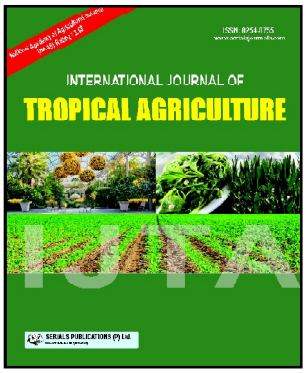

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Characterization of Onion (Allium Cepa L.) Genotypes Using Molecular Markers

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Abstract: A polymerase chain reaction (PCR) based approach, namely inter simple sequence repeat (ISSR) analysis was applied to 20 genotypes of onion (Allium cepa L.) in order to assess the degree of polymorphism within the genes and to investigate if this approach was suitable for genetic studies of onion. For this study, twenty onion genotypes were evaluated for variability using a set of 15 ISSR primers. The polymorphisms in PCR amplification products were subjected to the unweighed pair group method for arithmetic averages (UPGMA). The dendogram constructed from the similarity data grouped the genotypes into different clusters. Considering all the amplified primers a total of 87 scorable amplification products were obtained out of which 75 were polymorphic bands and 12 bands were monomorphic. In this study, it was found that maximum diversity was observed between the genotypes N-2-4-1 and BRBO 1005. Whereas, maximum similarity was observed between the genotypes BRBO 1026 and BRBO 1025.

Key words: ISSR, onion, genetic diversity, polymorphism.

INTRODUCTION

The knowledge of onion genetic diversity and resources is limited mainly due to a paucity of public markers and germplasm resources and their outbreeding, and biennial habit (McCallum and Havey 2006). A comparison of plant phenotypes is the simplest approach to the characterization of genotypes and the assessment of genetic diversity, however, phenotypic evaluation is influenced by environment and may not distinguish between closely related accessions (Rodriguez *et al.* 1999). Molecular markers have proved as valuable tools in the characterization and evaluation of genetic diversity within and between species and populations. It has

been shown that different markers might reveal different classes of variation (Russell *et al.* 1997). ISSR primers are highly polymorphic and are used in studies of genetic diversity (Reddy *et al.* 2002 and Gupta *et al.*2010).

MATERIALS AND METHODS

The study was conducted during Rabi 2012 at State Level Biotechnology Centre, MPKV, Rahuri.

Plant Material

A total of 20 onion genotypes were used for the study, which were collected from Scheme for Research on Onion Storage, MPKV, Rahuri.

Isolation of DNA

Total DNA was extracted from the leaves of Onion genotypes by CTAB (Cetyl Tri-methyl Ammonium Bromide) method as described by Doyle and Doyle (1990) with some modifications. The purified DNA was dissolved and stored in TE buffer.

ISSR Analysis

Amplification was carried out using 15 ISSR primers obtained from Operon Technologies, USA. Twenty ìl of the isolated DNA were used for PCR amplification. Amplification was performed in a 0.2 ml PCR tube having 20 ml PCR reaction mixture volume as described by Jin *et al.* (2013) with some modifications. The amplification cycles were carried out; the first cycle of denaturation of DNA carried at 95°C for 5 min; the second cycle at 94°C for 1 min, primer specific annealing temperature for 1 min, and extension synthesis at 72°C for 1 min, this second cycle was repeated for 35 times. The third cycle of extension was carried out at 72°C for 5 min. PCR products of ISSR primers were separated on 1.2% agarose gel. The DNA profiles were visualized on a UV transilluminator in Gel Documentation System (Flour chem. ™ Alpha innotech, USA). The clearly resolved amplicons of onion genotypes were scored manually for their presence (1) and absence (0) in the data sheet. Data were analyzed and similarity matrix was constructed from binary data with Dice similarity coefficients, which were calculated as per model suggested by Nei and Li (1979). Unweighted Pair Group Method using Arithmetic Averages (UPGMA) was employed for cluster analysis. The analysis was carried out using the software package NTSYSpc 2.02i (Rohlf, 1998).

Table 1
Details of the ISSR primers used for amplification of genomic DNA of onion.

Sr. No.	*Details*	*ISSR primer number*
1.	Number of polymorphic markers	15
2.	Total number of bands	87
3.	Total number of polymorphic bands	75
4.	Total number of monomorphic bands	12
5.	Percentage of polymorphic bands	86.20

RESULTS AND DISCUSSION

Among various techniques available for assessing the genetic diversity and relatedness among crop germplasm, molecular markers, which portray genome sequence composition and enabling detection of differences in the genetic constitution among different individuals are highly useful to assess the genetic variability for breeding programme. Unlike morphological markers, they are not influenced by environmental factors and growth practices. Thus, these techniques have been increasingly used to characterize and identify a novel germplasm for uses in the crop breeding process (O'Neill *et al.*, 2003). Twenty onion genotypes were assessed for molecular diversity using ISSR markers.

Considering all the amplified primers a total of 87 scorable amplification products were obtained out of which 75 were polymorphic bands and 12 bands were monomorphic. Maximum scorable bands were observed using the primer ISSR-857 (9 bands),

Table 2
Per cent polymorphism shown by different ISSR primers in onion.

Sr. No.	*Primer*	*Total no of bands generated*	*Total no of monomorphic bands*	*Total no of polymorphic bands*	*Per cent Polymorphism (%)*	*PIC*
1.	ISSR-807	4	0	4	100	0.72
2.	ISSR-809	5	0	5	100	0.74
3.	ISSR-810	5	0	5	100	0.77
4.	ISSR-812	5	2	3	60	0.61
5.	ISSR-813	6	1	5	83.33	0.82
6.	ISSR-815	5	2	3	60	0.79
7.	ISSR-821	7	2	5	71.42	0.83
8.	ISSR-823	7	1	6	85.71	0.83
9.	ISSR-826	6	0	6	100	0.80
10.	ISSR-827	6	1	5	83.33	0.82
11.	ISSR-834	5	1	4	80	0.68
12.	ISSR-841	4	0	4	100	0.65
13.	ISSR-854	7	0	7	100	0.78
14.	ISSR-856	6	0	6	100	0.80
15.	ISSR-857	9	2	7	77.77	0.87

followed by ISSR-821. The least number of bands were reported in the primer ISSR-807 and ISSR-841 (4 bands each). PIC values ranged from 0.61 to 0.87. Seven primers showed 100 per cent polymorphism. All the genotypes evaluated for the molecular diversity with 15 ISSR primers showed variation in the banding pattern. The maximum number of bands were observed in Sel 18 (67 bands), followed by ARBO 1003 and N-2-4-1 (66 bands each) and Sel 14 (63 bands). Whereas BRBO 1021 (47 bands) reported least number of bands followed by BRBO 1005 (51 bands). These results are in conformity with findings of Jin *et al.* (2013) where out of 132 bands generated 109 bands were polymorphic.

Similarity analysis of the ISSR markers revealed moderate to high diversity among the onion genotypes, with the similarity index value ranging from 0.467 to 0.883. Maximum diversity was observed between the genotypes N-2-4-1 and BRBO 1021 (0.467) followed by N-2-4-1 and BRBO 1005 (0.481). Maximum similarity was observed between the genotypes BRBO 1026 and BRBO 1025 (0.883) followed by ARBO 1003 and Sel 18 (0.873) (Table 3). Low to moderate diversity in 91 onion genotypes was reported by Mallor *et al.* (2013).

On Unweighted Pair Group Method Using Arithmetic Averages (UPGMA) based clustering analysis all the onion genotypes were distinguishable from each other in both the seasons. These genotypes were broadly grouped into five mega-clusters. Cluster I contained highest 11 genotypes sub-grouped into four subclusters, followed by cluster IV (6 genotypes) sub-grouped into three subclusters, whereas clusterII, III and V were monogenotypic. (Table 4). Mallor *et al.* (2013) grouped 91 onion genotypes into six clusters based on molecular diversity analysis. These results were in conformity with Jin *et al.* (2013) and Mir *et al.* (2013). DNA based markers serve as powerful tools for discriminating variation within

Table 3
Similarity coefficient values based on ISSR marker data of 20 onion genotypes

Genotype	*BRBO 1001*	*BRBO 1004*	*BRBO 1005*	*BRBO 1011*	*BRBO 1018*	*BRBO 1019*	*BRBO 1021*	*BRBO 1023*	*BRBO 1024*	*BRBO 1025*	*BRBO 1026*	*BRBO 1031*	*Sel 12*	*Sel 14*	*Sel 15*	*Sel 16*	*Sel 18*	*ARBO 1003*	*ARBO 1007*	*N-24-1*
BRBO 1001	1.000																			
BRBO 1004	0.820	1.000																		
BRBO 1005	0.800	0.810	1.000																	
BRBO 1011	0.766	0.692	0.672	1.000																
BRBO 1018	0.803	0.783	0.857	0.758	1.000															
BRBO 1019	0.742	0.721	0.729	0.672	0.793	1.000														
BRBO 1021	0.677	0.712	0.750	0.635	0.754	0.633	1.000													
BRBO 1023	0.634	0.592	0.618	0.575	0.600	0.528	0.514	1.000												
BRBO 1024	0.723	0.723	0.703	0.758	0.734	0.758	0.641	0.625	1.000											
BRBO 1025	0.797	0.750	0.703	0.727	0.734	0.703	0.694	0.625	0.871	1.000										
BRBO 1026	0.750	0.677	0.656	0.734	0.688	0.656	0.672	0.541	0.866	0.883	1.000									
BRBO 1031	0.762	0.688	0.810	0.692	0.814	0.641	0.712	0.662	0.750	0.807	0.730	1.000								
Sel 12	0.710	0.691	0.647	0.671	0.702	0.697	0.612	0.622	0.776	0.776	0.706	0.769	1.000							
Sel 14	0.622	0.581	0.541	0.566	0.568	0.583	0.487	0.627	0.681	0.704	0.662	0.648	0.747	1.000						
Sel 15	0.667	0.600	0.580	0.629	0.609	0.603	0.522	0.520	0.681	0.681	0.662	0.647	0.700	0.729	1.000					
Sel 16	0.739	0.692	0.698	0.778	0.758	0.754	0.689	0.575	0.839	0.754	0.682	0.692	0.721	0.608	0.606	1.000				
Sel 18	0.722	0.658	0.616	0.640	0.667	0.616	0.541	0.680	0.736	0.712	0.627	0.704	0.803	0.733	0.712	0.640	1.000			
ARBO 1003	0.708	0.644	0.625	0.649	0.653	0.648	0.548	0.689	0.722	0.676	0.613	0.667	0.814	0.697	0.747	0.671	0.873	1.000		
ARBO 1007	0.657	0.592	0.594	0.643	0.623	0.594	0.536	0.595	0.671	0.671	0.652	0.638	0.690	0.694	0.721	0.667	0.658	0.736	1.000	
N-2-4-1	0.557	0.519	0.481	0.564	0.506	0.520	0.468	0.582	0.590	0.610	0.592	0.584	0.649	0.767	0.747	0.544	0.705	0.737	0.689	1.000

Table 4
Distribution of 20 onion genotypes into different clusters by ISSR markers.

Cluster Number	*Sub-Cluster*	*No of genotypes*	*Genotype*
I	Ia	5	BRBO 1001, BRBO 1004, BRBO 1005, BRBO 1018, BRBO 1031
	Ib	3	BRBO 1024, BRBO 1025, BRBO 1026
	Ic	2	BRBO 1011,Sel 16
	Id	1	BRBO 1019
II		1	BRBO 1021
III		1	BRBO 1023
IV	IVa	2	Sel 14, N-2-4-1
	IVb	3	Sel 12, Sel 18, ARBO 1003
	IVc	1	Sel 15
V		1	ARBO 1007
Total		20	

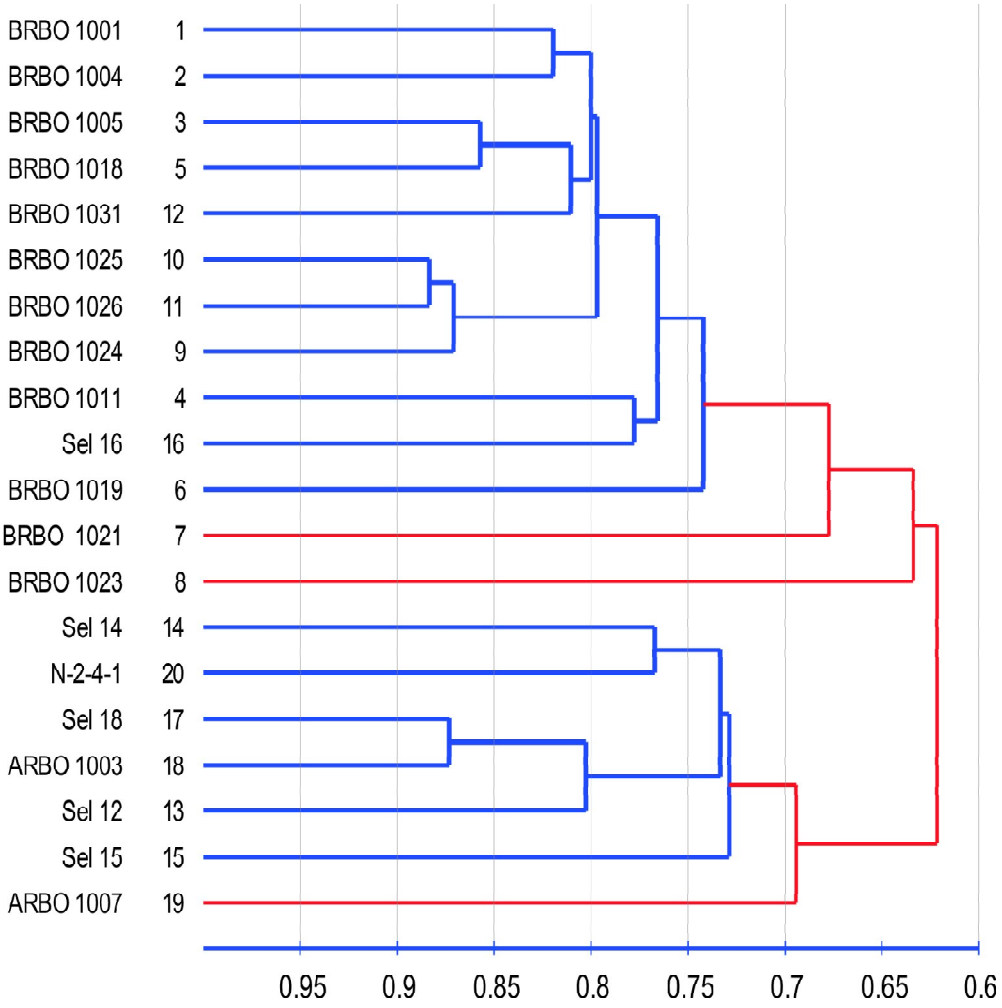
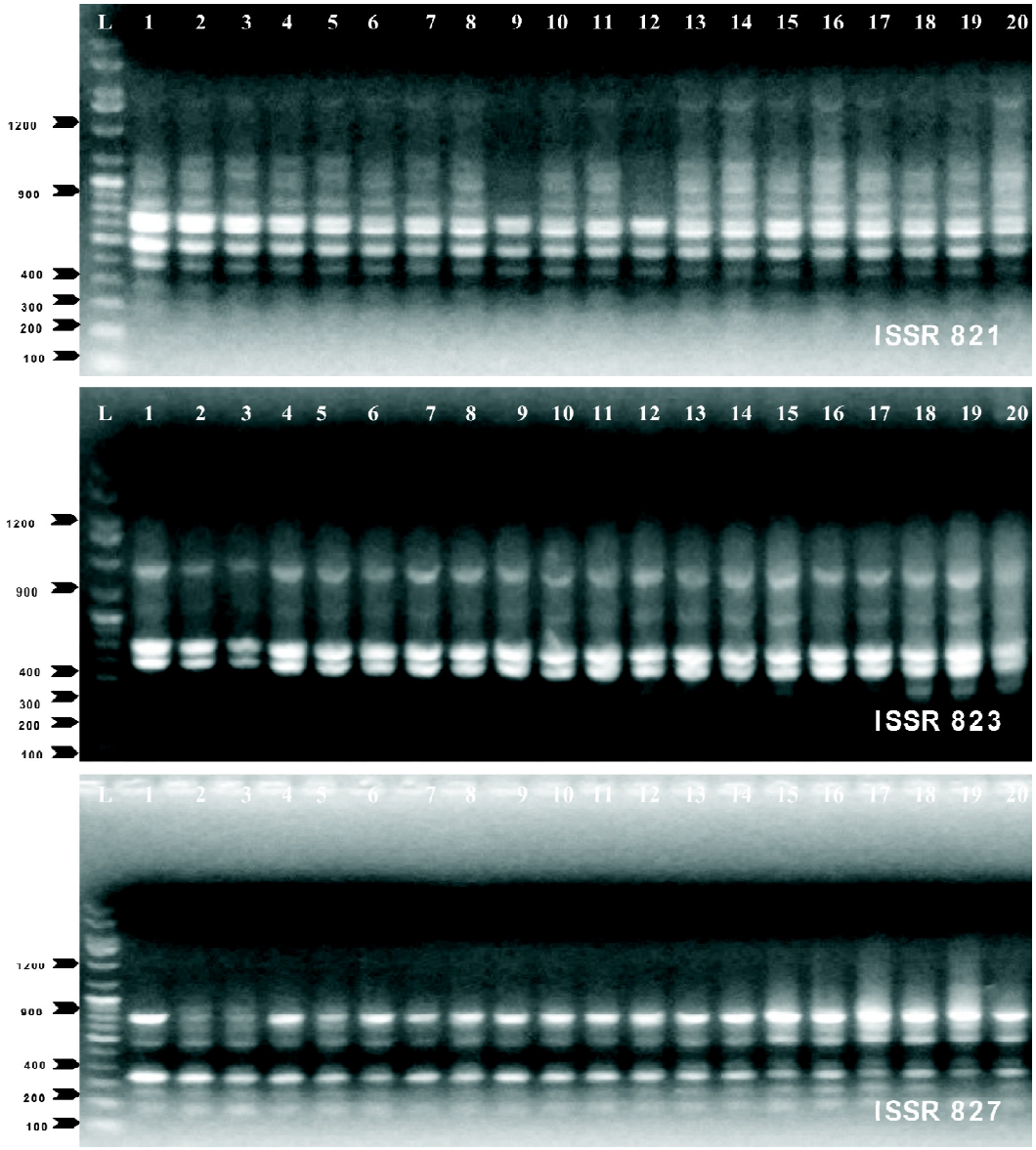


Figure 1 : UPGMA based dendrogram of 20 onion genotypes using ISSR markers



L	DNA Ladder	6	BRBO 1019	12	BRBO 1031	18	ARBO 1003
1	BRBO 1001	7	BRBO 1021	13	Sel 12	19	ARBO 1007
2	BRBO 1004	8	BRBO 1023	14	Sel 14	20	N-2-4-1
3	BRBO 1005	9	BRBO 1024	15	Sel 15		
4	BRBO 1011	10	BRBO 1025	16	Sel 16		
5	BRBO 1018	11	BRBO 1026	17	Sel 18		

Figure 2. Polymorphism pattern obtained using ISSR markers

crop germplasm and for studying evolutionary relationships (Gepts, 1993). The diversity at molecular level can be used for selecting parents for attempting better hybrid combinations.

CONCLUSION

These results demonstrate the potential value of ISSR markers for the assessment of genetic diversity. Considering the results obtained in the present study,

the genetic diversity of the onion genotypes used in this study was moderate to high. The results in this study demonstrate that most of the studied genotypes should be considered different, and maintained separately in the germplasm collection. The molecular analysis performed in this study provides valuable information to researchers and plant breeders for future studies.

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