Breeding Strategy for Resistance to *Striga Asiatica* Based on Genetic Diversity and Population Structure of Tropical Maize Lines

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Abstract

Maize (*Zea mays* L.*)* is a major staple crop in southern Africa and is produced on millions of hectares. However, its yield is greatly reduced by *Striga* spp, a parasitic weed which is causing US$ 7 billion losses annually. Use of host resistance could be an effective way of controlling *Striga* and resistance to *Striga* is quantitative, mainly controlled by additive gene action. Understanding the population structure and genetic diversity is therefore key in designing an effective breeding program targeting grain yield heterosis and resistance to *Striga*. The aim of this study was to determine the genetic diversity and population structure of the key germplasm from tropical Africa. This information could guide in the identification of heterotic groups and potential testers required to kick start a maize breeding program for *Striga asiatica* in southern Africa. A total of 222 maize inbred lines from IITA and CIMMYT were used in this study. The materials were genotyped using the genotyping-by-sequencing method. A total of 45 000 SNP markers were revealed, and these were subjected to analysis of molecular variance, structure analysis and clustering using the Gower's distance and neighbor joining algorithm. Molecular variance was lager within individuals (91%) than among populations (9%). The inbred lines clustered into three major groups, with the IITA germplasm clustering separately from CIMMYT germplasm. A breeding strategy for *Striga asiatica* resistance was proposed with the aim of increasing genetic gains in both the resistance and grain yield.

Introduction

Maize is the main preferred staple in southern Africa with consumption rate averaging about 100kg per capita per annum (Epka, 2019). However, *Striga spp* is one of the major biotic factors affecting maize production in Africa (Ejeta and Gressel, 2007) and is considered among the world's worst weeds (Shayanowako et al., 2018) causing up to US$ 7 billion loss annually in Africa (Rubiales et al., 2009). *Striga spp* is an obligate parasite that draws nutrients and water from its host (Ejeta and Gressel, 2007). There are two types of *Striga* spp which are *Striga asiatica* and *Striga hermonthica* that are prevalent in southern Africa and the rest of Africa, respectively. The widely reported cultural, biological and chemical control options for *Striga spp* are not feasible for the resource limited farmers in sub-Saharan Africa (Joel et al., 2007). Use of host resistance has been effective in controlling pests, diseases and weeds in various crops.

The International Institute of Tropical Agriculture (IITA) managed to incorporate resistance from wild relatives of maize, *Zea diploperennis* and *Tripsacum dactyloides* (Rispail et al., 2007). Resistance was also sourced from maize populations in east Africa where the cereals have co-existed with this parasite for long. Subsequently, a number of inbred lines and hybrids with resistance to *Striga* hermonthica were developed and were shared across various maize breeding programs in the rest of Africa. Resistant genotypes usually show few *Striga* root attachments and little *Striga* germination stimulant (strigolactones) production (Rank et al., 2004). Other resistance mechanisms include reduced flowering and reduced seed set of the *Striga* species (Awad *et al.*, 2006). These mechanisms of resistance were

found to be effective in controlling *Striga asiatica* in southern Africa (Gasura *et al*. In Press). Resistance to *Striga* spp was found to be controlled mainly by additive gene action (Gasura *et al*. In Press).

Germplasm from IITA has novel sources of resistance to *Striga* spp while germplasm from the International Maize and Wheat Improvement Centre (CIMMYT) is widely adapted to many regions including southern Africa. In order to maximize heterosis using germplasm from IITA and CIMMYT, there is need to understand the population structure and genetic diversity of these materials (Mengesha et al., 2017). This information is crucial in the identification of potential testers and prediction of potential heterotic groups, which are some key determinants of an effective breeding program in maize (Laborda et al., 2005; Nyombayire, 2016).

Plant breeders need to cross inbred lines that are from different heterotic groups to maximize heterosis. The longest and expensive period during a hybrid production is when selecting parents that when crossed produces superior crosses (Moose and Mumm, 2008). Genetic distances play a very important role in hybrid vigor (Stinard et al., 2008). According to Acquaah (2012), a heterotic group is a group of related genotypes or distant genotypes from the same or different populations, which have similar combining ability when crossed to complementary germplasm groups. In contrast, a heterotic pattern refers to heterotic groups that complements one another and showing high heterosis when crossed (Jannink et al., 2010). Maize breeders postulated the concept of heterosis, when they observed that, inbred lines from different heterotic groups were producing superior hybrids when crossed (Suwarno et al., 2014). Having information on heterotic groups and patterns, a breeder can fully utilize the available germplasm by exploiting complementary lines to produce better performing hybrids (Sibiya et al., 2013). From some studies conducted it was shown that the intergroup hybrids out yields intragroup hybrids in maize (Mengesha et al., 2017).

To begin a maize hybrid program one has to use a well-documented germplasm with well-known heterotic groups and patterns (Moose and Mumm, 2008). Methods such as geographical information, phenotypic traits, pedigree information, combining ability and the use of molecular markers (Wende et al., 2013) have been widely used to classify genotypes. In the temperate regions, the Reid * Lancaster heterotic pattern has been developed using pedigree analysis and also in Europe, the European flint * Maize Belt dent have been developed based on phenotypic markers (endosperm types) (Paschold et al., 2010; Fischer et al., 2010). In France F2*F6 heterotic pattern derived from open pollinated cultivars was reported while in the tropical regions many patterns have also been developed including the ETO-compote*Tuxpeno and the Suwani*tuxpeno pattern (Acquaah, 2012; Reid et al., 2011). The use of molecular markers especially the single nucleotide polymorphism (SNP) markers has shown advantages over other methods (Acquaah, 2012, Leal et al., 2010, Mammadov et al., 2012, Moose and Mumm, 2008, Xu and Crouch, 2008). The advantages of SNP markers include speed (Leal et al., 2010), locus specificity, codominance, high genomic abundance, high throughput, lower genotyping error rates and low cost per data point (Paschold et al., 2010, Richard et al., 2018, Semagn et al., 2012). The aim of this study was to determine the genetic diversity and population structure of the key germplasm from CIMMYT and IITA

using SNP markers. This information could guide in the identification of heterotic groups and potential testers required to kick start a maize breeding program for *Striga asiatica* in southern Africa.

Materials And Methods

Plant materials

A total of 222 maize inbred lines comprising of 192 inbred lines from CIMMYT, Harare, Zimbabwe and 30 inbred lines from IITA, Ibadan, Nigeria were used in the study. The names and pedigrees of this germplasm is provided in Table 1.

Genomic DNA isolation

Seeds were shipped to the Biosciences for East and Central Africa (BeCA) at the International Livestock Research Institute (ILRI) (BeCA-Hub-ILRI) Kenya. The seeds of the 222 inbred lines were germinated and the DNA was extracted from fresh tissues that were one week old using the modified CTAB method (Saghai-Maroof *et al.*, 1986). The DNA was checked for quality using the agarose gel and quantity using a spectrophotometer. Genotyping was done using Genotyping-By-Sequencing (GBS) platform according to the protocol of the Integrated Genotyping Support Services (IGSS) (Delannay et al., 2012) at BeCA-Hub-ILRI, Kenya.

Data analysis

A total number of 45 000 SNP markers were sampled in this study. Genotypic data were subjected to analysis of molecular variance (AMOVA) using GenAlex software version 6.5 (Meirmans, 2012; Peakall and Smouse, 2012). Structure software (Pritchard et al., 2003) with a burnin length of 500 and MCMC of 1000 was used to determine the number of groups among the inbred lines. The online genetic software Structure Harvester (Earl, 2012) visualized the structure analysis results following the Evanno approach. RStudio software was then used for cluster analysis to depict the inferred groups using the Gower's distance (Gower, 1971) and neighbor joining algorithm. The silhouette plots using RStudio like structure results also suggested three groups and the dendrogram was then sub-divided into the 3 groups using the cutree option in RStudio (Team R, 2015).

Results

Genetic diversity among genotypes and populations

The total molecular variation was partitioned into among population and within populations. Larger genetic variability (91%) was attributed to variation within populations and the remaining 9% variation was explained by variation among populations (Table 2).

Table 2
Analysis of molecular variance for the 222 maize inbred lines based on SNP markers

	Degrees of freedom	Sums of squares	Mean squares	Estimated variance	Percentage
Among Pops	1	13944.298	13944.298	232.417	9%
Within Pops	220	489505.580	2225.025	2225.025	91%
Total	221	503449.878		2457.443	100%

Population structure, cluster analysis and genetic distances

The population structure of the germplasm was suggested following the admixture model. From the prosed K = 10 groups, the Evanno criterion (Earl, 2012) suggested three distinct groups (Fig. 1).

The dendrogram generated using the Neighbor joining algorithm based on the 45 000 SNP markers grouped the 222 inbred lines into three major clusters (Fig. 2) as suggested by the silhouette plots and the Evanno criterion. The first cluster had two inbred lines, one from CIMMYT (T396-326) and another one from IITA (TZEI4). The remaining 220 inbred lines belonged to the second and the third clusters that are also partitioned clearly into IITA and CIMMYT maize lines. These two groups had some several sub-groups within them. Inbred lines within some sub-groups were also clustered based on their heterotic groups for example CML 395 in group A, clustered separately from CML 444 in group B. Furthermore, in most cases inbred lines in CIMMYT heterotic group A grouped together while inbred lines that are in CIMMYT heterotic group B also grouped separately within their sub-cluster.

Discussion

In a panmictic population, the among population variance is expected to be minimal or absent (Meirmans, 2006). The small among population variance observed could be due to the different selection systems being conducted by IITA and CIMMYT since they target different breeding agro-ecological regions. This could explain why the IITA materials grouped separately from the CIMMYT materials, thus the groups are in total agreement with the sources of germplasm. The occurrence of T396-326 and TZEI4 in the same group yet they come from different sources is an example of some common inconsistencies in clustering. Although mutation, selection and genetic drift can lead to the alignment of inbred lines from different sources into the same groups (Wende et al., 2013; Hu et al., 2021) a possible explanation could be different in this case. The most probable reason for failure to distinctly separate inbred lines is caused by developing lines from crosses between different groups, leading to having lines with mixed origin (Moose and Mumm, 2008). There are some situations where IITA and CIMMYT exchange germplasm, for example in this case where IITA materials should be donors of *Striga* resistance. The few number of

clusters obtained are in agreement with a genetic diversity study of tropical maize inbred lines from Rwanda where Nyombayire *et al* (2016) found two main clusters.

The high within population genetic variation observed is mainly due to the enrichments efforts to widen the genetic base of the breeding materials of these organizations. Indeed, within each group there were many sub-clusters that shows the existence of huge variation within the group. Inbred line TZEI4 that clustered with only one CIMMYT line T396-326 shows that it has unique properties as compared to all other inbred lines from IITA. This shows that TZEI4 can be crossed to the rest of CIMMYT lines in this collection and still expressing high heterosis. However, it is unclear to predict the heterotic groups of this line together with the rest of the CIMMYT lines because most of the IITA lines and CIMMYT lines clustered separately. We expected lines of the same heterotic groups to cluster together thus showing the relationship between IITA and CIMMYT germplasm. One possibility could be that all the IITA materials belong to a heterotic group completely different to that of CIMMYT, hence the existence of these two major groups.

Genetic diversity information enables breeders to take stock of available genetic variation, conserve and efficiently utilize their materials in various breeding programs (Bidhendi et al., 2012). Resistance to *Striga* was shown to be controlled by mainly additive gene action, suggesting that resistant lines must be crossed when formulating hybrids. However, grain yield, the final target trait, is mainly controlled by non-additive gene action. To guide the future breeding programs that aim at the simultaneous improvement of *Striga* resistance and grain yield in maize, the following program is proposed for tester identification, as well as line and hybrid development;

1. Cross eight (8) IITA materials from different sub-cluster (including TZEI4 that is highly distinct) to 20 CIMMYT lines in different sub-clusters using a line x tester scheme
2. Evaluate the 160 testcross materials under optimum condition to get the specific combini ability (SCA) effects for grain yield. Also evaluate the 160 materials in the laboratory and glasshouse to get the *Striga asiatica* resistance parameters.
3. Select two testers one for group A lines and two testers for group B lines. Lines with negative SCA effects are in the same group, while lines with positive SCA effects are in different groups. The testers must have positive general combining ability (GCA) effects for *Striga asiatica* resistance.
4. Line improvement will be done within heterotic group using *Striga asiatica* resistance materials from IITA that are within that group.
5. Line selection will be done by testcross performance evaluation using a tester from a different heterotic group. A desirable line must have both positive GCA effects for grain yield and negative GCA effects for *Striga* resistance parameters.
6. Desirable hybrids will be made by crossing lines from different heterotic groups but having positive GCA effects for grain yield and negative GCA effects for *Striga* resistance parameters.
7. Hybrids will be evaluated for *Striga asiatica* resistance in the laboratory and greenhouse. Only hybrids with minimum thresholds in terms of resistance to *Striga* will be taken to the field for

preliminary yield trials thus reducing the number of hybrids to be taken to the field. These will be followed by multi-locational testing on at least five locations for two seasons as required by the variety release committee.

The ultimate goal of every breeding program is to improve its efficiency as determined by the number of hybrids produced per unit time. In this regard, to improve the selection efficiency and genetic gains, we propose the use of molecular markers for screening of *Striga* resistance, major diseases, and then complement that information from the field evaluation of grain yield performance based on the combining ability of the testcross hybrids.

Conclusions

Molecular genetic diversity has been clearly seen in this study and is largely located within individuals (91%) with 9% among populations. The inbred lines were clustered into three major groups, with the IITA germplasm clustering separately from CIMMYT germplasm. A breeding strategy for *Striga asiatica* resistance was proposed with the aim of increasing efficiency in genetic gains for both the resistance and maize grain yield.

Declarations

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Conflicts of interest/Competing interests

There are no conflicts of interest

Availability of data and material

Data can be provided if required

Code availability

The code and Softwares are publicly available

Authors' contributions

Edmore Gasura and Martina Kyalo generated the data, Edmore Gasura and Brian Nyandoro wrote the manuscript, Stanford Mabasa and Peter Setimela reviewed the work, Peter Setimela helped in germplasm

acquisition, Nasser Yao supervised the whole work and helped in fundraising for the project.

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Table

Table 1 not available with this version.

Figures

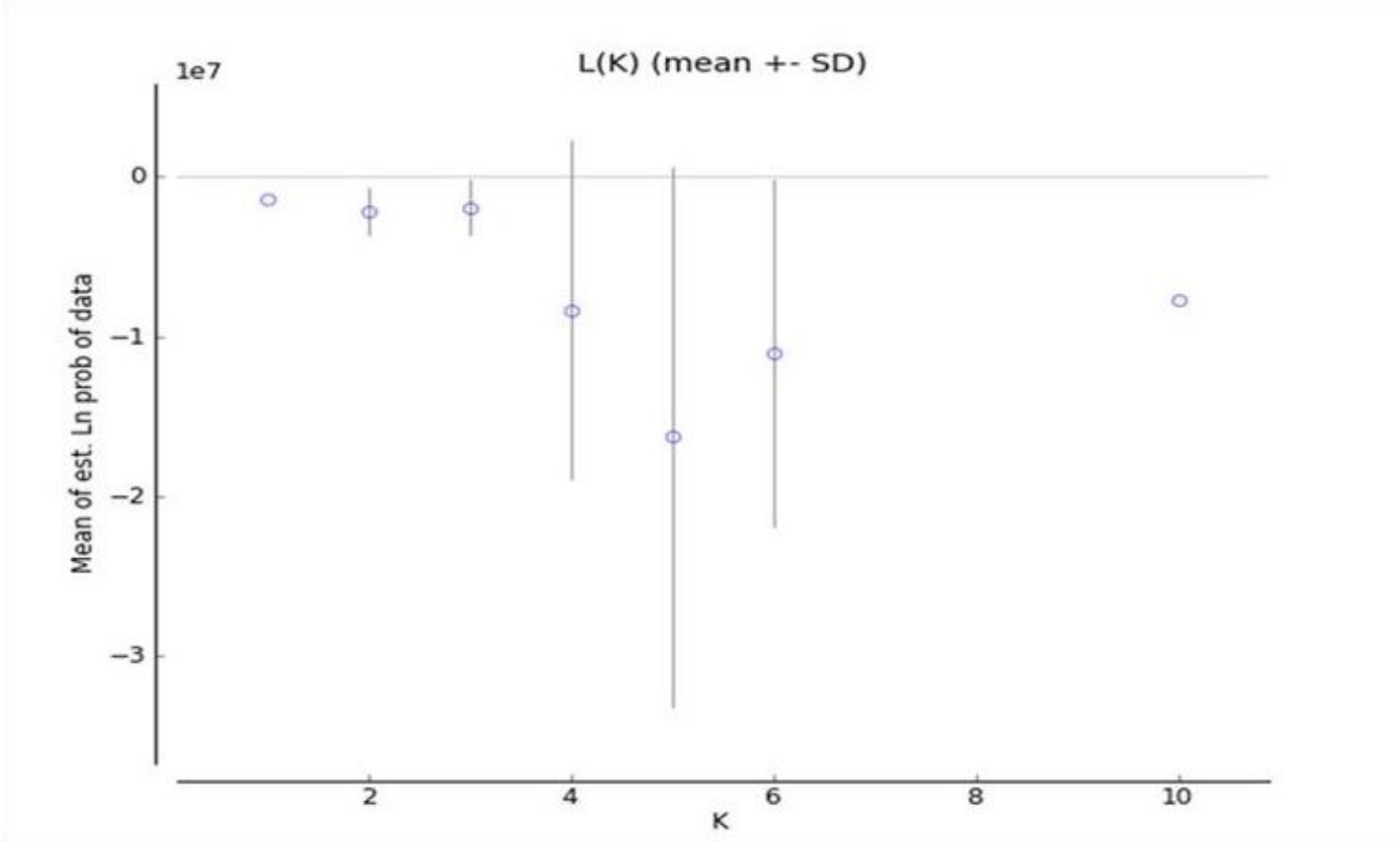

Figure 1

Number of groups inferred from structure software

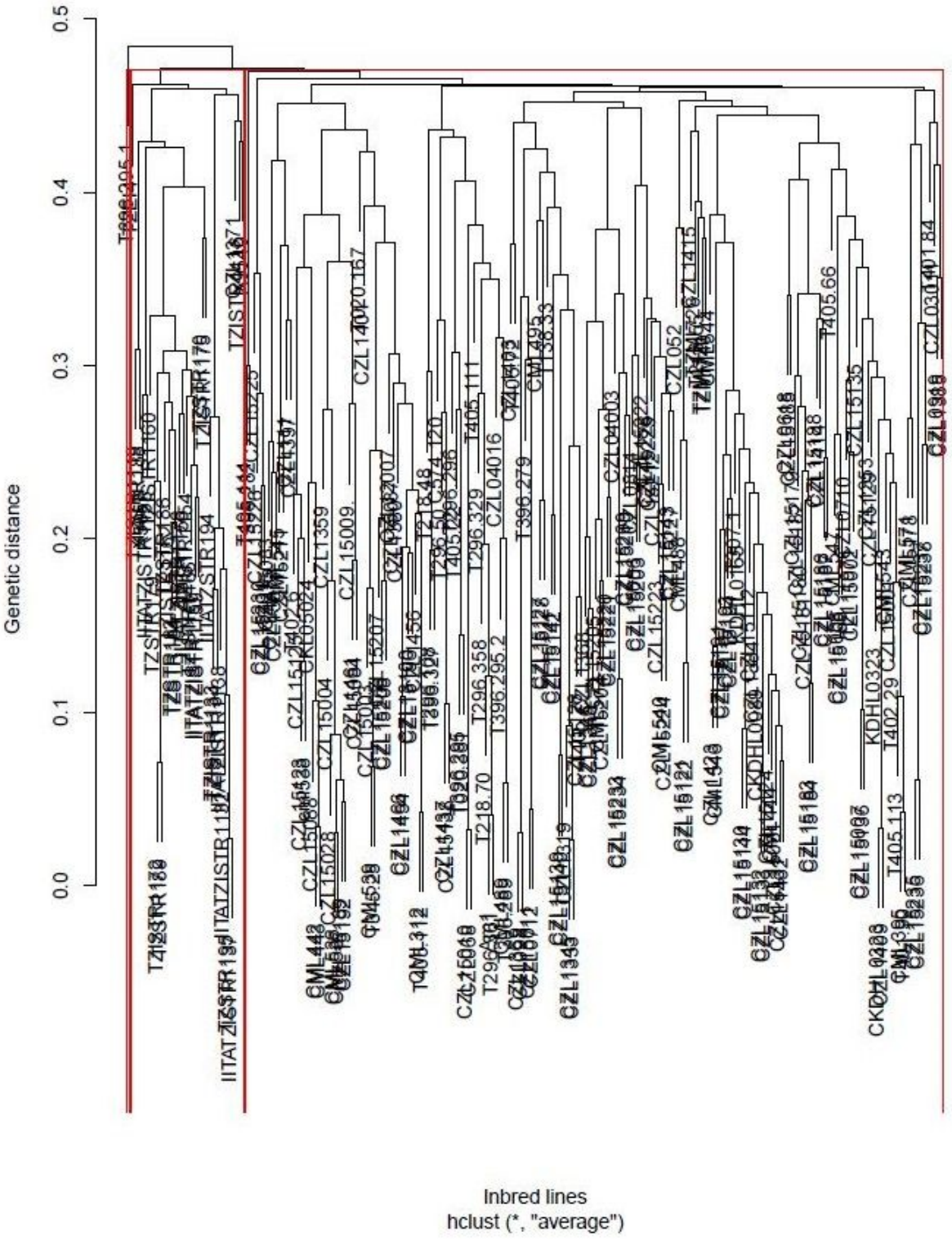

Figure 2

Inferred groups and clustering of the 222 IITA and CIMMYT maize inbred lines based on 45 000 SNP markers using the Gower's distance and the neighbor joining algorithm.