which on air oxidation gives (7). Direct air oxidation of the dienolate (5) to the dione (7) is also possible under the reaction conditions before neutralization. A similar air oxidation of dihydro-azulene to azulene has been reported[7].

The rearrangement product (7) shows two low-field protons at δ 8.5 when compared with the diol (3). This difference can be accounted for since structure (7) has two periprotons of the naphthalene rings proximate to two carbonyl groups[8].

All melting points are uncorrected. IR spectra were recorded on a Schimadzu 8201 instrument. PMR spectra were recorded on Bruker DPX-200, 200 MHZ FT NMR instrument employing tetramethyl silane as internal standard. Mass spectra were recorded on Varian Mat CH-7 Mass Spectrometer and also Schimadzu Q.P.5000 mass spectrometer.

Phenanthrene-9,10-dione[4] (2) was prepared by the oxidation of phenanthrene with chromium trioxide in conc. H_2SO_4.

For the preparation of 9,10-dihydroxy-9,10-di-1[I]-naphthyl phenanthrene (3). To a solution of α-naphthyl magnesium bromide prepared from magnesium (1.92 g, 0.08 g atom) and α-bromonaphthalene (16.6 g, 0.08 m) in dry THF (100 ml) was added a slurry of phenanthrene-9,10-dione (4.16 g, 0.02 m). After refluxing for 2 h, saturated ammonium chloride solution (50 ml) was added with stirring. Then the organic layer was separated and the aqueous layer was extracted with ether. The combined organic layer was dried. On removal of the solvent, a viscous oil (5.91 g) was obtained.

This viscous oil was chromatographed over a column of silica gel. Elution with ethyl acetate : petrol (1 : 4) gave a white solid, melting point 210–212°C. Yield based on reacted dione (2) was 3.2 g (61%). Further elution with ethyl acetate : petrol (1 : 3) gave unreacted phenanthrene-9,10-dione (1.8 g).

Let us consider the rearrangement of 9,10-dihydroxy-9,10-di-1[I]-naphthyl phenanthrene (4). To a suspension of potassium hydride (0.2 g, 0.005 m, 0.53 g of a 30% dispersion in mineral oil washed with 10 ml portions of dry petrol) in dry THF (40 ml) was added a solution of diol (3) (0.29 g, 0.0001 m) in THF (25 ml). The mixture was refluxed in an atmosphere of nitrogen for 2 h. The solvent was removed and saturated ammonium chloride (10 ml) was added to the residue. The aqueous solution was extracted with chloroform (3 × 5 ml). The chloroform extract was washed with water (150 ml), brine (100 ml) and dried. Removal of the solvent under reduced pressure gave a white crystalline solid, melting point 164°C (ethylacetate : petrol), yield 0.2 g (69%).

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RAPD markers reveal narrowing genetic base of Indian tomato cultivars

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Genetic diversity of 27 tomato cultivars grown in India was analysed with RAPD markers, generated by 42 random primers. The overall high levels of pairwise similarity (Jaccard's mean = 0.825) and low levels of marker diversity (mean = 0.165) implied the existence of limited genetic variation in the investigated materials. Interestingly, old introductions and locally developed cultivars of the 1970s exhibited significantly greater genetic variation than the ones released during the 1990s. Reduction in the genetic diversity among modern tomato cultivars may be attributed to the recent trend towards breeding for similar plant and fruit characteristics.

TOMATO (*Lycopersicon esculentum* Mill.) is one of the most important vegetable crops grown in India. With an annual production of 5.4 million Mt, the country is the sixth largest tomato producer in the world[1]. Though concrete historical records of its first introduction into the country from its primary centre of diversity in South America do not exist, tomato is presumed to have been brought here during the second half of the 16th century through Far Eastern countries[2]. Nineteenth century plant

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explorers in India noted the plant to be very common, highly variable and growing as a cultivated crop as well as an escape[3–5]. These materials formed the base of the first indigenous selections released as improved cultivars in the middle of the 20th century. Major boost to tomato cultivation in the country was provided by the introduction of high-yielding exotic cultivars like Sioux, Roma and Marglobe from 1950 onwards[6,7]. Over the years, indigenous high-yielding cultivars have been bred from the old local cultivars, the early introductions (referred to here as *Introduced cultivars*) and, more significantly, the newly introduced cultivars and breeding lines. Majority of these new and now popular cultivars have come from three breeding centres – Indian Agricultural Research Institute, New Delhi (*Pusa cultivars*) and Punjab Agricultural University, Ludhiana (*Punjab cultivars*) in north India; and Indian Institute of Horticultural Research, Bangalore (*Arka cultivars*) in south India.

A number of studies have been carried out on biochemical and molecular variation in *L. esculentum* accessions collected from primary and secondary centres of diversity. These studies have revealed that the species has undergone considerable reduction in genetic diversity during the course of its domestication and breeding for improved types[8–12]. However, there are also evidences of novel variations occurring in the secondary centres[12,13]. There is no information whether similar changes have taken place in tomato in India during the long history of its cultivation and breeding. Since knowledge of genetic diversity is essential for evolving systematic breeding and conservation strategies, the present molecular diversity analysis using RAPD technique was carried out on 27 tomato cultivars of diverse origins grown in India.

Twenty-seven tomato cultivars belonging to the four above-defined groups were used in the present study (Table 1). The exact years of release of the introduced cultivars in their respective countries of origin are not available. However, the period ranges between 1920s and 1960s.

Fresh young leaves pooled from five plants per sample were used for DNA extraction by modified cTAB method[14]. DNA was treated with bovine pancreatic RNase and extracted once each with phenol : chloroform (1 : 1) and chloroform : iso-amyl alcohol (24 : 1). DNA was quantified in a fluorometer and dissolved to appropriate dilution in TE buffer.

Amplification reactions contained 3 mM $MgCl_2$; 50 mM KCl; 10 mM Tris-HCl (pH 9.0); 0.1% Triton X-100; 200 µM of each dNTPs; 0.4 µM primer; 25 ng template DNA; and 2 units Taq DNA polymerase (Perkin-Elmer) in a reaction volume of 25 µl. After a pre-denaturation step of 4 min at 94°C, amplification reactions were cycled 40 times at 94°C for 1 min, 35°C for 1 min and 72°C for 2 min. A final amplification was allowed for 5 min at

Table 1. Tomato cultivars used in the study

Cultivar/accession	Group	Year of release	Pedigree/source
Arka Abha	Arka	1990	Pure line selection from VC-8-1-2-1, AVRDC, Taiwan
Arka Abhijit	Arka	1998	F1 hybrid of the cross 15SBSB × IIHR 1334
Arka Ahuti	Arka	1990	Pure line selection from Ottawa 60, Canada
Arka Alok	Arka	1992	Pure line selection from CL-114-5-1-0, AVRDC, Taiwan
Arka Ashish	Arka	1990	Selection from UC 82-B line, USA
Arka Meghali	Arka	1996	Pedigree selection (F8) of the cross Arka Vikas × IIHR 554
Arka Shreshta	Arka	1996	F1 hybrid of the cross 15 SBSB × IIHR 1614
Arka Vikas	Arka	1990	Pure line selection from an American variety Tip-Top
Punjab Chhuara	Punjab	1978	Derivative of the cross Punjab Tropic × EC 55055
Punjab Kesri	Punjab	NA	Derivative of the cross Punjab Tropic × EC 55055
Punjab Tropic	Punjab	1978	Selection from USA-216-17-BK-D5-D3-03-DBK CAVSTMIO (related to USA cultivar Tropic)
Selection –12	Punjab	1975	Product of mutation breeding (γ-irradiation) of Sioux
TH-802	Punjab	NA	Healani × Acc. No. 2
Pusa 120	Pusa	1970	Selection from Hawaiian introduction Anahu
Pusa Divya	Pusa	1985	–
Pusa Gaurav	Pusa	1981	Derivative of Glamour × Watch
Pusa Hybrid 2	Pusa	1993	F1 hybrid of the cross Pusa 120 × Pusa Gaurav
Pusa Hybrid 4	Pusa	1995	F1 hybrid of the cross Pusa 120 × A breeding line from USA
Pusa Ruby	Pusa	1985	Derivative of Improved Meeruti × Sioux
Pusa Sheetal	Pusa	1990	Derivative of Balkan (Bulgaria) × S-699 (Russia)
Pusa Uphar	Pusa	1994	Selection from Taiwan breeding line
Best of All	Introduced		
Healani	Introduced		
Marglobe	Introduced		
Roma	Introduced		
Sioux	Introduced		
VFN-8	Introduced		

NA, Not available

72°C. Upon completion of the amplification, reaction composition was loaded onto a 1.2% agarose gel and electrophoresed at 4 V/cm in Tris-borate buffer. Bands were visualized by ethidium-bromide staining and sizes of the identified bands were derived relative to 100 bp DNA ladder (MBI Fermentas).

Out of 60 random decamer primers (Operon) initially tested, 42, selected on the basis of robustness of the amplification, clarity and scorability of banding patterns, were employed for diversity analysis.

Amplicons were scored as discrete variables, using 1 to indicate presence and 0 to indicate absence. Polymorphism was confirmed by repeating the experiment twice. Markers were not scored as polymorphic unless at least more than two well-amplified samples exhibited the variant condition. This may have resulted in slightly conservative estimates of polymorphism. A pairwise similarity matrix was determined using Jaccard's coefficient. UPGMA cluster analysis was performed to develop a dendrogram. Principal coordinate analysis (PCO) of the similarity matrix was also used to estimate relationships among cultivars. These computations were performed using the program NTSYS-PC Ver 1.7 (ref. 15). Genetic diversity among groups of cultivars was estimated by marker diversity[12], a measure analogous to Nei's gene diversity. Mean marker diversity = $(1/N)\Sigma[2p_iq_in_i/(n_i - 1)]$, where p_i is the frequency of presence and q_i is the frequency of absence of ith RAPD marker in n_i accessions and N is the total number of RAPD markers.

The 42 selected primers generated 174 bands of different sizes in the 27 cultivars (Figure 1). The number of bands per primer ranged up to eight, with a mean of 4.1. The number of polymorphic bands per primer ranged between zero and seven, with a mean of 2.6. Interestingly, the frequency of polymorphic markers (63.8%) was much higher than that reported earlier[11] (37.2%) in a range of tomato accessions and wild *Lycopersicon* species. In another study of tomato cultivars[16], 12 out of 27 (44.4%) RAPD markers were polymorphic. These workers observed three polymorphic markers per primer using primers pre-selected for generating one or more polymorphisms. Taking into account the 33 such primers in the present study, the frequency of polymorphic markers works out to 3.4 per primer.

Pairwise similarities between the cultivars calculated on the basis of Jaccard's coefficient ranged between 0.610 and 0.976, with a mean of 0.825 (Figure 2). Mean marker diversity among the cultivars was 0.165. Overall high levels of pairwise similarity and low value of mean marker diversity, suggested the existence of limited genetic variation in tomato cultivars grown in India. However, similar results have been obtained in tomato accessions from other regions of the world, including both primary and secondary centres of diversity[8–12]. Existence of very low genetic diversity within cultivated tomatoes has been attributed to self-pollination, artificial selection and founder effect[8,9].

Table 2 provides results of the group-wise analysis of diversity in the present material. Mean marker diversity was highest in Punjab cultivars and least in Arka cultivars. Inter-group comparisons revealed that the groups

Table 2. Intra-group and inter-group RAPD marker variation. Numbers in the lead diagonal indicate within-group mean marker diversity (bold face) and mean pairwise similarity (normal face). Numbers above lead diagonal (italics) represent *P*-values obtained by comparing mean marker diversities between groups using *t*-test for paired observations. Numbers below lead diagonal represent between-group mean pairwise similarities

Cultivar group	Arka	Pusa	Introduced	Punjab
Arka	**0.068**/0.946	*< 0.001*	*< 0.005*	*< 0.001*
Pusa	0.915	**0.118**/0.905	*0.460*	*< 0.001*
Introduced	0.808	0.802	**0.116**/0.899	*< 0.001*
Punjab	0.750	0.744	0.796	**0.265**/0.832

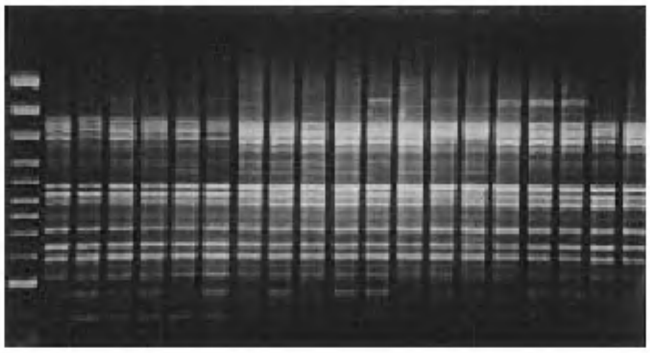

Figure 1. Typical RAPD electrophoretic pattern of tomato cultivars obtained by primer OPY-18. Left extreme lane represents 100 bp DNA ladder.

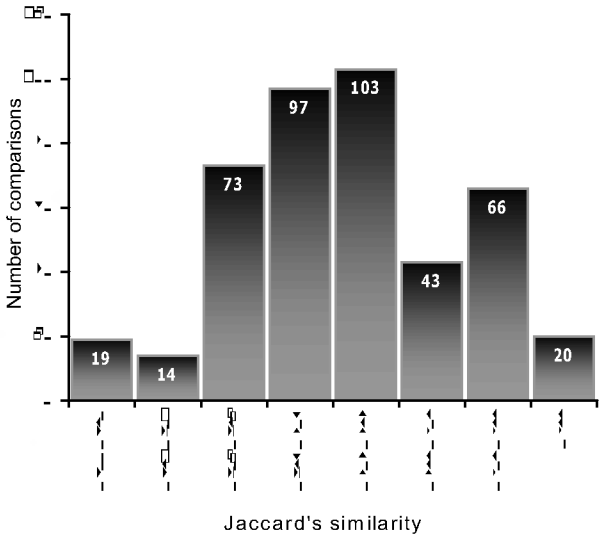

Figure 2. Distribution of similarity values obtained from pairwise comparison of RAPD markers in 27 tomato cultivars.

differed significantly in the mean marker diversity, except Pusa–Introduced comparison. Within-group mean Jaccard's pairwise similarity was highest in Arka cultivars followed in that order by Pusa cultivars, Introduced cultivars and Punjab cultivars. Mean similarity within groups was higher than that obtained from their inter-group comparisons, the only exception being Pusa–Arka comparison in which the mean similarity (0.915) was higher than that of Pusa cultivars (0.905). The existence of greater genetic homogeneity within groups could be due to shared pedigrees of at least some of their cultivars.

UPGMA clustering and principal coordinate analysis of the similarity indices (Figures 3 and 4) while

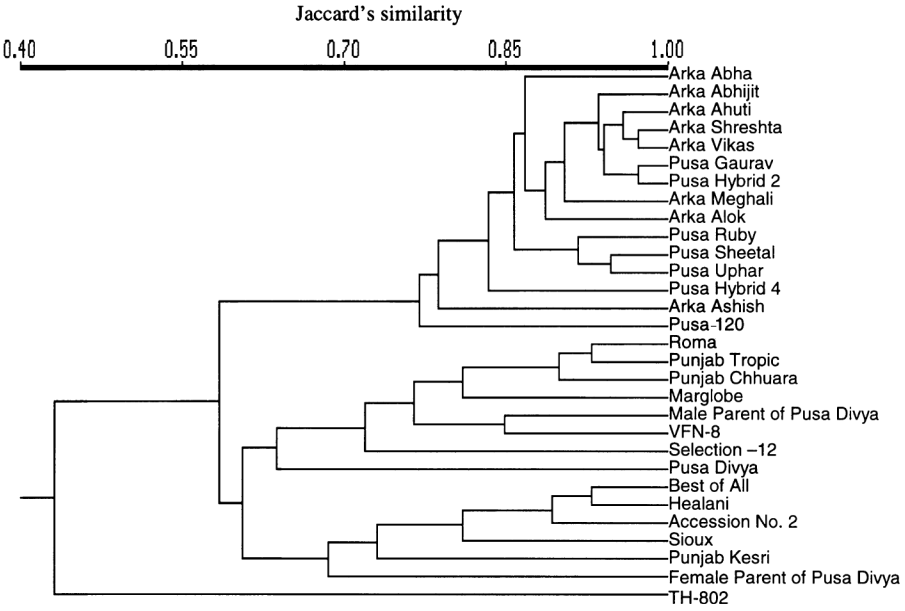

Figure 3. UPGMA dendrogram of tomato cultivars.

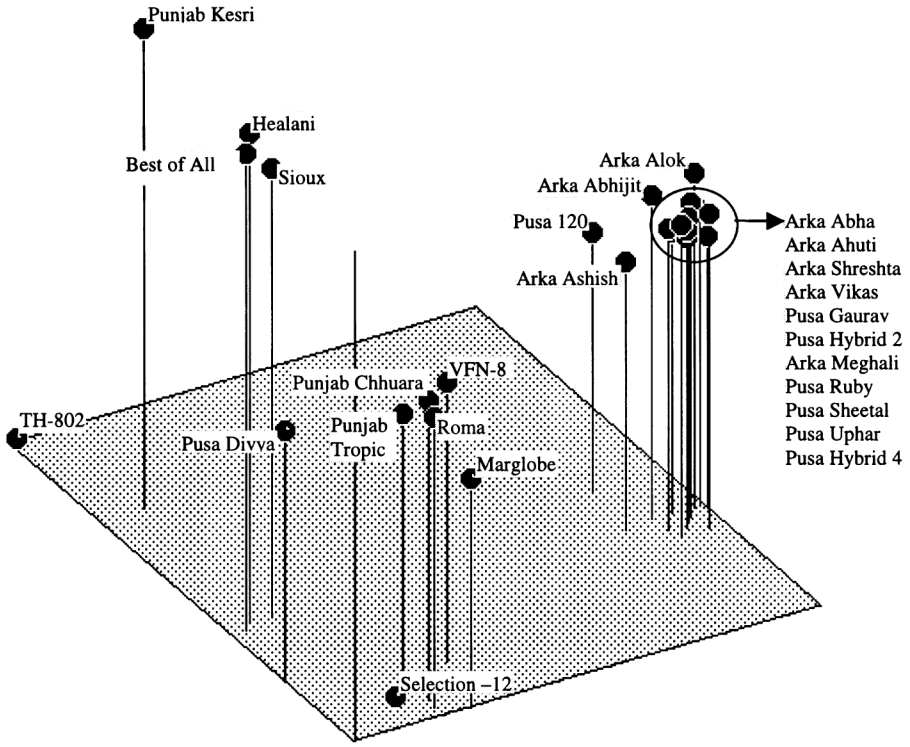

Figure 4. Distribution of tomato cultivars along first three principal coordinate axes based on RAPD similarities. (The axes represent 30.5, 12.3 and 9.9 per cent of the variation.)

supporting the above results provided further insight into interrelations among the cultivars. Arka and Pusa cultivars intermingled into a tight cluster, while Introduced and Punjab cultivars were more widely dispersed. However, Pusa Divya stood out prominently from the other Pusa cultivars, while TH-802 and Selection-12 were the most distantly related accessions of Punjab cluster. Pusa Divya, bred at Katrain, is a cultivar suitable for temperate regions, while all other Pusa cultivars have been bred at Delhi under subtropical conditions. Since TH-802 is a nematode-resistant cultivar, its greater distance could be due to introgression of genetic material from the wild species *L. peruvianum*, the source of nematode resistance in tomato. Previous studies have revealed high levels of RAPD polymorphism between UCT-5, a genotype with disease resistance genes from *L. peruvianum*, and other modern tomato cultivars[17]. A four-fold increase in polymorphic RAPD loci of modern cultivars over vintage cultivars observed in another study was attributed to introgression of disease-resistance genes from wild sources[11]. However, this does not seem to be the only reason for presently observed distinctness of TH-802, since three other cultivars, Pusa-120, Healani and VFN-8 also bear root knot nematode resistance genes from *L. peruvianum*, but are not as distantly placed as TH-802.

RAPD polymorphism of the above three cultivars notwithstanding, the present study points to the considerable reduction in the genetic base of cultivated tomato during the last three decades of public-sector breeding in India. Arka cultivars released during 1990s and majority of the Pusa cultivars released during the same period, exhibited prominently narrower genetic base than the older Punjab cultivars and Introduced cultivars. This is despite the fact that both Pusa and Arka cultivars have been bred from a diverse range of parents. For example, ten different parental lines, including local collections and introductions from USA, Canada and Taiwan were used to develop the eight presently investigated Arka cultivars (Table 1). However, the recent trend towards breeding for a specific plant and fruit type seems to have brought about considerable genetic uniformity among the modern cultivars, despite the use of different parents. Thus, all the eight Arka cultivars are of determinate habit and bear oval-round firm fruits that are high in total soluble solids and lycopene content. In contrast, the older cultivars range from determinate to indeterminate, and bear fruits of diverse shapes, sizes and having different levels of juiciness and acidity.

The trend towards reduction of genetic diversity in modern Indian cultivars as revealed by the present study, has implications on the future programmes of management and use of tomato genetic resources in India. Obviously, search for new genes and breeding for hybrid vigour would best be achieved with the use of new and more diverse materials. The latter could also include local landraces and old varieties that have gone out of cultivation.

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