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Assessment of Genetic Diversity of Niger Plant (*Guizotia abyssinica* L.) in Moiben Sub County, Kenya, Using Inter Simple Sequence Repeat Markers

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ABSTRACT

Niger plant (*Guizotia abyssinica*), exhibits phenotypic plasticity in different environments. There is need to assess its genetic diversity since guizotia species has a high number of species which may be confused amongst themselves. To achieve this, inter simple sequence repeat (ISSR) markers were used to estimate genetic diversity among 12 wild populations of Niger plant from Moiben sub-county. Total genomic DNA was extracted as per the cetyltrimethylammonium bromide (Ctab) method and subjected to ISSR analysis using 20 primers. None of the primers produced unique banding patterns. ISSR data were used to calculate a squared-euclidean distance matrix. All the twenty primers (100%) gave polymorphic bands thus they were all considered for further analysis. The allele frequency of all the primers was below 0.95 indicating that they were all polymorphic in character. Gene diversity was high ranging from 0.3550 to 0.7337 with a mean value of 0.6302. The ISSR based upgma clustering produced four clusters. Niger plant within Moiben sub-county was found to be genetically diverse though heterozygosity was not noticed. The study recommends further analysis of Niger plant so as to form a basis for further development of the plant.

Keywords- Guizotia, diversity, heterozygosity, phenotypic, DNA.

I. INTRODUCTION

Niger plant (*Guizotia abyssinica*) is exclusively diploid (2n=30) and a completely outcrossing species with self-incompatibility. It is an annual plant originating from Ethiopia where it is under cultivation for edible oil. The plant has bright yellow flowers, pollinated by insects, mainly bees. Taxonomically, Niger plant belongs to the family Asteraceae (Compositae), tribe Heliantheae and sub tribe Coreopsidinae.

Niger plant exhibits a high variability of morphological characteristics as influenced by the prevailing environmental conditions such as rainfall, temperature, altitude, growing period and edaphic factors. This makes morphological identification of varieties difficulty since these characters are not discrete. DNA markers provide a powerful tool for genetic evaluation and marker-assisted breeding of crops and especially for cultivar identification (Rai *et al*., 2010). DNA-marker technology detects more polymorphisms and is not influenced by prevailing environmental conditions. These DNA markers can identify many genetic loci simultaneously with an excellent coverage of the entire genome, are phenotypically neutral and can be applied at any development stage (Genet *et al*., 2005). ISSR markers, just like any other PCR-based markers, are rapid and require only a small amount of the template DNA. ISSR information does not require genome sequence information but produce highly polymorphic pattern.

Genetic diversity is a product of interplay of biotic factors, physical environment, artificial and plant factors (Frankel *et al*., 1995). It refers to the total number of genetic characteristics in the genetic makeup of a species and serves as a way for populations to adapt to changing environments. With more variation, it is more likely that some individuals in a population will possess variations of alleles that are suited for the environment thus those individuals are more likely to survive to produce offspring bearing that allele (NBII, 2011). Knowledge of genetic diversity and relationship among sets of germplasm is beneficial to all phases of crop improvement (Geleta *et al*., 2007).

The purpose of the present study was to investigate, through the use of ISSR markers, the genetic diversity of randomly collected Niger plant in Moiben sub-county. This is the first attempt to estimate genetic variability among Niger plant in Kenya in an effort to provide some information as a basis for future research.

II. MATERIALS AND METHODS

Study site

The plant material studied was collected from all the administrative wards in Moiben sub-county. Moiben sub county stands at an altitude of 2163 m above sea level and at 0°49′N - 0.82°N and 35°23′E and 35.38°E. The area has a bimodial rainfall pattern where long rains are experienced between April to July and short rains between September and November. The area dominantly falls under upper midland agro ecological zone with agriculture as the main economic activity. Maize and wheat are the main crops grown for both commercial and subsistence purposes. Here, cattle rearing is also practiced.

Plant material

Tender leaves of twelve Niger plant wild populations were collected randomly from all administrative wards within Moiben sub-county. A single collection from one site was considered a population. Since it was not possible to obtain a sample of characterized Niger plant, sunflower (*Helianthus annus*) was used as a check since the two belong to the same family. The samples were collected into zip lock bags, packed into a cool box and transported to the University of Eldoret where they were stored in a freezer until use. Certified sunflower seed was planted in University of Eldoret green house for one month. Tender leaves were harvested for genetic analysis.

DNA extraction procedure

This procedure was proposed by Doyle *et al.*, 1987. Tender leaves that had been kept in the freezer were ground into fine powder using a motor and pestle under an extraction buffer. The extracts were transferred to appendorf tubes each containing 500 µl of 2 x CTAB mecaptoethanol extraction buffer and the samples transferred to ice. This followed incubation in a water bath at 65^{o}C for 1 hour. The tubes were shaken and inverted every fifteen minutes. This was followed by addition of 500 µl of chloroform-isoamyl alcohol (24:1) and inversion for 5 minutes at room temperature to mix. The mixture was then centrifuged at 14000 rpm for 10 minutes. Without disturbing the bottom layer, 400 µl of the top clear layer was pipetted into fresh labelled appendorf tubes and 250 µl of isopropanol added. The contents were then gently mixed by inversion and then incubated at room temperature for 10 minutes.

The mixture was the centrifuged at 14000 rpm for 10 minutes to pellet the DNA. The supernatant was then gently discarded using the yellow tips and 320 µl of 1 x TE was added. The samples were placed on ice. A further 40 µl of magnesium chloride was added and the contents incubated on ice for 10 minutes followed by a centrifugation to 14000 rpm for 10 minutes and the supernatant discarded. The pellet was then vacuum dried for 5 minutes before adding 5 µl of R-nase enzyme and placed on a water bath set at 37^{o} C for 2 hours. 40 µl of sodium acetate was added followed by 250 µl of isopropanol and the contents incubated for 15 minutes at room temperature. This was followed by a 10 minute centrifugation of 14000 so as to re-pellet the DNA and the supernatant was discarded.

A 1 ml aliquot of 70 % ethanol was then added to the pellet followed by another centrifuge of 14000 rpm for 5 minutes. The supernatant was discarded followed by a quick spin for 2 minutes. The supernatant was then gently discarded and any liquid from the tube drained off using a clean tissue paper. The pellet was then dried for 3 minutes to remove any remaining liquid and the DNA pellet re-suspended in 50 µl of 1 x TE. It was then left to stand for 10 minutes at room temperature before storing at 4^{o} C.

DNA quantification

The quality and quantity of the DNA was verified by electrophoresis on a 0.8 % (w/v) agarose gel, for 45 minutes at 80 volts. Lambda phage DNA was used as the standard. After electrophoresis, the gel was stained in ethidium bromide (10 mg/ml) for 30 minutes and later de-stained in distilled water for 20 minutes before viewing under ultraviolet transilluminator. The concentrations of the samples were determined by comparing band sizes and intensities of the test DNA with those of standard lambda DNA. Between 0.5 µl and 1 µl of high quality DNA was obtained and was diluted to 0.02 µg/µl with TE buffer water for the PCR amplification. The procedure was proposed by Allagher (2011).

PCR amplification

The PCR cycles consisted of 94^{o} C for 3 minutes for initial denaturation, 94^{o}C for 3 minutes for actual denaturation, annealing at 56^{o}C for 30 seconds, extension at 72^{o}C for 1 minute followed by 34 cycles of 30 seconds at 94^{o}C, 1 minute at 56^{o} C, for 1 minute at 72^{o} C and a final extension step of 5 minutes at 72^{0} C.

The DNA fragments were separated on 4 % agarose gel run at 100 volts (V) for 2 hours using 0.5 M TBE buffer. The DNA fragments in gel were visualized by staining in 0.5 µl/mg ethidium bromide for 30 minutes and rinsed in distilled water for 20 minutes, visualized and photographed on ultraviolet trans-illuminator at 312 nm. Allele sizes were scored using a 1000 base pairs (bp) molecular size ladder. This procedure was proposed by Allagher (2011).

Data scoring and statistical analysis

Each ISSR fragment was considered as a simple bi-allelic locus with one amplifiable and one null allele. PCR amplification profiles of the 12 Niger plant genotypes and 1 sunflower genotype that was used as a check from the same family were scored by visual observation. The presence of amplified bands at each position was recorded as 1 (one) and its absence as 0 (zero). The pair-wise genetic similarities were computed using Jaccard's similarity coefficient and a corresponding dendogram of genetic relatedness was constructed by applying Unweighted Pair Group Method with Arithmetic Mean (UPGMA) clustering algorithm.

Determination of genetic similarities

Similarity matrices for ISSR were subjected to Unweighted Pair Group Method with Arithmetic Mean (UPGMA). A dendogram was constructed from molecular data by the Unweighted Pair Group Method (UPGM) and clustering using sequential agglomerative hierarchical nested (SAHN) program and tree plot of Numerical Taxonomy Multivariate Analysis System Package (NTSYS-pc) software, version 2.1.

Polymerase Chain Reaction (PCR) and agarose gel electrophoresis

The ISSR primers used for polymerase chain reaction were as shown in Table 1 below.

Table 1: List of primers and annealing temperatures

No.	Primer number	No. of pb	Primer sequence	Annealing temp (°C)
1	2903	14	ACACACACACACACYT	43
2	2904	5	BDBCACACACACACA	39
3	2906	5	HVHTGTGTGTGTGTG	40
4	2909	15	AGAGAGAGAGAGAGAGC	44
5	2910	8	GAGAGAGAGAGAGAT	41
6	2911	13	AGAGAGAGAGAGAGC	44
7	2922	9	AGAGAGAGAGAGAGC	44
8	2923	8	AGAGAGAGAGAGAGG	44
9	2924	5	GAGAGAGAGAGAGAT	41
10	2934	5	GTGTGTGTGTGTGTGC	44
11	2939	5	ACACACACACACACT	41
12	2941	10	ACACACACACACACACG	44
13	2955	18	GAGAGAGAGAGAGAGAYC	47
14	2956	7	GAGAGAGAGAGAGAGAYG	47
15	2961	9	CACACACACACACACARCG	48
16	2964	9	GTGTGTGTGTGTGTGTYC	45
17	2976	11	AGCAGCAGCAGCAGCAGC	52
18	2998	4	HBHAGAGAGAGAGAGAG	39
19	2999	5	BHBGAGAGAGAGAGAGA	39
20	3013	8	ACTTCCCCACAGGTTAACACA	48

III. RESULTS AND DISCUSSION

Microsatellite (ISSR) analysis

The study involved the use of twenty primer sets to amplify DNA extracts from 12 wild Niger plant populations and 1 sunflower genotype. All the twenty primers (100%) gave polymorphic bands thus they were all considered for further analysis. This percentage was higher than that reported by Yohannes *et al.*, (2011) who obtained 89.83%. A total of 73 alleles were detected. The number of alleles detected per locus ranged from 2 to 5 with an average of 3.65 (Table 2).

Table 2: Polymorphism, diversity and frequency results

Marker	Major allele frequency	Sample size	Allele no.	Gene diversity	PIC
P2903	0.4615	13	5	0.7101	0.6731
P2904	0.4615	13	4	0.6746	0.6197
P2906	0.3846	13	4	0.7219	0.6727
P2909	0.7692	13	2	0.3550	0.2920
P2910	0.7692	13	2	0.3550	0.2920
P2911	0.4615	13	3	0.6154	0.5353
P2922	0.3846	13	4	0.6982	0.6409

P2923	0.4615	13	5	0.6982	0.6560
P2924	0.6154	13	3	0.5444	0.4836
P2934	0.3846	13	4	0.7101	0.6580
P2939	0.5385	13	3	0.5562	0.4652
P2941	0.4615	13	5	0.7101	0.6731
P2955	0.3846	13	4	0.7219	0.6727
P2956	0.5385	13	3	0.6036	0.5361
P2961	0.3846	13	4	0.7101	0.6580
P2964	0.3846	13	4	0.6746	0.6130
P2976	0.3077	13	4	0.7337	0.6841
P2998	0.5385	13	3	0.6036	0.5361
P2999	0.6154	13	3	0.5444	0.4836
P3013	0.4615	13	4	0.6627	0.6039
Mean	**0.4885**	13	**3.6500**	**0.6302**	**0.5725**

Polymorphic information content (PIC) of markers

The markers with high polymorphic information content of more than 0.5 were P2976, P2941, P2903, P2955, P2906, P2961, P2934, P2923, P2922, P2904, P2964, P3013, P2998, P2956 and P2911 with 0.6841, 0.6731, 0.6731, 0.6727, 0.6727, 0.6580, 0.6580, 0.6560, 0.6409, 0.6197, 0.6130, 0.6039, 0.5361, 0.5361 and 0.5353 PIC respectively. Markers P2999, P2924, P2939 and P2909 had a PIC of 0.4836, 0.4836, 0.4652 and 0.2920 respectively (Table 2).

The polymorphic information content (PIC) value was calculated to characterize the capacity of each primer to detect polymorphic loci which ranged from 0.2920 to 0.6841 with a mean of 0.5725. The result showed that most of the primers were highly informative and can be used to study phylogenetic relationship and genetic diversity in future. The allele frequency of all the primers was generally below 0.95 indicating that they were all polymorphic in character Asare *et al*., (2011). Gene diversity was high ranging from 0.3550 to 0.7337 with a mean value of 0.6302.

An Unweighted Pair Group Method with Arithmetic Average (UPGMA) dendrogram based on pair wise comparison of genetic distance of 12 Niger plant genotypes based on ISSR data was as shown below (Figure1). Populations from Chepkoilel, Kimoning, Kaptuktuk, Merewet, Tembelio, Kapsaos and Kimumu were grouped together in one cluster, while Sergoit formed its own distinct cluster. Three populations (Huruma, Moiben and Sigot), were in the same cluster leaving out Cheplaskei with its own cluster. The genetic distance revealed that the closest genotypes were Sunflower, Merewet and Tembelio as shown by Figure 1.

The high genetic diversity exhibited by the Niger plant can be ascribed to the outcrossing mode of pollination exhibited by the plant. Niger plant is pollinated by insects, mainly bees Getinet and Sharma, 1996. There was no heterozygosity observed from the results. This is in contrast with Zakir *et al.*, (2015) who did a study in Ethiopia using ISSR and obtained a heterozygosity ranging from 0.245 (in primer 2976) – 0.497 (in 2939 and 2904). Lack of heterozygosity is a subject of further research with different primers to ascertain the true position. Since Niger plant grows wild in the area of study, breeding is not controlled and the plant relies on natural pollination. Growing in diverse environmental conditions within Moiben sub-county, the plant might have been forced to evolve differently for its survival thus the high genetic diversity.

While the general trend in the UPGMA clustering is that of grouping populations by region of origin and proximity of geographic location of the collection sites, not all populations, however, belonging to the same region were grouped together in the same cluster (Figure 1). This observation was also made by Yohannes *et al*., (2011). This could be because of the continuity of the Niger growing areas which makes the transfer of seed materials from one region to the other possible. Being perceived as a weed, Niger plant growth in the region remains uncontrolled. The fact that Niger plant is strictly cross pollinated aggravates the situation.

The genetic distance between Cheplaskei population and those from other areas is large indicating some sort of genetic isolation of the Cheplaskei population from those of other regions. This led to Cheplaskei populations forming a separate cluster in the dendogram clustering.

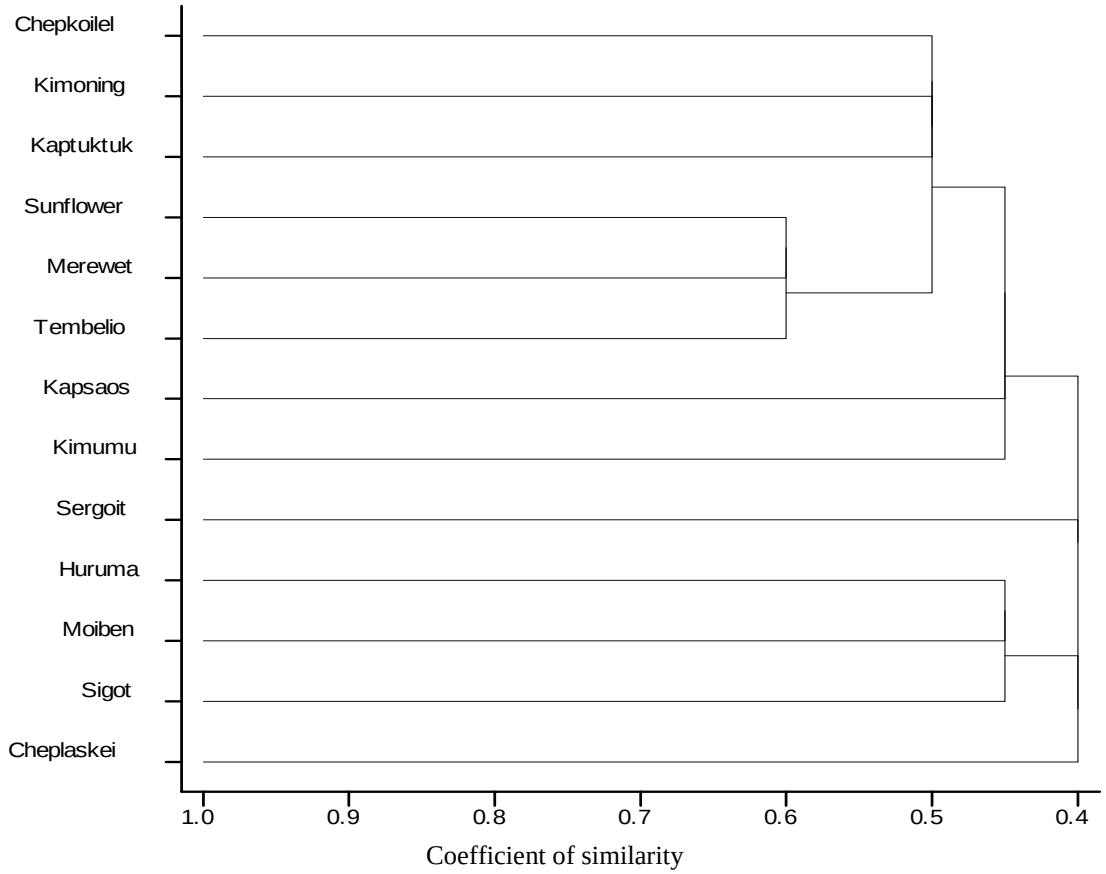

Figure 1: Clustering by region for Niger plant from Moiben sub-county

Sunflower* is not a site but is a plant that was used as a check because it is in the same family with Niger plant.

IV. CONCLUSIONS AND RECOMMENDATIONS

Conclusions

From the findings of the study, it can be concluded that Niger plant populations within Moiben sub-county were genetically diverse. A deviation from the earlier researches was that there was no heterozygosity in the Niger plant populations studied.

Recommendation

Further study should be carried out in other areas with different agro-ecological zones to so as to have reliable results thus conclude appropriately.

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