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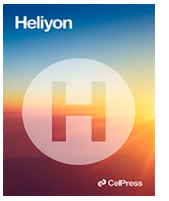

Research article

Deciphering genetic diversity phylogeny and assembly of *Allium* species through micro satellite markers on nuclear DNA

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ABSTRACT

The genus *Allium* is the most diverse, with cultivated crops such as onion, garlic, bunching onion, chives, leeks, and shallots, and several wild and semi-domesticated *Allium* species utilized as minor vegetables. These minor species are the genetic resources for various abiotic and biotic stresses. To employ underutilized species in breeding programmes, the magnitude of the genetic background of cultivated and semi-domesticated alliums, the phylogeny and diversity of the population must be known. In this study, nineteen SSR markers were employed to study the divergence and population structure of 95 *Allium* accessions which includes species, varieties, and interspecific hybrids, yielded 92 polymorphic loci, averaging 4.84 loci per SSR. PIC values range between 0.24 (ACM 018) and 0.98 (ACM 099). The cross transferability of ACM markers among *Allium* species ranges from 1.33 to 10.53 per cent, which is relatively low. The genotypes investigated were clustered into four primary clusters A, B, C, and D with 13 sub clusters I-XIII, conferring to the clustering results. The population structure investigations also found that K is a peak at value 4, implying that the population is predominantly segregated into four distinct groups, which associates the clustering pattern. The employed SSR markers adeptly unravel the complexities of diversity within alliums, holding promise for refining future breeding programs targeting elite progenies.

1. Introduction

The term "*Allium*" originates from the *Celtic* term "*All*," meaning "pungent," and the crops are given the *Allium* name because they contain sulphur compounds with a pungent flavour [1]. *Allium* is categorized taxonomically under the family Alliaceae, encompassing the subfamily Allioideae, within the order Asparagales [2]. Its primary center of origin is postulated to be within South-West Asia, with a subsequent expansion into the Mediterranean region, indicating a secondary origin [3]. The genus *Allium* contain numerous ancient cultivated plant species [4]. The genus *Allium* is the largest among monocots, with over 1000 species adaptable to diverse climatic

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conditions worldwide [5]. *Allium* has a variety of economically important crop and decorative species, including bulb onion (*Allium cepa*), garlic (*Allium sativum*), chives (*Allium schoenoprasum*), Chinese chives (*Allium tuberosum*), rakkyo (*Allium chinense*), bunching onion (*Allium fistulosum*) and leek (*Allium porrum*). Minor *Allium* species employed for vegetables in a particular area were *A. macaranthum, A. clarki, A. tuberosum, A. chinense, A. hookeri, A. angulosum, A. roylei, and A. ochinni* [6–8]. Wild species are exclusively found in the northern hemisphere and are mostly found in open grassland, shrub land, or desert environments [9]. Wild *Allium* species are distributed in the alpine and Himalayan regions of India [4,10].

Bulb onion is widely planted and used around the world, ranking third in the category of exporting fresh vegetable crops and second among the top ten vegetable crops produced globally [11]. Onions are in high demand because of the world's rapidly rising population [7]. Onions are difficult to breed due to their biennial life cycle, high frequencies of inbreeding, and cross-pollination [5,12]. The distinct features that aid in resistance and tolerance to various stresses challenges are found in wild and semi-domesticated *Allium*

Fig. 1. *Allium* species maintained at National Active Germplasm Site for onion, garlic and allies, India a) *Allium altaicum* CGN14769, b) *Allium angulosum* EC32846, c) *Allium cepa* var. *aggregatum,* d) *Allium chinense* MMK130, e) *Allium fistulosum* EC461748, f) *Allium hookeri* NG3255, g) *Allium macranthum* NMK3227, h) *Allium przewalskianum* MMK110, i) *Allium schoenoprasum* NR6 NGB5969, j) *Allium tuberosum* MKG84 and *l) Allium cepa* var. Bhima Red.

species [1]. The resistance characters can be imported from wild relatives through various genetic enhancement methods such as interspecific hybridization [1]. The variation and divergence study are extremely useful in developing a prolific variety with superior in quality and yield.

The *Allium* species indigenous to India found as a potential reservoir of immunity against afflictions such as purple blotch, garlic mosaic virus, and Stemphylium blight [13,14]. *Allium* species with the vital important gene pools are *A. cepa, A. galanthum, A. fistulosum, A. roylei, A. altaicum, A. oschaninii, A. vavilovii, and A. pskemense* [1,15]. Several infections contribute to the low productivity of cultivated alliums in India, causing the average yield to reduce significantly [16]. Wild alliums are high in flavonoids and volatile organic compounds, which aid in growth and development, attract insect pollinators, prevent plant illnesses, and improve weed control [17]. The Indian species of *Allium* was found to be a source of resistance to the diseases caused by the garlic mosaic virus, stemphyllium blight, and purple blotch [14]. But sources of tolerance or resistance to several diseases, including black mould, basal rot, and neck rot, are still unknown. Breeders now need to concentrate on developing sources of resilience to various pressures, including heat, cold, frost, drought, and flood, in the scenario of inevitable climate change. The most important gene pool species among alliums have been determined to be *A. roylei, A. vavilovii, A. galanthum, A. fistulosum, A. altaicum, A. pskemense, and A. oschaninii* [15,18]. Thus, these stresses can be decreased by transferring wild and semi-domesticated genes into cultivated species.

Genetic diversity holds significance in plant breeding as it empowers breeders to develop superior hybrids with innovative traits, imparting resistance to a multitude of abiotic and biotic challenges [13,19]. Diversity analysis is crucial for investigating the evolutionary links among the populations investigated [20–24]. Analysing genetic variation in germplasm and determining how much of it is heritable is critical for controlling and using diversity. There are several ways for studying population divergence, including morphological, biochemical, and molecular methods [18–20]. Among these, the molecular tools stand out as the most pertinent and precise tool for studying divergence at the gene level, which is unaffected by external environmental influences. Moreover, these genotypes undergo comprehensive DNA-level screening, demonstrating enhanced efficiency and a swifter process. Molecular markers are utilized to assess heterozygosity and are acknowledged for their contribution in establishing genetic profiles to identify germplasm [25]. To determine the evolutionary relationships between the collected accessions of shallot and *Allium* × *wakegi*, as well as to determine the origin of *A.* × *wakegi*, RAPD and PCR-RFLP studies were performed at Fukuoka, Japan [23], SNP-markers developed at Wageningen, The Netherlands [26], The establishment of intron length polymorphic (ILP) markers in onions and the cross-species transferability of these markers in wild close relatives and garlic was revealed in India [20] and simple sequence repeats (SSRs) were used to study the diversity among bunching onion (*Allium fistulosum*) in Japan [27], *Allium mongolicum* in china [28], garlic in India [29] and onion in India [21,30]. Among several markers, the SSR markers are high polymorphism, and co-dominant genetic markers, and were widely used in studies of genetic diversity studies [17]. Since, SSR markers exhibit considerable transferability and reproducibility of results, SSR markers emerged as powerful tool in plant genetics [31]. Aim of present study is to determine genetic divergence and population structure, and capture phylogenetic relationships between 95 *Allium* species, both wild and farmed, at the molecular level employing nineteen SSR markers. It also intends to create the framework for future efficient utilisation of the genotypes under investigation in breeding programmes.

2. Material and methods

2.1. Plant material

Present study encompasses ninety-five distinct accessions of *Allium* species, encompassing various varieties and interspecific hybrids, encompassing both cultivated and wild specimens [Fig. 1(a-l)]. These accessions were curated and preserved at the NAGS (National Active Germplasm Site) for Alliums, located at ICAR- Directorate of Onion and Garlic Research in Pune, India.

2.2. DNA isolation

Slightly modified Cetyl Trimethyl Ammonium Bromide method is employed for the extraction of DNA from fresh, meristematic leaf tissue [32,33]. The evaluation of integrity and yeild of the extracted DNA is quantified using NanoDrop, while the DNA purity is evaluated through 1.0 % agarose gel electrophoresis. Subsequently, the final concentration of DNA is adjusted to 25 ng/μl, stored at 4 °C, and used as a template for PCR applications.

2.3. PCR amplification

A set of nineteen SSR markers (Table S1) were employed to PCR amplify DNA from different *Allium* species [30]. PCR amplification is conducted within a 10 μl reaction mixture containing 1.5 μl 10 × PCR buffer with $MgCl_2$, 0.8 μl forward primer (10 mM), 0.8 μl reverse primer (10 mM), 0.2 μl dNTPs (2.5 mM), 0.1 μl Taq DNA polymerase (5U/μl) and 1 μl DNA as a template (Gowd et al., 2023b; Khade et al., 2022). The PCR thermocycling protocol as follows: initial denaturation at 94 °C for 2 min, followed by 35 cycles denaturation at 94 °C for 30 s, annealing at 50.3–58.0 °C for 1 min, and extension at 72 °C for 1 min [30]. Thermocycled products were electrophoresed at 140 V on 1.5 % agarose gel stained with 0.5 g/ml Ethidium Bromide in 1 × TBE buffer, amplicons were compared the 1 kb Plus DNA ladder, and documentation was done using UV protected gel documentation system.

2.4. Scoring and analysis of data

The gel images were manually assessed employing a binary system, where a value of 1 represented the presence of a band and 0 indicated its absence. This binary data was employed to construct the Jaccard's similarity matrix in NTSYS-pc Version 2.02i [34]. This matrix was then utilized to compute genetic distances by subtracting from unity. The resultant genetic distance matrix was employed to construct a dendrogram using the UPGMA method (Unweighted Pair Group Method with Average) through Molecular Evolutionary Genetics Analysis v11 (MEGA11) software [35]. Additionally, GenAlEx 6.5 software was used to calculate the PIC (Polymorphism Information Content) heterozygosity and band frequency [36].

2.5. Population structure

Structure version 2.3.4 was utilized to reveal Bayesian clustering and investigate the population structure. The range of populations (ΔK) was varied from 1 to 15 as default setting, with 10 separate simulations conducted for each ΔK value. Every iteration comprised a preliminary burn-in phase of 250,000 steps, followed by 106 repetitions of the Markov Chain Monte Carlo (MCMC) process. The data underwent analysis using the Structure Harvester v.6.93 web server to identify the optimal K value, which unveiled a notable peak of ΔK values.

Table 1
Genetic analysis parameter of 19 SSR markers among *Allium* species.

Sl. No	Marker	Amplicons per marker	Loci (bp)	PIC	Loci	Average Band Frequency	Avg. He	Average uHe
1	ACM 004	104	210, 280, 350, 380, 400, 550	0.536	6	0.182	0.154	0.155
2	ACM 008	200	210, 250, 300, 350, 450, 500, 700, 720	0.335	7	0.301	0.272	0.273
3	ACM 018	103	200, 250	0.245	2	0.542	0.359	0.361
4	ACM 034	171	250, 350, 400, 480, 500, 700	0.267	6	0.300	0.258	0.259
5	ACM 038	131	300, 350, 400, 480, 500, 530, 550	0.633	8	0.172	0.159	0.16
6	ACM 052	69	210	0.472	1	0.726	0.499	0.502
7	ACM 054	68	190, 250, 300, 350, 510	0.886	5	0.143	0.137	0.138
8	ACM 066	175	180, 200, 250, 280, 300, 350, 450, 650, 700	0.443	11	0.167	0.152	0.153
9	ACM 069	179	200, 230, 300, 350, 400, 450, 500, 550, 600, 680, 720	0.360	10	0.188	0.168	0.169
10	ACM 078	151	250, 450, 520, 600, 700, 750	0.561	7	0.227	0.209	0.210
11	ACM 081	108	200, 210, 280, 800	0.376	3	0.379	0.294	0.295
12	ACM 091	37	100, 130, 350	0.871	2	0.195	0.175	0.176
13	ACM 099	12	195	0.984	1	0.126	0.122	0.123
14	ACM 115	204	210, 260, 340, 400, 550, 600, 650, 700	0.289	9	0.239	0.214	0.215
15	ACM 138	65	150, 210, 280	0.819	3	0.087	0.210	0.211
16	ACM 151	44	200, 250, 300, 540	0.944	4	0.116	0.112	0.113
17	ACM 154	54	200, 420, 700	0.886	3	0.189	0.179	0.18
18	ACM 229	26	500, 550, 565	0.945	3	0.091	0.086	0.087
19	ACM 305	52	100	0.700	1	0.547	0.440	0.443
Total		1953	–	–	92	–	–	–
Average		102.79	–	0.608	4.842	0.259	0.221	0.222

PIC = Polymorphic Information Content; He = Expected Heterozygosity; uHe = Unbiased Expected Heterozygosity.

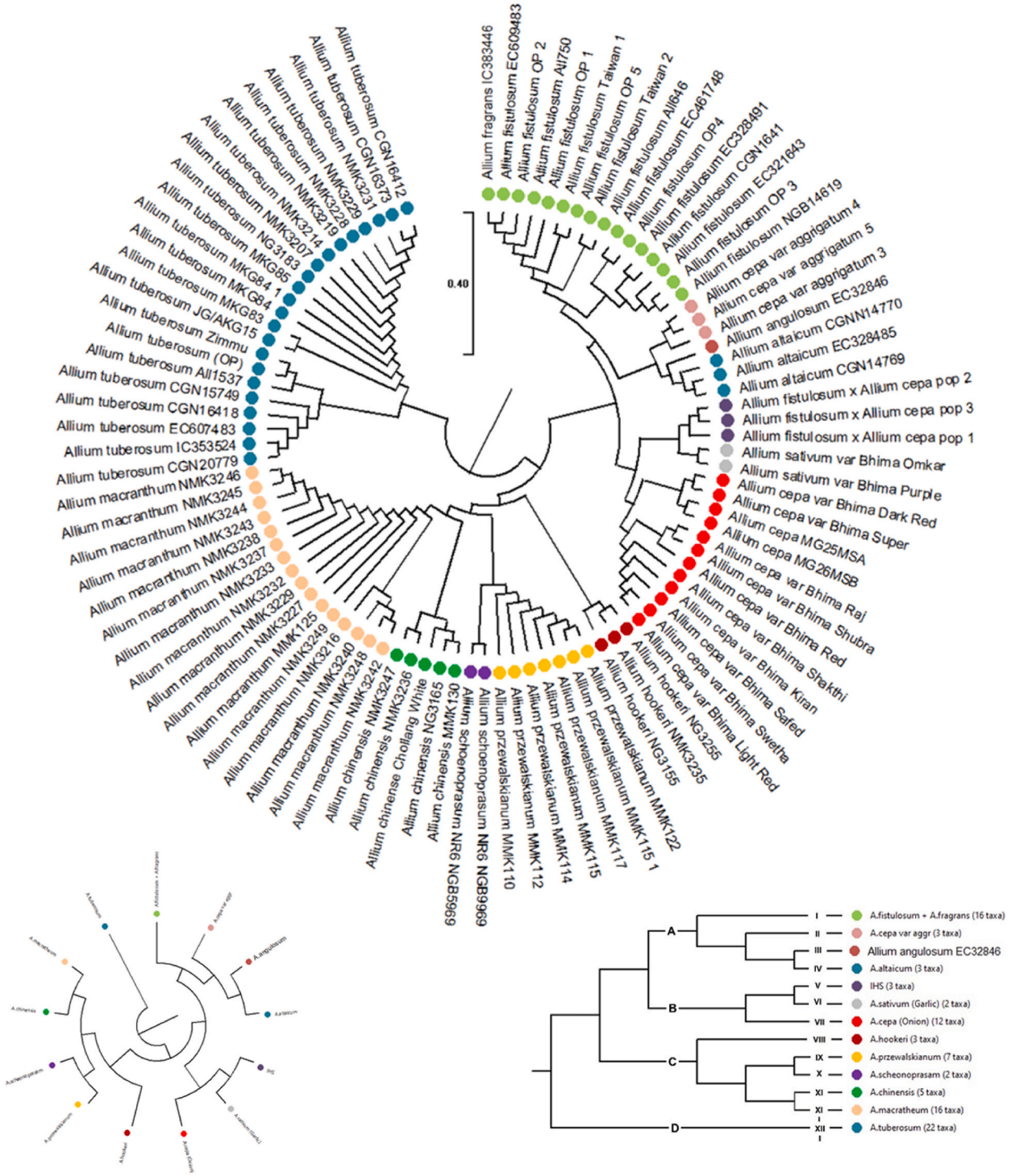

Fig. 2. Genetic diversity of *Allium* species produced by SSR markers in MEGA11 software through Unweighted Pair Group Method with Average (UPGMA) based on the dissimilarity matrix developed from jaccard's similarity matrix generated through NTSYS-pc Version 2.02i.

3. Results

3.1. Divergence analysis

Divergence offers valuable insights into the nature and degree of genetic variation, thereby it aids in the selection of genotypes for a successful breeding program. Genetic diversity stands out as a crucial factor in crop improvement. It was identified by SSR marker analysis among 95 *Allium* species, and results revealed significant divergence within the *Allium* population. The molecular characterization of the 95 *Allium* population using nineteen SSR markers revealed that the highest count, 11 loci, was observed in ACM066. The number of amplicons per marker ranged from 12 to 204 with a mean of 102.79 and total amplicons of 1953; the lowest and greatest amplicons were seen in markers ACM099 and ACM115. The average band frequency spans among the SSR marker ranged from 0.087 to 0.726, with an average of 0.259, the ACM138 marked had the lowest and ACM052 had the highest (Table 1). The observed PIC values for polymorphic markers varied between 0.245 and 0.984, with the lowest recorded in ACM018 and the highest in ACM099. In ACM299 and ACM052, the average predicted heterozygosity ranges from 0.086 to 0.499 (Table 1). SSR marker cross-transferability ranges from 1.33 to 10.53 % and Polymorphism (%) per genotype ranges from 9.78 % to 39.13 % (Table S2).

The clustering of 95 *Allium* genotypes revealed that they were grouped into 4 major clusters (Fig. 2), among four cluster, cluster A includes 23 genotypes, cluster B includes 17 genotypes, cluster C includes 33 genotypes, and cluster D includes 22 genotypes. Major cluster A is composed of four subclusters, I, II, III, and IV. Subcluster, I include sixteen genotypes of bunching onions (15 genotypes total; *Allium fistulosum* OP 1, EC321643, CGN1641, EC609483, All646, OP-2, OP-4, Taiwan 2, OP-3, Taiwan 1, All750, EC328491, EC461748, NGB14619, OP-5, and *A. fragrans* IC383446 grouped with 1 genotype of false garlic. Three genotypes of *A. cepa* var *aggregatum*, a potato onion or multiplier onion, make up sub cluster II (5, 4, 3). *A. angulosum* EC32846 is the sole genotype found in sub cluster III. Three genotypes (*Allium altaicum* CGN14770, CGN14769, and EC328485) contribute to sub cluster IV.

Major cluster B consist of 3 subclusters V, VI and VII (Fig. 2), in which sub cluster–V comprises interspecific hybrids between onion and welsh onion (*Allium fistulosum* × *Allium cepa* pop 1, pop 2, and pop 3). Sub cluster–VI comprises 2 genotypes of garlic (*Allium sativum* var. Bhima Omkar, Bhima Purple). Sub cluster–VII comprises of 12 genotypes of cultivated onion varieties (*Allium cepa* var. Bhima Shakthi, Bhima Kiran, Bhima Safed, Bhima Swetha, Bhima Light Red, Bhima Red, Bhima Raj, Bhima Shubra, Bhima Dark Red, Bhima Super, MG25MSA and MG26MSB).

Major cluster C consist of 5 subclusters VIII, IX, X, XI and XII (Fig. 2), in which sub cluster–VIII comprises 3 genotypes (*Allium hookeri* NG3255, NG3155, and NMK3235). Sub cluster–IX comprises 7 genotypes (*A. przewalskianum* MMK 115-1, MMK 117, MMK 122, MMK 110, MMK 112, MMK 114 and MMK 115). Sub cluster–X comprises 2 genotypes (*Allium schoenoprasum* NR6 NGB9969, and NR6 NGB5969). Sub cluster–XI consists of 5 genotypes (*Allium chinense* NMK3247, NG3165, MMK130, NMK3236 and Chollang White). Sub cluster–XII comprises of 16 genotypes (*Allium macranthum* NMK3243, NMK3244, NMK3249, NMK3248, NMK3246, NMK3238, NMK3242, NMK3233, NMK3240, NMK3227, NMK3247, NMK3229, NMK3237, NMK3216, MMK125, NMK3232, and NMK3237).

Major cluster D consist only one subcluster XIII, in which Sub cluster–XIII comprises 22 genotypes (*Allium tuberosum* CGN16418, NMK3219, MKG85, NMK3229, NG3183, JG/AKG15, CGN16412, MKG84, MKG83, CGN20779, CGN15749, IC353524, NMK3207, Zimmu, MKG84-1, OP, NMK3231, NMK3228, CGN16373, EC607483, All1537, and NMK3214). The clusters in the current study are established based on species, as seen in the clustering pattern in Fig. 2.

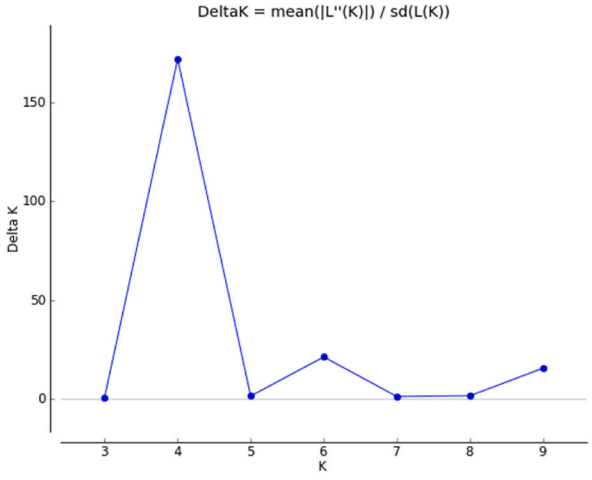

Fig. 3. Structure results, comparison of ΔK values for *Allium* using SSR; the maximum value of ΔK at K = 4 was considered to be the appropriate number of populations.

3.2. Population structure analysis

The bayesian clustering and investigate the population structure range of populations (ΔK) was varied from 1 to 15, with 10 separate simulations conducted for each ΔK value and identify the optimal K value at 4, which unveiled a notable peak at ΔK = 4. This signifies the division of the studied population into four distinct genetic clusters. The barplot is obtained with the ΔK value at 4, it illustrates the accurate clusters of the populations by the program (Fig. 3). Structure results, comparison of ΔK values for *Allium* using SSR; ΔK the maximum value of ΔK at 4 was considered to be the appropriate number of populations. The population represented by a bar plot from 1 to 7 was shared by *Allium altaicum, Allium angulosum*, and *Allium cepa* var *aggregatum*, bar plot from 8 to 12 contains *Allium chinense*, bar plot from 8 to 12 had *Allium chinensis*, bar plot from 13 to 27 with *Allium fistulosum*. Bar plot from 28 to 32 had shared by *Allium hookeri* and *A. fragrans*. Bar plot from 32 to 47 had *Allium macranthum*. Bar plot from 48 to 54 had *Allium przewalskianum*. Bar plot from 55 to 56 had *Allium schoenoprasum*, Bar plot from 57 to 78 had *Allium tuberosum*. Bar plot from 79 to 81 had interspecific hybrids *Allium fistulosum* × *Allium cepa*. Bar plot from 82 to 83 had *Allium sativum* and bar plot from 84 to 95 had *Allium cepa* (Fig. 4).

4. Discussion

The molecular markers employed for identifying variety, disclose differences in genetic makeup, phylogenetic relationships, and genotype classification with the greatest precision [5,7,37–40]. Despite of commercial importance of cultivated alliums, it is witnessing minimal research on genetic revolution paradigm of alliums. and hence it must be accomplished by utilising wild and semi-domesticated species [4,41–44]. The assessment of genetic diversity in *Allium* species is critical in many ways, including selecting the proper genotypes for the breeder, avoiding genotype duplication, and introgression breeding [22]. Polymorphism was found in SSR markers among the *Allium* species (Fig. S1). This high level of polymorphism associated with SSR markers can be attributed to the unique replication slippage process, as well as the loss or gain of nucleotides throughout evolution that are responsible for SSR allelic variability [45]. In this study, 19 SSR markers were utilized to show the diversity of 95 *Allium* genotypes from 13 different species including interspecific hybrids between *A. fistulosum* and *A. cepa*. The study found that the SSR markers (Table S1) chosen are extremely polymorphic; of the 19 SSRs chosen, 16 were shown to be highly polymorphic, with PICs greater than 0.5. These results were supported by 7 polymorphic SSRs out of 15 that were highly polymorphic in 96 onion accessions [22]. Furthermore, 16 SSRs employed in the study were highly polymorphic (84 %), which is higher than 43 % [24], 37 % [21], 82 % [46], and 92 % [47]. With the aid of SSR polymorphic markers, *Allium* genotypes can be well characterized. And it is a possible reason for exploration of diversity of *Allium* species.

The average PIC found was 0.608, which was consistent with the previous studies PIC of 0.64 [47] and 0.7 [46], but higher than the

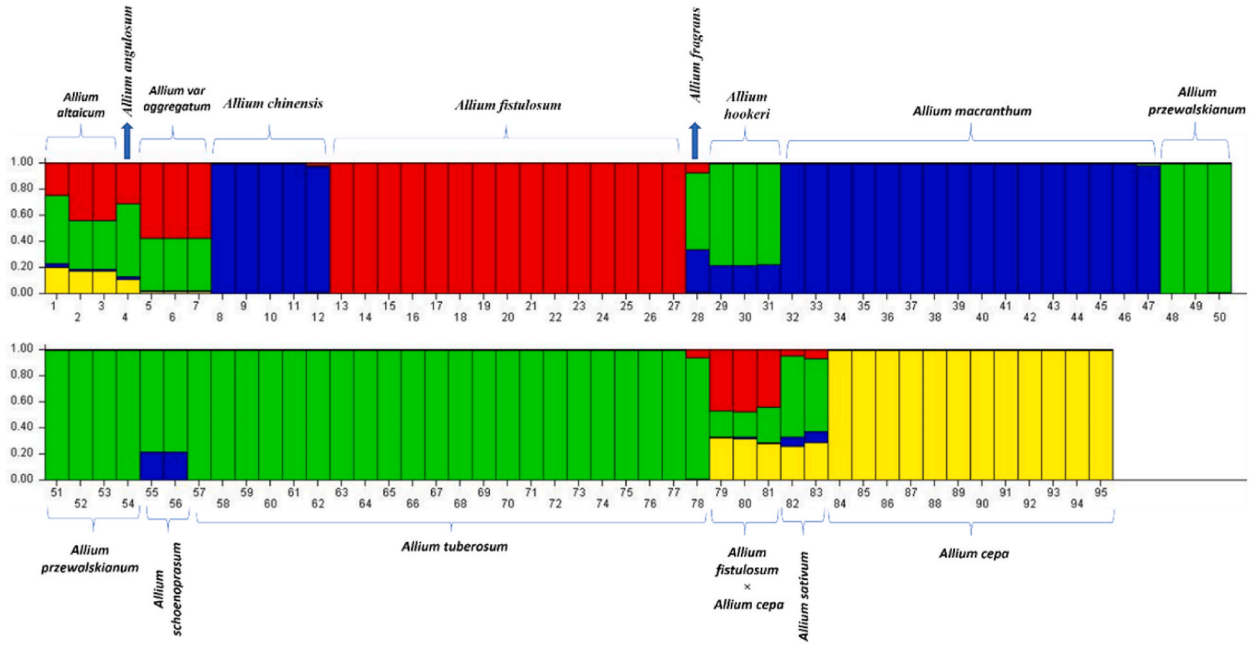

Fig. 4. Bayesian grouping of the population represented by using a barplot (1–7: *Allium altaicum, Allium angulosum* and *Allium cepa* var *aggregatum*. 8–12: *Allium chinensis*. 13-27: *Allium fistulosum*. 28–32: *Allium fragrans* and *Allium hookeri*. 32–47: *Allium macranthum*. 48–54: *Allium przewalskianum*.55-56: *Allium schoenoprasum*. 57-78: *Allium tuberosum*. 79–81: *Allium fistulosum* × *Allium cepa*. 82–83: *Allium sativum*. 84-95: *Allium cepa*). *Same colours indicate the sharing of genome counterparts. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

average PIC 0.45 and 0.51 [48,49]. The 95 *Allium* genotypes are divided into four major clusters: A, B, C, and D. Major cluster A includes the bunching onion, *A. altaicum*, *A. cepa* var *aggrregatum* (multipler onion), *A. fragrans*, and *A. angulosum*, which are cultivated and semi-domesticated species, although *Allium cepa* var *aggregatum* are multiplying or aggregating onions, very similar to shallots (*Allium cepa* var *ascalonicum*) and Bulb onion (*Allium cepa* var *cepa*), morphological traits have been suggested for differentiating aggregating onions, shallots and bulb onions [50]. In this context, genotyping with SSR markers aided in the grouping *Allium cepa* var *aggregatum* from other *Allium* species. *A. fistulosum* and *A. altaicum* share morphological similarities for leaf blades and psuedostem, but *A. fragrans* and *A. angulosum* share few morphological similarities for leaf blades and psuedostem. The major cluster B have the onion, garlic, and other interspecific hybrids between bunching onion and onion are found in the main cluster B. Whereas, Major Cluster B consists of widely farmed species that are genetically related to Major Cluster A. The major cluster C includes wild and less cultivated species such as *A. przewalskianum, A. macranthum*, *A. hookeri,* and *A. chinensis* that are morphologically similar in certain characteristics such as short stature, leaf blades and psuedostem. The major cluster D includes only one species, *A. tuberosum*, which is a semi-domesticated minor vegetable that is separated from other *Allium* species. Although, few species share wide morphological similarities [Fig. 1(a-l)], but clustering pattern not influenced by morphological traits of all cultivated, semi-domesticated, and wild species [46,47,51–53]. In the population structure research, we discovered the K to be highest at the value 4, indicating that the population investigated is generally divided into four genetic groupings, which is associated to the four major clusters in the dendrogram of divergence investigations. The population structure is clear regarding the subsequent subgrouping, which is consistent [22], where K = 1, 2, and 10 were not visible (Fig. 3), this could be either due to the limited polymorphism across the populations of genotyping markers, or population admixtures, and also may be due to wild species evolved by natural interspecific hybridization and natural sections. The cross-transferability of these SSR producers is found to be poor, ranging from 1.33 to 10.53 per cent, which was consistent with the low cross-transferability [30]. These polymorphic cross-transferable SSR markers have the potential to be used in phylogenetic investigations and long-term management of *Allium* species [30]. We discovered a high degree of genetic divergence in cultivated and wild *Allium* species; wild relatives are being potential sources of resistance to diverse biotic and abiotic challenges. This offers researchers the possibility of transferring unique traits from wild cousins to cultivated alliums in future breeding programmes.

5. Conclusion

The wild and cultivated *Allium* accessions possess genes that are specific to certain traits, which could be harnessed to enhance cultivated *Allium* species, by attempting interspecific hybridization based on the present study findings of divergence. To showcase the effectiveness of simple sequence repeat (SSR) markers have been identified that distinctly indicate genetic variations among different *Allium* species. By employing these SSR markers, breeding and improvement could be ascertained for disease resistance, yield, and quality in Alliums. Additionally, they can play a role in safeguarding genetic resources through germplasm characterization and conservation in the future. Currently, limited cultivated *Allium* species would be empowered by systematic broadening and diversification of the genetic basis, as demonstrated by the analyzed genetic variation among the alliums. Considering the diversity and evolutionary relationships of the *Allium* species leads to future breeding efforts to improve the quality and resilience traits.

Ethical statement

The manuscript was not submitted elsewhere, and the findings were presented accurately and without distortion, falsification, or data manipulation. Research poses no threat to public health or national security.

Data availability statement

All the research data presented in this manuscript and supporting data has been provided as supplementary raw data and gel images.

CRediT authorship contribution statement

Talamarla Yeswanth Mahidar Gowd: Writing – original draft, Investigation, Formal analysis, Data curation. **Chandra Deo:** Supervision, Project administration. **Dalasanuru Chandregowda Manjunathagowda:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Vijay Mahajan:** Supervision, Resources. **Ram Dutta:** Visualization, Resources. **Nangsol Dolma Bhutia:** Supervision, Project administration. **Barun Singh:** Visualization, Supervision. **Vadde Mounika:** Writing – review & editing, Visualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e31650.

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