In Upland cotton (*Gossypium hirsutum* L.), the Pee Dee germplasm program represents one of the most historically significant Upland cotton breeding programs and is known as a key source of fiber quality genes for commercial cultivars. The foundation of the Pee Dee germplasm is known to represent a wide array of genetic diversity involving the hybridization of *G. hirsutum* L., *G. barbadense* L., and triple hybrid strains (*G. arboreum* L. × *G. thurberi* Todaro × *G. hirsutum* L.). In this study, we characterized genetic relationships within the Pee Dee germplasm collection using molecular marker and field performance data. A set of 82 genotypes was selected for this study to represent the history of the long time Pee Dee germplasm enhancement program. Molecular marker and field performance data showed the Pee Dee germplasm collection maintains useful amounts of genetic diversity. Cluster analyses of the molecular and field performance data effectively separated the Pee Dee germplasm into smaller groups based on genetic diversity. Cotton breeders can use the data presented to make informed parental line selection decisions.