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SHORT COMMUNICATION

DNA Profiling of Pomegranate (*Punica granatum* L.) Field Genebank Semi-feral Collection by Using ISSR Markers

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Molecular genetic diversity estimation among 68 semi-feral accessions of pomegranate (*Punica granatum* L.) that were collected from Uttarakhand and Himachal Pradesh was carried out using 43 ISSR markers. All the markers were polymorphic with mean pairwise similarity of 39%. The screening showed that all the 68 accessions were distinct genotypes and the markers grouped them into two major groups and 11 clusters.

Key Words: DNA profile, ISSR, JMP, Pomegranate

Pomegranate (*Punica granatum* L.), a woody perennial shrub or small tree, belongs to the Punicaceae family and is one of the oldest known edible fruits (Damania, 2005). India is the largest producer of pomegranates in the world. Moreover, India produces finest edible quality of pomegranates which are available almost throughout the year. India's target for the year 2025 is to increase pomegranate production by 10 folds and export by 6.97 folds (Chandra *et al.,* 2010). However, pomegranate suffers from bacterial blight, which may cause up to 60-80% yield losses (Ramesh *et al.,* 1991). Searching for novel genetic backgrounds that are resistant to diseases needs broadening of the genetic base of pomegranate germplasm requiring collecting, characterization and genetic diversity analysis of germplasm imperative. Genetic diversity analysis of Indian pomegranate field genebank collections comprising of varieties and germplasm accessions employing morphometric markers and microsatellite based DNA markers has been reported (Archak *et al.,* 2014). The present study was undertaken to obtain a qualitative insight into the extent of genetic diversity present among the semi-feral pomegranate collections from Uttarakhand and Himachal Pradesh using ISSR markers.

Sixty-eight pomegranate accessions (Table 1) mainly belonging to Uttarakhand (35 accessions) and Himachal Pradesh (14 accessions) conserved in the field genebank at ICAR-National Research Centre on Pomegranate, Solapur, Maharashtra (17° 68' N, 75° 91' E, 457m above msl) were used for the study. Young and healthy leaves were excised from a late *hasta-bahar* crop (October-November flowering) from well-established five year old trees and were fixed in liquid nitrogen and stored at –80 °C. DNA extraction, purification and ISSR analyses were carried out as explained by Archak *et al.* (2014). Five ISSR primers — Pome_$(TA)_5(GT)_5$, Pome_DAMD_M13, Pome_$(AT)_5(GT)_5$, Pome_DAMD_HBV, and Pome_$(GACA)_4$ with annealing temperatures (in °C) of 50, 52, 50, 44 and 50 respectively were employed to generate DNA profiles. The DNA profiles were manually scored from gel photographs for the presence (1) or absence (0) of amplicons. Binary data were prepared as rectangular data matrix and pairwise distance (D_{KL}) was computed using Ward's method as:

$$D_{KL} = \frac{\|\bar{X}_K - \bar{X}_L\|^2}{\frac{1}{N_K}+\frac{1}{N_L}} D_{KL} = \frac{\|\bar{X}_K - \bar{X}_L\|^2}{\frac{1}{N_K}+\frac{1}{N_L}}$$

where D_{KL} is the distance or dissimilarity measure between clusters C_K and C_L where C_K is K^{th} cluster, subset of {1,2,3…,n} having N_K number of observations. ‖x‖ is the square root of the sum of the squares of the elements of x (the Euclidean length of the vector x) and $d(x_i, x_j)$ is ‖x‖. Statistical analyses were carried out using JMP Genomics version 5.1 (SAS, 2012).

Five ISSR primers — Pome_$(TA)_5(GT)_5$, Pome_DAMD_M13, Pome_$(AT)_5(GT)_5$, Pome_DAMD_HBV,

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Table 1. List of pomegranate field genebank accessions analysed in the study

S.No.	Genebank ID	Source (District)	S.No.	Genebank ID	Source (District)
1	IC318702	Mandi	35	IC556886	Tehri Garhwal
2	IC318706	Mandi	36	IC556887	Tehri Garhwal
3	IC318707	Mandi	37	IC556888	Tehri Garhwal
4	IC318716	Mandi	38	IC556889	Tehri Garhwal
5	IC318724	Mandi	39	IC556890	Tehri Garhwal
6	IC318733	Mandi	40	IC556891	Tehri Garhwal
7	IC318734	Mandi	41	IC556892	Tehri Garhwal
8	IC318735	Mandi	42	IC556893	Uttarkashi
9	IC318740	Mandi	43	IC556894	Uttarkashi
10	IC318743	Mandi	44	IC556895	Uttarkashi
11	IC318744	Shimla	45	IC556897	Dehradun
12	IC318749	Shimla	46	IC556898	Dehradun
13	IC318766	Solan	47	IC556899	Dehradun
14	IC318793	Solan	48	IC556900	Dehradun
15	IC444198	Narendranagar	49	IC556901	Dehradun
16	IC444200	New Tehri	50	R-18(H2)P-934	
17	IC524026	Nainital	51	R-21(H2)P-1121	
18	IC524030	Almora	52	R-9(H1)P-528	
19	IC524031	Chamoli	53	RC/PB/1181	
20	IC548192	Nainital	54	RC/PB/1261	
21	IC548193	Almora	55	RC/PB/1287	
22	IC548194	Almora	56	RC/PB/1288	
23	IC548195	Bageshwar	57	RC/PB/1290	
24	IC548196	Chamoli	58	RC/PB/1291	
25	IC548197	Chamoli	59	RC/PB/1292	
26	IC548198	Chamoli	60	RC/PB/1294	
27	IC548199	Chamoli	61	RC/PB/1295	
28	IC548200	Chamoli	62	RC/PB/1296	
29	IC548202	Nainital	63	RC/PB/1298	
30	IC548203	Nainital	64	RC/PB/1299	
31	IC556880	Pauri Garhwal	65	H-5	
32	IC556882	Tehri Garhwal	66	H-6	
33	IC556884	Tehri Garhwal	67	H-10	
34	IC556885	Tehri Garhwal	68	H-14	

and Pome_$(GACA)_4$ — amplified 8, 9, 11, 8 and 7 amplicons respectively. All the 43 amplicons were found to be polymorphic. Average number of polymorphic amplicons per primer was 8.6. All the 68 pomegranate genotypes were grouped into two major groups and 11 clusters (Fig 1). The clusters in no way reflected the collection sites. Pomegranate is an introduced fruit to India. Pomegranate breeders often use well-established cultivars for inter-varietal crosses. However, recent focus on the bacterial blight resistance (www.icar.org.in/en/node/8271) meant that genotypes occurring in semi-feral conditions needed to be collected and characterized. Before detailed assay for disease resistance, it was important to ascertain that the genotypes were actually genetically distinct. In the present study, the preliminary screening has shown that all the 68 accessions were distinct genotypes. The results were encouraging to plan detailed morphological, molecular and trait-specific evaluations of the semi-feral genotypes that remain till-date unexplored.

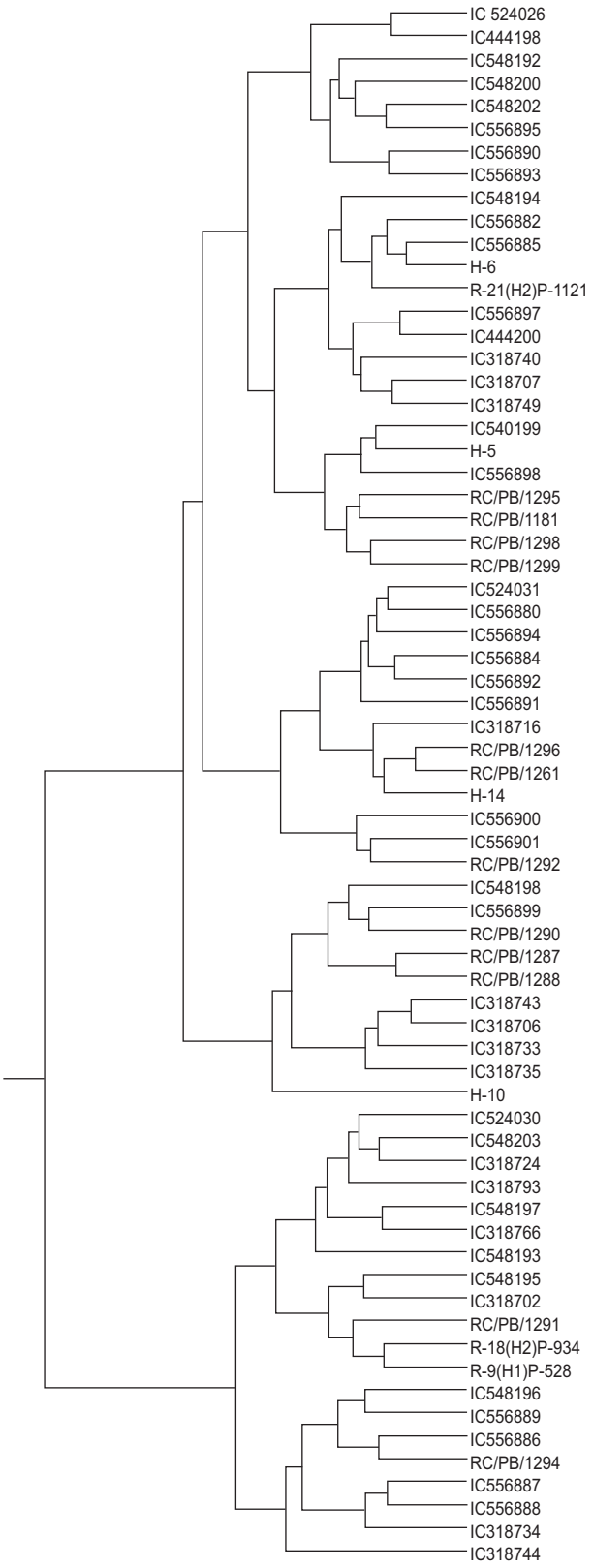

Fig. 1. Genetic clusters of 68 pomegranate germplasm accessions based on 43 ISSR markers

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