Molecular Characterization of Global Finger Millet (*Eleusine coracana*, L. Gaertn) germplasm Reaction to *Striga* in Kenya

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Authors’ contributions

This work was carried out in collaboration between all authors. Author SPN designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors DOG and ODA managed the analyses of the study. Authors WDS and OC managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Finger millet (*Eleusine coracana*, L. Gaertn) is an important food crop in Africa and Asia. The parasitic weed *Striga hermonthica* (Del.) Benth limits finger millet production through reduced yield in agro-ecologies where they exist. The damage of *Striga* to cereal crops is more severe under drought and low soil fertility. This study aims to determine genetic basis for reaction to *Striga hermonthica* among the selected germplasm of finger millets through genotyping by sequencing (GBS). One hundred finger millet genotypes were evaluated for reaction to *Striga hermonthica* infestation under field conditions at Alupe and Kibos in Western Kenya. The experiment was laid out...
INTRODUCTION

Food security exists when all people have physical, social, and economic access to sufficient, safe, and nutritious food that meets their dietary needs and food preferences for an active and healthy life [1]. According to Fahey [2], food security could improve if focus could be on the locally important crops such as finger millet, commonly known as orphan crops. Finger millet has been the most important minor millet in the tropics and is grown in more than 25 countries where Africa and Asia, accounts for 12% of the global millet area [3]. The demand of finger millet is high in Kenya and fetches prices of over twice that of sorghum and maize in local markets [4]. The major biological constraint to increased finger millet production by small holder (SH) sector in Africa is attack by Striga or witch weeds [5]. Striga hermonthica is particularly harmful to sorghum, maize and millet. It is also increasingly being found in sugar cane and rice fields [6]. Crop yield losses may be up to 100% when susceptible cultivar is grown under high level of infestation [7]. Parasitic weeds such as Striga hermonthica compete with crop for nutrients, water and also by harbouring disease causing organisms [8]. The parasitic weeds lack their own root system and therefore compensate this by penetrating the roots of the host plants, depleting them of essential nutrients for growth resulting to stunted growth and finally low yields [9].

In Kenya Striga infects about 210,000 ha of land, causing crop losses of US$ 40.8 million annually [10]. These losses largely depend on the level of infection, crop variety, soil fertility and rainfall patterns. The greatest impact is on the infertile soils and most affected being subsistence farmers [11]. The control of Striga hermonthica in cereals has proven elusive. The presence of Striga and its interaction with host plant leads to high yield loss of 10-70%, especially under heavy infestation depending on crop cultivar [12]. Economically feasible and effective technologies are still to be developed for the cash strapped subsistence farmers in most Striga stricken areas [13]. Research on Striga control has been carried out for a long time and a wide range of technologies developed that have not been widely adopted due to mismatch between technologies and the farmers’ socio-economic conditions [6]. The control of the weed has proven difficult because of its high fecundity and it’s biology that allows the seed to remain viable underground for more than 10 years allowing it to persist and increase in magnitude [14]. Moreover complete control of Striga on cereals has been a challenge to scientists for a long time and therefore the need to search for farmer satisfying strategies. For a long time crop improvement through conventional breeding has been going on but it’s slow, especially for traits controlled by quantitative gene action like Striga resistance and given the fact that the plant mainly is self-fertile with some amount of cross pollination (<1%) mediated by wind Jansen and Ong [15] and seldom by insect pests [16].

The major challenge therefore is to develop methods or varieties that will help small scale farmers control Striga effectively within a sustainable and profitable farming systems [17]. According to Scholes and Press [18], the use of resistant crop cultivars is considered one of the most effective strategies; however, their effective deployment has been limited due to lack of understanding of genetic and phenotypic basis of

Keywords: Striga hermonthica; genotyping; genome; susceptible; genetic diversity.
adaptation of *Striga* population to their new host resistance phenotypes. Considering the wide range of distribution of *Striga* spp., limited studies on genetic diversity have been conducted in Kenya [19]. Similarly finger millet genotypes tolerant to *Striga* infestation have not been developed. Therefore knowledge of the extent and distribution of genetic variation within finger millet could be an important tool for efficient collection, conservation and development of improved varieties against *Striga*.

Evolution of molecular markers has primarily been driven by the through put and cost of detection method and the level of reproducibility [20]. Among the most popular markers used in plant genetics are; RFLP, AFLP, RAPD and SSR. GBS was selected for this study because it’s low cost for reduced representation sequencing, highly polymorphic, amenability to automation and robust simplicity for genome wide profiling of complex populations. In addition it uses a wide range of restriction enzymes to reproducibly capture a targeted region of the genome, allowing for high level of multiplexing while obtaining sufficient sequencing coverage [21], whose applications include genetic mapping, assaying genetic diversity/germplasm characterization, population structure, and genomic selection [22].

The method has also the potential to simultaneously discover and score segregating markers in populations of interest. An approach incorporating most resistance mechanisms and screening approaches becomes the best way forward to the overall management of *Striga*. Similarly, identification and adoption of *Striga* resistant genotypes could be a feasible cost-effective solution to finger millet production in soils infested by *Striga*. The main objectives of this study was to determine genetic basis for reaction to *Striga hermonthica* among the selected germplasm of finger millet through (GBS). The study screened Kenyan and Internationally sourced selected finger millet accessions for *Striga* resistance, study the mechanisms of resistance and determine the overall genetic diversity among the finger millet germplasm using GBS protocol. The main aim for field experiment was to investigate the effect of *Striga* on morphological traits, analyze and categorize the genotypes as either resistant or susceptible to *Striga* based on population of *Striga* emerging as per the genotype. This was followed by molecular analysis through genotyping by sequencing to confirm the source of variation among the genotypes in response to *Striga* reaction and finally determine the genetic diversity in the selected germplasm.

2. MATERIALS AND METHODS

One hundred finger millet genotypes of unknown genetic background to *Striga* reaction both local and international accessions obtained from breeding programme in Kenya at Kenya Agricultural and Livestock Research Organizations (KALRO) Kakamega. They were grown to both under *Striga* and no *Striga* conditions at two agroecological environmental conditions during two rainy seasons at Kibos and Alupe. *Striga* seeds were collected from the experimental localities and used as inoculum for artificial inoculation. Alupe lies at an altitude of 1189 m above sea level, latitude of 0°29’ N and longitude of 34°08’ E. The soil is Ferralo-orthic Acrisol with pH of 5.0 [23]. Kibos lies at an altitude of 1135 m above sea level, latitude 0°S and longitude 34°49’ E. The soil is black cotton with clay loamy and pH of 6.55. The two study sites are located in regions that are *Striga* endemic.

Field screening for *Striga* resistance was done in two seasons, during long and short rainy season. The seeds of finger millet in long rainy season were planted on 10th June, 2012 at Alupe and on 20th June, 2012 at Kibos. After harvesting, the collected seeds were planted at KALRO Alupe on 19th September 2012 and at Kibos on 23rdSeptember for the second rainy season trials.

The experimental design was a 10 x 10 triple lattice. A plot was made of three rows of 2 meters length spaced 30 cm apart between rows and later thinned to intra-row spacing of 15cm. Plots were spaced 50 cm apart and replications separated by 1 m paths. Planting was in shallow furrows where DAP basal fertilizer was applied followed by seed drill before being loosely covered. For the inoculated plots, a *Striga* seed and sand mixture were applied by drill before fertilizer and seed application. Because *Striga* seeds are too tiny (200 to 400 µm), they were mixed with ½ kg of sterilized sand to serve as the carrier before being drilled into furrows of respective plots for the purpose of providing adequate volume of *Striga* seeds for rapid infestation [24]. Three weeks after germination of finger millet, the rows were thinned to 15 cm intra-row spacing. Weeding was done three times throughout the crop season. However, the removal of weeds from finger millet plots...
Table 1. The 100 finger millet accessions used in the experiment

| Entry no | Geno type | Entry no | Geno type | ENTRY no | Geno type | Entry no | Geno type | Entry no | Geno type |
|----------|-----------|----------|-----------|----------|-----------|----------|-----------|----------|-----------|
| 1        | I.E 4491  | 21       | GBK000463 | 41       | KACIMI20  | 61       | GBK008278 | 81       | GBK029798 |
| 2        | I.E 6165  | 22       | GBK027300 | 42       | KACIMI6  | 62       | GBK008292 | 82       | GBK029820 |
| 3        | I.E 4497  | 23       | I.E 4816  | 43       | KACIMI65 | 62       | GBK008299 | 83       | GBK033414 |
| 4        | I.E 6537  | 24       | I.E 2217  | 44       | KACIMI17 | 64       | KACIMI77  | 84       | GBK033416 |
| 5        | OMUGA-P   | 25       | KACIMI7   | 45       | GBK008292 | 65      | GBK029199 | 85       | GBK039217 |
| 6        | KACIMMI15 | 26       | KACIMMI47 | 46       | KACIMMI24 | 66      | GBK029678 | 86       | GBK043268 |
| 7        | I.E 4115  | 27       | VL 149    | 47       | KACIMMI49 | 67      | GBK029715 | 87       | GBK000369 |
| 8        | GBK029661 | 28       | GBK043081 | 48       | KACIMMI72 | 68      | GBK029722 | 88       | UFM 138   |
| 9        | I.E 5870  | 29       | OKHALE-1  | 49       | KACIMMI42 | 69      | GBK029724 | 89       | GBK000482 |
| 10       | KACIMMI11 | 30       | OMUGA-G   | 50       | GBK000516 | 70      | GBK03821  | 90       | GBK000909 |
| 11       | I.E 5306  | 31       | P 224     | 51       | GBK000692 | 71      | GBK040568 | 91       | GBK008348 |
| 12       | I.E 2957  | 32       | P224 CV   | 52       | GBK008339 | 72      | GBK000409 | 92       | GBK033446 |
| 13       | PR 202    | 33       | P 283     | 53       | GBK029701 | 73      | GBK000449 | 93       | U15XP283  |
| 14       | GBK000451 | 34       | P4C3      | 54       | GBK029793 | 74      | GBK000462 | 94       | GBK000784 |
| 15       | I.E 5873  | 35       | SERERE-1  | 55       | GBK029805 | 75      | GBK000493 | 95       | GBK000831 |
| 16       | I.E 4795  | 36       | U-15      | 56       | GBK029821 | 76      | GBK000568 | 96       | GBK026992 |
| 17       | I.E 2606  | 37       | N-BROWN   | 57       | GBK029847 | 77      | GBK0011082 | 97     | GBK000900 |
| 18       | I.E 2440  | 38       | GULU-E    | 58       | KACIMMI36 | 78      | GBK011113 | 98       | GBK000549 |
| 19       | I.E 6337  | 39       | BUSIBW-1  | 59       | GBK000802 | 79      | GBK011126 | 99       | GBK029807 |
| 20       | KACIMMI30 | 40       | KACIMMI73 | 60       | GBK000828 | 80      | GBK029744 | 100      | GBK000520 |

Key: I.E = International Eleusine, CV = Chakol Variant, U = Uganda, P = Purple
N = Nanjala, GBK = Gene Bank Kenya, G = Green, KACIMMI = KARI African Centre for Crop Improvement Mc Knight Foundation Millet
inoculated with *Striga* was by hand pulling with effect from second weeding. Duduthrin pesticide was applied at two weeks interval to prevent crop attack by shoot fly and the stalk borer. Calcium Ammonium Nitrate (C.A.N) fertilizer (27:0:0) was used to top dress the crop three weeks after thinning.

### 2.1 Data Collection

#### 2.1.1 DNA extraction and genotyping-by-Sequencing

After 110 days during season two the crop was harvested and eight seeds of each finger millet genotype planted in pots in a glasshouse at International Centre for Research in Agroforestry (ICRAF) campus, Gigiri Nairobi for the purpose of molecular analysis. DNA was extracted from seedlings after a week of germination using ISOLATE II Plant DNA Kit (Bioline) protocol. The DNA was then subjected to electrophoresis at 80 V for 45 minutes and quantification of each sample done using Quibit® 2.0 Fluorometer (Invitrogen by Life technologies corporation, USA). The quantified DNA of the 95 genotypes was packed into the 96-plex/wells together with one blank and sent to Institute of Genomic Diversity (Cornell University, Ithaca, New York, USA from which libraries were prepared using *ApeK*I restriction enzyme for genomic digestion. The barcoded samples were then pooled in 96-plex and sequenced in 1 lane of Illumina Hiseq 2500 (Illumina, San Diego, CA, USA Inc.).

Single-Nucleotide-Polymorphism Calling was used to align sequencing tags for SNP calling since finger millet does not have reference genome. Association between phenotypic and GBS data was determined by running on UNEAK (Universal network enabled analysis Kit) production pipeline as explained in Elshire et al. [25 a,b] with *ApeK*I restriction enzyme for genomic digestion. The barcoded samples were then pooled in 96-plex and sequenced in 1 lane of Illumina Hiseq 2500 (Illumina, San Diego, CA, USA Inc.).

TASSEL GBS pipeline [28]. The TASSEL Universal Network Enabled Analysis Kit (UNEAK) filter was used to align reads in absence of reference genome [26]. Raw SNPs were filtered to include only sites with 80% coverage across sample and minor allele frequencies ≥ 0.05, and only samples with ≥ 25% coverage across the remaining sites.

#### 2.1.2 Determination of population structure analysis

Population structure was determined using the program fast structure [29] an updated version of the program structure [30] designed to handle large SNP data set rapidly.

### 3. RESULTS

#### 3.1 Genetic Diversity Screening of Finger Millet for *Striga* Resistance Using Molecular Markers

Genotyping by sequencing was performed on 95 genotypes which comprised of a set of 77 land races from Gene banks of Kenya and 18 land races from different regions of the world. Libraries were prepared using *ApeK*I restriction enzyme because it cuts frequently and has history of performing well for GBS in many different grass species. It is thus methylation sensitive and produces overhangs i.e. it does not cut in the major repetitive fraction of the genome. After assigning reads, Single-nucleotide polymorphisms (SNPs) were called using the TASSEL GBS pipeline [28]. The TASSEL UNEAK filter [26] was used to align reads in absence of reference genome. Raw SNPs were filtered to include only sites with ≥ 80% coverage across sample and minor allele frequencies P ≥ 0.05, and only samples with ≥ 25% coverage across the remaining sites. A total of 17 GB Fastq.gz (~ 60 GB fastq.txt) raw sequence data was obtained from Cornell University laboratories Ithaca New York, USA from which 117,542 SNPs single-end 64-bp reads were obtained from raw GBS dataset that were used for genome wide association studies (GWAS) to analyse for *Striga* resistance (Tables 2 and 3).

For population structure, the fast structure program [29] was used which is an updated version of the program structure [30] designed to handle large SNP data set rapidly.
Table 2. Finger millet hap map file before filtering

| File  | Data  | Filter | Analysis | Results | GBS  | Help |
|-------|-------|--------|----------|---------|------|------|
| HapMap_chr02-154455_Collapsed_22350.mapped | PC for HapMap_chr02-154455_Collapsed_22350.mapped | Eigenanalysis for HapMap_chr02-154455_Collapsed_22350.mapped | Eigenanalysis for HapMap_chr02-154455_Collapsed_22350.mapped | Eigenanalysis for HapMap_chr02-154455_Collapsed_22350.mapped | PC for HapMap_chr02-154455_Collapsed_22350.mapped | Help |

Number of sequences: 96
Number of sites: 22350
Loci: 0

Table 3. Finger millet filtered hap map genotype file

| File  | Data  | Filter | Analysis | Results | GBS  | Help |
|-------|-------|--------|----------|---------|------|------|
| HapMap_chr02-154455_Collapsed_22350.mapped | PC for HapMap_chr02-154455_Collapsed_22350.mapped | Eigenanalysis for HapMap_chr02-154455_Collapsed_22350.mapped | Eigenanalysis for HapMap_chr02-154455_Collapsed_22350.mapped | Eigenanalysis for HapMap_chr02-154455_Collapsed_22350.mapped | PC for HapMap_chr02-154455_Collapsed_22350.mapped | Help |

Number of sequences: 96
Number of sites: 22350
Loci: 0
Filter Alignment
Size Filter
Base Type: ContGenotypeTable
Panel: Poly
3.2 Phylogenetic Analysis of Finger Millet Genotypes on Molecular Data

Genetic diversity analysis was done on the 95 finger millet genotypes using molecular data. The dendrogram was generated through neighbor-joining method of TASSEL software. The genotypes were grouped into three major clusters (A, B and C) based on reaction to Striga (Fig. 1). Cluster A comprised 32 genotypes of which 27 were Kenyan and 5 were exotic genotypes from; India (1), Uganda (2), Malawi (1), and Zambia (1). Cluster B comprised 56 genotypes of finger millet. Cluster B was further divided into two sub-clusters: B1 and B2. Of the 34 accessions in sub-cluster B1, 28 were from eastern Africa (Kenya 27 and Uganda 1), two from southern Africa (Zimbabwe), one from western Africa (Nigeria), two from Asia (India and Nepal) and one from Europe (Germany). Cluster B2 had 22 genotypes of which 21 were from eastern Africa (Kenyan 20 and Uganda 1) and India (1). Cluster C had seven genotypes in total, out of which 4 genotypes were from southern Africa Zimbabwe 1 and 3 from Kenya.

3.3 Cluster Analysis for the 95 Inbred Lines of Finger Millet Genotypes

In Table 4, the genotypes that showed low resistance to Striga were mostly from cluster A which included; GBK000549, GBK000462, GBK029715 and GBK029744. Two were from sub-cluster B1 (GBK011113 and GBK008292) and one from cluster C which was I.E 5306. All genotypes that showed high resistance to Striga belonged to cluster B. They included; I.E 2217, I.E 6537 and GBK000516 from sub-cluster B1 while genotypes I.E 4115, I.E 4491, KACIMMI 24, KACIMMI 30, KACIMMI 36, KACIMMI 47 from sub-cluster B2. Similarly the genotypes that were tolerant belonged to cluster B. They were high yielders despite supporting high population of Striga at maturity and included GBK003821, GBK000568, I.E 4816 KACIMMI 17 and KACIMMI 73. The clustering pattern revealed highly diverse nature of composite collection based on racial and regional diversity.

Fig. 1. Phylogenetic analysis of 95 finger millet genotypes generated through neighbor-joining method of TASSEL software in response to Striga in two agroecological environments in Western Kenya, Alupe and Kibos. The genotypes are represented by entry numbers.
Table 4. Finger millet membership cluster for the 95 inbred lines from phylogeny tree

| Cluster A (Red) | Cluster B1 (Green) | Cluster B2 (Blue) | Cluster C (Dark blue) |
|----------------|--------------------|-------------------|----------------------|
| KACIMMI 77     | GBK000568          | KACIMMI 17        | I.E 4497             |
| P4C3           | I.E 6165           | KACIMMI 49        | GBK039217            |
| GBK000520      | GBK011113          | I.E4816           | GBK043268            |
| GBK000451      | GBK008292          | KACIMMI 73        | I.E 4491             |
| GBK008299      | I.E 5873           | KACIMMI 47        | I.E 5306             |
| GBK029805      | GBK000784          | BUSIBWABO-1       | KACIMMI 11           |
| GBK000549      | I.E 2217           | KACIMMI 36        | I.E 5870             |
| GBK008339      | GBK000516          | KACIMMI 30        |                     |
| KACIMMI 7      | GBK029821          | OMUGA G           |                     |
| GBK029744      | U15 X P283         | OMUGA P           |                     |
| U-15           | GBK029798          | OKHALE-1          |                     |
| KACIMMI 22     | I.E 2957           | KACIMMI 6         |                     |
| GBK000462      | GBK029199          | SERERE-1          |                     |
| GBK029793      | P 224 CV           | KACIMMI 72        |                     |
| GBK000463      | GBK029678          | KACIMMI 42        |                     |
| GBK000493      | I.E 6537           | I.E 4115          |                     |
| UFM 138        | VL149              | KACIMMI 20        |                     |
| PR 202         | GBK027300          | KACIMMI 24        |                     |
| GBK000409      | GBK000692          | P 283             |                     |
| I.E 2606       | I.E 6337           | GBK000900         |                     |
| GBK000802      | GBK008292          | GBK000831         |                     |
| GBK029715      | GBK029701          | GBK026992         |                     |
| KACIMMI 15     | GBK008348          |                   |                     |
| GBK029724      | GBK033446          |                   |                     |
| GBK011126      | GBK040568          |                   |                     |
| P 224          | GBK029847          |                   |                     |
| GBK033416      | GBK000369          |                   |                     |
| GBK029820      | GBK033414          |                   |                     |
| GBK000828      | GBK011082          |                   |                     |
| GBK029661      | GBK043081          |                   |                     |
| NANJALA-BROWN  | GBK000449          |                   |                     |
| GBK029807      | GBK000909          |                   |                     |
|                |                    |                   |                     |

3.4 SNP Markers Showing Association with Striga Resistance

This was performed using general linear model (GLM) and mixed linear model (MLM). GLM performs association analysis using a least squares fixed effects where TASSEL utilizes a fixed effect linear model to test for association between segregating sites and phenotypes. It accounts for population structure using covariates that indicate degree of membership in underlying population. A MLM is one which conducts analysis using both fixed and random effects giving it the ability to incorporate information about relationship among individuals. Some markers that were detected using mixed linear model (MLM) analysis were similarly detected in general linear model (GLM) analysis (Table 5). This confirmed the reliability of MLM in genome wide association studies (GWAS). The markers identified were TP85424 and TP88244 as highlighted in bold (Table 5).

3.5 Population Structure of the 95 Inbred Lines of Finger Millet Genotypes

The first three Principal component analysis (PCA) of the twelve components results showed cumulative proportion of 8% (Fig. 2 and Table 6 in bold). These results provided evidence for genetic variation in response to Striga in finger millet for the first time ever. Although only 95 accessions were used, there is likelihood that more novel sources of resistance to Striga is available within cultivated and wild germplasm.
Table 5. Presentation of SNP markers showing significant association with Striga resistance using GLM and MLM in finger millet crop

| Trait  | Marker    | Locus_pos | Marker_F | Marker_p | Perm_p | Marker R² |
|--------|-----------|-----------|----------|----------|--------|-----------|
| AlupSfree | TP11346   | 11346     | 11.77614 | 8.71E-05 | 0.966  | 0.2876    |
| AlupSfree | TP16436   | 16436     | 13.46379 | 1.87E-05 | 0.54   | 0.28372   |
| AlupSfree | TP25285   | 25285     | 11.43916 | 9.62E-05 | 0.973  | 0.28983   |
| AlupSfree | TP53302   | 53302     | 12.4427  | 5.71E-05 | 0.885  | 0.34173   |
| AlupSfree | TP68225   | 68225     | 15.04937 | 6.97E-06 | 0.271  | 0.30923   |
| Alupinoc | TP68225   | 68225     | 14.36346 | 1.87E-05 | 0.343  | 0.30987   |
| Alupinoc | TP86696   | 86696     | 18.17384 | 7.98E-05 | 0.93   | 0.21652   |
| kibosSfree | TP7986   | 7986      | 12.58671 | 9.04E-04 | 0.26291 | 1.19121   |
| kibosSfree | TP53302  | 53302     | 22.36557 | 2.44E-07 | 0.006  | 0.40889   |
| KibosIno  | TP85424   | 85424     | 14.12507 | 1.26E-05 | 0.388  | 0.31724   |
| KibosIno  | TP88244   | 88244     | 11.76539 | 5.74E-05 | 0.871  | 0.27191   |

GLM 60% filter 0.05

| Trait  | Marker    | Locus_pos | Marker_F | Marker_p | Perm_p | Marker R² |
|--------|-----------|-----------|----------|----------|--------|-----------|
| kibosIno | TP70567   | 70567     | 8.0447   | 9.04E-04 | 0.26291 | 1.19121   |
| KibosIno  | TP78789   | 78789     | 8.03945  | 9.93E-04 | 0.27239 | 1.19121   |
| KibosIno  | TP85424   | 85424     | 9.72326  | 2.59E-04 | 0.31777 | 1.19121   |
| KibosIno  | TP88244   | 88244     | 8.51908  | 6.09E-04 | 0.27301 | 1.19121   |

Table 6. The PCA values

| PC  | Eigen values | Individual proportion | Cumulative proportion |
|-----|--------------|-----------------------|-----------------------|
| 1   | 5741.6       | 0.035188              | 0.035188              |
| 2   | 3771.7       | 0.023115              | 0.058303              |
| 3   | 3578.5       | 0.021931              | 0.080234              |
| 4   | 2753.3       | 0.016874              | 0.097108              |
| 5   | 2707.5       | 0.016593              | 0.1137                |
| 6   | 2624.5       | 0.016085              | 0.12979               |
| 7   | 2537.5       | 0.015551              | 0.14534               |
| 8   | 2456.3       | 0.015054              | 0.16039               |
| 9   | 2422.4       | 0.014846              | 0.17524               |
| 10  | 2410.3       | 0.014772              | 0.19001               |
| 11  | 2368         | 0.014512              | 0.20452               |
| 12  | 2337.3       | 0.014325              | 0.21884               |
| 13  | 2317.1       | 0.014201              | 0.23305               |
| 14  | 2293.2       | 0.014054              | 0.2471                |
| 15  | 2261.6       | 0.01386               | 0.26096               |
| 16  | 2236.7       | 0.013708              | 0.27467               |
| 17  | 2232.7       | 0.013684              | 0.28835               |
| 18  | 2201.2       | 0.013491              | 0.30184               |
| 19  | 2180.6       | 0.013364              | 0.31521               |
| 20  | 2172.1       | 0.013312              | 0.32852               |

The values generated during PCA including the Principal Components, Eigen values and the two last Eigen vectors are presented in Table 6.

3.6 Multidimensional Scaling: A Confirmation of Population Structure

The purpose of multi-dimensional scaling (MDS) in this case was to provide a visual representation of the pattern of proximities (i.e. similarities or distances) among individual within the data set. It was performed on the data set to show the population structure. It is one among the many multivariate techniques that aim to reveal the structure of data set by plotting points in one or two dimensions. The 95 genotypes were not clearly classified into three broad groups (Fig. 3) however the clusters are collinear.
with the population structure. It is extremely similar to principal component analysis (PCA), with the main difference being that for MDS the raw SNP scores are first converted into matrix of distances between all samples (Fig. 3). The conversion is necessary because PCA does not function on data sets where some elements are missing and the stochastic nature of GBS ensures that essentially every data set will have at least some missing data [31]. The MDS plot provides a bit of some separation of the accessions into subpopulations, a confirmation of population structure and the clustering pattern that was observed in phylogenetic analysis (Fig. 1).

Fig. 2. PCA graphical presentation of individual and cumulative proportion of finger millet genotypes

Fig. 3. Multiple dimensional scaling for the entire collection of finger millet genotypes. Colours depict corresponding subpopulations
3.7 Genome Wide Association Studies

GWAS also called association mapping studies focuses on polymorphism in candidate genes that are suspected to have roles in controlling phenotypic variations for one specific trait of interest [32]. Using the few genotypes from the HapMap shows that diversity within inbred lines of finger millet were as a result of copy number variation (CNV) in response to reaction to Striga (Fig. 4). These variations involved deletion, insertions and duplication as can be observed below in the consensus sequence among the eight genotypes as follows:

1. KACIMMI 43
   CAGCAAAACGCCAAGCACAGA<TGGG>CAACTGCTCGGGCAGAAAAAAAAAAAAAAAAAAAAA
   KACIMMI 43
   CAGCAAAGCCCAAGCACCGA<TGGG>CAACTGCTCGGGCAGAAAAAAAAAAAAAAAAAAAAA
   KACIMMI 49
   CAGCAGGCTACGGGAGAAAACCAACCTGCACACTTGGGCTCAGCAACGGAAAAAAA
   AAA AA

2. KACIMMI 49
   CAGCAAGCTACGGGAGAAAACCAACCTGCACACTTGGGCTCAGCAACGGAAAAAAA
   AAA AA

3. GBK000516
   CACGAAACACGAGGTCTGATCGCTCCCTACTTCTACTTTTGGCTCAGCTGA
   AAA

4. KACIMMI 36
   CAGCAAGGCCAGTTTTTCATCCCGAGAAAACCTCAAGGTTCAACGAGATGTGCTAGCTGA
   AAAA

5. KACIMMI 24
   CAGCAAGGGCGGGAAGCGCGAAGGCTTCCCCGACGGGCTGGCTGAAAAAAA
   AAA

6. BUSIBWABO-1
   CAGCAACGGGTCCGAGTCTGCGTGGAGCATGACGCCGAGCAGGAAGGAA
   AAAA

BUSIBWABO-1
   CAGCAACGGGTCCGAGTCTGCGTGGAGCATGACGCCGAGCAGGAAGGAA
   AAAA

7. KACIMMI 6
   CAGCAAGGCTCAGAGCAGCGGAGGAGGAGGATGTGGCTGGGCTGA
   AAAA

KACIMMI 6
   CAGCAAGGCTCAGAGCAGCGGAGGAGGAGGATGTGGCTGGGCTGA
   AAA A

8. KACIMMI 65
   CAGCAAGCTACAGGAGAGGAGGAGGAGGATGTGGCTGGGCTGA
   AAA A

Fig. 4. Eight paired end reads trimmed to 64 bp arrangement of SNPs among the 95 finger millet genotypes that showed high resistance to Striga
4. DISCUSSION

4.1 Variation in Finger Millet Genotypes for Striga Resistance

The clustering of the 95 genotypes with respect to reaction to Striga is an indication that resistance is genetically controlled and occurring in particular gene loci. According to Bush and Moore [33], genome wide association studies typically identifies common variants with small effect on sizes. Similarly the variants that were tolerant to Striga belonged to the same cluster B an indication that susceptibility to the weed and its effect occurs when the gene is in homozygous recessive state. Similar results were reported by Vogler et al. [34], who observed that a single nuclear recessive gene controls this mechanism in sorghum variety SRN 39.

4.2 Population Structure and Phylogenetic Analysis

Population structure analysis with fast structure [29] separated the finger millet genotypes along into three primary clusters. Phylogenetic analysis closely corresponds with the structure analysis, whereby the inferred clusters matched major branch points in the phylogeny.

The results also provided evidence for genetic variation in response to Striga in finger millet which is the first study reported so far. It revealed three groups depending on the germplasm with the resistant genotypes being separated from the susceptible ones. This separation was due to differences in the reaction of the 95 types of germplasm to Striga infestation. These findings are consistent with results of Menkir et al. [35] who found that Striga – resistant hybrids were separated from Striga tolerant hybrids but contrary to the results of Badu-Apraku and Lum [36] who found that the clustering of inbred lines were independent of the genetic background of genotypes. Even though only 95 accessions were used, there was likelihood that more novel sources for resistance to Striga could be available within cultivated and wild germplasm.

Of the eight genotypes that were selected for resistance all were from same cluster B implying the high reliability of the results obtained in field screening and verification by molecular work. Therefore the molecular markers that were obtained through General Linear Model (GLM) and Mixed Linear Model (MLM) with respect to resistance to Striga confirm the similar findings.

The resistance might have come about as a result of a number of variations through insertion and deletion.

5. CONCLUSION

The genetic diversity analysis based on molecular markers revealed;

i) From the GBS analysis, finger millet genotypes inoculated with Striga at Kibos had the markers TP 85424 and TP 88244 present in both GLM and MLM. This indicates that the two markers were stringent, hence confirming the reliability of GBS in genome wide association studies.

ii) The population structural analysis divided the genotypes into three sub-populations (A, B and C) where all the three sub-populations had an admixture of alleles. Cluster A consisted of susceptible genotypes which included; GBK000549, GBK000542, GBK029715 and GBK029744 agreeing with results from agronomic traits.

All genotypes that showed high resistance to Striga were in cluster B. They include I.E 2217, I.E 6537, I.E 4115, KACIMMI 24, KACIMMI 30 and KACIMMI 47. Similarly the tolerant genotypes equally belong to cluster B and include GBK003821, GBK000568, I.E 4816, KACIMMI 17 and KACIMMI 73. At least two of the susceptible genotypes were also found in cluster B1 (i.e. GBK027300, GBK011113, GBK040568 and one of them I.E 5306 was found in cluster C). Cluster C also comprised of susceptible genotypes and include I.E 4497, GBK039217, GBK043268, I.E 4491, KACIMI 11 and I.E 5870.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. FAO. Trade Reforms and food security: Conceptualizing the Linkages. Rome; 2003.
2. Fahey JW. Underexploited African grain crops: A nutritional resource. Nutrition Review. 1998;56:282-285. [CrossRef] [PubMed]
3. ICRISAT. Archival Report; 2008. Available:http://intranet/ddg/admin%20Pages2009/Documents Archival (Accessed on 23/06/2017)
4. Obilana AB, Manyasa EO, Kibuka JG, Ajanga S. Finger millet blast (fmb) samples collection in Kenya: Passport data, analyses of disease incidence and report of activities. ICRISAT, Nairobi, Kenya; 2002.

5. De Vries, Toenniessen, De Vries J, Toenniessen G. Securing the harvest biotechnology, breeding and seed systems for African crops. CABI Publishing, New York; 2001.

6. Atera EA, Itoh K, Onyango JC. Evaluation of ecologies and severity of Striga weed on rice in sub-Saharan Africa. Agricultural and Biological Journal of North America. 2011; 2:752-760.

7. Haussmann BIG, Geiger HH, Welz HG. Improved methodologies for breeding Striga-resistant sorghums. (Review Article). Field crops Research. 2000b;66: 195-201.

8. Press MC, Graves JD. Parasitic plants. Chapman and Hall, London; 1995.

9. Parker C. Observations on the current status of Orabanche and Striga problems worldwide. Pest Management Science. 2009;65:453-459.

10. Vanlauwe B, Kanampiu F, Odhiambo GD, De Groote H, Wadhams HJ, Khan ZR. Integrated management of Striga hermonthica, stem borers, and declining soil fertility in Western Kenya. Field Crops Research. 2008;107:102-115.

11. Kabambe V, Katunga L, Kapewa T, Ngwira AR. Screening legumes for integrated management of witchweeds (Alectra vogelli and Striga asiatica) in Malawi. African Journal of Agriculture Research. 2008;3:708-715.

12. Lagoke ST, Parkinson V, Agiunbiade RM. Parasitic weeds and control methods in Africa. In: Kim SK. (ed). Combating Striga in Africa: Proc. International Workshop organized by IITA, ICRISAT and IDRC; 1988. IITA, Ibadan, Nigeria. 1991;3-15.

13. Debrah SK. Socio-economic constraints to the adoption of weed control techniques: The case of Striga control in West African semi-arid tropics. Institute of Insect Pest Management. 1994;40:153-158.

14. Hearne SJ. Control – The Striga conundrum. Pest Management Science. 2009;65:603-614.

15. Jansen PCM, Ong HC. Eleusine coracana (L) Gaertner cv. Group finger millet. Grubben I, GJH, Partohardjono S. (Editors). Plant resources of south-east Asia No. 10 Cereals. Bakhuys Publishers, Leiden, Nethelands. 1996;90-95.

16. Duke JA. Ecosystematic data on economic plants. Quarterly Journal of Crude Drug Research. 1979;17:91-110.

17. Doggett H. Witchweed (Striga) In: Wingley G (ed.) Sorghum. Second (ed). Longman Scientific and Technical. London. 1988; 368-404.

18. Scholes JD, Press MC. Striga infestation of cereal crops-an unsolved problem in resource limited agriculture. Current Opinion in Plant Biology. 2008;11:180-186.

19. Gethi JG, Smith, ME, Mitchell SE, Kresovich S. Genetic diversity of Striga hermonthica and S. asiatica populations in Kenya. Weed Resource. 2005;45:64–73.

20. Bernado R. Molecular markers and selection for complex traits in plants: Learning from the last 20 years. Crop Science. 2008;48(5):1649-1664.

21. Davey JW, Hohenlohe PA, Etter PD, Boone JQ, Catchen JM. Genome-wide genetic marker discovery and genotyping using next-generation sequencing. Nat Rev Genet. 2011;12:499-510. DOI: 10.1038/nrg3012 [PubMed: 21681211]

22. Chen Q, Ma Y, Yang Y, Chen Z, Liao R. Genotyping by genome reducing and sequencing for outbred animals. Plos One. 2013;8:e67500.

23. FURP. Fertilizer Use Recommendation Project, (FURP). Find report annex III. Phase I. ministry of agriculture, Nairobi, Kenya. National Agricultural Research Laboratories. Nairobi, Kenya; 1987.

24. Doggett H. Sorghum. Longman’s, London; 1970.

25. (a) Elshire RJ, Glaubitz JC, Sun Q, Poland JA, Kawamoto K, Buckler ES, Mitchell SE. A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. PloS One. 2011a;6(5):e19379. DOI:1371/journal.pone.0019379
(b) Elshire RJ, Glaubitz JC, Sun Q, Poland JA, Kawamoto K, Buckler ES, Mitchell SE. Power point presentation for rapidly genotyping highly diverse species; 2011b.

26. Lu F, Lipka AE, Elshire RJ, Glaubitz JC, Cherney J. Switch grass genomic diversity, ploidy and evolution: Novel insights from a network-based SNP discovery Protocol. PloS Genet. 2013;9: e1003215.
27. Danecek P, Auton A, Abecasis G, Albers CA, Banks E. The variant call format and VCF tools. Bioinformatics. 2011;27:2156-2158.

28. Glaubitz J, Casstevens T, Lu F. TASSEL-GBS: A high capacity genotyping by sequencing analysis pipeline. PLoS One. 2014;9(2):e90346.

29. Raj A, Stephens M, Prichard JK. Fast structure: Variation inference of population structure in large SNP datasets. Genetics. 2014;197:573-589. DOI: 10.1534/genetics.114.164350

30. Prichard JK, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. Genetics. 2000;155:945-959.

31. Wallace JG, Upadyaya HD, Vetriventhal M. The genetic makeup of global barnyard millet germplasm collection. Plant Genome; 2015. DOI: 10.3835/plantgenome2014.10.0067

32. Thornsberry JM. Dwarf 8 polymorphisms associate with variation in flowering time. Nat. Genet. 2001;28:286-289. Western Kenya. Field Crops Res. 2001;107:102-115.

33. Vogler RK, Ejeta G, Buttler LG. Inheritance of low production of Striga germination stimulant in Sorghum. Crop Science. 1996;36:1185-1191.

34. Bush WS, Moore JH, Lewitter, Fran; Kann, Maricel, eds. "Chapter 11: Genome-wide association studies". PLoS Comput Biol. 2012;8(12):e1002822. DOI: 10.1371/journal.pcbi.1002822. [PMC 3531285] [PMID 23300413]

35. Menkir AD, Makumbi, Franco J. Assessment of reaction patterns of hybrids to Striga hermonthica (Del.) Benth. Under artificial infestation in Kenya and Nigeria. Crop Sci. 2012b;52:2528–2537. DOI: 10.2135/cropsci2012.05.0307

36. Badu-Apraku B, Lum AF. Agronomic performance of Striga resistant early-maturing maize varieties and inbred lines in the savannas of West and Central Africa. Crop Sci. 2007;47:737–750. DOI: 10.2135/cropsci2006.04.0245

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