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Characterization of *Allium* germplasms for conservation and sustainable management using SSR markers

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Allium species are very important due to their medicinal values. Quercetin and allicin are medicinally important compound of onion and garlic, respectively which are proved useful to treat various diseases. However, highly heterozygous nature, self-incompatibility and long gestation period limits genetic improvement of *Allium* species. Further, the existing germplasms in Indian subcontinents are largely cultivated ones with poor genetic characterization, which limits the germplasm conservation and future management. A total of thirty polymorphic Simple Sequence Repeats (SSRs) were utilized for characterisation of popular onion germplasms and their cross-transferability revealed relatedness with fifteen garlic and wild relatives. Average number of alleles per SSR locus, PIC and heterozygosity was found to be 3.9, 0.51 and 0.57,respectively.Overall genetic diversity recorded was higher in wild relative compared to cultivated *A. cepa*, possibly because most of the *A. cepa* variety is derived by domestication but wild relatives are open pollinated and undergoes extensive gene pool shuffling leading to higher heterogeneity. In this study, SSR markers were successfully utilized to assess genetic variations in popular Indian *A. cepa*, *A. sativum* and establish genetic relationships with wild *Allium* species. These markers can be harnessed for molecular breeding, varietal identification and planning germplasm conservation strategies in future.

Keyword: *Allium cepa, Allium sativum*, Cross transferability , Genetic diversity , SSR

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Allium species have been employed for a long time in traditional medical practice to treat a variety of diseases[1]. Among them, onion (*Allium cepa* L.), garlic (*Allium sativum* L.), and wild relatives have been used for centuries for their pungency, flavouring value, and medicinal properties[2]. Onion (*A. cepa*) contain flavonoid quercetin and other secondary metabolites, which prevents several human diseases[3]. Further, garlic (*A. sativum*) has a wide range of pharmacological effects including antimicrobial, cardiovascular, anti-inflammatory, anticancer, and immune-modulatory properties imparting health benefits to human[4]. However, highly heterozygous nature, self-incompatibility and long gestation period limits genetic improvement of *Allium* species. Further, the existing germplasms in Indian subcontinents are largely cultivated ones with poor genetic characterization, which limits the germplasm conservation and future management. Moreover, wild *Allium* species are underutilized for improving quality and stress tolerance of elite cultivars. It is therefore, envisaged that efforts shall be made to unravel the complexities of the *Allium* genome including the wild relatives for conservation and sustainable management of *Allium* germplasms. Few reports exist on phenotypic variations and diversity using morphological and biochemical markers, providing useful information about germplasm of *Allium* spp[5, 6]. However, major limitations with such markers are that they are limited in number and influenced by environmental change. Among various marker systems, genetic markers are superior due to robustness, higher polymorphism, stability to environmental variations. These markers enable estimation of genetic diversity on the basis of various parameters like heterozygosity, allele richness. Microsatellites or SSRs are among the most popular genetic markers due to their robustness, high

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polymorphism potential, genome-wide distribution and sequence specificity[7, 8]. SSR derived from transcribed region (EST) are more cross-transferable to related species and may have prominent role in regulation of plant metabolism and development[9, 10]. These markers could be utilized to study of gene flow and genetic drift[11, 12]. Further, SSRs are proved to be very useful in cases where the aim is restoration and translocation of plant species[13]. With these characteristics and advantages over other marker systems, SSR markers emerge as a powerful tool to study evolutionary trend and conservation management of *Allium* germplasm[10, 11]. Few SSR markers were utilized to evaluate genetic relatedness in few onion and wild germplasms in India[14-17], still a large number of popular onion and garlic varieties with improved traits remain uncharacterized and their genetic relatedness with wild *Allium* needs evaluation. To fill the existing lacuna in *Allium* research, current study aims at characterization of selected Indian garlic and onion germplasms and wild relatives using thirty SSRs mined from public domain. These markers will be beneficial for cultivar identification, molecular breeding and conservation of diverse medicinally important *Allium* germplasms.

Materials and Methods

Plant Material

In the current study twenty-three germplasms of onion, garlic and wild relatives conserved at the ICAR-DOGR Pune, India gene bank was evaluated using SSR markers (Table 1). Of these twenty-three a total of eight individuals of *A. cepa* (onion), and fifteen related species of *Allium* (7 *A.sativum or* garlic) and 8 wild species) were utilized for genetic characterization.

DNA Isolation

DNA from young and freshly procured leaves of *Allium* was isolated as previously described by Murray and Thompson[18]with minor modifications. The estimation of DNA concentration was done spectro-photometrically on Nano Drop 2000 (Thermo Scientific, USA) and quality was checked on 0.8% agarose gel. The DNA dilution of 20 ng/µl was done for performing PCR.

Table 1 — Details of germplasms used for validation and transferability of Onion SSR markers

Variety/Accession number	Species	Ploidy*	Individual code
B. Red/ IC 561258	*Allium cepa*	2n=2x=16	*AcB-Red*
B. Raj/ IC 561257	*Allium cepa*	2n=2x=16	*AcB-Raj*
B. Shakti/ IC 572769	*Allium cepa*	2n=2x=16	*AcB-Shakti*
B. Kiran/ IC 572766	*Allium cepa*	2n=2x=16	*AcB-Kiran*
B. Shubhra/ IC 572763	*Allium cepa*	2n=2x=16	*AcB-Shubhra*
B. Dark red/ IC 572765	*Allium cepa*	2n=2x=16	*AcB-D.red*
B.Super/ IC 561259	*Allium cepa*	2n=2x=16	*AcB-Super*
B. Shweta/ IC 572761	*Allium cepa*	2n=2x=16	*AcB-Shweta*
B. Omkar/ IC 569789	*Allium sativum*	2n=2x=16	*AsB-Omkar*
B. Purple/ IC 570742	*Allium sativum*	2n=2x=16	*AsB-Purple*
Godavari GY Mut RI	*Allium sativum*	2n=2x=16	*As-RI*
RG- 321	*Allium sativum*	2n=2x=16	*As-321*
COL-AC-38-382	*Allium sativum*	2n=2x=16	*As-382*
GOI-Vr-GR	*Allium sativum*	2n=2x=16	*As-GR*
ACC-183	*Allium sativum*	2n=2x=16	*As-183*
A. tuberosum	*Allium tuberosum* (Wild)	2n=4x=32	*Atub*
A. fistulosum NGB-14619	*Allium fistulosum* (Wild)	2n=2x=16	*Afis*
*A. cepa*var. aggregatum 5 Manipur	*Allium cepa* var. aggregatum (Wild)	2n=2x=16	*AcAg5M*
A. cepavar.aggregatum 3 Meitei Tilou	*Allium cepa var*. aggregatum (Wild)	2n=2x=16	*AcAg3MT*
A. altaicumpell(Ausdauernd) All-284	*Allium altaicum* (Wild)	2n=2x=16	*Aalt-284*
*A. cepa*var. Aggr.4 EshingTilou	*Allium cepa* var. aggregatum (Wild)	2n=2x=16	*Ac-Ag4ET*
*A. ampeloprasum*L. Balody CGN-18724	*Allium ampeloprasum* (Wild)	2n=4x=32, 6x=48	*Aamp-18724*
*A. ampeloprasum*Blue green autumn Neptune Leek EC- 609483	*Allium ampeloprasum* (Wild)	2n=4x=32, 6x=48	*Aamp-609483*

Table 2 — Details of SSR markers used for Allium germplasm evaluation

Marker Name	Forward Primer Sequence (5'–3')	Reverse Primer Sequence (5'–3')	Ta	Observed product size	Alleles	He	PIC
ACM004	TCGTTCTTTAGAACACGTTAGGAA	TGTCGGCGGATATAGTGACA	52.3	100-600	2	0.48	0.365
ACM008	GCCGGAAGAGGAGAAGAAGT	CATAATTCCCATGGCTTTGC	50.3	100-800	4	0.62	0.549
ACM054	GAGTGAGAGGGGAAATGGAA	AAAGATGGTTTGTTGGTGGC	50.3	100-500	4	0.4852	0.4505
ACM066	CTCCCCGCAACCAGTAATAA	GCTTGGGTTTTGTTTCTCCA	50.3	100-600	4	0.5383	0.497
ACM069	TTCTGCGCTCTTCCCAGTAT	CAAGCGGTTTGAAAAAGGAG	50.3	100-800	7	0.5444	0.5181
ACM080	GCATTATGCAGTAACGGGCT	GCAGCAGCATTTGATTGAAC	50.3	200-800	6	0.7244	0.6865
ACM093	GCCAACAGTTTTCGTAAGTTGA	ATTCTCTTCGGCTTTCGTGA	50.3	100-800	6	0.7486	0.7137
ACM154	CGATGAATACACCGATGACG	CTTGTTTTGGCAGTTGGGAT	50.3	100-300	2	0.6644	0.5902
ACM018	GGGGAATGGTGGAGAATAGA	AACAGAGGCAAGAGGAGCG	52.3	100-600	4	0.6639	0.6001
ACM033	CCTTCTCCCCATTCTCTTCC	ATCATCGTCCTCGTCCTCAT	52.3	100-600	4	0.6593	0.5949
ACM034	CACCTTGGACCGTGAAGAAC	CTGCTGTTTGGAGATGTGGA	52.3	100-600	4	0.5067	0.4323
ACM038	ATGCCAGACTACGACAACGA	ACGCCTACCAACCTTCAATG	52.3	200-400	4	0.6811	0.6166
ACM047	CATTCATCTACTCTCTTCTTCAGCC	GAGGTCATTGGTTTGGTTAGC	52.9	200-700	3	0.5536	0.4525
ACM077	AAATTATGGGCCACCTCCTC	CAAGATTGTCGACTCCCCAT	52.3	100-800	5	0.7603	0.7196
ACM081	CTGAAAAGAAACCCGCAGAG	TCAGGATGCACTTGCTTCAG	52.3	200-300	4	0.6446	0.5862
ACM180	CCTTCAGACCCTAAAAGGGC	CAAAGGACATTGGCAAGTGA	50.3	100-600	3	0.546	0.4986
ACM78	CGCAGAATCTCGTCCTTTTT	AATGGTTTGGAGGTCAGTCG	50.3	100-400	6	0.7111	0.6628
ACM94	GATGATGGCGAAGACACAGA	AAAAACGGCTTAGGAATTTAACG	50.3	200-600	6	0.7639	0.7265
ACM119	TTTCAGCAACATAGTATTGCGTC	TCTTCGGGATTGGTATGGAG	52.1	100-400	2	0.18	0.1638
ACM146	ATGTCCCAATTCGACCAGAG	CGTTACGGCTGAGAACTTCC	52.3	100-400	3	0.6243	0.5537
ACM171	AATATAGAAGAGTCCGTGTGCG	GTCACATCATCAAGCAACCG	52.3	100-400	3	0.4388	0.3862
ACM147	CACTTTCCCGTCTAATCGACA	TTCCCACAATCAAAACACCA	48.2	100-600	2	0.2449	0.2149
ACM168	TGGACTGGCCATGAGACATA	TGCAAGAAGAGAAATTGCCA	48.2	100-300	2	0.4938	0.3719
ACM133	CCACATGGATGAAAAACACAA	CGCTGGTAGCTGAAGCAAAT	49	100-600	4	0.5624	0.5192
ACM125	AAAAAGGGTTTTATCAGTCGCA	CCGCTGTTGAAATATGGGTT	49.7	100-200	2	0.3967	0.318
ACM151	TGTCAGACAAGCAACTCCTCC	AGGTGAGGCTTAGATGGGGT	54.4	100-800	6	0.8	0.7716
ACM229	TACGAGCGGAGGTATGAGC	GCCAGGAAGGCGAGTAGTAA	53.8	200-400	2	0.1528	0.1411
ACM170	TTCTGCAATGAAAACACATTGA	ATCCAACTGAGTCGGCAATC	47.8	100-600	3	0.48	0.4122
ACM016	ATGGAAGCCTCGGGTCTG	GCCGTAAGTCGAGGGTAGAA	53.2	100-800	5	0.6224	0.5874
ACM115	TCCATCTATGCATCTGCCAC	CTATTCTTCCACTGGGGCAA	52.3	100-800	5	0.6667	0.5926

SSR analysis

Genetic characterization of 23 *Allium* germplasms done using 30 SSR (Table 2) markers derived from public domain[19-22]. PCR amplification competence for all markers in study was checked in 10µL reaction volume containing 20 ng of template DNA. The PCR program comprised of one denaturation step at 94°C for 4 min, subsequent 35 cycles of 94°C for 1 min, annealing at optimum temperature (Ta) (Table 2) for 1 min and extension at 72°C for 1 min. The final extension was done at 72°C for 5 min in I-Cycler (Bio-Rad) as previously described[7, 8]. The amplified PCR products were then separated and visualized on 3% Agarose gel, and amplicon size were estimated based on 1 kb Plus DNA ladder (O'GeneRuler) as reference.

Molecular data analysis

The amplification profiles produced using SSR markers were scored based on their presence (1) or absence (0) crossways in all individuals as earlier reported[7,8]. The markers producing same sized fragments across all individuals were termed as monomorphic, while individuals producing variable sized fragments were termed polymorphic. Individuals not producing any amplification under standard conditions were allocated null alleles. Cross-transferability and amplicon size was recorded for all the species to perform PCoA and draw anunweighted neighbour joining tree based on Jaccard's coefficient using DARwin6[23]. Polymorphism information content and heterozygosity of all the primers was calculated using PIC calculator[24]available online at https://www.liverpool.ac.uk/~kempsj/pic.html.

Result

SSR marker selection

We selected 80 best SSR markers from public domain[19-22]. Eight *A. cepa* individuals were selected

for SSR marker screening and validation. Among 80 SSR markers only 30 were polymorphic and cross transferable in *Allium* species and others either did not amplify due to variation in primer binding site or large non-amplifiable regions[25] or were monomorphic. A total of 117 alleles ranging from 2 to 7 were observed in *Allium* germplasms. Average number of alleles per SSR locus was found to be 3.9. PIC ranged from 0.14 to 0.77 with an average of 0.51. Heterozygosity averaged 0.57 which ranged from 0.15to 0.8.

Cross transferability

Cross-transferability of SSR markers producing successful amplification was tested in fifteen related species of *Allium* (7 *A sativum* and 8 wild related species) (Table 3). Cross-transferability of markers ranged from 4. 3 to 56.5 (Avg. 36.8). Maximum transferability (56.5%) was recorded by ACM69, ACM81, ACM180, ACM78, and ACM146 markers.

Genetic diversity and phylogenetic analysis

Thirty polymorphic microsatellite markers that showed positive amplification were deployed for genetic diversity and phylogenetic analysis of 23 *Allium* spp. germplasms. All the cultivated *A.cepa* and *A. sativum* germplasms, and their wild relatives fall under three separate clusters based on PCoA (Fig. 1) and NJ dendogram (Fig. 2). *A. tuberosum and A. ampeloprasum* are wild relatives have polyploidy chromosome 2n=4x=32 and 2n=6x=48, respectively, and detected high level of genetic diversity. Among the onion varieties, B. Dark Red and B. Super were most similar (0.036) and B. Shakti and B. Super were most diverse (0.29). Similarly, in case of garlic, B. Omkar and ACC-183 showed maximum dissimilarity (0.92). Interestingly, two wild relatives*, A. tuberosum and A. fistulosum,* reported tolerant to various biotic including against diseases and abiotic stresses are

Table 3 — Cross-transferability of SSR markers in 7 garlic and 8 wild germplasms

	Garlic								Wild								
Marker	AsB-Omkar	AsB-Purple	As-RI	As-321	As-382	As-GR	As-183	Atub	Afis	AcAg5M	AcAg3MT	Aalt-284	Ac-Ag4ET	Aamp-18724	Aamp-609483	Percent Transferability
ACM04	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	30.4
ACM08	-	-	+	+	+	+	+	-	-	+	+	+	+	+	+	47.8
ACM54	+	+	+	+	+	+	+	+	-	+	+	-	-	+	+	52.2
ACM66	-	-	+	+	+	+	+	+	-	+	+	+	+	+	+	52.2
ACM69	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+	56.5
ACM80	-	-	+	+	+	+	+	+	-	+	+	+	+	+	+	52.2
ACM93	-	-	-	-	-	-	-	-	-	+	-	-	+	-	+	13.0
ACM154	-	-	+	+	+	+	+	-	-	+	+	+	+	+	+	47.8
ACM18	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	34.8
ACM33	+	-	+	+	+	+	-	-	+	+	+	+	+	+	+	52.2
ACM34	+	+	+	+	+	-	-	+	-	+	+	+	+	+	+	52.2
ACM38	-	-	+	+	+	+	-	-	-	+	+	+	+	+	-	39.1
ACM47	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	4.3
ACM77	-	-	-	+	+	-	-	-	+	-	+	-	+	+	-	26.1
ACM81	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	56.5
ACM180	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	56.5
ACM78	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	56.5
ACM94	+	-	+	+	+	+	-	+	-	+	+	+	+	+	+	52.2
ACM119	-	-	-	+	+	-	-	+	-	+	+	+	+	+	+	39.1
ACM146	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	56.5
ACM171	-	-	+	-	-	-	-	-	-	+	+	+	-	+	+	26.1
ACM147	+	+	-	-	-	-	-	+	-	+	+	+	+	-	-	30.4
ACM168	-	-	-	-	-	-	-	+	-	+	-	+	+	+	-	21.7
ACM133	-	-	+	+	+	+	+	-	-	+	+	+	-	+	+	43.5
ACM125	-	-	-	-	-	-	-	-	-	+	+	-	+	-	-	13.0
ACM151	+	+	+	-	-	-	-	+	+	-	+	-	-	-	-	26.1
ACM229	+	+	-	-	-	-	-	+	+	-	-	-	-	-	-	17.4
ACM170	-	-	-	-	-	-	-	-	+	+	-	-	+	-	-	13.0
ACM16	-	-	-	-	-	-	-	+	+	+	-	+	-	-	-	17.4
ACM115	-	-	-	-	-	-	-	-	+	-	+	-	-	+	+	17.4

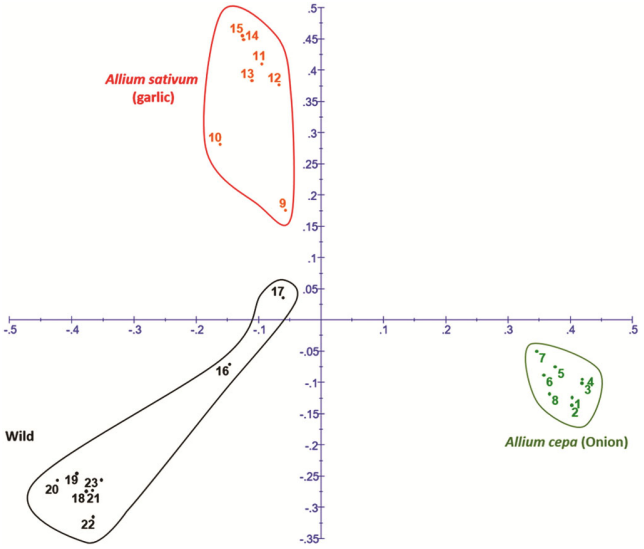

Fig. 1 — Principal coordinate analysis depicting genetic relatedness of 23 *Allium* species using thirty SSR markers.

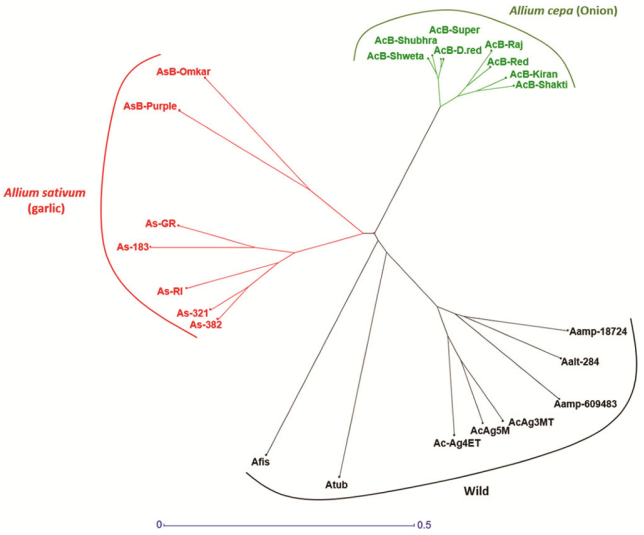

Fig. 2 — Phylogenetic relationship of *Allium* species using 30 SSR markers.

show clear divergence from other wild species. Further, B. Omkar and B. Purple (*A sativum*) variety reveal clear genetic divergence from other *Allium sativum* variety. B. Shubhra and B. Shweta a white onion (*A. cepa*) variety have separate group (Fig. 2) within *A. cepa* compared to other red onion variety which clearly reveal significance and authenticity of these SSR markers (Fig. 3).

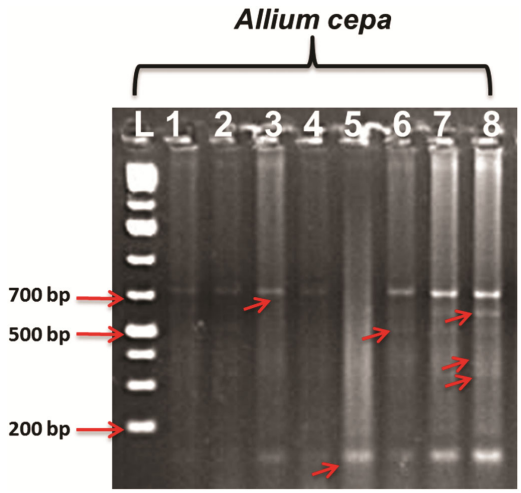

Fig. 3 — PCR amplification profile of eight *Allium* germplasm using ACM093 marker along with ladder.

Discussion

Allium comprises of several medicinally important species like onion and garlic. However, for efficient management, utilization and conservation of elite and wild germplasms, studies are largely limited for Indian germplasms. Lack of genomic resources and reliable markers severely obstruct genetic characterization, breeding and conservation efforts. Although, efforts were made to study genetic relationships in few *Allium* species[15-17], several popular onion and garlic varieties remains uncharacterized. In current study, SSR markers were mined from public domain and utilized for evaluation of popular Indian *Allium* germplasms. Average number of alleles per SSR locus and PIC was found lower as compared to previous report[16], may be because different set of marker and germplasms used in their study. Low level of cross transferability (4.3 to 56.5) detected by these SSR markers in the present work is comparable with our unpublished ILP markers in *Allium*. These polymorphic cross-transferable microsatellite markers reveal their potential use in sustainable *Allium* spp. management and phylogenetic studies. Overall genetic diversity recorded was higher in wild relative compared to cultivated *A. cepa*, possibly because most of the *A. cepa* variety is derived by domestication but wild relatives are open pollinated and undergoes more gene pool shuffling leading to higher heterogenity. *A. tuberosum and A. ampeloprasum* formed separate group possibly due to different ploidy level from another *Alliums* spp. Further, *A. tuberosum and A. fistulosum, a* wild *Allium* spp. reported tolerant to

various diseases also form different subgroup within wild relative and therefore provide opportunities to bring these traits in elite cultivar of *A. cepa* and *A. sativum*. Additionally, B. Omkar and B. purple (*A. sativum*), the ICAR-DOGR Indian varieties also formed subgroup within *Allium sativum* (garlic group) due to their different geographical origin and unique morphological characteristic feature like purple colour clove of B. Purple. B. Shubhra and B. Shweta, the white colour onion (*A.cepa*) varieties of ICAR-DOGR showing separate subgroup from other red colour onion (*A.cepa*) due to their different colour and other unique features further confirm uniqueness of ICAR-DOGR varieties and significance of these SSR markers.

Conclusion

SSR markers were successfully utilized to assess genetic variations in popular Indian *A.cepa*, *A. sativum* and establish genetic relationships with wild *Allium* species. These markers can be harnessed for molecular breeding, varietal identification and planning germplasm conservation strategies in future.

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Conflict of interest

The authors declare that they have no conflict of interest.

Reference

1 Devi, A., Rakshit, K. & Sarania, B. 2014. Ethnobotanical notes on *Allium* species of Arunachal Pradesh, India. Indian Journal of Traditional Knowledge 13(3): 606-612

2 Keusgen M, Fritch RM, Hisoriev H, Kubornova PA, Khassanov FO. Wild *Allium* species (Alliaceae) used in folk medicine in Tajikistan and Uzbekistan. *J Ethno b Ethnom* 2006; 2:18.

3 Liguori L, Califano R, Albanese D, Raimo F, Crescitelli A, Di Matteo M (2017) Chemical composition and antioxidant properties of five white onion (*Allium cepa* L.) landraces. J Food Qual article ID 6873651:9

4 L. Bayan, P.H. Koulivand, A. Gorji Garlic: a review of potential therapeutic effects Avicenna J Phytomed, 4 (2014), pp. 1-14

5 Gemma AC, Terry LA, White PJ (2007) Effect of controlled atmosphere storage on abscisic acid concentration and other biochemical attribute of onion bulbs. Postharvest Biol Tec. 39:233-242.

6 Adsul GG, (2009) Genetic diversity analysis of some onion germplasm lines (*Allium cepa* L.), Ph.D. thesis submitted to North Maharashtra University, Jalgaon

7 Bhandawat A, Sharma V, Sharma H, Sood A, Sharma RK, (2015) Development and cross transferability of functionally relevant microsatellite markers in *Dendrocalamus latiflorus* and related bamboo species. *Journal of genetics*, *94*(1), pp.48-55.

8 Bhandawat A, Sharma H, Nag A, Singh S, Ahuja PS, Sharma RK (2015). Functionally relevant novel microsatellite markers for efficient genotyping in Stevia rebaudiana Bertoni. *Journal of genetics*, *94*(1), pp.75-81.

9 Moxon ER, Wills C. 1999. DNA microsatellites: agents of evolution? Scientific American 280(1):94–99 DOI 10.1038/scientificamerican 0199-94.

10 Jayaswall, K., Mahajan, P., Singh, G., Parmar, R., Seth, R., Raina, A., et al. (2016). Transcriptome analysis reveals candidate genes involved in blister blight defense in tea (*Camellia sinensis* (L) Kuntze). *Sci. Rep.* 6:30412. doi: 10.1038/srep30412

11 Kumar R., Kuldip, Ahuja P.S., Sharma R.K. Status and opportunities of molecular breeding approaches for genetic improvement of tea Molecular Breeding for Sustainable Crop Improvement, Springer (2016), pp. 101-125

12 Oliveira EJ, Pa´dua JG, Zucchi MI, Vencovsky R, Vieira MLC. 2006. Origin, evolution and genome distribution of microsatellites. Genetics and Molecular Biology 29(2): 294–307

13 Hedrick PW. 2014. Conservation genetics and the persistence and translocation of small populations: bighorn sheep populations as examples. Animal Conservation 17(2):106–114

14 Klaas, M. and N. Friesen. (2002). Molecular markers in *Allium*, p. 159–186. In: Rabinowitch, H.D. and L. Currah (eds.). *Allium* cropscience: Recent advances. CABI Publishing, New York, NY.9

15 Khosa JS, Negi KS, Dhatt AS, Khar A. Utility of Simple Sequence Repeat (SSR) markers to realize worth of germplasm in genus *Allium*. Indian J. Plant Genet. Resour, 2014; 27:238-45.

16 Khosa JS, AS Dhatt, KS Negi and K Singh (2013) Characterization of *Allium* germplasm using bulb onion derived SSR markers. Crop Improv. 40: 69-73

17 Mahajan V, Jakše J, Havey MJ, Lawande KE (2009) Genetic fingerprinting of Indian onion cultivars using SSR markers. Indian J Hortic 66:62–68

18 Murray M G, Thompson W F (1980). Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res. 8, 4321-4325. https://www.ncbi.nlm.nih.gov/pubmed/7433111

19 Kuhl JC, Cheung F, Yuan Q, *et al.* 2004. A unique set of 11 008 onion expressed sequence tags reveals expressed sequence and genomic differences between the monocot orders Asparagales and Poales. *The Plant Cell* 16, 114 –125.

20 Martin WJ, McCallum J, Shigyo M, Jakse J, Kuhl JC, Yamane N, Pither-Joyce M, Gokce AF, Sink KC, Town CD, et al: Genetic mapping of expressed sequences in onion and in silico comparisons with rice show scant colinearity. Mol Genet Genomics. 2005, 1-8.

21 McCallum J, Clarke A, Pither-Joyce M, Shaw M, Butler R, Brash D, Scheffer J, Sims I, van Heusden S, Shigyo M, Havey MJ (2006) Genetic mapping of a major gene affecting onion bulb fructan content. TheorAppl Genet 112:958–967

22 McCallum J, Pither-Joyce M, Shaw M, Kenel F, Davis S, Butler R, Scheffer J, Jakse J, Havey MJ (2006) Genetic mapping of sulfur assimilation genes reveals a QTL for onion bulb pungency. Theor Appl Genet 114:815–822

23 Perrier X, Jacquemoud-Collet J. (2006). DARwin software http://darwin.cirad.fr/

24 Jan 2002. Available online at https://www.liverpool.ac.uk/~kempsj/pic.html.

25 Thiel T, Michalek W, Varshney R, Graner A (2003). Exploiting EST databases for the development and characterization of gene-derived SSR-markers in barley (*Hordeum vulgare* L.). *Theoretical and Applied Genetics* 106 (3): 411-422. doi: 10.1186/1471- 2229-10-278