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**Abstract:** DNA genetic variation among eleven selected Jordanian Barley landraces that showed significant morphological differences and the three long-term checks, in addition to two improved varieties (Rum and Acsad-176) was estimated utilizing DNA marker-based Random Amplified Polymorphic DNA (RAPD). Using a set of 5 primers, a total of 349 data points were scored over all of the landraces. The scored data points corresponded to a total of 40 RAPD markers of which 32 markers were polymorphic with a percentage of 80%. A genetic similarity matrix based on Jaccard coefficient was constructed using the generated RAPD data to assess the genetic relatedness. The mean similarity indices ranged from 0.92 to 0.30 with an average of 0.60, which indicated a high DNA polymorphism occurrence among the landraces. Clustering based on genetic similarity indices basically showed clustering of the same row type regardless to collection sites, which indicated that there was agreement between classical classification based on agronomic traits and those generated by RAPD analysis. Of the 16 bulked samples used, there was an average of 96% reproducibility in the two to three replications using five primers. This analysis demonstrated that the RAPD-PCR has proved to be a useful tool to determine the extent of genetic diversity among barely landraces.