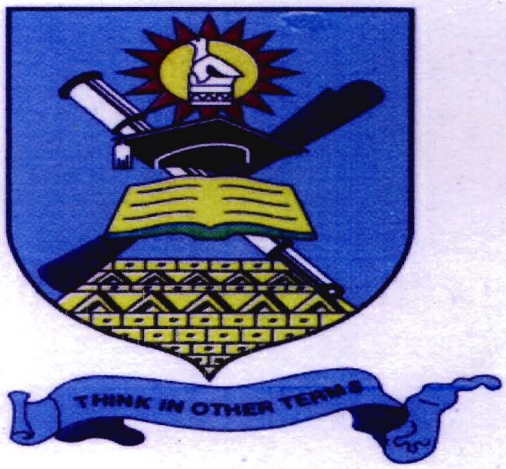

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APPLICATION OF ISSR AND IRAP MOLECULAR MARKERS TO ASSESS GENETIC VARIATIONS IN KUTSAGA TOBACCO VARIETIES

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ABSTRACT

Molecular markers are excellent tools to investigate genetic relations among varieties. Inter-simple sequence repeat (ISSR) and Inter Retrotransposon Amplification Polymorphisms (IRAP) have proven to be useful in differentiating closely related plant cultivars but have also become extremely useful for studies of natural populations of plants, fungi, insects, and vertebrates. In this study fifteen ISSRs and five IRAPs were investigated for their ability to differentiate among ten tobacco varieties. All the primers used were able to amplify the DNA indicating their usefulness but only a few primers produced good polymorphic patterns. The best ISSR primers showing a high number of amplification polymorphic fragments were UBC 826 (85.7%), UBC 834 (83.1%), UBC 845 (97.7%) and UBC 856 (83.3%) and the IRAPs were RTR-2 (77.8%), RTR-7 (64.3%) and RTR-1 (64.2%). Collectively ISSRs had higher amplification polymorphic fragment percentages than IRAPs indicating that ISSRs were the better primers for distinguishing the tobacco varieties. The Principal Coordinate graph summarised the general genetic groupings created among the varieties, with most of the flue cured closely clustered (KRK 26, KRK 28,K30,T61 and T66), KRK 1 a flue-cured variety clustered with BRK 2 and Xanthi. Bikita clustering on its own and *Nicotiana repanda* furthest from the rest. This corresponds with the data generated by the collective dendrogram for both ISSRs and IRAPs which pictorially represents the four major clusters. It was therefore shown that the tobacco germplasm is very narrow in its diversity hence the varieties are closely related making it difficult to differentiate and manipulate them, thus there is need to efficiently identify the distinctive IRAPs and ISSRs from a large pool for efficient tools for differentiation and to generate clear DNA profiles. This can be done through intensive optimisation of PCR reaction conditions and agarose gel percentages.