Mohapatra PP *et al.* (2023)
Notulae Botanicae Horti Agrobotanici Cluj-Napoca
Volume 51, Issue 3, Article number 13369
DOI:10.15835/nbha51313369
Research Article

Efficiency of RAPD and SSR markers in assessing genetic diversity in summer onion (*Allium cepa* L.) genotypes

Priyadarshani P. MOHAPATRA[1*], Mahmoud F. SELEIMAN[2*], Rupsanatan MANDAL[3], Kartik PRAMANIK[4], Tapan Kumar MAITY[5], Jayanta TARAFDAR[6], Subhashree P. DAS[7], Chandini A. RAJ[8], Chinmaya JENA[9], Mekhled M. ALENAZI[2], Sumit SOW[10], Shivani RANJAN[10], Awais AHMAD[2], Harun GITARI[11], Naeem KHAN[12]

[1]*Central Agricultural University, College of Community Science, Tura, Meghalaya 794005, India; lipi.pragati@gmail.com (*corresponding author)*
[2]*King Saud University, College of Food and Agriculture Sciences, Department of Plant Production, P.O. Box 2460, Riyadh 11451, Saudi Arabia; mseleiman@ksu.edu.sa (*corresponding author); amekhled@ksu.edu.sa; arehmatullah@ksu.edu.sa*
[3]*Uttar Banga Krishi Viswavidyalaya, Department of Genetics and Plant Breeding, Pundibari 736165, West Bengal, India; rup.biotech@gmail.com*
[4]*Odisha University of Agriculture and Technology, Department of Vegetable Science, Bhubaneswar 751003, Odisha, India; kartikouat@gmail.com*
[5]*Central Agricultural University, College of Agriculture, Lembucherra 799210, West, Tripura, India; proftkmaity@gmail.com*
[6]*Bidhan Chandra Krishi Viswavidyalaya, Department of Plant Pathology, Mohanpur 741252, West Bengal, India; jayanta94bckv@gmail.com*
[7]*Department of Agriculture and Farmers Empowerment, Dasarathpur, Jajpur 755006, Odisha, India; shubhashree23@gmail.com*
[8]*Dr. Y.S.R. Horticultural University, College of Horticulture, Venkataramannagudem, West Godavari 534006, Andhra Pradesh, India; chandua91@gmail.com*
[9]*Odisha University of Agriculture and Technology, Department of Fruit Science and Horticultural Technology, Bhubaneswar 751003, Odisha, India; chinmaya.jena@cutm.ac.in*
[10]*Dr. Rajendra Prasad Central Agricultural University, Department of Agronomy, Pusa, Samastipur 848125, Bihar, India; sumitsow19@gmail.com; ranjanshivani54@gmail.com*
[11]*Kenyatta University, School of Agriculture and Environmental Sciences, Department of Agricultural Science and Technology, Nairobi P.O. Box 43844-00100, Kenya; harun.gitari@ku.ac.ke*
[12]*University of Florida, Institute of Food and Agricultural Sciences, Agronomy Department, Gainesville FL, 32611, USA; naeemkhan@ufl.edu*

Abstract

The genetic diversity assessment of agricultural crops is crucial for breeding programs aimed at enhancing crop yield, resistance to diseases, and adaptation to changing environmental conditions. In the present investigation, a comparative genetic relationship in sixteen onion genotypes was assessed utilizing dominant (RAPD) and co-dominant (SSR) marker systems. Ten RAPD and nine SSR markers showed genetic diversity remarkably and produced 503 and 107 amplicons respectively. Spearman rank correlation was used to compare the different efficiency parameters in two marker systems with respect to sixteen onion genotypes.

Received: 25 Aug 2023. Received in revised form: 06 Sep 2023. Accepted: 12 Sep 2023. Published online: 14 Sep 2023.
From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

The genetic relationship based on similarity matrix values between a pair of cultivars was higher for SSR markers than for the RAPD marker system. OPC-04 (RAPD primer) and ACM-004 (SSR primer) witnessed the highest poly-morphic bands along with other polymorphic markers that proved to be useful in grouping onion genotypes. Finally, dendrograms were constructed and compared following the mantel test to find out the genetic diversity among the germplasms. This study will be effective for a selection of efficient primers and suitable marker systems to distinguish the onion genotypes in the future.

Keywords: amplicons; genetic diversity; RAPD; Spearmen rank correlation; SSR; summer onion

Introduction

Onion (*Allium cepa* L.) (2n =16) is one of the important bulbs producing vegetable crops having great economic demand all over the world due to its nutritional and therapeutic value. Summer onion is grown in the rainy season in different parts of the world and has a great role in controlling high market demand and price in the shortfall of winter onion stock in the market. So, the global cultivation of summer onions is on the rise, highlighting the ongoing need for increased emphasis on varietal improvement. Onion is a highly cross-pollinated crop attributed to its high genetic variability and diversity. The knowledge of genetic diversity in a crop species is fundamental to unlocking its genetic potential for improvement (Camargo *et al*., 2013; Morales *et al*., 2013; Mariano *et al*., 2019). Although wide genetic diversity is exhibited in onion, progress in crop improvement is not up to the mark as compared to other monocots (McCallum *et al*., 2007).

Utilization of morphological characterization along with modern-day throughput techniques *i.e*., PCR-based techniques have emerged as promising which are fast, reliable, and require a minimal amount of tissue for investigation (Semagn *et al*., 2006). The various marker tools like random amplified polymorphic DNA (RAPD), inter-simple sequence repeat (ISSR), microsatellite or simple sequence repeat (SSR) are used for molecular dissection in onion (Xu *et al*., 2011; Sangeeta *et al*., 2006; Qijiang and Jia, 2007; Mahajan *et al*., 2009; Maniruzzaman *et al*., 2010; Jabbes *et al*., 2011). Anandhan *et al*. 2014 evaluated seven open-pollinated varieties of onion for varietal identity using SSR primers. The molecular markers have varied utility *i.e*., characterization of the gene pool, DNA fingerprinting, phylogenetic analysis, molecular dissection of complex traits, characterization of genome organization (Arumuganathan and Earle, 1991; Al-Zahim *et al*., 2005), and disease resistance (Ganesh and Veeregowda, 2007; Chowdappa *et al*., 2012). Anandhan *et al.* 2014 evaluated seven open-pollinated varieties of onion for varietal identity using SSR primers. The characteristics of genomic microsatellites (gSSR) are abundant in plant genomes, with reproducibility, high levels of polymorphism, and co-dominant inheritance (Nicot *et al*., 2004). The genetic fidelity of *A. ampeloprasum* L. and *A. sativum in vitro* regenerated clones was studied by Gantait *et al.* 2010 using 10 ISSR primers. Jakse, 2005 identified 398 SNP, indels, and SSRs which distinguished 35 elite onion populations. The diversity assessment of tropical Indian onion and cross-amplification of genomic and expressed sequence tag (EST)-SSR markers in distantly related native wild species were estimated (Khar *et al*., 2010). These DNA markers offer several advantages over traditional phenotypic markers, as they provide data that can be analysed objectively.

Onion production statistics revealed a doubling of bulb production in the last 50 years due to the availability of F1 hybrids and improved OP varieties which become futile due to current climate change and population pressure (Brewster, 2008). The information on the use of molecular marker in explaining onion genetic diversity is limited due to a scarcity of markers available in the public domain and germplasm resources, outbreeding and biennial habit (McCallum *et al*., 2008). The evaluation of A. cepa biodiversity provides onion breeders with great prospects to obtain plants with high resistance to environmental factors, remarkable yield, and product quality. Genetic diversity assessment of a collection of nine short- and long-day onion landraces

and varieties originated from different Iranian regions, using ISSR markers and UPGMA dendrograms (Kiani *et al*., 2023).

Therefore, in the present investigation, an attempt was made to evaluate different marker efficiency parameters and efficacy of two marker systems (RAPD and SSR) in the molecular characterization of onion to support germplasm evaluation, its conservation and selection to strengthen productivity, quality, adaptability, storage, resistance to biotic and abiotic stresses.

Materials and Methods

The present study comprised 16 genotypes of summer onion that were collected from different sources (Table 1 and Figure 1) as per their adaptability to the summer season and popularity among onion growers and subjected to characterization using molecular markers. The genotypes were planted in the research field of the All-India Network Research Project on Onion and Garlic (ICAR), "C" Block Farm, Bidhan Chandra Krishi Viswavidyalaya, Kalyani in New Alluvial Zone, West Bengal situated at 22°40′ N latitude, 88°18′ E longitude and 7 m AMSL. The site belongs to a sub-humid and sub-tropical agro-ecological zone with an average temperature varies 27-33 °C, and an average annual rainfall of 1560 mm, 80% of which falls during June–September, 2015-17 due to the southwest monsoon. Recommended good agricultural practices (GAPs) were followed to raise the experimental materials. The molecular experiments were done in the Molecular Biology Laboratory under AICRP on Tuber Crops (ICAR), Kalyani, Nadia, West Bengal. The main assumption for the success of breeding programme is the proper selection of parental genotypes. (Owiti *et al*., 2023).

Table 1. List of sixteen summer onion genotypes

Sl. No.	Genotypes	Source
1	'Agrifound Dark Red'	NHRDF, Nashik
2	'Agrifound Rose'	NHRDF, Nashik
3	'Bhima Super'	DOGR, Rajgurunagar
4	'Red Diamond'	Jindal Crop Science Pvt. Jalna
5	'Light Red'	Jindal Crop Science Pvt. Ltd., Jalna.
6	'N-53'	Jindal Crop Science Pvt. Ltd., Jalna.
7	'Kohinoor-09'	Jindal Crop Science Pvt. Ltd., Jalna.
8	'Fursungi'	Jindal Crop Science Pvt. Ltd., Jalna.
9	'Arka Niketan'	IIHR, Bangalore
10	'Arka Kalyan'	IIHR, Bangalore
11	'Arka Bheem'	IIHR, Bangalore
12	'Arka Bindu'	IIHR, Bangalore
13	'Indam Marshall'	Indo-American Hybrid Seeds, Bangalore
14	'Indam Gulab'	Indo-American Hybrid Seeds, Bangalore
15	'Indam Hybrid-04'	Indo-American Hybrid Seeds, Bangalore
16	'Indam Red Stone'	Indo-American Hybrid Seeds, Bangalore

Total genomic DNA was extracted from the young leaves of each genotype following the standard cetyl trimethyl ammonium bromide (CTAB) method with some modifications. The quality and quantity of the extracted DNA were verified on 0.8% TBE agarose gels and were stored at -20 °C. A total of ten RAPD primers

and nine SSR primer pairs were used for this study (Table 2). PCR amplifications were performed in 25 µl reaction mixtures containing 1 U *Taq* polymerase (Biotools), 2.5 µl 10X PCR buffer, 1 µl 2.5 mM of each dNTPs, 2 µl 10 µM of RAPD primer or 1 µl 10 µM of each forward and reverse primer (for SSR) and approximately 10 ng genomic DNA. Cycling conditions were 5 min at 94 °C, followed by 35 cycles of 45 s at 94 °C, 45 s annealing at 37° (for RAPD) or 50°-60 °C (for SSR), a 1 min extension at 72 C and a final extension step of 2 min at 72 °C. Final PCR products were resolved on a 1.5% agarose gel and visualized by Ethidium Bromide. Each primer received a score (1 for presence and 0 for absence of bands in each accession), and a binary matrix was generated (Saini *et al*., 2010). All the analyses were done by using iMEC tools and R package (V. 3.6.1). Genomic DNA was successfully isolated from all the sixteen genotypes and the quality of isolated DNA was studied by 0.8% agarose gel. The concentrated genomic DNA samples were diluted to 10 ng/µl based on stock DNA concentration for the RAPD and SSR profiling. Cluster analyses were implemented by UPGMA method, and the corresponding dendrogram was constructed. The capacity of each primer to distinguish among the genotypes studied was evaluated by the Resolving power (RP) (Prevost and Wilkinson, 1999), Marker index (MI) (Powell *et al*., 1996), and the polymorphic information content (PIC) (Weising *et al*., 2005).

Figure 1. Images of sixteen summer onion genotypes

Table 2. Details of used RAPD and SSR primers

Sl. no.	Primer Id	Primer sequence (5′-3′)	Tm (°C)	Ta(°C)
RAPD				
1	Oligo-1	CTTCACCCGA	32	28
2	Oligo-2	TCGGCGATAG	32	28
3	Oligo-3	CAATCGCCGT	32	30
4	Oligo-4	CAAACGTCGG	32	27
5	Oligo-5	GTTGCGATCC	32	28
6	OPA-06	GGTCCCTGAC	34	28
7	OPA-09	GGGTAACGCC	34	27
8	OPA-11	CAATCGCCGT	32	27
9	OPA13	CAGCACCCAC	34	27
10	OPA-17	GACCGCTTGT	32	27
11	OPA-18	AGGTGACCGT	32	27
12	OPA-20	GTTGCGATCC	32	29
13	OPAD-05	ACCGCATGGG	34	27
14	OPC-04	CCGCATCTAC	32	27
15	OPC-08	TGGACCGGTG	34	28
16	OPC-10	TGTCTGGGTG	32	27
17	OPC-13	AAGCCTCGTC	32	27
18	OPD-03	GTCGCCGTCA	34	29
19	OPG-13	CTCTCCGCCA	34	28
20	OPO-1	GGCACGTAAG	32	28
21	OPO-5	CCCAGTCACT	32	28
22	OPO-18	CTCGCTATCC	32	25
23	OPO-19	GGTGCACGTT	32	25
24	OPQ-05	CCGCGTCTTG	34	27
25	OPQ-06	GAGCGCCTTG	34	30
26	OPQ-20	TCGCCCAGTC	34	27
SSR				
27	ACM004	F-TCGTTCTTTAGAACACGTTAGG	59.9	58
		R-GTCGGCGGATATAGTGACA	61.2	
28	ACM018	F-GGGGAATGGTGGAGAATAGA	62.6	57
		R-AACAGAGGCAAGAGGAGCG	64.7	
29	ACM046	F-TCCTCGTCACCACCACAG	63.8	57
		R-CTGAAAGGGAGTAGCGGAG	61.6	
30	ACM068	F-GAAGGTGAAGGTGTACGGT	59.0	57
		R-CAAATGGCTGCAATAAGCAA	63.6	
31	ACM187	F-GTACTCGGGCAGTGGAGGTA	64.0	59
		R-GGAGCTGTCCAAATGCTAGG	63.7	
32	ACM240	F-GTGCAACTCCAAGAGAAGGG	63.8	58
		R-AATATAAAGGCGTTGGCCTG	62.6	
33	ACM300	F-AGGTGCAGTTTCGTGGTAGG	64.0	58
		R-TTAGCCCCTGGTAAGTGTGG	63.8	
34	ACM318	F-TCCTCCTTCCAAACCACATC	63.9	57
		R-GATCAGAAACAGCAGCGTC	61.2	

35	ACM326	F-AAACCAGCAACAACCAATG	61.0	57
		R-AAAATTGGAGAGCAGGCAAA	63.5	

Tm =Primer melting temperature; Ta = Primer annealing temperature

Results and Discussion

RAPD and SSR profiling and polymorphism

The high level of genetic diversity within onions is confirmed by several researchers using different molecular markers like ISSR, RAPD, and SSR (Brahimi *et al*., 2022). The amplification products of RAPD primers showed a distribution of amplified fragments unique for each primer. The population-specific bands could be discerned from the fragment patterns generated. These bands were treated as genetic loci. Each band produced by the primers was distinct and reproducible. The ten RAPD primers produced bands ranging in size from (100 bp to 1.5 kb). The data collected from the random amplification of polymorphic DNA with 10 arbitrary primers produced a total of 55 loci with 503 amplicons. The number of alleles ranged from two in Oligo-03 to twelve in OPC-04. The average number of alleles was 3.56 per locus (Table 3). Out of ten primers, seven primers showed 100% polymorphism (Oligo-01, Oligo-02, Oligo-03, Oligo-04, OPC-04, OPQ-06, and OPG-13) Oligo-05 had (88.9%) moderate polymorphism and OPD-03 and OPA-09 had the lowest polymorphism (75%). In this study, nine SSR primers were also used which had been developed by Mc Callum *et al*., 2008. Simple sequence repeats (SSRs), which are codominant markers, are preferred for the determination of genetic diversity because they are highly polymorphic, multi-allelic, highly reproducible, and have good genome coverage (Özkan *et al*., 2022) Among the nine primers tested, eight exhibited varying degrees of efficacy in distinguishing distinct multiband phenotypes within a pool of 16 genotypes. Nineteen out of the twenty bands generated were polymorphic, accounting for 88.89%, while only one band exhibited a monomorphic characteristic (Table 3). Primer ACM-004, ACM-068, ACM-187, ACM-326, ACM-046, and ACM-240 produced more bands and primer ACM-318, ACM-018 and ACM-300 amplified the fewest. The agarose gel picture of RAPD and SSR primes is presented in Figures 2 and 3.

Table 3. Different marker efficiency parameters revealed by RAPD and SSRs marker system

Marker ID	TSB	NTB	NPB	PPB Value (%)	PIC	MI	DI	RP
RAPD								
Oligo-01	19	3	3	100	0.39	0.6	0.83	0.63
Oligo-02	41	4	4	100	0.4	1.08	0.83	0.66
Oligo-03	23	2	2	100	0.39	0.26	0.84	0.56
Oligo-04	43	4	4	100	0.38	1.02	0.84	0.59
Oligo-05	80	9	8	88.9	0.31	4.27	0.88	0.47
OPC-04	100	12	12	100	0.42	10.14	0.82	0.68
OPD-03	53	4	3	75	0.17	0.27	0.95	0.21
OPA-09	45	4	3	75	0.34	0.91	0.85	0.53
OPQ-06	53	6	6	100	0.34	2.03	0.86	0.52
OPG-13	46	7	7	100	0.39	3.23	0.83	0.61
Mean	50.3	5.5	5.2	93.89	0.35	2.38	0.85	0.55
SSR								
ACM004	16	3	3	100	0.44	1.08	0.89	0.67
ACM018	5	3	3	100	0.19	0.46	0.99	0.21
ACM046	14	3	3	100	0.34	0.84	0.88	0.5
ACM068	16	2	2	100	0.38	0.41	0.69	0.5
ACM187	16	1	0	0	0.5	0.14	0	0

ACM240	12	3	3	100	0.37	0.92	0.94	0.5
ACM300	3	1	1	100	0.31	0.08	0	0
ACM318	10	2	2	100	0.31	0.34	0.84	0.5
ACM326	15	2	2	100	0.42	0.99	0.88	0.52
Mean	11.89	2.22	2.11	88.89	0.36	0.59	0.68	0.38

TSB: Total Scorable Band; NTB: Number of Total Band; NPB: Number of Polymorphic Band; PIC: Polymorphism Information Content; MI: Marker Index; DI: Diversity Index; RP: Resolving Power

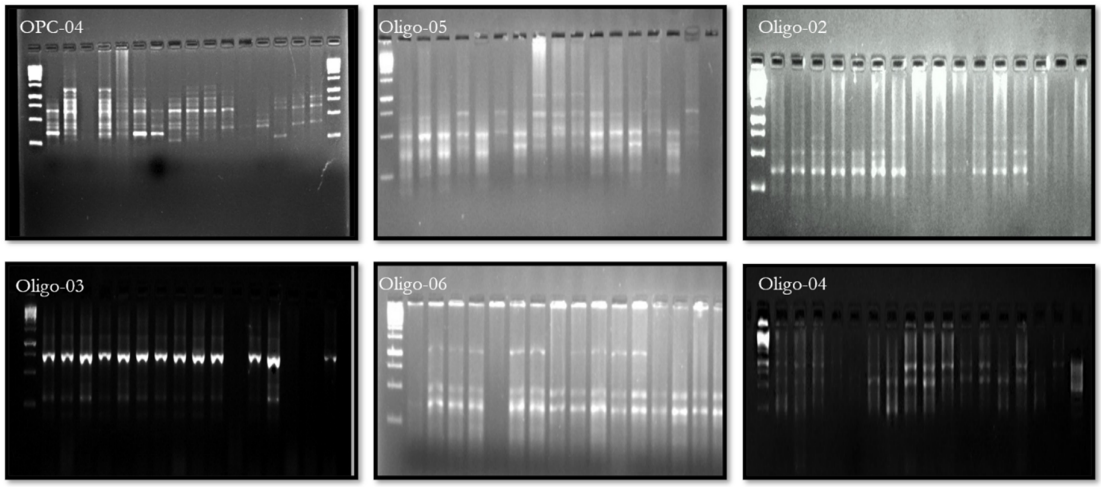

Figure 2. RAPD Profile of 16 onion genotypes using primers OPC-04, Oligo-05, Oligo-02, Oligo-03, OPQ-06 and Oligo-04

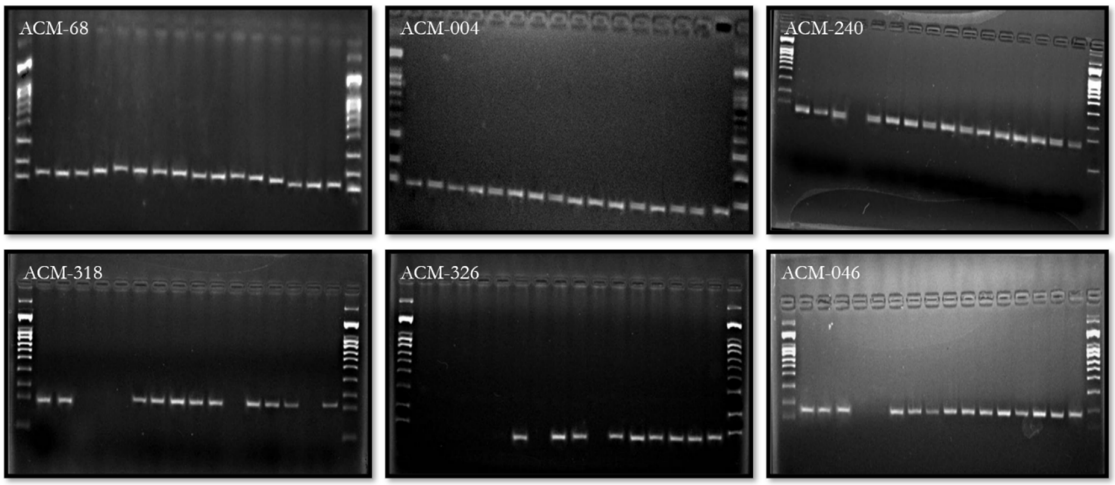

Figure 3. SSRs Profile of 16 onion genotypes using primers ACM-068, ACM-004, ACM-240, ACM-318, ACM-326, ACM-046

Comparison of marker efficiency of different maker systems

The range of the PIC value (which denotes allelic diversity and frequency) was 0.17 in OPD-03 to 0.42 in OPC-04 in the RAPD maker system and 0.19 in ACM-018 to 0.44 in ACM-004 in SSR profiling (Figure 4). The highest PIC value indicated its usefulness in differentiating individuals and presented high information content compared to other primers. The average MI for RAPD is 2.38 followed by 0.58 in the SSR marker system. The diversity index value ranged from 0.82 (OPC-04) to 0.95 (OPD-03) with an average of 0.85 in the RAPD marker system and ranged from 0.00 (AMC-187) to 0.99 (AMC-018) with an average of 0.68 in SSRs. The estimated average resolving power is lower in SSR (0.38) compared to the RAPD marker system (0.55). These results indicated that the studied marker system efficiently discriminated against all sixteen onion genotypes. A high degree of polymorphism was witnessed in onion genotypes by using RAPD primers. For vivid and effective trait identification in onion genotypes necessity of a large number of primers with polymorphic products were emphasized (Arifin *et al*., 2000; Tanikawa *et al*., 2002; Madoka *et al*., 2004; Mei *et al*., 2015). Anandhan *et al*. 2014 evaluated seven open-pollinated varieties of onion for varietal identity using SSR primers.

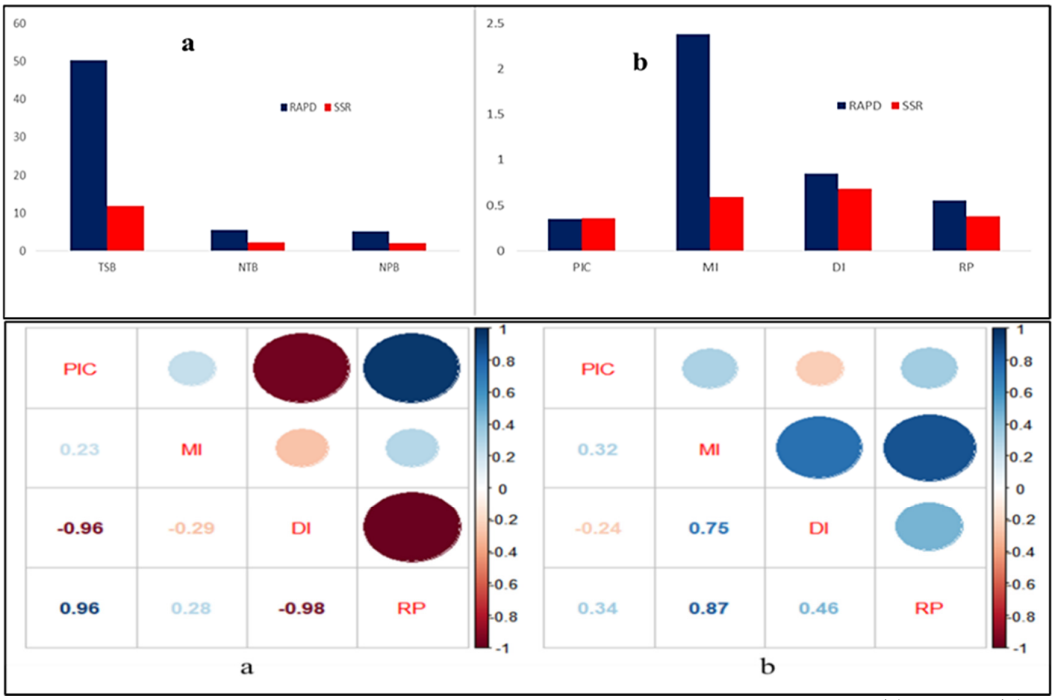

Figure 4. Spearman rank correlation of different efficiency parameters revealed by RAPD (a) and SSR (b) marker system

Relationships among efficiency parameters were analyzed following Spearman rank correlation in both markers systems (Figure 5). In the case of the RAPD marker system, DI is negatively correlated with other parameters but in SSRs, DI is positively correlated with MI and RP except for PIC. RP and MI showed a positive relationship with other parameters except for DI in the RAPD marker system but they showed a positive correlation with all the efficiency parameters in SSRs. The value of Mantel's test correlation showed a positive (r=0.35) non-significant (p=0.29) relationship between the two marker systems to compare their efficiency parameters. These results implied that all efficiency parameters were not equally important to asserting the onion genotypes in both of the marker systems. These results also indicated that RP is the most efficient parameter followed by MI and DI is the less efficient parameter in both the marker system.

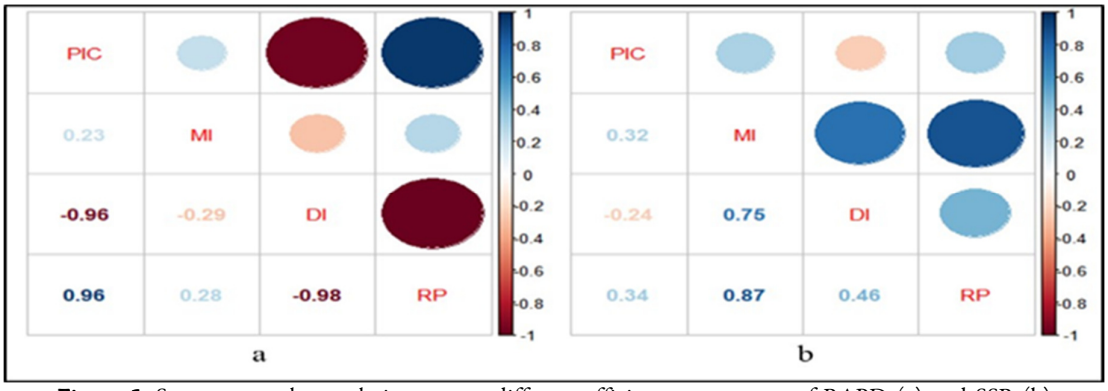

Figure 5. Spearman rank correlation among different efficiency parameters of RAPD (a) and SSR (b) markers

Comparison of genetic relationships analysis revealed by RAPD and SSR profiling

The genetic relationship is studied in both of the marker systems based on Jaccard's Similarity Coefficient. Jaccard's Similarity Matrix generated by RAPD primer was found to be the highest between Arka Kalyan with Bhima super and Arka bheem (0.81) followed by Agri found dark red with N-53 (0.80) and Fursungi with Agri found rose (0.80) while it was lowest between Indam gulab with N-53 and Arka bindu (0.46), Light red with Agri found dark red (0.46). Other genotypes were moderately similar. The entries in this study were grouped into four clusters. Cluster I consisted of five genotypes whereas Cluster II included three and Cluster III had four genotypes and Cluster IV included four genotypes (Figure 4). On the other hand, through SSR profiling, the similarity was noticed to be lowest between Agrifound Rose with Bhima super, N-53 with Arka Niketan, and Indian Gulab with Indian Red Stone whereas, highest between Agri Dark Red with Arka bindu. As the RAPD marker system, SSR profiling also grouped sixteen genotypes into four clusters based on their similarity matrix. Cluster I consisted of five genotypes whereas Cluster II included seven and Cluster III and Cluster IV had two genotypes (Figure 6).

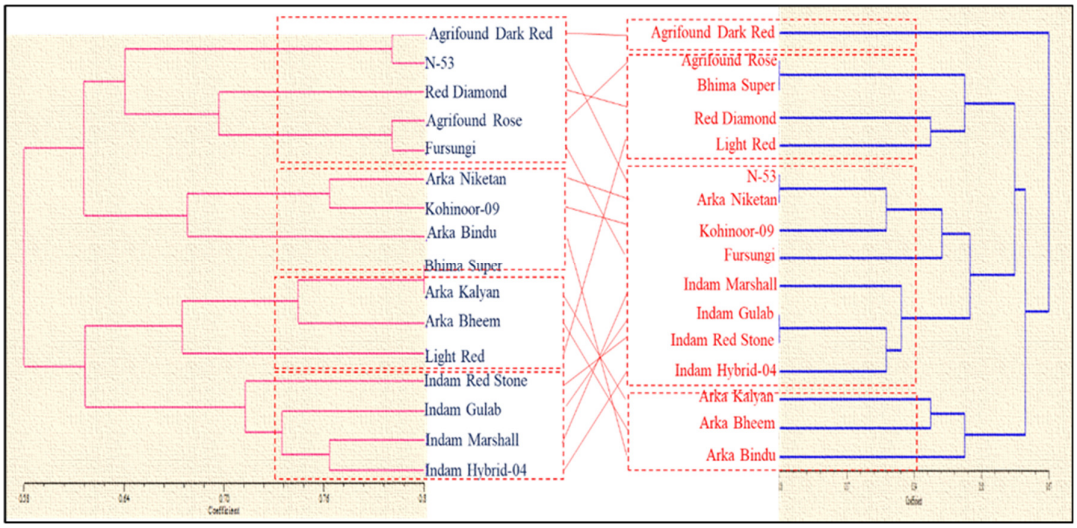

Figure 6. Comparison of UPGMA cluster analysis revealed by RAPD and SSR marker system

On the other hand, through SSR profiling, the similarity was noticed to be lowest between Agrifound Rose with Bhima super, N-53 with Arka Niketan, and Indian Gulab with Indian Red Stone whereas, highest between Agri Dark Red with Arka bindu. As the RAPD marker system, SSR profiling also grouped sixteen genotypes into four clusters based on their similarity matrix. Cluster I consisted of five genotypes whereas Cluster II included seven and Cluster III and Cluster IV had two genotypes (Figure 6). The genetic similarity values between the pair of cultivars were higher for SSR markers (1.00) followed by the RAPD marker system (0.81). The value of Mantel's test correlation showed a positive (r=0.34) and significant correlation (p=0.001) between two similarity matrix data derived from the RAPD and SSR profiling. Maniruzzaman *et al*. 2010 also reported on *allium* species and the dendrogram constructed from the similarity data showed that all the cultivars analyzed were related. Genetic diversity among twenty-seven Egyptian garlic (*Allium sativum* L.) cultivars and ecotypes were studied based on morphological characteristics and ISSR molecular markers. Euclidean distances and Ward's method for cluster analysis were performed among studied genotypes using 14 morphological characters (Elsharkawy *et al*., 2021)

Conclusions

Molecular marker-based investigations utilizing RAPD and SSRs effectively characterized the genetic diversity among the studied onion genotypes. Among the markers, the RAPD primer OPC-04 yielded the highest number of polymorphic bands, which, along with other polymorphic markers, proved valuable for genotype differentiation and categorization. Similarly, the SSR primer ACM-004 also produced the highest number of polymorphic bands. The grouping of genotypes based on SSR markers exhibited similarity to the phenotypic and RAPD-based groupings. While these various studies did not provide precise differentiation for average genotypes, they distinctly grouped the best genotypes based on various parameters. The construction of a UPGMA dendrogram resulted in four clusters (I to IV) for the studied onion genotypes, indicating that genotypes with higher resemblance clustered together in less divergent subclusters and sub-subclusters. The investigation was primarily focused on the selection of suitable primers based on marker efficiency parameters and an efficient DNA marker system which are beneficial to the critical assessment of genetic relationships among the summer onion genotypes. The major finding of this investigation is that resolving power and marker index are the most efficient parameters for the selection of primers in onion crops. Furthermore, this investigation demonstrates that SSR markers are particularly effective in distinguishing among onion genotypes, offering valuable insights for molecular-level genotype screening. The knowledge acquired through this study is poised to play a pivotal role in the integration of molecular markers within onion improvement programs.

Authors' Contributions

Conceptualization: TKM, JT and PPM; methodology: PPM; software: RM, MFS; validation: TKM, PPM, JT and RM; formal analysis: RM; investigation: PPM; resources: TKM and JT; data curation: RM; writing—original draft preparation: PPM and MFS, writing—review and editing: KP, SPD, ACR, CJ, SS, SR, HG, MMA, AA and NK; visualization: JT and TKM; supervision: TKM, JT and MMA.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

Acknowledgements

The authors gratefully acknowledge Department of Plant Pathology and Laboratory of Molecular Biology, Bidhan Chandra Krishi Viswavidyalaya (BCKV), West Bengal and ICAR-AINRP on Onion and Garlic, BCKV-Kalyani Centre, West Bengal for providing essential support to accomplish the research. The authors also acknowledge Researchers Supporting Project number (RSPD2023R751), King Saud University, Riyadh, Saudi Arabia for supporting this work.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Al-Zahim MA, Ford-Lloyd BV, Newbury HJ (2005). Detection of somaclonal variation in garlic (*A. sativum* L.) using RAPD and cytological analysis. Plant Cell Reports 18:473-477.

Anandhan S, Mote SR, Gopal J (2014). Evaluation of onion varietal identity using SSR markers. Seed Science and Technology 42:279-285. *https://dx.doi.org/10.15258/sst.2014.42.2.16*

Arifin NS, Ozaki Y, Okubo H (2000). Genetic diversity in Indonesian shallot (*Allium cepa* var. *ascalonicum*) and *Allium* x *wakegi* revealed by RAPD markers and origin of *A*. x *wakegi* identified by RFLP analyses of amplified chloroplast genes. Euphytica 111(1):23-31. *http://doi.org/10.1023/A:1003732403402*

Arumuganathan K, Earle E (1991). Nuclear DNA content of some important plant species. Plant Molecular Biology Reports 9:208-218. *https://doi.org/10.1007/BF02672069*

Brahimi A, Landschoot S, Bekaert B, Hajji L, Hajjaj H, Audenaert K, ... Mazouz H (2022). Exploring the genetic and phenotypic diversity within and between onion (*Allium cepa* L.) ecotypes in Morocco. Journal of Genetic Engineering and Biotechnology 20:96. *https://doi.org/10.1186/s43141-022-00381-w*

Brewster JL (2008). Onions and Other Vegetable Alliums. CABI, Oxfordshire, UK.

Camargo LKP, Mogor AF, Resende JTV, Da-Silva PR (2013). Establishment and molecular characterization of a sweet potato germplasm bank of the highlands of Parana State, Brazil. Genetic and Molecular Research 12:5574-5588. *http://doi.org/10.4238/2013.November.18.7*

Chowdappa P, Sandhya H, Bhargavi BR (2012). Diversity analysis of *Alternaria porri* (Ellis) Cif-causal organism of purple leaf blotch of onion. International Journal of Innovative Horticulture 1:11-17.

Elsharkawy GA, Hegazi HH, Azab E, Gobouri AA, Sayed SA (2021). Assessment of genetic diversity among Egyptian garlic landraces based on morphological characteristics and ISSR markers. European Journal of Horticultural Sciences 86(6):579-589. *https://doi.org/10.17660/eJHS.2021/86.6.1*

Ganesh CT, Veeregowda R (2007). Fingerprinting and genetic diversity of purple blotch disease resistant and susceptible genotypes of onion (*Allium cepa* L.) using RAPD markers. In Recent trends in horticultural biotechnology, ICAR national symposium on biotechnological interventions for improvement of horticultural crops: Issues and strategies, Vellanikkara, Kerala, India, New India Publishing Agency, 11:599-611.

Gantait S, Mandal N, Bhattacharyya S, Das PK (2010). Determination of genetic integrity in long-term micro-propagated plantlets of *A. ampeloprasum* L. using ISSR markers. Biotechnology 9:218-223. *https://doi.org/10.3923/biotech.2010.218.223*

Jabbes N, Clerc V, Dridi B, Hannechi C (2011). Inter simple sequence repeat fingerprints for assess genetic diversity of Tunisian garlic populations. Journal of Agricultural Science 3:77-85. *http://doi.org/10.5539/jas.v3n4p77*

Jakse J (2005). Single nucleotide polymorphisms, Indels, Simple Sequence Repeats for onion cultivar identifications. Journal of the American Society for Horticultural Science 130(6):912-915. *http://doi.org/10.21273/JASHS.130.6.912*

Khar A, Lawande KE, Negi KS (2010). Microsatellite marker-based analysis of genetic diversity in short day tropical Indian onion and cross amplification in related *Allium* spp. Genetic Resources and Crop Evolution 58:741-752. *http://doi.org/10.1007/s10722-010-9616-y*

Kiani Z, Mashayekhi K, Golubkina N, Mousavizadeh SJ, Nezhad KZ, Caruso G (2023). Agronomic, physiological, genetic and phytochemical characteristics of onion varieties influenced by daylength requirements. Agriculture 13:697. *https://doi.org/10.3390/agriculture13030697*

Madoka H, Satoko K, Ritsuka U, Chifuyu Y, Kazumi T, Shoji H (2004). Identification of onion cultivars by RAPD method with small-scale isolation of the total DNA. Journal of Nishikyushu University and Saga Junior College 34:15-49.

Mahajan V, Jakse J, Havey MJ, Lawande KE (2009). Genetic fingerprinting of onion cultivars using SSR markers. Indian Journal of Horticulture 66:62-68.

Maniruzzaman ME, Haque MM, Haque MA (2010). Molecular characterization of onion (*A. cepa* L.) using RAPD markers. Bangladesh Journal of Agricultural Research 35:313-322. *http://doi.org/10.3329/bjar.v35i2.5894*

Mariano LC, Zchonski FL, da Silva CM, Da-Silva PR (2019). Genetic variability in a Brazilian apple germplasm collection with low chilling requirements. Peer J. 6:e6265. *https://doi.org/10.7717/peerj.6265*

McCallum J, Pither-Joyce M, Shaw M, Kenel F, Davis S, Butler R, ... Havey MJ (2007). Genetic mapping of sulphur assimilation genes reveals a QTL for onion bulb pungency. Theoretical and Applied Genetics 114:815-822. *https://dx.doi.org/10.1007/s00122-006-0479-8*

McCallum J, Thomson S, Pither-Joyce, M, Kenel, F, Clarke, A., Havey MJ (2008). Genetic diversity analysis and single-nucleotide polymorphism marker development in cultivated bulb onion based on expressed sequence tag-simple sequence repeat markers. Journal of the American Society for Horticultural Science 133(6):810-818. *https://doi.org/10.21273/JASHS.133.6.810*

Mei Z, Zhang C, Khan MA, Zhu Y, Tania M, Luo P, Fua J (2015). Efficiency of improved RAPD and ISSR markers in assessing genetic diversity and relationships in *Angelica sinensis* (Oliv.) Diels varieties of China. Electronic Journal of Biotechnology 18:96-102. *http://doi.org/10.1016/j.ejbt.2014.12.006*

Morales RGF, Resende JTV, Resende FV, Delatorre CA, Figueiredo AST, Da-Silva PR (2013). Genetic divergence among Brazilian garlic cultivars based on morphological characters and AFLP markers. Genetic and Molecular Research 12:270-281. *https://doi.org/10.4238/2013.february.4.1*

Nicot N, Chiquett V, Gandon B, Amilhat L, Legeai F, Leroy P, ... Sourdille P (2004). Study of simple sequence repeat (SSR) markers from wheat expressed sequence tags (ESTs). Theoretical and Applied Genetics 109:800-805. *https://doi.org/10.1007/s00122-004-1685-x*

Owiti AA, Bargul JL, Obiero GO, Nyaboga EN (2023). Analysis of genetic diversity and population structure in Yam (Dioscorea Species) germplasm using start codon targeted (SCoT) molecular markers. International Journal of Plant Biology 14:299-311. *https://doi.org/10.3390/ijpb14010025*

Özkan G, Haliloglu K, Türkoglu A, Özturk HI, Elkoca, E, Poczai, P (2022). Determining genetic diversity and population structure of common bean (*Phaseolus vulgaris* L.) landraces from Türkiye using SSR markers. Genes 13:1410. *https://doi.org/10.3390/genes13081410*

Powell W, Morgante R, Andre C, Hanafey M, Vogel J, Tingey S, Rafalsky A (1996). The Comparison of RFLP, RAPD, AFLP and SSR (Microsatellite) Markers for Germplasm Analysis. Molecular Breeding 2(3):225-238. *https://doi.org/10.1007/BF00564200*

Prevost A, Wilkinson MJ (1999). A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. Theoretical and Applied Genetics 98:107-112. *https://doi.org/10.1007/s001220051046*

Qijiang CC, Jia T (2007). Genetic diversity analysis of onion (*A. cepa* L.) germplasm by ISSR markers. Chinese Agricultural Science Bulletin 23:126-130.

Saini M, Sing S, Hussain Z, Sikka VK (2010). RAPD analysis in mungbean [*Vigna radiate* (L.) Wilczek.] II: A comparison of efficiency parameters of RAPD primers. Indian Journal of Biotechnology 9:276-282.

Sangeeta KM, Gowda RV, Lalitha A (2006). Analysis of genetic diversity among Indian short-day onion (*A. cepa* L.) cultivars using RAPD markers. Journal of Horticultural Science and Biotechnology 81:774-777. *http://doi.org/10.1080/14620316.2006.11512136*

Semagn K, Bjørnstad A, Ndjiondjop MN (2006). An overview of molecular marker methods for plants. African Journal of Biotechnology 5:2540-2568.

Singh PR, Mahajan V, Verma A, Sagar R, Jayaswal K, Shukla N, ... Singh M (2021). Identification and characterization of white onion (*Allium cepa* L.) genotypes for high total soluble solid content through molecular markers. Genetic Resources and Crop Evolution 68:957-967. *https://doi.org/10.1007/s10722-020-01037-9*

Tanikawa T, Takagi M, Ichii M (2002). Cultivar identification and genetic diversity in onion (*Allium cepa* L.) as evaluated by random amplified polymorphic DNA (RAPD) analysis. Journal of Japan Society for Horticultural Sciences 71(2):249-251. *http://doi.org/10.2503/jjshs.71.249*

Weising K, Nybon H, Wolff K, Kahl G (2005). Applications of DNA fingerprinting in plant sciences. In: Taylor & Francis eds. DNA Fingerprinting in Plants. Principles, Methods and Applications (2nd edition), CRC Press Boca Raton, London, New York, Singapore, p. 235-276.

Xu P, Liu H, Gao Z, Srinivas P, Yang CY (2001). Genetic identification of garlic cultivars and lines by using RAPD assay. Acta Horticulturae 555:213-220. *https://doi.org/10.17660/ActaHortic.2001.555.31*