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DOI: 10.9734/ajbgmb/2018/v1i2493

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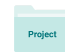

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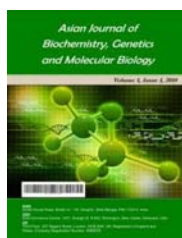

Asian Journal of Biochemistry, Genetics and Molecular Biology

1(2): 1-14, 2018; Article no.AJBGMB.43924

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.

Article Information

DOI: 10.9734/AJBGMB/2018/43924
Editor(s):
(1) Dr. Ahmed Medhat Mohamed Al-Naggar, Professor, Department of Agronomy, Faculty of Agriculture, Cairo University, Egypt.
Reviewers:
(1) R. Mahalakshmi, India.
(2) Esin Oluk, Ege University, Turkey.
(3) Nebi Bilir, Suleyman Demirel University, Turkey.
Complete Peer review History: http://www.sciencedomain.org/review-history/26090

Original Research Article

Received 20th June 2018
Accepted 26th August 2018
Published 4th September 2018

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

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in a randomized complete block design (RCBD) consisting of 10 x 10 square (triple lattice) under *Striga* (inoculated) and no *Striga* conditions and plant growth monitored to maturity after 110 days. All genotypes were genotyped by genotyping by sequencing (GBS) and data analyzed using the non-reference based Universal Network Enabled Analysis Kit (UNEAK) pipeline. Genome wide association studies (GWAS) were done to establish the association of detected Single Nucleotide Polymorphisms (SNPs) with *Striga* reaction based on field results. In molecular analysis 117,542 SNPs from raw GBS data used in GWAS revealed that markers TP 85424 and TP 88244 were associated with *Striga* resistance in the 95 genotypes. Principal Component Analysis revealed that the first and third component axes accounted for 2.5 and 8% of total variance respectively and the genotypes were distributed according to their reaction to *Striga* weed. Genetic diversity analysis grouped the 95 accessions into three major clusters containing; 32 (A), 56 (B), and 7 (C) genotypes. All finger millet genotypes that showed high resistance to *Striga* in the field were from cluster B while the most susceptible genotypes were from clusters A and C. Results revealed genetic variation for *Striga* resistance in cultivated finger millet genotypes and hence the possibility of marker –assisted breeding for resistance to *Striga*.

Keywords: Striga hermonthica; genotyping; genome; susceptible; genetic diversity.

1. 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 habouring 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 loses 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

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	KACIMMI17	64	KACIMMI77	84	GBK033416
5	OMUGA-P	25	KACIMMI7	45	KACIMMI22	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	KACIMMI 11	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) for genotyping with GBS. Library preparation and sequencing followed the protocol described by 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.).

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] to align sequencing tags for SNP calling. Full description of UNEAK protocol was obtained by logging to (http://www.maizegenetics.net/gbs-bioinformatics). Quality filtering was performed primarily using built in function in VCF tools [27]. All bioinformatics and Subsequent analysis were performed on High Computing Machine Workstation with 60 GB of RAM running Ubuntu. After assigning reads, Single-nucleotide polymorphisms (SNPs) were called using the 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_chr0_20-134055_Collasped_22350_imputed
PC for HapMap_chr0_20-134055_Collasped_22350_in
Eigenvectors for HapMap_chr0_20-134055_Collaspec
Eigenvalues for HapMap_chr0_20-134055_Collasped_
PC for HapMap_chr0_20-134055_Collasped_22350_in
Sequence
HapMap
HapMap_chr0_20-134055
Tree
Tree:HapMap_chr0_20-134055

Number of sequences: 96
Number of sites: 117542
Loci: 0

Physical Positions / Site Numbers / Locus / Site Name / Alleles — MajorMinorAllele — (Enter physical position)

1 14896 29791 44686 59581 74476 89371 104266 119161

Table 3. Finger millet filtered hap map genotype file

File Data Filter Analysis Results GBS Help

HapMap_chr0_20-134055_Collasped_22350_imputed
PC for HapMap_chr0_20-134055_Collasped_22350_in
Eigenvectors for HapMap_chr0_20-134055_Collaspec
Eigenvalues for HapMap_chr0_20-134055_Collasped_
PC for HapMap_chr0_20-134055_Collasped_22350_in
Sequence
HapMap
HapMap_chr0_20-134055
Tree
Tree:HapMap_chr0_20-134055

Number of sequences: 96
Number of sites: 22350
Loci: 0
FilterAlignment...
Site Filter
Base Type: CoreGenotypeTable
Point Poly.

Physical Positions / Site Numbers / Locus / Site Name / Alleles — MajorMinorAllele — (Enter physical position)

20 14912 29804 44696 59588 74480 89372 104264 119156

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, 1.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.

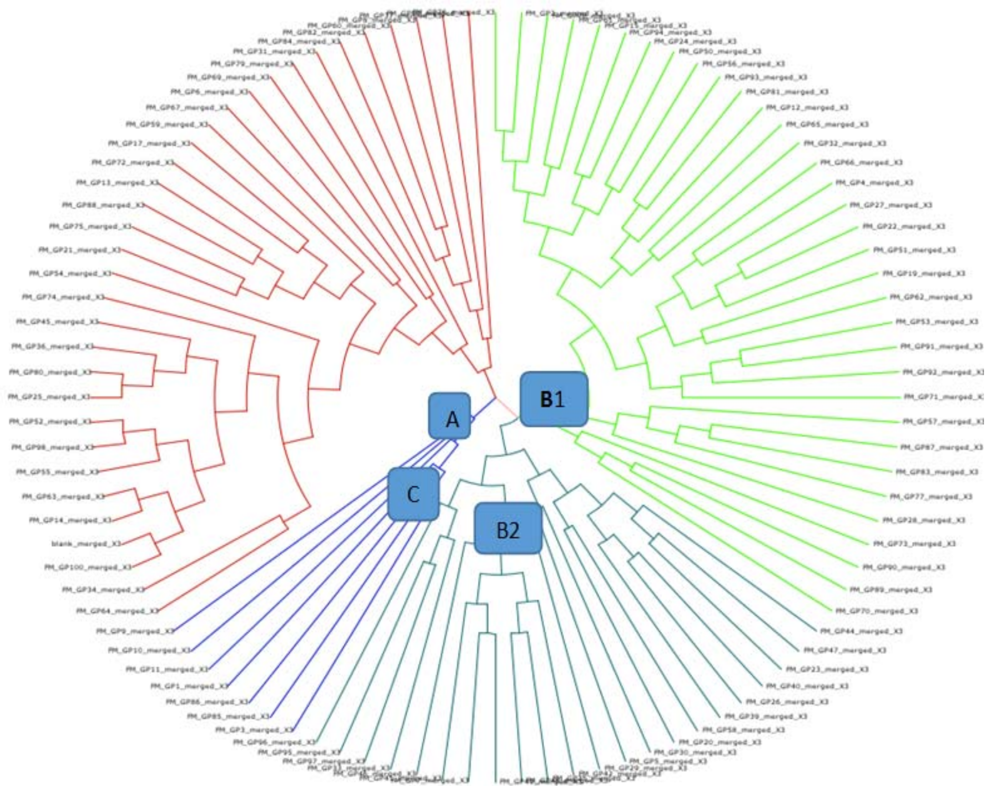

Fig. 1. Phylogenetic analysis of 95 finger millet genotypes generated through neighbor-joining method of TASSEL software in response to *Striga* in two agroecological environments in Western Kenya, Alupe and Kibos. The genotypes are represented by entry numbers

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		
	GBK000482		
	GBK003821		
32	34	22	7

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

GLM 60% filter 0.05						
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	1.08E-05	0.343	0.30987
Alupinoc	TP86696	86696	18.17384	7.98E-05	0.93	0.21652
kibosSfre	TP7986	7986	12.58671	6.76E-05	0.864	0.33936
kibosSfre	TP53302	53302	22.36557	2.44E-07	0.006	0.40889
KibosIno	TP14093	14093	14.49574	1.25E-05	0.384	0.33984
KibosIno	**TP85424**	**85424**	**14.12507**	**1.26E-05**	**0.388**	**0.31724**
KibosIno	**TP88244**	**88244**	**11.76539**	**5.74E-05**	**0.871**	**0.27191**
MLM 60% filter 0.05						
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**	**85425**	**9.72326**	**2.59E-04**	**0.31777**	**1.19121**
KibosIno	**TP88244**	**88244**	**8.51908**	**6.09E-04**	**0.27301**	**1.19121**

Key: Alupsffree = Alupe Striga free, Alupinoc = Alupe inoculated with Striga, Kibosfree = Kibos Striga free, KibosInoc = Kibos inoculated with Striga

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).

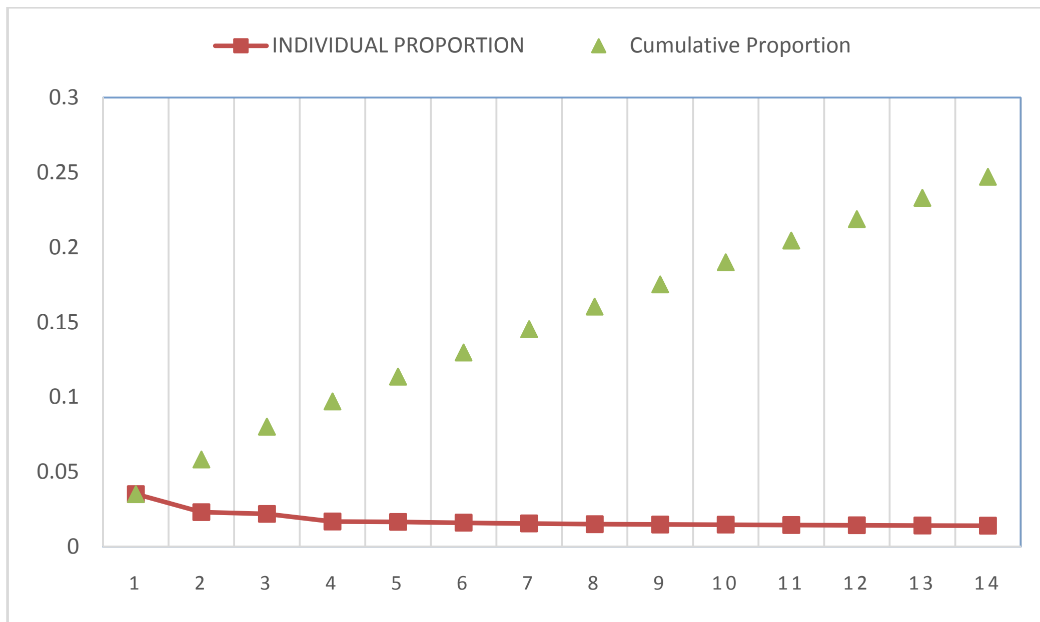

Fig. 2. PCA graphical presentation of individual and cumulative proportion of finger millet genotypes

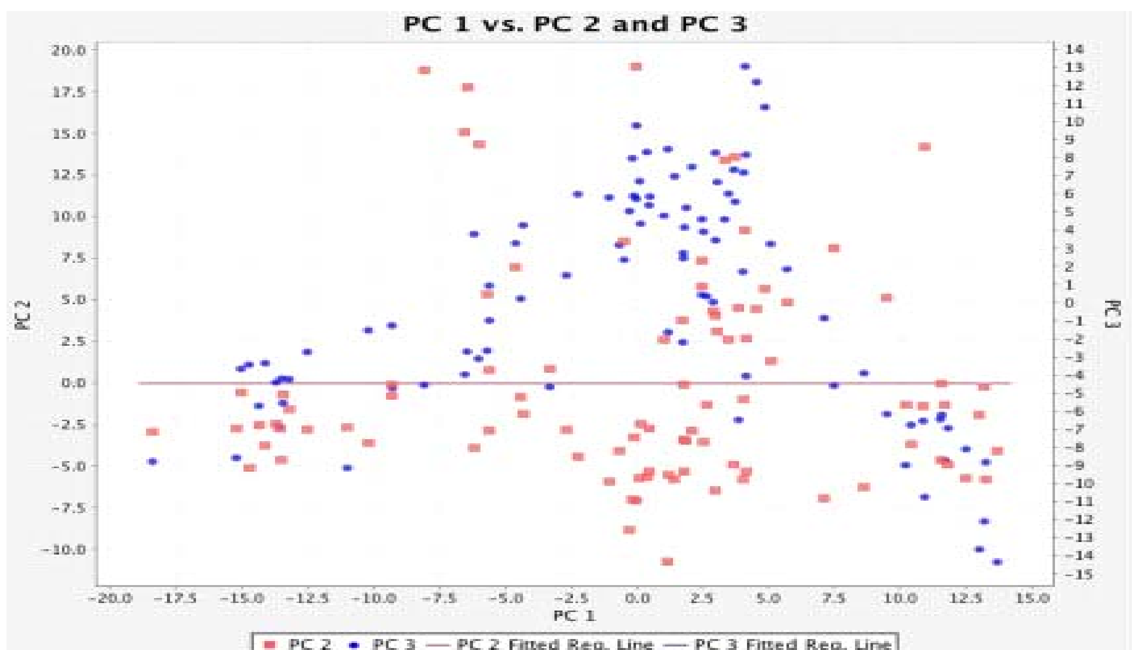

Fig. 3. Multiple dimensional scaling for the entire collection of finger millet genotypes. Colours depict corresponding subpopulations

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**CAACTGCTCGGG**C**AGAAAAAAAAAAAAAAAAAAA
AAAAA
KACIMMI 43
CAGCAAAACGCCAAGCACGGA**TGGG**CAACTGCTCGGG**C**AGAAAAAAAAAAAAAA
AAAAAAAAA AA
2. KACIMMI 49
CAGCAAGCTACGGGAGAAAACCAACCTCGCCAC**TGGGG**CCGAAGCAGAAAAAAAAAAAAAAAA
KACIMMI 49
CAGCAGGCTACGGGAGAAAACCAACCTCGCCAC**TGGGG**CCGAAGCAGAAAAAAAAAAA
AAAA AA
3. GBK000516
CAGCAAACACGAGGTCTGATCGCTCCCTCTCACTTT**TGG**CTCCACTGCTGAAAAAAAAAAAAA
GBK000516

CAGCGAACACGAGGTCTGATCGCTCCCTCTCACTTT**TGG**CTCCACTGCTGAAAAAAAAAA
AAAA
4. KACIMMI 36
CAGCAAGGCAGTTTTTCCATCCCGAGAAACCTCAAGCTTCCAACAGA**TGTG**TCAGCTGAAAAAA
KACIMMI 36

CAGCAAGGCAGTTTTTCCATCCCGAGAAACCTCAAGCTTCCAACGGA**TGTG**TCAGCTGA
AAAAA
5. KACIMMI 24
CAGCAAAGGGGGGAAGCAGAAGGCGTTCCCCGACGGGCGG**TGG**CTGAAAAAAAAAAAAAAAA
A
KACIMMI 24
CAGCAAAGGGGGGAAGCGGAAGGCGTTCCCCGACGGGCGG**TGG**CTGAAAAAAAAAAAA
AAA AAA
6. BUSIBWABO-1

CAGCACCGTCGAGTCG**TGG**AGCGATGACGGCGGGAGCAGAAAAAAAAAAAAAAAAAAA
AAAAAA
BUSIBWABO-1
CAGCACCGTCGAGTCG**TGG**AGCGATGACGGCGGGGGCAGAAAAAAAAAAAAAAAAAAA
AAAAAA
7. KACIMMI 6
CAGCAAGCCTCGGCAGAGCGGAGAGGGA**TGG**C**GG**CAA**GG**CAGAAAAAAAAAAAAAAAA
AAAAAA
KACIMMI 6
CAGCAAGCCTC**GG**CAGAGC**GG**AGAGGGG**TGG**CGGCAAGGCAGAAAAAAAAAAAAAAA
AAA AAA
8. KACIMMI 65
CAGCAAGCTACAGCAGGAGAGATGAGCTG**TGGG**CGCACTGCAGAAAAAAAAAAAAAAAAAAAA
KACIMMI 65
CAGCAAGCTACAGCAGGAGAGATGAGCTG**TGGG**CGCCCTGCAGAAAAAAAAAAAAAAA
AAA AA

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. Phylogenic 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.

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