Durum wheat (Triticum durum spp.) is an allotetraploid crop widely grown in the Mediterranean region, especially in Italy, where with bread wheat, it represents the main source of carbohydrates that characterize the local diet. Modern breeding programs have greatly improved yield and technological quality of this plant sometimes resulting in a reduction of genetic variability and potentially making crops more vulnerable to disease and climate change. Therefore novel sources of genetic variability are needed to be employed in future breeding programs. Collections of old varieties and landraces may hold novel variability not present in modern varieties, therefore evaluation of the genetic variation within collections and local landraces is crucial. In this study, nearly 130 old Italian landraces and 20 modern varieties of durum wheat were genotyped using the Axiom® Wheat breeder’s Array with 35 K features. One plant from each accession, grown in a peat based soil inside a glasshouse, was chosen and its Genomic DNA was isolated from the third leaf using a Phenol-Chloroform extraction method treated with RNase-A. Plants were then grown until the ripening of the ear and the seeds produced were collected and will be used as single seed descent lines for the future work. Samples were genotyped with Affymetrix GeneTitan® system and SNP callings were assigned using the software package GTC developed by the same company. SNP data were elaborated to analyze the genetic relationship between the different lines, then a Population structure analysis was inferred with a Bayesian model-based clustering method. Our results show that landraces contain a substantial amount of novel genetic diversity that should be considered in the current breeding program. Collection of old varieties and landraces represent a potentially important source of variability that should be characterized and conserved.