# SUMMARY

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**Assessing the Genetic Variability of Basil *(Ocimum)* Cultivars Using ISSR Markers.**

**MSC. In Agricultural Biotechnology (Plant)**

Basil *(Ocimum)* is a well-established plant whose economic importance, agronomic value and ecological impetus is deserving research. To understand *Ocimum* in an impactful approach, it`s clear that researchers have to define it`s taxonomic boundaries. Basil hails from the Lamiaceae family, it is documented to have about 50 to 150 species (Avetisyan et al. 2017). The taxonomical intricacies in basil are attributed to several factors like crossbreeding in basil species that allows gene flow (Avetisyan et al. 2017). Its uses span from pharmaceutical therapy, antimicrobial activity, culinary and also in the ornamental industry (Cohen, 2014). While there are numerous examples of basil species, the following are the most common; *Ocimum sanctum, Ocimum gratissimum, Ocimum viride, Ocimum baslicum, Ocimum americanum, O. kilimandscharicum Guerk.* Different molecular markers present a plethora of novel platforms to significantly study the variation in species and cultivars, in this study, ISSR primers were the choice, the technique is based on the variation of the regions between microsatellites (György, 2022). Our study had the following objectives; (i) to assess the genetic variation within 26 *Ocimum* cultivars using 19 ISSR primers. (ii) To understand if there could be any morphological resemblance between clustered species. The overall hypothesis was that there was a clear delimitation between the studied cultivars either based on the species or morphological traits. The plant materials used in this experiment were obtained from the MATE Institute of Horticulture gene bank. The ISSR primers produced DNA fingerprints in *Ocimum* cultivars with an average polymorphism of 94.85% with only 2 of the tested primers being monomorphic. ISSR 888 generated the highest number of bands with the average count of bands per primer being 6. The average PIC value was 0.32 and this ranged from 0.285 to 0.419. The typical range of the band sizes was between 250 bp to 2500 bp measured against a 1kb ladder. Detailed analysis was done as cluster analysis and PCoA. The hierarchical clustering was obtained by calculating the Jaccard similarity index between the cultivars whereby the genetic variation between the 18 selected accessions was high with the Jaccard similarity distance values ranging between 0.289 to 0.897. The maximum similarity index (0.897) was observed between ‘Red Rubin’ (*O. basiclicum)* and ‘Purple Ruffles’ (*O. basiclicum*) while ‘Corsican’ (*O. basiclicum*) and ‘Rama Tulsi’

(*O. sanctum/ tenuiflorum*) showed the smallest index (0.289). This then meant that 'Red Rubin' and 'Purple Ruffles' were comparatively closely related while 'Corsican' and 'Rama Tulsi' cultivars were far relatives. The PCoA analysis showed a largely congruent visualization of clusters as depicted in the earlier analysis on the UPGMA dendrogram and also the Jaccard similarity indices. The accessions were clustered in the same groupings as explained in the dendrogram. Of the 26 samples, 18 showed a good amplification using 11 primers out of the 19. It was observed that; (i) the PCoA clustering had the *Ocimum basilicum* in 2 subtle clusters with other *O. basilicum* accessions spread throughout the plot. (ii) Despite the observed clustering there were no clear morphological traits that would point out commonality. The other species were clearly separated from the rest but their position could not be attributed to traits because they were individual species and no average result could be gotten from them. It was clear that using the selected ISSR markers, the genetic relationship between the selected cultivars could be clustered however this needs a large sample of data and a high number of replicates just to be sure of the occurrence as there were slight overlaps between the species and also no distinct clustering of same species. In this study, 11 markers worked well, they had good reproducibility with 71 fragments and very high polymorphism averaging at 94.85%. The ISSR markers could isolate the different species though with a few overlaps that could be attributed to the subjectivity of gel scoring. From this study it was also observed that a large sample size and high replication of both the DNA and the primers is needed for a clear analysis. It is also important to take care of amplification efficiency while running the experiment.