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Genetic diversity of pran (*Allium cepa* var. *prolliferum*) in Kashmir

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Abstract

Ten pran genotypes were evaluated using RAPD markers to determine the genetic relationships among genotypes showing morphological variations. Fourty three decamer primers were used in this study. Genetic diversity in the genotypes studied was analyzed using several variability parameters. Genotypes were grouped into two main clusters based on jaccard similarity coefficient. A dendrogram based on UPGMA analysis grouped the ten genotypes into two main clusters. Genotypes representing cluster-I are superior over genotypes in cluster-II with respect to yield attributes. Some genotypes did not show any similarity with either cluster, depicting their diverse nature. The grouping that was obtained with the RAPD analysis was at par with the morphological grouping based on yield attributes. The results of Principal Co-ordinate (PCO) analysis were comparable to the cluster analysis. Finally, the investigation of the genetic variation of pran with RAPD indicated that this marker is a suitable tool for assessing polymorphism and estimating the genetic similarity.

Keywords: bunching onion, molecular markers, pran, RAPD

Introduction

The genus *Allium* L. (*Alliaceae*) exhibits great diversity in various morphological characters, particularly in life form (bulb) and ecological habitat. It is of major economic importance as a vegetable and herbal crop and ornamental plant (Ricroch *et al.* 2005). This genus consists mostly of perennial and bulbous plant and it is widely distributed over Holarctic regions from the dry subtropics to the boreal zone (Stearn 1992). This species was used in traditional pharmacopoeia for its expectorant properties (Marcucci & Tornadore 1997). Contrary to the cultivated garlic (*Allium sativum* L.) which is very well studied in terms of its morphological, agronomic and molecular aspects (Baghalian *et al.* 2006; Singh *et al.* 2011), studies about rosy garlic (*A. roseum* L.) are few and limited to morphophenology (Jendoubi *et al.* 2001) and cytocaryology (Marcucci & Tornadore 1997).

In the genetic improvement process, it is desirable to use molecular markers for evaluating genetic diversity of accessions, choosing of parents and selection of progeny. Diversity analyses of different garlic accessions have been carried out using RAPD, AFLP and Microsatellite markers for detection of putative duplicates in germplasm collections (Ipek *et al.* 2003) or grouping with respect to photoperiodism (Maass 1997; Singh *et al.* 2011).

Pran, also known as bunching or Egyptian onion, is one of the high value spice of Kashmir used for culinary seasoning and to flavor foods. The range of foods that have been spiced with pran is wide, including cheese, chicken and meat and different Kashmiri cuisine favours the use of pran. While morphological differences between pran cultivars can be observed, only a few morphological traits are used for grouping pran, and these can be affected by environmental conditions. Even with the introduction of new molecular marker systems during the last years, reports of these markers in pran have been scarce (Tsukazaki *et al.* 2008). But further molecular characterization of pran is necessary to associate the desirable traits with the molecular markers which can be further used in pran improvement programmes. In the present study, diverse pran accessions have been evaluated with respect to yield parameters and characterized at molecular level for identifying the most divergent accessions which can further be used for crossing and development of superior cultivars.

Material and methods

The present experiment was carried out during 2010–11 in which ten pran selections (CITH-Pran-1, CITH-Pran-2, CITH-Pran-3, CITH-Pran4, CITH-Pran-5, CITH-Pran-6, CITH-Pran-7, CITH-Pran-8, CITH-Pran-9, CITH-Pran-10) maintained at the Central Institute of Temperate Horticulture, Srinagar (J&K) under open field conditions were used. These genotypes were used with respect to different traits like plant height, bulb length, bulb thickness, neck thickness, average bulb weight, number of bulbs/plant and plant yield. Genomic DNA was extracted from freshly emerged leaves of pran genotypes using the CTAB method (Murray & Thompson 1980). Approximately 0.5 g tissue samples from each plant species were snap-frozen in liquid nitrogen. DNA was purified and quantified spectrophotometrically using uncut lambda DNA fragment as control. Final concentration of 20 to 40 ng μL^{-1} was used for PCR. Samples were screened for RAPD variation using standard 10-base primers supplied by Operon. DNA from an individual plant of each pran accession was screened with 45 RAPD primers. The PCR reaction (25 µL) contained the following: 1x reaction buffer (20 mM Tris-Cl pH 8.4, 50 mM KCl), 0.2 mM dNTPs, 2 mM $MgCl_2$, 10 pM primer, 1.0 Unit of Taq DNA polymerase and 25 to 50 ng genomic DNA. The DNA was amplified in a thermal cycler (Takara Thermal Cycler) that was programmed as follows: initial DNA denaturation for 5 min at 94°C; 45 cycles of 60 sec at 94°C (denaturation), 60 sec at 37°C (annealing) and 120 sec at 72°C (extension) and a final extension for 7 min at 72°C. All primers tested using all cultivars and markers were checked three times for reproducibility. The RAPD amplified-DNA was analyzed by electrophoresis on 2% agarose gel in a 0.5 TBE buffer. The gels were stained with ethidium bromide (0.5 µg L^{-1}) and visualized under UV light.

In order to determine the utility of these markers, polymorphic information content (PIC), effective multiplex ratio (EMR)/resolving power (Rp) and marker index (MI) were computed. PIC for genetic markers was calculated from the sum of the squares of allele frequencies: $Din=1-\sum pi_2$ (where pi is the allele frequency of the ith allele). The arithmetic mean heterozygosity was calculated for each marker class: $Diav=\sum Din/n$, (where n is the number of markers (loci) analyzed). The DI for polymorphic markers is: (Diav) $p=\sum Din/np$ (where np is the number of polymorphic loci and n is the total number of loci). EMR (E) is the product of the fraction of polymorphic loci and the number of polymorphic loci for an individual assay; EMR (E)=np (np/n). MI is defined as the product of the average diversity index for polymorphic bands in any assay and the EMR for that assay, MI=Diavp * E. Prominent DNA bands that were amplified by a given primer were scored as present (1) or absent (0). The PIC values of individual primers were calculated based on the formula PIC=2 × F (1–F) (Anderson *et al.* 1993). The Jaccard's similarity index was calculated using NTSYS-pc version 2.02e (Applied Biostatistics, Inc., Setauket, NY, USA) package to compute pair-wise Jaccard's similarity coefficients (Jaccard 1908) and this similarity matrix was used in

cluster analysis using an unweighted pair-group method with arithmetic averages (UPGMA) and sequential, agglomerative, hierarchical and nested (SAHN) clustering algorithm to obtain a dendrogram. To judge the confidence of the group revealed in the dendogram, bootstrap analysis was performed using the WINBOOT program (Yap & Nelson 1996) with 1000 replications.

Results and discussion

Evaluation of pran genotypes

The performance of ten pran/bunching onion genotypes during the year 2010–2011 with respect to traits like plant height, bulb length, bulb thickness, neck thickness, average bulb weight, number of bulbs plant^{-1} and yield are given in Table 1. Average bulb weight varied from 22.97 g in CITH-Pran-1 to 49.93 g in CITH-Pran-10. Yield plant^{-1} varied from 214 g plant^{-1} in CITH-Pran-6 to 404 g plant^{-1} in CITH-Pran-2. Number of bulbs was maximum (15 plant $^{-1}$) in CITH-pran-2 and minimum (5 plant^{-1}) in CITH-Pran-10.

RAPD analysis and genetic diversity

All the chosen primers amplified with the number of amplified fragments varying in size from 200–1200 bp. Out of 64 amplified bands, 46 were found polymorphic, with an average number of bands/polymorphic bands per primer as 1.42. The respective values for overall genetic variability for PIC, Rp and MI across all the 10 genotypes are given in Table 2. PIC is a feature of a primer and therefore the PIC values were calculated for all primers. Highest PIC value (0.24) was observed for the primer OPP-10 and lowest PIC value (0.05) was recorded for the primer OPJ-5 (Table 1). Average PIC value was 0.18. The RP is a feature of the primer that indicates the discriminatory potential of the primer. RP ranged from 0.05 to 0.93 with an average of 0.29 per primer. MI is a feature of a marker and therefore the MI values were calculated for all markers. MI values ranged from 0.23 (OPJ-5) to 1.07 (OPP-10, OPJ-11, OPP-4, OPB-20, OPA-15, OPB-2, OPB-18, OPV-15 and OPP-5) with an average value of 0.83 across all the primers. Similar results have been obtained earlier in *Allium sativum* using RAPD markers (Abdoli *et al.* 2009; Singh *et al.* 2011). Jaccard's coefficient showed that the highest similarity was observed between cv. CITH-Pran-10 and CITH-Pran-9 (0.90), implying that these are genetically closer than the other cultivars. Lowest similarity was

Table 1. Evaluation of pran genotypes

Variety	Plant height (cm)	Bulb length (cm)	Bulb dia. (cm)	Neck thickness (cm)	Avg. bulb wt. (g)	No. of bulbs plant^{-1}	Plant yield (g plant^{-1})	Total yield (q ha^{-1})
CITH-Pran-1	46.50	8.5	1.88	0.76	22.97	14	321.60	536.00
CITH-Pran-2	53.00	8.9	1.72	0.72	26.96	15	404.40	674.00
CITH-Pran-3	63.00	9.6	2.42	0.70	24.02	13	312.30	520.50
CITH-Pran-4	58.50	9.9	2.13	0.88	28.15	11	309.60	516.00
CITH-Pran-5	58.50	11.5	2.49	1.26	49.03	7	343.20	572.00
CITH-Pran-6	57.00	10.2	2.05	1.03	35.70	6	214.20	357.00
CITH-Pran-7	54.50	8.8	2.20	0.86	34.07	10	340.66	567.77
CITH-Pran-8	56.70	8.7	2.70	0.87	40.04	8	320.33	533.88
CITH-Pran-9	60.10	8.0	2.80	0.99	39.11	9	352.00	586.66
CITH-Pran10	52.8	10.4	2.80	1.30	49.93	5	249.66	416.10
CD @ 5%	3.626	1.388	N.S.	0.209	3.076	2.505	5.765	6.393
SE(d)	1.713	0.655	3.244	0.099	1.453	1.183	2.723	3.020
SE(m)	1.211	0.463	2.294	0.070	1.027	0.837	1.925	2.135
CV(%)	3.746	8.465	131.772	12.876	5.143	14.787	1.054	0.727

Table 2. Polymorphic profile of 43 RAPD primers across 10 genotypes of pran

Primer	PIC	Rp	MI	Primer	PIC	Rp	MI
OPJ-1	0.17	0.59	0.86	OPP-5	0.21	0.73	1.07
OPJ-4	0.16	0.17	0.78	OPV-3	0.21	0.49	0.64
OPJ-5	0.05	005	0.23	OPV-6	0.18	0.61	0.91
OPJ-7	0.17	0.39	0.52	OPV-15	0.21	0.24	1.07
OPJ-9	0.20	0.22	0.97	OPV-14	0.20	0.22	0.97
OPJ-3	0.20	0.93	0.81	OPV-10	0.18	0.20	0.88
OPZ-J-6	0.21	0.71	1.04	OPZ-3	0.19	0.41	0.55
OPJ-9	0.07	0.07	0.352	OPZ-4	0.16	0.17	0.78
OPJ-11	0.21	0.24	1.07	OPA-15	0.21	0.24	1.07
OPP-15	0.20	0.22	0.97	OPA-16	0.18	0.20	0.88
OPP-16	0.18	0.20	0.88	OPA-17	0.20	0.44	0.58
OPP-6	0.21	0.24	1.07	OPA-19	0.18	0.20	0.88
OPP-13	0.18	0.20	0.88	OPA-8	0.20	0.22	0.97
OPP-1	0.20	0.22	0.97	OPB-13	0.14	0.15	0.67
OPP-2	0.16	0.17	0.78	OPB-1	0.18	0.20	0.88
OPP-3	0.20	0.22	0.97	OPB-2	0.21	0.21	1.07
OPP-4	0.21	0.24	1.07	OPB-5	0.14	0.29	0.40
OPP-7	0.16	0.17	0.78	OPB-6	0.20	0.22	0.97
OPP-9	0.18	0.20	0.88	OPB-10	0.09	0.10	0.46
OPP-10	0.24	0.24	1.07	OPB-11	0.11	0.12	0.57
OPB-19	0.20	0.22	0.97	OPB-18	0.21	0.24	1.07
OPB-20	0.21	0.73	1.07	OPO-19	0.16	0.17	0.78
OPO-15	0.21	0.49	0.64				

observed between cv CITH-Pran-7 and CITH-Pran-3 with Jaccard's similarity coefficient of 0.56. The average genetic similarity of 0.75 among the cultivars clearly showed that significant genetic diversity exists among the pran cultivars. The low level of genetic diversity present among these cultivars clearly suggested that they must have originated from genetically less divergent parents or have a short history of adaptation to their respective microclimatic regions. Earlier studies using RAPD (Maniruzzaman *et al.* 2010) techniques also showed lack of genetic variations among different onion cultivars. But similar studies using RAPD (Abdoli *et al.* 2009; Singh *et al.* 2011) and AFLP (Ipek *et al.* 2003) techniques in garlic showed large genetic variation among cultivars from different countries. A dendrogram based on UPGMA analysis grouped the 10 Pran genotypes into two main clusters (I to II; Fig 1). Cluster I was represented by CITH-Pran-1, CITH-Pran-2, CITH-Pran-4 and CITH-Pran-5 with an average similarity co-efficient of 0.84. Cluster II was represented by CITH-Pran-8, CITH-Pran-9 and CITH-Pran-10 with an average similarity co-efficient of 0.86. CITH-Pran-3, CITH-Pran-6 and CITH-Pran-7 form separate sub-clusters. Genotypes representing cluster-I are superior over genotypes in cluster-II with respect to yield attributes (Table 1). Average number of bulbs of cluster-I genotypes was higher (12 plant^{-1}) than in cluster-II genotypes (7 plant^{-1}). Also average yield was 344 g plant^{-1} in cluster-I genotypes which is much higher than average yield per plant of 307 g plant^{-1} in cluster-II genotypes. Average similarity between cluster-I and cluster-II was 0.82, which is very similar

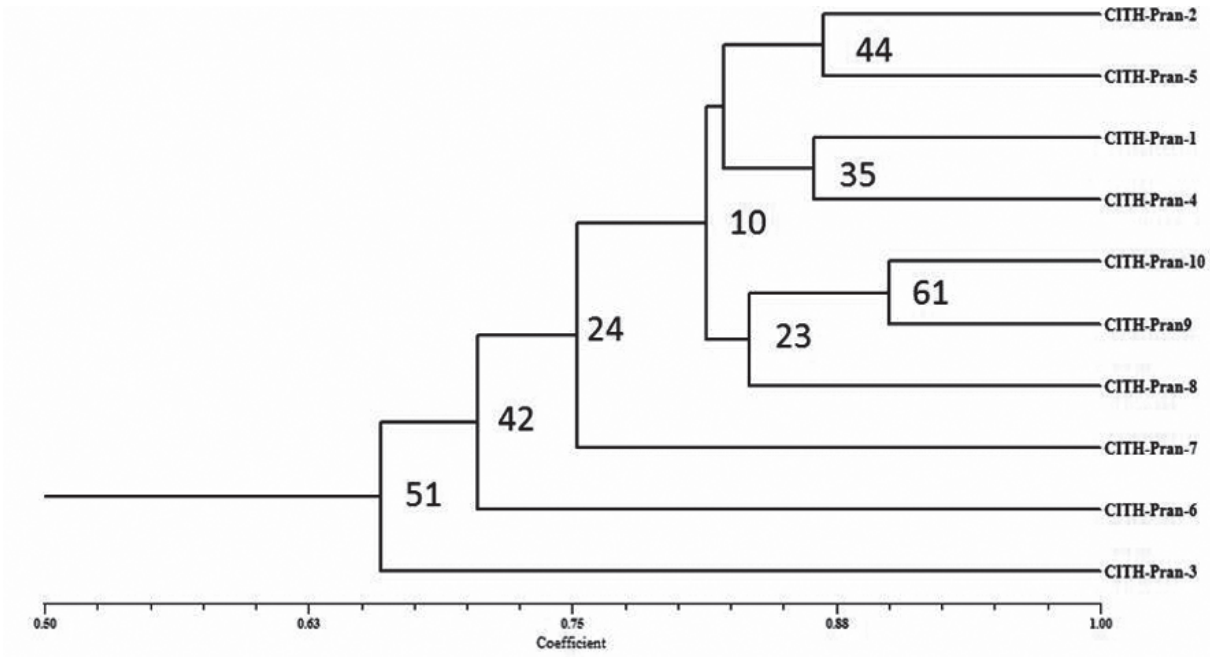

Fig 1. UPGMA dendrogram showing clustering pattern of pran genotypes. The bootstrap values are given on the nodes

to average similarity within cluster-I (0.84) and within cluster-II (0.86). Although there is little divergence between cluster-I and cluster-II, genotypes CITH-Pran-3, CITH-Pran-6 and CITH-Pran-7 showed least similarities with rest of the genotypes, average similarity coefficients between CITH-Pran-3, CITH-Pran-6 and CITH-Pran-7 with rest of the genotypes were 0.66, 0.68 and 0.68, respectively.

The genetic relationship between the accessions was clearly depicted in the dendrogram which was constructed from the DNA profile and the confidence of the cluster was further confirmed by bootstrap analysis. The grouping that was obtained with the RAPD analysis was at par with the morphological grouping based on yield attributes. The results of PCO analysis were comparable to the cluster analysis (Fig 2). Overall 89% of the variability observed was explained by the first three coordinates, PCO1, PCO2 and PCO3 accounted for 78.63%, 5.82% and 3.99% of total variability, respectively. Molecular markers have been applied in classification, identification and mapping in bunching onion (Tsukazaki *et al.* 2008). The present study clearly identified the genotypes having better fruit quality. Improvement of these genotypes and inclusion of these genotypes in breeding programs for further quality enhancement can be done with the aid of molecular markers. First step in breeding is the identification of closely related genotypes

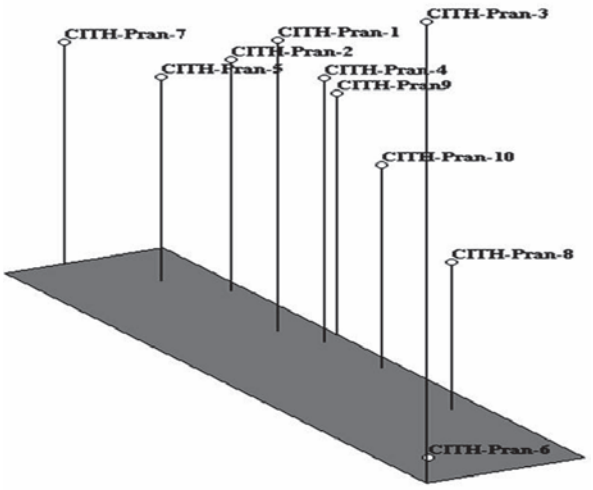

Fig 2. Three dimensional PCO (principal co-ordinate analysis) scaling of 10 pran genotypes using 43 RAPD markers

and the study of genetic relationships. To assess origin and genetic relationships among pran cultivars by RAPD markers, a wider survey including additional genotypes belonging to all established eco-geographical groups and additional markers would be more desirable. Results from this study showed that RAPD markers are a useful tool for pran diversity studies, which provided the basic knowledge for improvement of pran quality through further breeding programmes involving diverse genotypes.

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