.60%	-			GUA	
<i>trnA</i>	269635	269671	73	43.84%	56.16%	-			UGC	
<i>trnI</i>	270710	270741	72	38.89%	61.11%	-			GAU	

indicating that it could be used for subsequent bioinformatic analysis of the mitochondrial genome. The total mitochondrial genome length of *A. fistulosum* was 382,053 bp (GenBank accession number OL347690), with a GC content of 44.98%. It consisted of 26 protein-coding genes (PCGs) (*atp1*, *atp4*, *atp6*, *atp9*, *ccmB*, *ccmC*, *ccmFN1*, *ccmFN2*, *ccmFC*, *cob*, *cox1*, *cox2*, *cox3*, *matR*, *mttB*, *nad1*, *nad2*, *nad3*, *nad4*, *nad4L*, *nad5*, *nad6*, *nad7*,

nad9, and two *rps12*), 14 tRNA genes, and 4 rRNA genes (Figure 2). All PCGs used ATG as the start codon, except for *cox1*, *nad1*, *nad4L*, and *rps12*, for which the start codons were GTG, ACG, ACG and ACT, respectively. The 14 tRNA-coding genes ranged in size from 67 bp to 83 bp (Table 1). With regard to stop codons, three PCGs (*atp6*, *atp9*, and *ccmFC*) were terminated with CAA or CGA, whereas the remaining 23 PCGs were

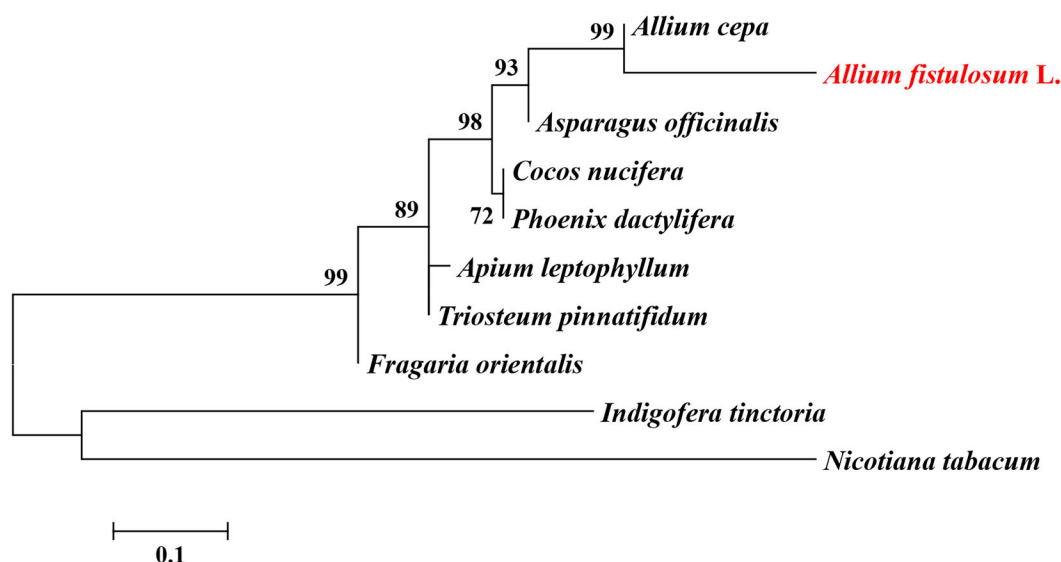


Figure 3. A maximum-likelihood (ML) tree was constructed based on ten species of mitochondrial genomes (mtDNA) with 1000 bootstrap replicates, with *Indigofera tinctoria* (MW448462) and *Nicotiana tabacum* (NC_006581) as outgroups. The newly sequenced species in this study is indicated in red. The following sequences were used: *A. cepa* AP018390 (Shigyo et al. 2018), *Asparagus officinalis* NC_053642 (Sheng 2020), *Cocos nucifera* NC_031696 (Aljohi et al. 2016), *Phoenix dactylifera* NC_016740 (Fang et al. 2012), *Apium leptophyllum* MZ328723, *Triosteum pinnatifidum* NC_064333, *Fragaria orientalis* NC_057524 (Liu et al. 2021), *Indigofera tinctoria* MW448462 (Choi et al. 2021), *Nicotiana tabacum* NC_006581 (Sugiyama et al. 2005). NCBI accession numbers are given for Genbank sequences.

terminated with routine termination codons (TAA, TAG, or TGA). Gene annotation prediction results showed that the genome contained 3 cis-splicing genes (*ccmFC*, *nad4*, and *nad7*) and 4 trans-splicing genes (*cox2*, *nad1*, *nad2*, and *nad5*) (supplemental Figures S2 and S3). And the PCR identification result of the *cox2* in *Asparagus*, *A. cepa* and *A. fistulosum* also showed that *cox2* was trans-splicing (supplemental Figure S4), which was similar to the findings of Kim et al. (2013). Additionally, the phylogenetic analysis showed that *A. fistulosum* was closely related to *A. cepa*, followed by *Asparagus officinalis* (Figure 3).

Discussion and conclusion

There are over 900 species in the genus *Allium* (Yang et al. 2023). Given the huge genome, molecular biology research on *A. fistulosum* has progressed slowly compared to that on other crops. Liao et al. (2022) published the genome of *A. fistulosum*, with a size of 11.27 Gb and pointed that *A. fistulosum* was the closest relative to *A. cepa*. In the present study, we assembled and analyzed the complete mitochondrial genome of *A. fistulosum* and found that *A. fistulosum* and *A. cepa* have a close genetic relationship. Not only that, the PCR identification result showed that *cox2* gene in mitochondrial genome of *A. fistulosum* was trans-splicing, which further proof that *A. fistulosum* are closely related to *A. cepa* (Kim et al. 2013). The complete mitochondrial genome of *A. fistulosum* was reported for the first time. Our study lays the foundation for the study of the mitochondrial genome of the *Allium* genus and contributes to the identification and genetic, evolutionary, and functional studies of Amaryllidaceae species.

Author contributions

Jiayi Xing and Yongqin Wang designed the experiments, analyzed the data and wrote the manuscript. Mingzhao Zhu and Huiying Liu revised

the manuscript and approved the version to be published. All authors agree to be accountable for all aspects of the work.

Ethical statement

The research and collection of plant material was carried out according to the guidelines of the Beijing Vegetable Research Center, Beijing Academy of Agricultural and Forestry Sciences. This study includes no endangered plant samples, and the sampling site is not located in any protected area.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was supported by the National Natural Science Foundation of China under Grant Number [31972409] and Science Innovation of the Beijing Academy of Agriculture and Forestry Sciences under Grant Number [KJXC20230123].

Data availability statement

The results of this study are authentic and effective, and the genome sequence data are publicly available in NCBI (<https://www.ncbi.nlm.nih.gov/>) under the accession number OL347690. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA782797, SRR17015068, and SAMN23401061, respectively.

References

- Aljohi H, Liu W, Lin Q, Zhao Y, Zeng J, Alamer A, Alanazi I, Alawad A, Al-Sadi A, Hu S, et al. 2016. Complete sequence and analysis of coconut palm (*Cocos nucifera*) mitochondrial genome. *PLoS One*. 11(10): e0163990. doi:10.1371/journal.pone.0163990.
- Borgström E, Lundin S, Lundeberg J. 2011. Large scale library generation for high throughput sequencing. *PLoS One*. 6(4):e19119. doi:10.1371/journal.pone.0019119.

- Chen J, Guan R, Chang S, Du T, Zhang H, Xing H. 2011. Substoichiometrically different mitotypes coexist in mitochondrial genomes of *Brassica napus* L. *PLoS One*. 6(3):e17662. doi:10.1371/journal.pone.0017662.
- Choi IS, Wojciechowski MF, Ruhlman TA, Jansen RK. 2021. In and out: evolution of viral sequences in the mitochondrial genomes of legumes (Fabaceae). *Mol Phylogenet Evol*. 163:107236. doi:10.1016/j.ympev.2021.107236.
- Fang Y, Wu H, Zhang T, Yang M, Yin Y, Pan L, Yu X, Zhang X, Hu S, Al-Mssallem I, et al. 2012. A complete sequence and transcriptomic analyses of date palm (*Phoenix dactylifera* L.) mitochondrial genome. *PLoS One*. 7(5):e37164. doi:10.1371/journal.pone.0037164.
- Gao S, Wang K, Li N, Lv Y, Cao B, Chen Z, Xu K. 2022. The growth and photosynthetic responses of white LEDs with supplemental blue light in green onion (*Allium fistulosum* L.) unveiled by Illumina and single-molecule real-time (SMRT) RNA-sequencing. *Environ Exp Bot*. 197: 104835. doi:10.1016/j.envexpbot.2022.104835.
- Greiner S, Lehwark P, Bock R. 2019. OrganellarGenomeDRAW (OGDRAW) version 1.3. 1: expanded toolkit for the graphical visualization of organellar genomes. *Nucleic Acids Res*. 47(W1):W59–W64. doi:10.1093/nar/gkz238.
- Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol*. 30(4):772–780. doi:10.1093/molbev/mst010.
- Kim B, Kim K, Yang TJ, Kim S. 2016. Completion of the mitochondrial genome sequence of onion (*Allium cepa* L.) containing the CMS-S male-sterile cytoplasm and identification of an independent event of the *ccmF_N* gene split. *Curr Genet*. 62(4):873–885. doi:10.1007/s00294-016-0595-1.
- Kim S, Bang H, Patil B. 2013. Origin of three characteristic onion (*Allium cepa* L.) mitochondrial genome rearrangements in *Allium* species. *Sci Hortic*. 157:24–31. doi:10.1016/j.scienta.2013.04.009.
- Liao N, Hu Z, Miao J, Hu X, Lyu X, Fang H, Zhou Y, Mahmoud A, Deng G, Meng Y, et al. 2022. Chromosome-level genome assembly of bunching onion illuminates genome evolution and flavor formation in *Allium* crops. *Nat Commun*. 13(1):6690. doi:10.1038/s41467-022-34491-3.
- Liu HB, Sun J, Sun R, Li ST, Zhong CF, Dong J, Chang LL, Wang GX, Zhang HL, Wei YQ, et al. 2021. The complete mitochondrial genome sequence of *Fragaria orientalis* (Rosaceae). *Mitochondrial DNA B Resour*. 6(7):1951–1952. doi:10.1080/23802359.2021.1920498.
- Sheng W. 2020. The complete mitochondrial genome of *Asparagus officinalis* L. *Mitochondrial DNA B Resour*. 5(3):2627–2628. doi:10.1080/23802359.2020.1780986.
- Shigyo M, Khar A, Abdelrahman M. 2018. The *Allium* genomes. Cham: Springer.
- Singh B, Ramakrishna Y. 2017. Welsh onion (*Allium fistulosum* L.): a promising spicing-culinary herb of Mizoram. *Indian J Hill Farming*. 30:201–208.
- Sugiyama Y, Watase Y, Nagase M, Makita N, Yagura S, Hirai A, Sugiura M. 2005. The complete nucleotide sequence and multipartite organization of the tobacco mitochondrial genome: comparative analysis of mitochondrial genomes in higher plants. *Mol Genet Genomics*. 272(6): 603–615. doi:10.1007/s00438-004-1075-8.
- Tang W, Wang G, Chen R, Liu X, Chen X, Shen X, Yin C, Mao Z. 2022. *Allium fistulosum* L. alleviates apple replant disease by suppressing *Fusarium solani*. *JoF*. 8(10):1071. doi:10.3390/jof8101071.
- Yang J, Kim SH, Gil HY, Choi HJ, Kim SC. 2023. New insights into the phylogenetic relationships among wild onions (*Allium*, Amaryllidaceae), with special emphasis on the subgenera *Anguinum* and *Rhizirideum*, as revealed by plastomes. *Front Plant Sci*. 14:1124277. doi:10.3389/fpls.2023.1124277.
- Zheng B, Cao L, Tang P, Achterberg K, Hoffmann A, Chen H, Chen X, Wei S. 2018. Gene arrangement and sequence of mitochondrial genomes yield insights into the phylogeny and evolution of bees and sphecid wasps (Hymenoptera: Apoidea). *Mol Phylogenet Evol*. 124:1–9. doi:10.1016/j.ympev.2018.02.028.