

## Comparison of Biochemical and Molecular Markers Genetic Analysis in Niger [*Guizotia Abyssinica* (L.F.) Cass.] Genotypes

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**ABSTARCT:** Variability in 35 accessions of niger (*Guizotia abyssinica*) was assessed for biochemical as well molecular markers. The biochemical parameters include test weight, oil and oil quality parameters. The analysis of variance showed significant differences among accessions for seed weight and oil parameters. Significant genetic diversity reported for test weight (0.44 g to 0.26 g), total oil content (40.47% - 35.16%), oleic acid (23.52-53.3%) and linoleic acid (32.03-58.28%). The range of Manhattan distance coefficient values based on biochemical parameters were 3.46 and 0.23 with a mean of 1.84. Molecular characterization was carried out using 25 ISSR primers. The range of mean genetic similarities between accessions based on Jaccard's similarity coefficient was significantly high (0.1 to 0.95) with mean value of 0.40. The UPGMA clustering was done based on Manhattan distance coefficient for biochemical traits as well as Jaccard's similarity coefficient values for ISSR markers. Although a significant genetic diversity was observed based on ISSR and biochemical markers, but only partial correlation between diversity based on these two parameters was observed. The comparison of genetic diversity based on both biochemical and molecular markers has been made to find out the suitable accessions diverse for use in niger improment programs.

**Key words:** Genetic diversity, *Guizotia abyssinica*, Biochemical parameters, ISSR, Molecular markers

*Guizotia abyssinica* (L.f.) Cass. (2n = 30), commonly known as niger, has been introduced in central parts of India as minor oilseed crop used for human consumption as well as for industrial uses<sup>1-4</sup>. High variability in morphological as well as oil parameters of Indian niger is an encouraging reality and it could be of great help in developing varieties to suit nutritional and industrial uses [5,6]. Niger, being of exotic oilseed crop can be utilized for enhancing food oil production in India. The increase in the quantity of oil per unit area of land is the most important objective in niger improvement program which can be achieved either through increasing the seed yield by breeding for high yielding varieties or through developing varieties with high oil content [5,6]. However, the narrow genetic base of improved niger cultivars has been a limitation [6]. To alleviate this, parents with broad genetic base will be required in the breeding programs. For this studies on genetic divergence for seed oil quality parameters and biochemical parameters in germplasm will be helpful

in finding out the suitable parents for breeding for oil and oil parameters in niger. However, in order to ensure the broad genetic base of parents, it requires further validation through genome analysis using more reliable molecular markers. In niger, ISSR-based markers have demonstrated reasonably high level of genetic diversity [5].

The comparative genetic diversity study for both biochemical as well as molecular markers will further validate the suitability of potential genotypes for niger crop improvement programs.

### MATERIALS AND METHODS

#### *Plant Material*

A total 35 accessions, selected for the present analysis were subjected to biochemical analysis as well as characterized using ISSR markers and found to be diverse for both parameters. The accessions with high variability for oil content, palmitic acid, stearic acid, oleic acid, linoleic acid were used for ISSR analysis.

### Diversity Analyses

The diversity analysis for oil parameters was done using Manhattan distance coefficient based dissimilarity matrix was used to construct dendrogram by unweighted pair group method for arithmetic mean (UPGMA) based Sequential Agglomerative Hierarchical and Nested (SAHN) clustering. was calculated in pairwise combinations. The Principal Component Analysis (PCA) based on the Euclidean coefficients of dissimilarity was done.

For ISSR markers, the pair-wise genetic similarities were computed using Jaccard's similarity coefficients and corresponding dendrogram of genetic relatedness was constructed by applying Unweighted Pair Group Method with Arithmetic mean (UPGMA) clustering algorithm. Principal coordinate analysis (PCoA) was performed to generate a correlation matrix. "Bootstrap" analysis was done using the software WINBOOT [11]. All these calculations were done using NTSYS-pc version 2.11 (USA) [18].

## RESULTS AND DISCUSSION

### Cluster analysis

The range of Manhattan distance coefficient values for all the 35 accessions were 3.46 and 0.23 with a mean value of 1.84. The range of coefficient values for accessions from Maharashtra were 0.31-2.75 with a mean value of 1.15 while for 8 accessions of Madhya Pradesh, the range was 0.52-2.11 with a mean value of 1.18 (Table 1). Pair-wise genetic similarities based on Jaccard's similarity coefficient ranged from 0.1 to 0.9 in all the accessions with mean values of 0.5. Genetic similarity among the group of genotypes

with a common geographic origin was also studied on the basis of Jaccard's similarity coefficient. The pairwise similarity range was 0.10-0.95 with a mean range of 0.40 for 35 accessions. The 22 genotypes from Maharashtra showed highest range of similarity (0.11 to 0.95) with a mean value of 0.4. Similarly, the range of similarity for four accessions from Jharkhand was 0.30 to 0.63 with a mean value of 0.4. Maharashtra state contributed maximum variability for biochemical parameters, followed by Madhya Pradesh, which have the major areas under niger cultivation. Similarly, for ISSR markers, diversity contributed by accessions from Maharashtra was maximum. This might be due to the reason that all the germplasm collected from within India as well imported from outside India has been maintained and evaluated at NBPGR Regional Station at Akola, Maharashtra. The same relationship was revealed in clustering based on oil parameters explained by Yadav *et al.* (2012) [6]. Comparison of matrices of ISSR and oil parameters data shows a low correlation between dendrograms following 500 random permutations with the Mxcomp procedure from the NTSYS program. The low correlation had been reported in other studies in *Jatropha curcas* [21]. Similar results were found in niger as differences between dendrograms generated from morphological and ISSR data were detected [7]. The morphological and molecular differences are apparently independent, due to different selection and evolutionary factors [7]. Although ISSR markers can cover a high proportion of the genome because of availability of the high number nucleotide repeats in genome, there is no guarantee that such bands fall in coding regions of the genome involved in traits

**Table 1. Comparison of Manhattan distance coefficient and Jaccard's similarity coefficient values of niger genotypes from various geographical regions**

Origin	Number of genotypes	Total Oil (%)	Palmitic acid (%)	Oleic acid (%)	Linoleic acid (%)	Manhattan distance coefficient		Jaccard's similarity coefficient	
						Mean	Range	Mean	Range
Total	35	35.4 - 40.1	8.3-9.4	26.1-53.0	32.3-55.6	1.84	0.23-3.46	04	0.10-0.95
MH	17	37.3-38.0	8.3-9.3	29.0-50.6	33.7-53.4	1.15	0.31 - 2.75	0.4	0.11 - 0.95
OT	18	35.2-40.5	8.4-9.5	23.5-47.8	36.4-58.2	1.18	0.53 – 2.11	0.4	0.3 – 0.63

Note: MH-Maharashtra, OT- Accessions from other state

related to oil parameters. Geographically isolated populations accumulate genetic differences as they adapt to different environments. A strong correlation between the genetic distances and the geographic distances between the populations of the taxa of niger has been reported [17].

The two most diverse accessions with highest genetic distance (3.46) were IC372586 and IC211078, however, for these accessions, the Jaccard's similarity coefficient value was 0.43, indicating no correspondence between two parameters. The most distant accession based on ISSR markers was IC268291, collected from Madhya Pradesh with Jaccard's similarity coefficient pairwise values (<0.20) with most of accessions. The various parameters of IC268291 viz., Oil (38.36%), Palmitic acid (7.75%), Oleic acid (35.14%), Linoleic acid (47.93%) and stearic acid (9.18%). These make it a suitable parent for further hybridization programs due to its high linoleic acid (47.93%) values and low values of oleic acid (35.14%) simultaneously with distant genetic base. Such genotypes help in broadening the genetic base of varieties developed from such crosses as such varieties have better potential for tolerance to various biotic and abiotic stresses.

The lowest Manhattan coefficient value (0.23) was found between genotypes IC372535 and IC372578. However, the Jaccard's similarity coefficient value was 0.59. Manhattan dissimilarity coefficient based clustering could discriminate the entire germplasm was grouped into three major clusters and the collections from eastern region were clearly separated from the genotypes collected from the central zones. Different genotypes were segregated into three clusters according to the geographical zones suggesting that some specific traits remain confined to certain geographical region than to other regions.

Principal Component Analysis (PCA) is a multivariate technique that allows one to find and plot the major patterns within a multivariate data set (e.g., multiple loci and multiple samples). PCA is a process by which the major axes of variation are located within a multidimensional data set. In order to assess the patterns of variation, PCA was done by considering all the 35 variables, simultaneously, two

of the principal components accounted for more than 81% of the total variation encountered. However, PCoA analysis revealed more uniform distribution of diversity across the principal components and a total of 11 PC accounted for 81% diversity.

There was low genetic variability in genotypes for the test weight and total oil content, generally used parameters for preparing the core collection, the variability in fatty acid parameters that are underestimated, was significantly high. Similarly, the ISSR study indicated significant genetic diversity. On the basis of present findings it is evident that lot of genomic diversity exists among the niger accessions which are diverse for the oil quality parameters as well as ISSR markers. The findings in the present study will be helpful in finding out robust genetic diversity analysis techniques for finding suitable genotypes for pre-breeding for generating variability for selection of superior lines for oil quality, validation of core based on morphological and biochemical parameters, developing the mapping populations, tagging genes for oil quality traits and devising effective germplasm exploration and conservation strategy [5-6].

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