Original Paper Open Access

Genetic diversity among maize inbred lines selected for the mid-altitudes and highlands of Rwanda

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Abstract

Understanding the genetic diversity and relationships among breeding materials is fundamentally considerable for any crop improvement program. This study was carried out to apply specific single nucleotide polymorphism (SNP) markers to determine the amount of genetic diversity prevailing among maize inbred lines selected for the mid-altitudes and highlands of Rwanda and classify the inbred lines according to their relationships for an effective hybrid breeding program. Seventy one maize inbred lines from different sources were genotyped with ninety two SNP markers. The unweighted pair group method with arithmetic mean (UPGMA) revealed that there was a random allocation of the inbred lines into different clusters and they were allocated into 2 major clusters regardless their origin. The highest (0.375) polymorphic information content (PIC) observed was exhibited by 3 markers; PZA00543_12, PZA00878_2, and PZA01735_1; while the lowest PIC value was revealed by the marker PZA01755_1 (0.1224).The PIC value (0.30) revealed in this study may confirm the potential for these SNP markers to discriminate between inbred lines from diverse origins and their usefulness for diversity analysis of maize inbred lines under this study. Genetic clustering information acquired from the current study would be suitable information not only for maize hybrid program establishment in Rwanda, but also for other collaborative tropical maize breeding programs. This might guide towards suitable heterotic patterns and groups as well as the combining ability of the inbred lines involved in this study.

Keywords: maize, genetic diversity, single nucleotide polymorphism

Introduction

Genetic distance among breeding materials is a key factor to consider when predicting genetic variability among parental combinations (Bertan et al, 2007; Laborda et al, 2005; Mohammadi and Prasanna, 2003; Semagn et al, 2012; Wende et al, 2013). High yielding as well as genetically distant genotypes might represent parent inbred lines with different loci controlling the character and probably with high combining ability. Therefore, information on germplasm diversity and relationships existing among breeding materials is a key to crop improvement. Evaluation of genetic diversity and relationships in a given set of germplasm is valuable for selecting parental combinations aiming at developing progenies with high genetic variability (Semagn et al, 2012).

Assessing genetic diversity and relatedness among breeding materials has a preponderant role in a breeding program. Development of improved inbred lines and identifying suitable parental combinations to generate high performing hybrids is the leading task of maize breeders (Semagn et al, 2012). Information related to genetic diversity and relationships among diverse germplasm is valuable to plant breeders as this information leads the decision making during selection of parents for crossing and is useful for broadening the genetic basis of different breeding programs (Laborda et al, 2005). Unfortunately, many maize breeding programs depend on phenotypic evaluations. However, the presence of favorable alleles is difficult to be detected among germplasm mainly due to environment effect. This was earlier revealed by Leal et al (2010), who reported that molecular markers have proved to have different advantages over other methods since they show genetic differences on a more detailed level without interferences from environmental factors and they involve techniques that provide fast results detailing genetic diversity. Therefore, for effective management of genetic diversity, there is need of well-characterized germplasm and genetic pools well classified into different clusters based on genetic diversity (Dhliwayo et al, 2009; Muhinyuza et al, 2015; Wende et al, 2013).

Genetic clustering of parental inbred lines will permit breeders to predict maize hybrid performance resulting from different intergroup crosses. However,

 RECEIVED 2/18/2016

the effectiveness of this will be depending on genetic backgrounds of the germplasm being documented. Generally, high diversity is expected from inbred lines resulting from different cluster while, low diversity is expected between two inbred lines within the same cluster. Not only genetic diversity assessment is useful to identify parents for making crosses but also in predicting heterotic groups. Increased allelic diversity will be responsible of the presence of discrete genetic groups among inbred lines, and this might result in high level of heterozygosity in the hybrid related to increased heterosis. However, confirming genetic grouping generated through molecular data is the most informative method and needs to be complemented with combining ability tests especially on yield and yield components (Adeyemo et al, 2012; Wende et al, 2013).

Various methods to identify the best progenitors for generating combinations and to cluster these progenitors to a given heterotic group have been reported (Bertan et al, 2007; Semagn et al, 2012): i) phenotypic performance for particular traits, ii) pedigree relationship, iii) adaptability and yield stability, iv) top crosses, v) diallel crosses, and vi) genetic distance assessed from morphological and molecular markers. Although each of these methods has its own advantages and disadvantages, using information resulting from them can contribute to identify the best hybrid combinations (Dhliwayo et al, 2009; Wende et al, 2013)

DNA markers can assist for assessing the amount of genetic diversity available in breeding materials (Adeyemo et al, 2012; Muhinyuza et al, 2015). They have been reported to increase the efficiency of conventional breeding by shortening the time allocated to variety development (Semagn et al, 2012; Wende et al, 2013). Genetic distance assessed from molecular markers can be estimated from different types of molecular markers, comprising amplified fragment length polymorphism (AFLP), restriction fragment length polymorphism (RFLP), simple sequence repeats (SSRs), and single nucleotide polymorphisms (SNPs) (Semagn et al, 2012). Of these markers, current advances in molecular technology have shown a shift heading to SNPs (Jones et al, 2007; Semagn et al, 2012). This is because of their various attributes such as; locus-specificity, low cost per data point, codominance, high genomic abundance, potential for high throughput analysis, and lower genotyping error rates (Chagné et al, 2007; Rafalski, 2002; Schlötterer, 2004; Semagn et al, 2012). In their findings, Semagn et al (2012) reported SNP markers as a powerful tool in genetic diversity studies and marker assisted breeding.

In the current study, SNPs markers were used to assess the magnitude of genetic diversity and relationships among maize inbred lines selected for the mid-altitudes and highlands of Rwanda. This will be useful for establishment of a hybrid breeding program in Rwanda. In different breeding programs, it was realized that many undesirable crosses could be avoided by allocating inbred lines into well-differentiated clusters (Wende et al, 2013; Muhinyuza et al, 2015) and molecular markers have been reported to play considerable role in characterizing inbred lines and then generating diverse clusters of genotypes based on genetic diversity (Melchinger and Gumber, 1998; Reif et al, 2005; Wende et al, 2013). Earlier studies, using molecular markers effectively allocated maize germplasm into different heterotic groups (Dubreuil et al, 1996; Lee et al, 1989; Livini et al, 1992; Wende et al, 2013).

Currently, the maize breeding program in Rwanda performs selection and genetic relationships of maize lines based on phenotypic characterization. No study exists on genetic diversity assessment among maize inbreds in Rwanda based on molecular data. Earlier studies focused mostly on evaluation for adaptability of new introduced genotypes form different collaborators such as International Maize and Wheat Improvement Center (CIMMYT) and International Institute of Tropical Agriculture (IITA). Therefore, there is need to explore the genetic interrelationships existing among maize inbred lines selected for the major agroecologies of Rwanda and find out specific clusters and relationships in order to establish a sustainable maize hybrid program in Rwanda. Consequently, the objectives of the current study were to apply selected SNP markers and determine the genetic distances and clusters among potential maize inbred lines selected for the mid-altitudes and highlands of Rwanda. This is for a solid foundation of maize hybrid breeding program, hence a basic understanding of the genetic diversity and relationships among these maize accessions was considered essential.

Materials and Methods

Plant materials

A total of 71 maize inbred lines; comprising 44 local inbred lines, 16 inbred lines from CIMMYT-Ethiopia and 11 lines from CIMMYT-Mexico, were used in the study (Table 1). Most of inbred lines from CIMMYT were of tropical origin and they differ in their response to different foliar diseases and heterotic grouping. On the other hand, the local inbred lines were from nine maize open pollinated varieties (OPVs) and some of these populations have been grown by farmers for their different attributes. All these inbred lines were selected based on disease resistance, vigor, and adaptability to local environments.

DNA sampling and isolation

DNA was extracted from inbred lines planted in a nursery at Nyagatare research station in 2014B growing season. Using the punch method, at 4 weeks after planting, leaf sample tissue of each individual inbred line was harvested at the 3-4 leaf stage. Two leaf discs from each inbred line were then placed into 2 labelled 96-well blocks and each well representing an

Table 1 - Description of maize inbred lines used in the study.

No	Code	Origin	No	code	Origin
1	E1	CIMMYT-Ethiopia	37	M8144	Rwanda
2	E3	CIMMYT-Ethiopia	38	ACR3	Rwanda
3	E4	CIMMYT-Ethiopia	39	ACRO4	Rwanda
4	E5	CIMMYT-Ethiopia	40	ACR4	Rwanda
5	E8	CIMMYT-Ethiopia	41	ACRO29	Rwanda
6	E9	CIMMYT-Ethiopia	42	ACR29	Rwanda
7	E10	CIMMYT-Ethiopia	43	ECA1	Rwanda
8	E11	CIMMYT-Ethiopia	44	ECA13	Rwanda
9	E12	CIMMYT-Ethiopia	45	ECA18	Rwanda
10	E14	CIMMYT-Ethiopia	46	ECA1ECA2	Rwanda
11	E15	CIMMYT-Ethiopia	47	ECA1ECA1S5	Rwanda
12	E17	CIMMYT-Ethiopia	48	ECA1ECA5	Rwanda
13	E18	CIMMYT-Ethiopia	49	ECA1ECA43	Rwanda
14	E19	CIMMYT-Ethiopia	50	ECAP3	Rwanda
15	E20	CIMMYT-Ethiopia	51	ECAP11	Rwanda
16	E21	CIMMYT-Ethiopia	52	ECAPO23	Rwanda
17	M351	CIMMYT-Mexico	53	ECAP23	Rwanda
18	M352	CIMMYT-Mexico	54	TQX7	Rwanda
19	M353	CIMMYT-Mexico	55	TQ7	Rwanda
20	M354	CIMMYT-Mexico	56	TQ8	Rwanda
21	M355	CIMMYT-Mexico	57	TQX31	Rwanda
22	M356	CIMMYT-Mexico	58	TQ31	Rwanda
23	M455	CIMMYT-Mexico	59	CM523	Rwanda
24	M456	CIMMYT-Mexico	60	CM506	Rwanda
25	M457	CIMMYT-Mexico	61	MZ3	Rwanda
26	M459	CIMMYT-Mexico	62	MZ4	Rwanda
27	M464	CIMMYT-Mexico	63	MZ5	Rwanda
28	R10164	Rwanda	64	POL1	Rwanda
29	R10127	Rwanda	65	POL2	Rwanda
30	R10141	Rwanda	66	POL3	Rwanda
31	RM8147	Rwanda	67	POL4	Rwanda
32	RM8119	Rwanda	68	POL5	Rwanda
33	M8147	Rwanda	69	POL6	Rwanda
34	M8119	Rwanda	70	POL7	Rwanda
35	RM8144	Rwanda	71	POL8	Rwanda
36	RM8115	Rwanda			

individual inbred line. Once the block was completed, a sheet of air-pore tape was put on the top of the block for sealing and then placed inside plastic bags together with 50 g of silica gel for drying purpose. The samples were then conveyed to DNA Landmarks laboratory, Canada for genotyping. DNA was extracted and isolated following a proprietary Sarkosyl Nitrogen based method at the DNA Landmarks laboratory (Blin and Stafford, 1976).

Genotypic data analysis

Based on previous research studies on maize at CIMMYT, a total of 100 SNPs (Table 2) were used in the study. However, 8 of them were not polymorphic with the genotypes involved in the study and therefore discarded from the analysis. For each SNP marker, number of alleles, allele frequency, number of genotypes, genotype frequency, observed heterogeneity, gene diversity, genetic distance, polymorphic information content (PIC), and cluster analysis based on similarity matrices obtained with Unweighted Pair Group Method with Arithmetic Average (UPGMA) to generate dendrograms were computed (Nei, 1991) using Power Marker version 3.25 (Liu and Muse, 2005).

Results and Discussion

SNPs characteristics and genetic polymorphisms

Of the 100 SNPs genotyped, 92 (92%) with missing data less than 10% and of good quality were used for subsequent analysis. Among the 71 maize inbred lines involved in the study, the 92 SNPs revealed a total of 184 alleles (with an average of 2 alleles per marker). Genetic diversity varied from 0.014 to 0.500 with an average of 0.385. As a measure of allelic diversity at a locus, expected heterozygosity (He) values varied from 0.00 to 0.19 with a mean of 0.08, while the PIC estimates ranged from 0.014 to 0.375 with a mean of 0.303.The ten SNPs (Table 2) exhibiting the highest PIC and their potential to detect differences between the inbred lines were; PZA00543_12 (0.3750), PZA00878_2 (0.3750), PZA01735_1 (0.3750), PZB00085_1 (0.3749), PZA00257_22 (0.3748), PZB01647_1 (0.3746), PZD00022_6 (0.3746), PZA02763_1 (0.3745), PZB02510_ (0.3742),

Table 2 - Details of the 92 successful SNPs markers used to genotype the 71 maize inbred lines.

Marker	Availability	He	PIC	Marker	Availability	He	PIC
PZA00106_10	0.9577	0.0588	0.3671	PZA03116_2	1.0000	0.1549	0.3498
PZA00136_2	0.9296	0.1212	0.3599	PZA03182_5	1.0000	0.0986	0.3584
PZA00223_2	0.9296	0.1061	0.3736	PZA03231_1	1.0000	0.1408	0.3362
PZA00257_22	0.9718	0.0580	0.3749	PZA03391_2	1.0000	0.1127	0.3362
PZA00266_7	0.9718	0.1304	0.3716	PZA03395_3	0.9859	0.0143	0.1906
PZA00309_2	0.9718	0.1014	0.3574	PZA03404_1	1.0000	0.1127	0.3726
PZA00343_31	0.9718	0.1159	0.3707	PZA03445_1	0.9859	0.0571	0.3091
PZA00352_23	0.9577	0.0882	0.3715	PZA03470_1	1.0000	0.0704	0.1886
PZA00455_16	0.9859	0.0429	0.3466	PZA03474_1	1.0000	0.0704	0.3111
PZA00543_12	0.9577	0.0882	0.3750	PZA03507_1	1.0000	0.0423	0.3700
PZA00726_8	1.0000	0.0563	0.3228	PZA03602_1	1.0000	0.0704	0.3392
PZA00827_1	1.0000	0.1127	0.3726	PZA03644_1	1.0000	0.0704	0.2049
PZA00878_2	0.9859	0.0571	0.3750	PZA03661_3	1.0000	0.0282	0.2777
PZA00881_1	0.9577	0.1029	0.2550	PZA03695_1	1.0000	0.0141	0.0139
PZA00920_1	0.9718	0.1884	0.3612	PZA03728_1	1.0000	0.1408	0.3421
PZA00947_1	0.9577	0.0147	0.0929	PZA03733_1	1.0000	0.0986	0.2606
PZA00948_1	0.9859	0.1143	0.3742	PZA03743_1	1.0000	0.1127	0.3522
PZA01142_4	1.0000	0.0704	0.3742	PZB00008_1	1.0000	0.0563	0.1007
PZA01292_1	1.0000	0.0986	0.3700	PZB00068_1	1.0000	0.0704	0.2049
PZA01304_1	0.9859	0.0429	0.2854	PZB00085_1	1.0000	0.1268	0.3748
PZA01315_1	0.9718	0.0725	0.3304	PZB00109_2	1.0000	0.1408	0.3738
PZA01342_2	0.9718	0.1014	0.3645	PZB00175_6	1.0000	0.0423	0.2203
PZA01396_1	0.9577	0.0588	0.3715	PZB00232_1	1.0000	0.0704	0.2203
PZA01447_1	1.0000	0.0563	0.2979	PZB00772_1	1.0000	0.0423	0.0405
PZA01735_1	1.0000	0.1127	0.3750	PZB00869_4	1.0000	0.0282	0.1969
PZA01755_1	1.0000	0.0563	0.1224	PZB01042_7	1.0000	0.1127	0.3689
PZA01804_1	0.9859	0.0714	0.3515	PZB01156_2	1.0000	0.0845	0.2979
PZA02019_1	0.9859	0.1857	0.3633	PZB01186_1	1.0000	0.0704	0.3025
PZA02027_1	0.9718	0.1159	0.3686	PZB01358_2	1.0000	0.0986	0.3620
PZA02068_1	1.0000	0.1127	0.3726	PZB01400_1	1.0000	0.0282	0.1800
PZA02113_1	0.9859	0.0857	0.2800	PZB01647_1	1.0000	0.0563	0.3746
PZA02148_1	1.0000	0.0000	0.1007	PZB02017_1	1.0000	0.1268	0.3700
PZA02212_1	0.9859	0.0429	0.2629	PZB02033_2	1.0000	0.1268	0.3742
PZA02367_1	0.9859	0.1000	0.2369	PZB02155_1	1.0000	0.0563	0.3069
PZA02386_2	1.0000	0.0141	0.1886	PZB02283_1	1.0000	0.0986	0.3448
PZA02450_1	1.0000	0.0563	0.3603	PZB02480_1	1.0000	0.0423	0.2346
PZA02564_2	0.9859	0.1286	0.3725	PZB02510_5	1.0000	0.0986	0.3742
PZA02585_2	1.0000	0.0845	0.2882	PZD00022_6	1.0000	0.0282	0.3746
PZA02589_1	1.0000	0.0563	0.3522	PZD00027_2	1.0000	0.1127	0.3474
PZA02606_1	1.0000	0.0423	0.1327	PZD00054_1	1.0000	0.0423	0.3678
PZA02676_2	0.9859	0.0429	0.3737	PZD00072_2	1.0000	0.0563	0.1224
PZA02683_1	0.9859	0.0714	0.3212	ZHD1_1	1.0000	0.0563	0.3474
PZA02763_1	0.9859	0.0714	0.3745	bt2_2	1.0000	0.0563	0.2665
PZA02890_4	1.0000	0.0000	0.0777	csu1171_2	1.0000	0.0563	0.2414
PZA02916_5	1.0000	0.0563	0.2882	sh1_2	1.0000	0.0563	0.3362
PZA02957_5	1.0000	0.0704	0.3498	umc128_2	1.0000	0.1268	0.3718

He and PIC means expected heterozygosity and polymorphic information content respectively;

and PZD00022_6 (0.3742). Contrary to this, the following ten SNPs (Table 2) exhibited the lowest PIC: PZB01400_1 (0.1800), PZA02606_1 (0.1327), PZA01755_1 (0.1224), PZD00072_2 (0.1224), PZA02148_1 (0.1007), PZB00008_1 (0.1007), PZA00947_1 (0.0929), PZA02890_4 (0.0777), PZB00772_1 (0.0405), and PZA03695_1 (0.0139).

As relative value of each marker with respect to the amount of polymorphism exhibited, the mean PIC value (0.303) observed in the current study was higher than the one reported in earlier findings. Using SNP markers for identification of functional genetic variations underlying drought tolerance in maize. Similar trend was also reported by Lu et al (2009) who reported a mean PIC value equivalent to 0.259 using 1034 informative SNPs and 770 maize inbred lines. Therefore, the high PIC value revealed in this study might be relevant indication confirming the potential for these SNP markers to discriminate between inbred lines from diverse origins. This was even proven by the fact the markers were able to disjoint closely related lines, indicating their usefulness for diversity analysis of maize inbred lines under the current study. On the contrary, when comparing SNPs and SSRs in assessment of genetic relatedness in maize, (Yang et al, 2011) reported a higher PIC (0.340). Similar trend

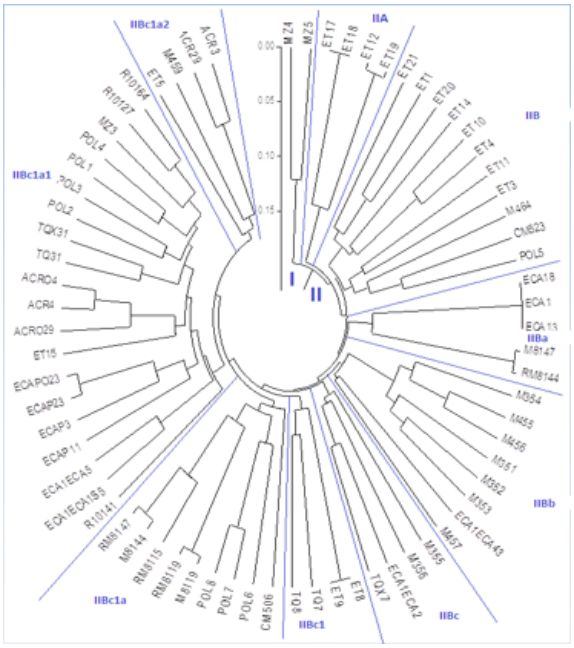

Figure 1 - Radial dendrogram showing genetic relationships among 71 maize inbred lines tested using 92 SNP markers. The two clusters are denoted from I to II while sub-clusters are denoted from IIA to IIBc1a2.

was also later revealed by Wende et al (2013), in their study on genetic interrelationships among medium to late maturing tropical maize inbred lines using selected SSR markers, a PIC of 0.54 was reported. However, according to Srinivasan et al (2004), the PIC values are dependent on the genetic diversity of the accessions chosen. Based on genetic diversity revealed in the current study in combination with the revealed PIC, it would contribute in minimizing the use of closely related maize germplasm in maize breeding program which would otherwise lead to genetic depression and reduced genetic variation. Therefore the current PIC demonstrates the usefulness of the SNPs and their potential to detect differences among the maize lines based on their genetic relationships.

Genetic distance and relationships

The dendrogram generated using the UPGMA clustering algorithm based on SNPs data grouped all the 71 inbred lines into 2 major clusters (Figure 1) with cluster one (I) having only 2 inbreds (MZ4 and MZ5) closely related in their pedigree information and originating from the same open pollinated variety. The remaining 69 inbred lines (97%) belonged to second cluster (II) also partitioned into many sub-clusters (from IIA-IIBc1a2) but also exhibiting distinct groupings within individual sub-clusters. Two major sub-clusters within cluster II; the first one (IIA) consisted of 4 lines (ET17, ET18, ET12, and ET19) of the same origin (CIMMYT-Ethiopia), while the second comprised all the rest (65) of the inbred lines. Of these 65 lines, 11 of them (IIB) fall in the same group and most of them (8) sharing the same origin (CIMMYT Ethiopia) and the remaining 54 (76%) formed another group except 5(IIBa) (from ECA18 to RM8144) lines from Rwanda forming their own group. The remaining 49 (69%) inbred lines (IIBb-IIBc1a2) formed another major group having many small groups in it, however, some of the inbred lines within these groups were aligned following their origin or their pedigree origin.

Generally, with some exceptions, there was a random allocation of the inbred lines into different clusters and / sub-clusters. Some of the inbred lines closely related were grouped in the same cluster or same sub-cluster (cluster I), confirming the presence of relationship between the pedigree and the SNPs marker groupings in this study. Though some of these inbred lines seemed to cluster according to their pedigree grouping (ECA18, ECA1, and ECA13), there were some inconsistencies; for instance: M355, M356, ECA1ECA2, and TQX7 clustered together despite being unrelated by pedigree. Similar findings were earlier reported (Dhliwayo et al, 2009; Semagn et al, 2012; Wende et al, 2013; Yang et al, 2011).

Discrepancies in classification of germplasm revealed when comparing molecular results with classification based on pedigree relatedness were earlier reported (Dhliwayo et al, 2009; Yang et al, 2011). They might resulted in the fact that all the local inbred lines involved in the current study were developed from maize open pollinated varieties selected from regional trials obtained from CMMYT-Kenya, therefore, there might be exchange of breeding materials among different CIMMYT breeding programs, justifying the alignment of some inbred lines from different origin in the same clusters or sub-clusters. Furthermore, these inconsistencies in inbred lines alignment may result also from the effects of mutation, selection, and genetic drift (Marsan et al, 1998; Senior et al, 1998; Wende et al, 2013).

Prasanna et al (2004) mentioned that effective and reliable discrimination of inbred lines not only helps in identification of genotypes, but also in promoting efficient utilization of genetic materials in breeding programs. This was also earlier pointed out by Hallauer and Miranda (1988) mentioning that the genetic divergence of parental varieties defines the manifestation of heterosis, and the heterotic pattern is determined by the genetic divergence of 2 parental lines. Therefore, crossing schemes comprising the more distant maize genotypes might allow for greater success in the production of genetic variability and thus might maximize the exploitation of heterosis and segregation (Molin et al, 2013). Consequently, the observed relationships in this study could be exploited accordingly in order to design a strong breeding maize hybrid program in Rwanda.

Conclusions

In overall, the SNPs markers disjointed the inbred lines into 2 major distinguishable clusters; this was disagreeing with the current pedigree records. However, this was not applied for sub-clusters; in some

of the sub-clusters, the SNPs markers partitioned the inbred lines into distinguishable clusters in alignment with the pedigree records. Furthermore, in addition to high PIC exhibited by some individual markers and their mean, the amount PIC observed under this study confirmed how useful are these SNPs markers for diversity investigation among these maize inbred lines under consideration. The acquired information from the current study regarding the amount of genetic diversity and relationships revealed in the maize inbred lines selected for the mid-altitudes and highlands of Rwanda in combination with combining ability and pedigree records would be explored to point out suitable heterotic patterns and group the inbred lines into specific heterotic groups. This genetic clustering would be suitable information for maize hybrid breeding program establishment in Rwanda, but also for other collaborative tropical maize breeding programs.

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