Short communication

RAPD profile based grouping of garlic *Allium sativum* germplasm with respect to photoperiodism

S.R. Singh, J.I. Mir*, N. Ahmed, Rizwan Rashid, Shabir. H. Wani, M.A. Sheikh, Hina Mohi ud din, Wajida Shafi, Nusrat Jan, and H. Mir

Central Institute of Temperate Horticulture, Rangreth, Srinagar190007, J & K, India.

Received 27 February 2011; received in revised form 2 June 2011; accepted 2 June 2011.

Abstract

Forty garlic genotypes were evaluated using RAPD markers to determine the genetic relationships among genotypes showing morphological variations. Eighteen decamer primers were used, all of which were polymorphic in the set of cultivars studied and allowed every genotype to be unambiguously distinguished. Genetic diversity in the population studied was analyzed using several variability parameters. A dendrogram based on UPGMA analysis grouped the 40 genotypes into three main clusters with Jaccard's similarity coefficient ranging from 0.00 to 0.75 with an average of 0.25. Cluster I represented short day garlic genotypes, cluster II day-neutral garlic selections, and cluster III long day garlic selections. Principal Coordinate (PCO) analysis was comparable to the results of cluster analysis. RAPD marker based grouping of garlic genotypes with respect to photoperiod requirement will be useful in garlic improvement.

Keywords: Molecular markers, Genotypes, Genetic diversity.

Clonal selection is a major breeding method for garlic (*Allium sativum* L.; Family Alliaceae), since plant sterility usually precludes crop improvement through cross hybridization. Nevertheless, garlic shows wide morphological and agronomic variations in colour and size of bulb, plant height, flowering, number and size of the cloves, days to harvesting, resistance to storage capacity, dormancy and adaptation to agroclimatic situations (Mario et al., 2008). Environmental stimuli and day length also influence bulb initiation in garlic. Generally day lengths of 9 to 12 h induce bulbing but it varies from genotype to genotype. Garlic varieties are classified according to the photoperiod requirement for bulbing into short day (bulb under 9 to 12 h day length regimes), long day (14 to 16 h day length) and day-neutral (having no day length specification) varieties. Diversity analyses of different garlic accessions have been carried out using RAPD and AFLP markers for detection of putative duplicates in germplasm collections (Ipek et al., 2003). However, no previous work has been reported regarding genetic diversity with respect to photoperiod requirement in garlic. With a goal to group garlic accessions based on photoperiod requirement, we evaluated different garlic genotypes using RAPD profiling.

The present experiment was carried out during 2010–2011 in which a total of 40 garlic selections maintained at the Central Institute of Temperate Horticulture, Srinagar (J&K) under open field conditions were used. Out of 40 accessions, 15 were short day (Bgld-RB0901, Bgld-RB0905, Bgld-RB0906, Bgld-RB0907, Bgld-RB0908, Bgld-RB0909, Cgld-RB0901, Cgld-RB0902, Cgld-RB0903, Cgld-RB0906, Agld-0902, Agld-0905, Local and G8), 18 were long day (Bgld-RB0902, Cgld-RB0904, Agld-0901, Agld-0903, CITH-G3, CITH-G4, CITH-G5, CITH-G6, Mukteshwar-S1, Mukteshwar-S2, Single-Clone, Garlic-6, GS-1, G-9, G4, G1, G3, G7 and

*Author for correspondence: Tel 0194-2305044, Fax 0194-2305045; Email <jimir@icar.org.in>.

G5), and seven were day-neutral (Bgld-RB0904, Cgld-RB0905, Cgld-RB0908, Agld-0906, CITH-G1, CITH-G2 and CITH-G7). Genomic DNA was extracted from freshly emerged leaves of garlic genotypes using the CTAB method (Murray and Thompson, 1980). Approximately 0.5 g tissue samples from each plant species were snap-frozen in liquid nitrogen. DNA was purified and quantified spectrophotometrically and using uncut λ DNA fragment. Final concentration of 20 to 40 ngμL^{-1} was used for PCR. Samples were screened for RAPD variation using standard 10-base primers supplied by Operon. The PCR reaction (25 μl) contained the following: 1*x* 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 (denatu-ration), 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*x* 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 i^{th} 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 $PIC = 2 \times F (1– F)$ (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 and Nelson, 1996) with 1000 replications.

All the 33 amplified bands were polymorphic, with an average of 1.83 bands per primer. Highest PIC value (0.44) was observed for the primer OPA-2 and OPA-20 and lowest PIC value (0.02) was recorded for the primer OPA-12 (Table 1). Average PIC value was 0.18. RP ranged from 0.02 to 0.68 with an average of 0.24 per primer. Similar results have been obtained earlier in garlic using RAPD markers (Abdoli et al., 2009). Jaccard's coefficient showed that the highest similarity

Table 1. RAPD profile of 40 garlic genotypes used for genetic diversity analysis.

Primer	PIC	Rp	MI
OPA1	0.321237	0.414634	0.321237
OPA2	0.449732	0.682927	0.449732
OPA4	0.20122	0.231707	0.20122
OPA3	0.156454	0.178862	0.156454
OPA5	0.323171	0.47561	0.323171
OPA9	0.244646	0.353659	0.244646
OPA7	0.103064	0.109756	0.103064
OPA8	0.069304	0.073171	0.069304
OPA11	0.342653	0.439024	0.342653
OPA12	0.024093	0.02439	0.024093
OPA14	0.135634	0.146341	0.135634
OPA17	0.055027	0.056911	0.055027
OPA18	0.232005	0.268293	0.232005
OPA19	0.086333	0.091463	0.086333
OPA20	0.441701	0.658537	0.441701
OPV2	0.092802	0.097561	0.092802
OPV3	0.047591	0.04878	0.047591
OPV7	0.047293	0.04878	0.047293

was observed between cv. Bgld-RB0907 and Bgld-RB0901 (0.75), implying that these are genetically closer than the other cultivars. The average genetic similarity of 0.25 among the cultivars clearly showed that significant genetic diversity exists among the garlic cultivars. The high genetic diversity present among these cultivars clearly suggests that they must have originated from genetically divergent parents or have a long history of adaptation to their respective micro-climatic regions. Earlier studies using RAPD (Abdoli et al., 2009) and AFLP (Ipek et al., 2003) techniques showed large genetic variations present among different garlic cultivars from different countries. A dendrogram based on UPGMA analysis grouped the 40 genotypes into three main clusters (I to III; Fig 1). Cluster I represented short day garlic genotypes with an average similarity co-efficient of 0.46. Cluster II represented all day-neutral garlic selections with average similarity coefficient of 0.48 and cluster III represented all long day garlic selections with average similarity coefficient of 0.14. Garlic selection 'Agld-0901' came in between clusters I and II, which represents that this selection may be developed after hybridization between day-neutral and short day garlic genotypes. The average similarity between the short day and long day accessions was 24% whereas within the short day it was 46% and within long day it was 14%. The average similarity between the short day and day-neutral accessions was

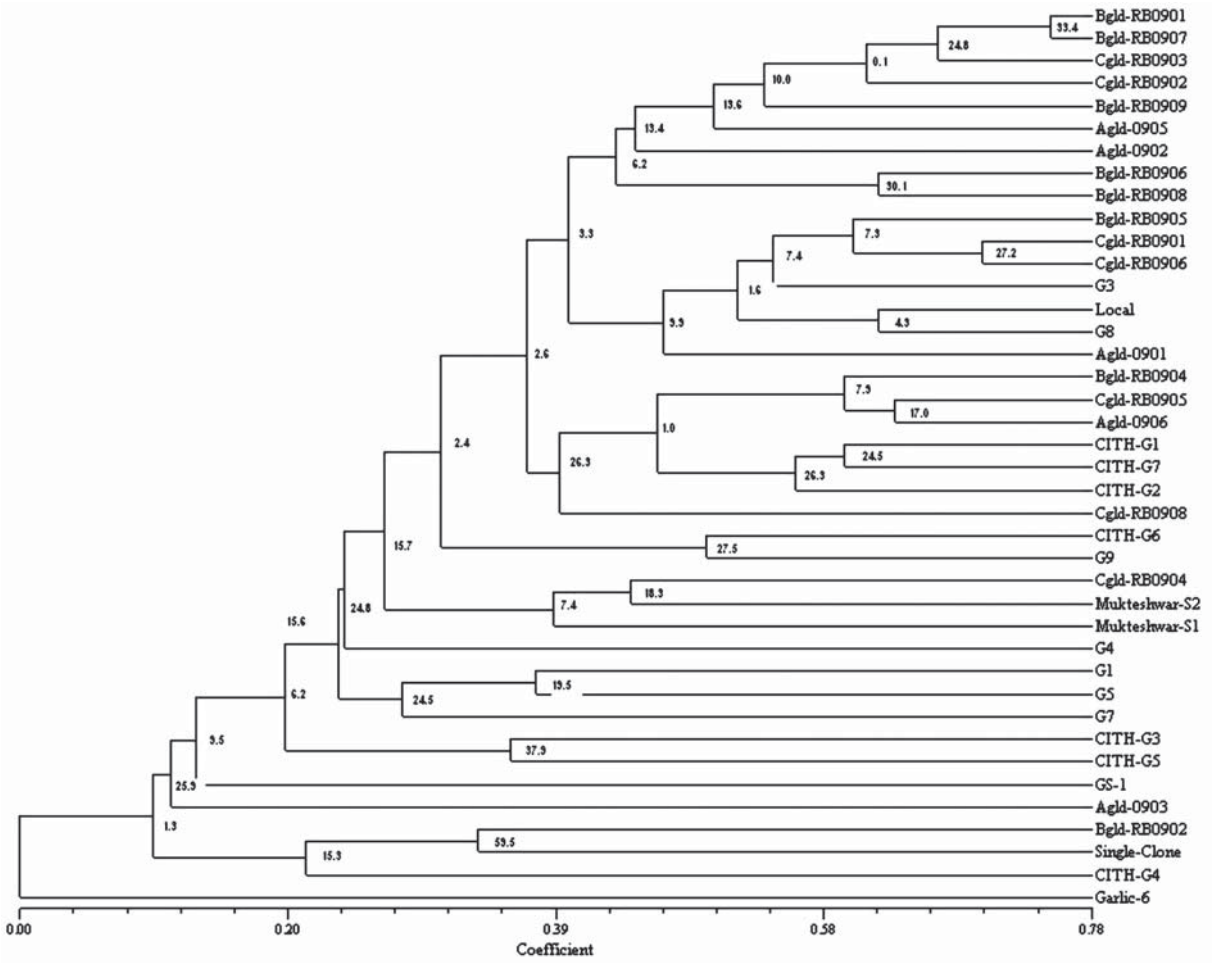

Figure 1. UPGMA dendrogram showing clustering pattern of Garlic, *Allium sativum* L. genotypes. The bootstrap values are given on the nodes.

42% whereas within the day-neutral it was 48%. The average similarity between the long day and day-neutral accessions was 19%. 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 day length.

On a final note, the present study evaluated the day length pattern of the selected accessions of garlic and correlated the results with molecular profile data. This study is the first effort to group the garlic accessions according to photoperiodism and will possibly pave the way for mapping population development followed by marker assisted breeding in garlic.

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