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Genetic diversity and population structure of Indian melon (*Cucumis melo L.*) landraces with special reference to disease and insect resistance loci

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With 3 figures and 3 tables

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Abstract

This study was aimed to examine the genetic diversity and population structure of Indian melon landraces with special reference to disease and insect resistance loci. Thirty-six simple sequence repeat (SSR) markers along with seven markers at disease and insect resistance loci were used for this purpose on a panel of 91 accessions available at Indian Institute of Horticultural Research, Bengaluru, India. Model-based structure analysis revealed the presence of four groups that were consistent with the results of principal coordinate analysis (PCoA). The delineation of populations was mostly based on geography with improved varieties as a separate group. Ten accessions have been identified to possess beneficial alleles at all the selected disease resistance loci and shall be useful for incorporating multiple disease resistance after phenotypic validation. The results obtained in the current study demonstrate the importance of the Indian melon group as a valuable genetic reservoir and the need to plan strategies for its conservation and utilization in breeding programmes.

Key words: melon — India — genetic diversity — resistance — simple sequence repeats

Melon (*Cucumis melo* L., 2n = 24) is an important commercial dessert crop grown in tropical and subtropical regions of the world in an area of 1.29 M ha with a production of 29.46 million tonnes (FAO, 2013). India is a major contributor of world melon production with 0.761 million tons from 0.037 million hectares (NHB, 2014). Recent literature suggests an Asian origin for *C. melo* with progenitor populations in the Himalayan region and high genetic diversity of land races in India and China (Schaefer et al. 2009, Sebastian et al. 2010). There are three main types of non-sweet melons cultivated as landraces in India, viz. *C. melo* ssp. *agrestis* Flexuous group, ssp. *agrestis* Acidulus group and ssp. *agrestis* Momordica group (Burger et al. 2010). A fourth group is formed by a semi-domesticated melon known as chibber or kachri used for pickles and chutneys (Dhillon et al. 2012). This is a feral form belonging to *C. melo* ssp. *agrestis* according to Pitrat et al. (2000). All of these groups are intercrossable and also to *C. melo* ssp. *melo*. Because of widespread adoption of improved varieties, there might have been frequent intercrossing between local landraces and improved varieties. Therefore, we find several intermediate forms being domesticated.

The Indian gene pool has gained relevance in global breeding programmes as a source of resistance to several biotic stresses including *Fusarium* wilt, downy mildew, powdery mildew, aphids and viruses. Furthermore, Indian snapmelon (ssp. *momordica*) accessions have also been used for creating mapping populations (Baudracco-Arnas and Pitrat 1996, Wang et al. 1997) and establishing taxonomic relationships with other melons (Silberstein et al. 1999, Stepansky et al. 1999, Akashi et al. 2002, Monforte et al. 2003).

Microsatellite markers or simple sequence repeat (SSR) markers are frequently used to evaluate the genetic diversity of germplasm collections because of their versatility, higher reproducibility and manifestation of a high degree of polymorphism (Gupta and Varshney 2000, Vignal et al. 2002). SSR markers were used to study genetic diversity of melons from Greece and Cyprus (Emmanouil et al. 2009), China (Kong et al. 2011, Hu et al. 2015), Spain (Escribano et al. 2012) and Turkey (Kacar et al. 2012).

India is the likely centre of origin and a major centre of diversity for melons. Several Indian melon accessions are maintained in major gene banks like USDA and VIR collected during their explorations in India. Few such explorations include the one by VIR in 1961 and USDA during 1992 (Staub and McCreight 1992). Among 2276 *C. melo* accessions curated at National Plant Germplasm System (NPGS), 716 accessions (33%) are from India, highlighting the importance of variability available in this region. A thorough analysis of the genetic diversity and structure of this germplasm is a fundamental requirement to effectively use it for breeding and crop improvement. Attempts have been made to understand the diversity of Indian melon germplasm especially during the last few years. However, these attempts were restricted to a few regions and botanical groups like the state of Punjab (Roy et al. 2012), Indo-Gangetic plains (Malik et al. 2014), snap melon collection of north-western India (Dhillon et al. 2007), states of Uttar Pradesh–Bihar (Singh et al. 2015) and Acidulus collection from southern India (Fergany et al. 2011). Hence, the current experiment was planned to understand the diversity and population structure of a pan-Indian melon landrace collection in comparison with improved varieties and collections from USDA and VIR gene banks as a reference set.

In melon, several resistance genes have been genetically characterized and tightly linked markers are available. The *Fom-1* (*Fusarium oxysporum f.* sp. *melonis*) gene confers monogenic resistance against soil-borne fungus Fom races 0 and 2, while the closely linked *prv* gene specifies resistance against PRSV (*Papaya ring spot virus*). *Fom-2* confers resistance to Fom races 0 and 1. Another R gene, *Vat,* confers a double resistance phenotype, viz. resistance to aphid (*Aphis gossypii*) infestation and also virus transmission by aphid. An allele of the same gene named *Pm-W* confers resistance to powdery mildew (*Podo-*

sphaera xanthii) (Dogimont et al. 2007). *Gsb-4*, a single dominant gene, governs resistance to gummy stem blight (*Didymella bryoniae*) in melon. Tightly linked markers have been reported for *Fom-2* (Joobeur et al. 2004), *Fom-1* and *prv* (Brotman et al. 2005), *Gsb-4* (Hong-ying et al. 2012) and *Vat*/*Pm-W* (Brotman et al. 2002). The current study tries to understand variability at these resistance loci available in Indian melon landrace collection.

Materials and Methods

Plant material: The Indian Institute of Horticultural Research (IIHR), Bengaluru, maintains about 280 germplasm lines of *C. melo L.* From this collection, a total of 91 accessions were selected for the present study with emphasis on landraces (49 accessions) along with improved varieties (28 accessions) being cultivated in India. These accessions were selected based on availability of passport information and due representation of all geographic regions of India according to their proportion of the original collection. Fourteen accessions collected from USDA (PI-371795, 614548, 614304, 164720, 145594, 614572, 123689, 123502, WI-998) and VIR (K-6217, 5817, 5614, 5622 and 6584) gene banks available at IIHR, Bengaluru, were selected as reference panel. The landraces were classified into three groups based on their region of collection, viz. east, west and south Indian accessions. The details of the germplasm lines along with their place of collection and details of improved varieties are presented in Table S1.

Original germplasm, maintained through sibling, was used for the molecular study. All the germplasm lines were grown in field during October 2011–January 2012. Leaf samples for analysis were collected from one plant per accession assuming a fair level of homogeneity due to several generations of controlled sibmating in the process of maintenance of these accessions. The leaf samples were used for genomic DNA extraction using cetyl trimethyl ammonium bromide (CTAB) method with some minor modifications (Doyle and Doyle 1987). The DNA concentration was determined using a UV spectrophotometer.

Markers: A total of 110 pairs of SSR primers were screened to genotype a subset of collection. Among them, 36 markers that showed high polymorphism were chosen for structure analysis (Table S2). Thirty of these markers were from the linkage map developed by Diaz et al. 2011 and remaining six markers were from map developed by Harel-Beja et al. 2010. Markers were selected to ensure an even distribution on the genome, satisfying no-linkage assumption for structure analysis.

Variability at the resistance loci, viz. *Fom-1, Fom-2, prv, Vat/Pm-W* and *GSB-4,* was studied across the germplasm panel. The details of markers used for this purpose are presented in Table 1.

PCR and gel electrophoresis assay: The volume of the PCR was 10 μl with 30 ng of template DNA, 1.5 mM $MgCl_2$, 1× PCR buffer, 200 μM of dNTPs (deoxynucleotide triphosphates), 0.25 μM of each primer and 0.5 U *Taq* DNA polymerase (Bangalore Genei, Bangalore, India). The PCR program used was 94°C for 4 min, 35 cycles of 94°C for 30 s, 51°C for 30 s, 72°C for 1 min and a 8-min final extension at 72°C. Amplified products were size separated by 6% polyacrylamide gel electrophoresis. Alleles were detected using image analysis software, UVI proplatinum 2.0.

Statistical analysis: Genetic diversity measures were computed as implemented in PowerMarker version 3.25 Bioinformatics Research Center, Campus Box 7566, North Carolina State University, Raleigh, NC 27695–7566, USA (Liu and Muse 2005). These measures include the average number of alleles, the major allele frequency, gene diversity, heterozygosity and polymorphic information content (PIC).

Estimation of population structure: Population structure was analysed using a model-based approach available in the software STRUCTURE 2.3.1. The Pritchard Lab, Mail Stop-5120 Stanford University Stanford, California 94305–5120 Data from 36 SSR markers listed in Table S2 was used for this purpose. The membership of each genotype was tested from $K = 2–10$ with admixture model. Each run was implemented with a burn-in period of 30 000 steps followed by 100 000 Monte Carlo Markov chain with three replications at each K value. The estimated log-probability of data [LnP(D)] from STRUCTURE overestimates the number of subpopulations. STRUCTURE HARVESTER (http://taylor0.biology.ucla.edu/structureHarvester), a web-based python program, was used to measure the ΔK to estimate the number of subpopulations (Evanno et al. 2005). The CLUMP software was used to align cluster assignment across replicates analysed. The run of the estimated subpopulation showing maximum likelihood was used to assign accession with membership probability ≥0.75 to groups. Accessions with membership probability less than 0.75 were assigned to an admixture group (Stich et al. 2005).

To investigate population differentiation, an analysis of molecular variance (AMOVA) and estimation of pairwise F statistics (F_{st}) among populations were performed using Genalex program (Peakall and Smouse 2006).

Results

Genetic diversity

A total of 159 alleles were detected at 36 SSR loci with an average of 4.42 alleles per locus (Table S2) ranging from 2 (CMAAAGN148, CMAGAN268, CMAGN249, CMCAAN253, CMCTTN174 and CMTTCN163) to 12 (CMAACN216) per locus. Moderate to high PIC values, a reflection of allele diver-

Table 1: List of markers used to study variability at disease/insect resistance loci in native Indian melon germplasm panel

S. No	Disease/insect	Primer name	Primer sequence	No of alleles	Allele sizes	References
1	Fusarium wilt (*Fom-2*)	SSR138	F: GACACGACCTTGATCCATGTG R: GCAACGACCTGATCCATGTG	2	138bp[1], 150 bp	Joobeur et al. 2004
2	Fusarium wilt (*Fom-2*)	SSR430	F: CCATCATGATTTGGAATGAATTAG R: CGTTGCAATTTGATCTTTTTAATG	2	400bp, 430bp[1]	Joobeur et al. 2004
3	Gummy stem blight (*Gsb-4*)	CMTA170a	F: TTAAATCCCAAAGACATGGCG R: AGACGAAGGACGGTTAGCTTT	2	125bp[1], 150 bp	Hong-ying et al. 2012
4	Aphids (*Vat*)	Vat 681	F:GGAATCTTGTTGAGGCCGAGAGGG R:GTTGTATATGGCTTCCCTGTAGCC	3	681bp[1], 435bp, 221 bp,	Brotman et al. 2002
5	Aphids (*Vat*)	Vat 1684	F:CAACAGGCTCAACAGTGTATTCGG R:GAAGAAGGTGACGAGAGAGATGCC	2	1684bp[1], 1350bp	Brotman et al. 2002
6	Papaya ring spot virus (*prv*) and Fusarium wilt (*Fom-1*)	62-CAPS	F: GGAGAAGATGCTAGAGCCATTC R: AATCGGGCATCCTGTTTTGG	2	330 bp[1], 420bp	Brotman et al. 2005
7	Papaya ring spot virus (*prv*) and Fusarium wilt (*Fom-1*)	NBS1 CAPS	F:TATTGCTAAAGCTGTTTTCAAAAGCG R: AACAAAAACTTTTCGATTTCCTAAGTT	2	205 bp, 250bp[1]	Brotman et al. 2005

[1]Resistance allele size.

sity and frequency were observed ranging from 0.257 to 0.784, with an average of 0.482. The overall gene diversity and mean heterozygosity were 0.553 and 0.122, respectively.

Population structure

The model-based approach (Pritchard et al. 2000) was performed to understand the population structure of 91 muskmelon accessions with 36 polymorphic SSR markers. Twenty-seven data sets were obtained by setting the number of possible clusters (K) from 2 to 10 with three replications each. The results for each K value were permuted using CLUMP software Rosenberg labs, Stanford University 371 Serra Mall Stanford, CA 94305–5020 USA. The exact K value could not be straightforwardly inferred because the estimated log-likelihood values appeared to be an increasing function of K for all examined K values (Fig. 1a). In contrast, the maximum of the ad hoc measure ΔK developed by Evanno et al. (2005) was observed for $K = 4$ (Fig. 1b), which indicated that the entire population could be divided into four subpopulations (Fig. 2). Similar results were obtained by PCoA using Genalex software Rod Peakall, School of Botany and Zoology, The Australian National University, Canberra ACT 0200, Australia. (Fig. 3).

Based on the membership probabilities of ≥0.75, Pop I consisted of 12 accessions, 8 of them belonging to west Indian states of Rajasthan and Gujarat (Table 2). Pop II contained 10 accessions which predominantly came from eastern Indian states (7 accessions). Pop III consisted of 9 accessions, all of which are improved varieties. The fourth population contained 22 accessions which predominantly included accessions from south Indian states of Andhra Pradesh and Karnataka (12 accessions) along with exotic collections (9 accessions). The remaining 38 genotypes had membership probabilities lower than 0.75 in any group and were classified into an admixture group. This group included 11 genotypes from south, 1 from east, 8 from west, 13 improved varieties and 5 exotic lines.

Population differentiation

Comparing the four populations, AMOVA results indicated that only 14% of the total genetic variation was partitioned among populations, 65% within populations and 21% within individuals. Further, the pairwise comparison on the basis of F_{st} values interpreted as standardized population distances between populations ranged from 0.267 between Pop I and Pop IV to 0.376 between Pop II and Pop III. The average pairwise F_{st} value in this study was 0.320.

Genetic diversity of populations

The genetic diversity for each population was evaluated (Table 2). Accessions in Pop IV whose primary origin is from south Indian states of Andhra Pradesh and Karnataka were more diverse with a higher mean allele number of 3.00 and gene diversity of 0.497 compared to the other populations. The Pop III with accessions belonging to improved varieties showed the least allele number (2.25) and heterozygosity (0.071) compared to Pop IV which showed the highest heterozygosity of 0.147.

Variation at disease and insect resistance loci

A total of 15 alleles were detected at the seven resistance loci with an average of 2.14 alleles per locus. The mean of resistant allele frequency across populations was nearly on par for all the four populations ranging from 0.72 (Pop III) to 0.75 (Pop I). The maximum diversity was observed in Pop IV (0.35) and least in Pop II (0.24) (Table 3).

Discussion

Landraces constitute genetically dynamic and diverse populations that are valuable sources to broaden the genetic base of cultivated types. The assessment of genetic diversity and structure of such a unique gene pool is a prerequisite for their efficient organization conservation and utilization for crop improvement. In the current study to assess the Indian melon diversity, a total of 91 accessions including 49 Indian landraces and 28 improved varieties being cultivated in India were genotyped at 36 SSR marker loci. This panel recorded an overall PIC and gene diversity of 0.482 and 0.551, respectively, with a mean heterozygosity of 0.122. Fergany et al. (2011) also reported a PIC value of 0.544 in a set of Indian melon accessions, which is in the range

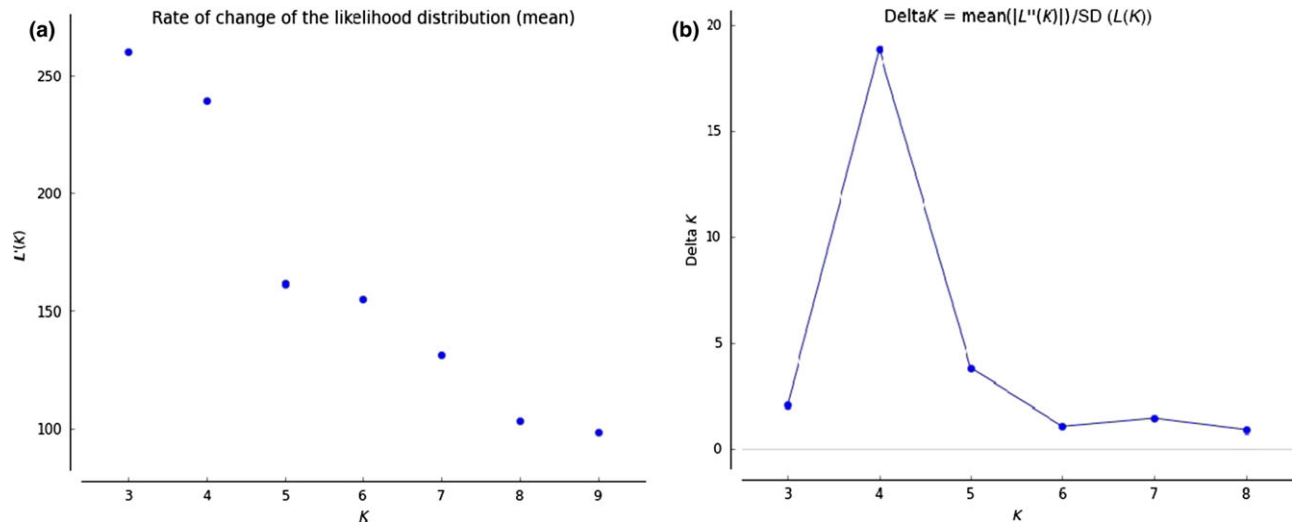

Fig 1: Two different methods for determining optimal value of K: (a) the *ad hoc* procedure described by Pritchard et al. (2000) where the K value is increasing (b) the second-order statistic (ΔK) developed by Evanno et al. (2005), the sharp peak of ΔK at $K = 4$ suggesting four sub populations

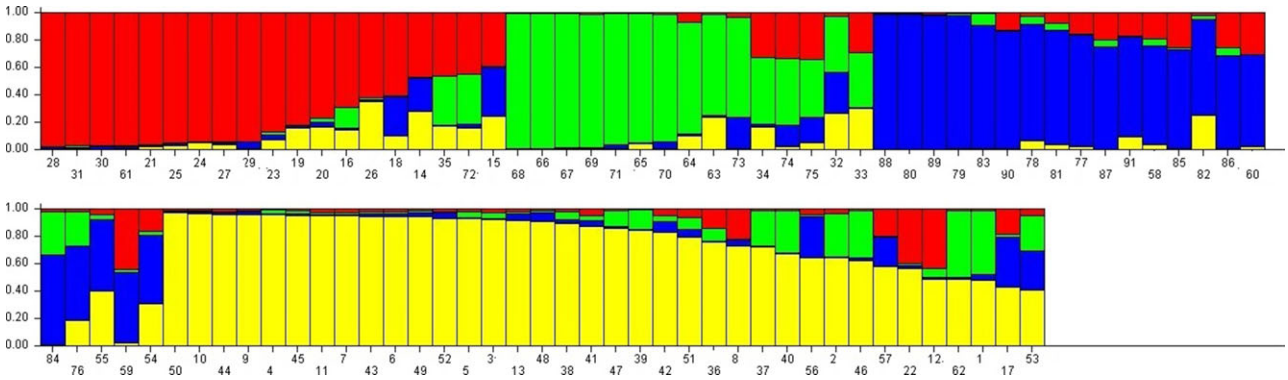

Fig 2: Model-based cluster membership of 91 germplasm accessions into four sub-populations identified with STRUCTURE using 36 SSR markers

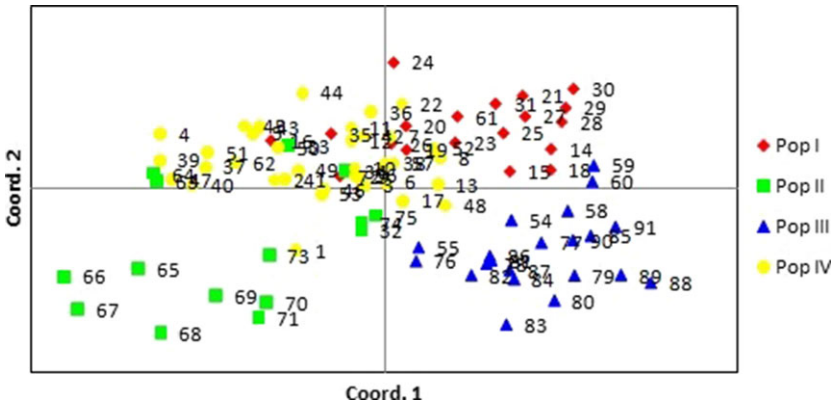

Fig 3: Principal coordinate analysis (PCoA) of the 91 accessions of muskmelon based on genetic distance estimates which shows the presence of four sub-populations

Table 2: Classification of 91 accessions of native Indian melon germplasm panel into populations, their respective regions of origin along with genetic diversity parameters

	Source of collection						Genetic diversity parameters				
Population	South India	East India	West India	Improved line/variety	Gene bank collections	Total	Allele number	Major allele frequency	Gene diversity	Heterozygosity	PIC
Pop I	1	–	8	3	–	12	2.41	0.714	0.380	0.097	0.323
Pop II	–	7	1	2	–	10	2.50	0.684	0.420	0.120	0.356
Pop III	–	–	–	9	–	9	2.25	0.743	0.327	0.071	0.285
Pop IV	12	–	–	1	9	22	3.00	0.592	0.497	0.147	0.420
Admixture	11	1	8	13	5	38	3.86	0.559	0.5483	0.131	0.480
Total	24	8	17	28	14	91	4.44	0.553	0.551	0.122	0.482

of results obtained in the current experiment. The average alleles per locus in Spanish melons ranged from 1.05 to 2.82 (Escribano et al. 2012) and 2.47 in Greek and Cypriot melons (Emmanouil et al. 2009), compared to 4.44 for Indian melon accessions in the current study. Although it would not be appropriate to compare results across these experiments as the number of accessions and markers employed would influence the allele number, the current findings provide a suggestive evidence of a high variability among Indian melon germplasm. McCreight et al. (2004) also determined that melon germplasm from southern and eastern India might contain allelic diversity not available in the germplasm collections held in various global gene banks.

Simple sequence repeats have been a marker of choice for understanding population structure owing to their desirable genetic attributes, including locus specificity, wide genomic distribution, codominant and multi-allelic nature resulting in high degree of reproducible polymorphisms. They have been used to interpret population structure and LD in maize (Remington et al. 2001), rice (Zhang et al. 2011), wheat (Hao et al. 2012) and other crops. A model-based approach implemented in the software STRUCTURE is the most frequently used method to understand population differentiation in a panel. However, in the current experiment, it was interesting to note that the first-order statistic of LnP(D) implemented in STRUCTURE could not detect the population structure. The LnP(D) value for each given K increased with the increase of K, without any abrupt change, and hence, the probable K value could not be inferred (Fig. 1a). Because the estimated log-probability of data [LnP(D)] from STRUCTURE overestimates the number of subpopulations, we used the ΔK measure to estimate the number of subpopulations

Table 3: Genetic diversity for markers at disease and insect resistance loci across the four populations of Indian melon germplasm

Marker	Allele number					Resistant allele frequency					Gene diversity					PIC				
	Pop I	Pop II	Pop III	Pop IV	Admixture	Pop I	Pop II	Pop III	Pop IV	Admixture	Pop I	Pop II	Pop III	Pop IV	Admixture	Pop I	Pop II	Pop III	Pop IV	Admixture
62-CAPS	2.00	2.00	2.00	2.00	2.00	0.47	0.82	0.75	0.55	0.55	0.50	0.26	0.28	0.49	0.49	0.37	0.22	0.24	0.37	0.37
NBS1-CAPS	2.00	1.00	2.00	2.00	2.00	0.75	1.00	0.89	0.96	0.85	0.38	0.00	0.20	0.04	0.21	0.31	0.00	0.18	0.04	0.19
CMTA170a	2.00	1.00	2.00	2.00	2.00	0.85	1.00	0.67	0.76	0.72	0.22	0.00	0.44	0.33	0.36	0.20	0.00	0.35	0.27	0.30
SSR 138	2.00	2.00	2.00	2.00	2.00	0.71	0.25	0.67	0.88	0.58	0.38	0.32	0.40	0.17	0.49	0.31	0.27	0.32	0.15	0.37
SSR 430	2.00	2.00	2.00	2.00	2.00	0.80	0.56	0.75	0.69	0.73	0.22	0.38	0.44	0.43	0.44	0.20	0.31	0.35	0.34	0.34
Vat681	2.00	3.00	2.00	3.00	3.00	0.75	0.56	0.44	0.70	0.76	0.47	0.57	0.35	0.57	0.52	0.36	0.48	0.29	0.48	0.41
Vat1684	2.00	2.00	2.00	2.00	2.00	0.92	0.90	0.89	0.68	0.84	0.15	0.18	0.20	0.43	0.27	0.14	0.16	0.18	0.34	0.23
Mean	2.00	1.86	2.00	2.14	2.14	0.75	0.73	0.72	0.74	0.72	0.33	0.24	0.33	0.35	0.40	0.27	0.21	0.27	0.29	0.32

(Evanno et al. 2005). Applying this second-order statistic, there was a sharp peak of ΔK at $K = 4$, suggesting four subpopulations (Fig. 1b). A similar pattern was reported by Ranc et al. (2008). The presence of four subpopulations was also confirmed with PCoA using Genalex software (Fig. 3). Values of F_{st} among the four subpopulations were significant, suggesting a real differentiation among them. The distribution of the melon accessions into populations fits very well with their geographic origin, demonstrating a clear spatial isolation among the gene pools. The reference panel of improved varieties and exotic accessions was found to be genetically distinct. Several accessions had partial ancestry in more than one background and were found to be admixtures. These accessions probably had a complex history involving intercrossing especially with the improved varieties. Pop III with improved varieties recorded the least genetic diversity. Repeated recycling of a relatively small number of genetically related melon lines in commercial breeding programmes has reduced the genetic diversity as observed in the current experiment. For example, Pusa Sharabati, Punjab Sunehri, Hara Madhu and Durgapura Madhu have been grown in India for more than two decades and are routinely used in breeding programmes. Hybrids developed using these genotypes include Pusa Rasraj (M3 X Durgapura Madhu), Punjab Hybrid1 (Ms-1X Hara Madhu) and MH10 (W1998X Punjab Sunehari). Pop IV from the southern states of Andhra Pradesh and Karnataka was the largest group with 22 accessions and displayed highest genetic diversity.

Many of the improved varieties released from different universities were actually selections from landraces, and hence, we observed these improved varieties to be clustering along with landraces of those regions. For example, Arka Jeet developed at IIHR, Bengaluru, is a selection from material collected around Lucknow (eastern India) and hence clustered in Pop II which contained landraces from eastern Indian states. Similarly, Hara Madhu developed at Punjab Agricultural University (PAU), Ludhiana, located in western India is a selection from local material and grouped along with other landraces coming from western India in Pop I. Several collections from USDA and VIR gene banks were distinctly grouping along with south Indian accessions. Similar observation was made by Malik et al. 2014 and opined that there exists affinity between Indian and USA melons, which could be because some group of melons moved from India to North America, or as a result of intercrossing of Indian landraces with the USA-derived cultivars.

Dhillon et al. 2012 reviewed melon landraces of India, highlighting their potential as sources of valuable agronomic and stress tolerance traits. Indian germplasm has proved to be a good source of alleles for biotic stress tolerance like powdery mildew (Zink and Thomas 1990, McCreight 2001), downy mildew and Fusarium wilt (Cohen and Eyal 1987), aphids (Dogimont et al. 2008) and viruses (Dogimont et al. 1997, McCreight 2000, Pitrat et al. 2000, Yousif et al. 2007, McCreight and Wintermantel 2008, McCreight et al. 2008, Fergany et al. 2011). Allelic variability was studied across the Indian melon panel for seven such resistance loci with established markers (Table 1). These markers revealed good variability with a total of 15 alleles at the seven loci in the Indian accessions. Although the mean resistant allele frequency was on par across different populations, significant differences were observed for specific loci among populations. The resistant allele frequency was highest in Pop II for *prv* and *Gsb-4* loci, and Pop I for *Fom-2* and *Vat* loci. Ten accessions were identified to possess beneficial alleles at all these loci (Table S1) and can be utilized in breeding programmes for

incorporating multiple disease resistance after phenotypic validation.

In the present case, south Indian accessions, with high genetic diversity and quite differentiated from the other groups, hold great promise. Hence, future explorations in this region can possibly add novel variation. Considering that the natural range of Indian melon landraces encompasses environments as diverse as river beds to arid regions, these may also be a potential source of beneficial alleles for abiotic stress tolerance.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. List of native Indian melon accessions along with improved varieties and exotic collections used in this study and their region of origin along with population.

Table S2. List of 36 SSR markers in positional order and their basic statistics across the 91 accessions of native Indian melon germplasm panel.