The genetic diversity and relationship of 42 tomato varieties sourced from different geographic regions was examined with EST-SSR markers. The genetic diversity was between 0.18 and 0.77, with a mean of 0.49; the polymorphic information content ranged from 0.17 to 0.74, with a mean of 0.45. This indicates a fairly high degree of diversity among these tomato varieties. Based on the cluster analysis using unweighted pair-group method with arithmetic average (UPGMA), all the tomato varieties fell into 5 groups, with no obvious geographical distribution characteristics despite their diverse sources. The principal component analysis (PCA) supported the clustering result; however, relationships among varieties were more complex in the PCA scatterplot than in the UPGMA dendrogram. This information about the genetic relationships between these tomato lines helps distinguish these 42 varieties and will be useful for tomato variety breeding and selection. We confirm that the EST-SSR marker system is useful for studying genetic diversity among tomato varieties. The high degree of polymorphism and the large number of bands obtained per assay shows that SSR is the most informative marker system for tomato genotyping for purposes of rights/protection and for the tomato industry in general. It is recommended that these varieties be subjected to identification using an SSR-based manual cultivar identification diagram strategy or other easy-to-use and referable methods so as to provide a complete set of information concerning genetic relationships and a readily usable means of identifying these varieties.