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Genetic Characterization Of Early Generation Lines Using SNPS Makers And Agronomic Traits For Resistance To Striga Improvement In Maize

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Abstract—The characterization of a greater number of lines hence potentially increase the efficiency of maize breeding programs. This study aims to assess the genetic variation and relationships existing within a population of 177 lines and the two parental lines, using 8,883 SNPs markers obtained from sequencing genotyping (GBS) and four agronomic traits. Two hundred S_1 lines and four checks including the two parents have been evaluated under *Striga hermonthica* infestation in Benin Republic and Nigeria for two years during 2018 and 2019 growing seasons using 51 x 4 lattice design with two replicates. The UPGMA phylogeny, was used to group the progenies based on their genetic similarity. The tested lines have displayed high genetic variability for all the agronomic traits. Analysis molecular revealed that the polymorphism information content has been varied from 0.047 to 0.50, with average of 0.37, and 63% of the SNP makers were highly polymorphic. The population has displayed a moderate diversity with average genetic diversity of 0.44. The estimated genetic distance has been varied from 0.01 to 0.79 and the highest distance has been observed between the two parental lines. UPGMA clustering based on the Gower dissimilarity matrix grouped the 177 lines into two clusters (I and II) at 30% genetic similarity threshold. The estimated genetic distances between lines showed that all the progenies were genetically related to the two parental lines; and have the potential to provide new favorable alleles for the development of high-performing, Striga-resistant and/or Striga-tolerant maize populations.

Keywords— Genetic variation, molecular markers, GBS, maize lines

I. INTRODUCTION

Maize (*Zea mays* L.) is one of an important food security and income generating crop in developing countries. In Sub-Saharan Africa (SSA), more than 80% of maize produced are used as food, providing at least 30% of the total calorie intake to the people, with intake ranging from 52 to 450 g/person/day [1]. In this region, maize is the only cereal crop with a remarkable potential production and high yield than other cereals; and constitute a major source of food security and economic development [2], [3]. Since 2010, its production in SSA has increased from 46 million tons to 744 million tons [4]. This strong increase in production is associated with the large increase in the area under maize production (55%), rather maize yields. The low productivity of maize in several maize-growing countries, compared to global average of nearly 5 tons ha^{-1}, is attributed to various socioeconomic, abiotic, and biotic constraints [1], [5]. Among the biotic constraints, *Striga hermonthica* is one of the important factors limiting maize production [2]. Depending on the level of infestation in the field and the susceptibility of the cultivar, grain yield losses of maize

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due to *S. hermonthica* can ranged from 70% to 100% [5], [6], [7], [8]. The Striga problem still persists because its infestation is greatly increasing in African soils. Besides this situation and climate resilience, maize varieties need to be improved with resistance or the tolerance to impact on the grain yields well-being of the farmers. Accelerated replacement of old climate-vulnerable maize varieties will go a long way in improving food security and livelihoods for several millions of smallholder farmers and their families [1], [9]. Efforts have been deployed by the breeders, to develop maize lines tolerant and resistant to *Striga*. These populations were developed from a cross containing inbred lines that have varied significantly in their responses under artificial *Striga hermonthica* infestation [10], [11]. Effective exploitation of these genotypes, needs the availability of the information on their genetic variation and relationship.

Information and utilization of knowledge on the genetic variation and of relationship among the breeding material impact on the identification of promising genotypes for use as source materials in breeding program [12]. The identification of lines for outstanding single crosses production as quickly as possible depend on the use of pedigree information and dependence on the morphological traits. The methods utilize morphological traits and are slow, laborious, greatly influenced by environment and several other factors limit their ability to estimate genetic diversity [12], [13]. Molecular markers are a useful tool for genetic diversity analysis at the DNA level in plant species because they are not sensitive to environmental factors and they reflect the actual level of genetic difference existing among the genotypes [14], [15], [16].

Classification of the genotypes in to different heterotic groups avoids the development and evaluation of the crosses that would be eventually be discarded [17]. Nyombayire et al. [18] and Mengesha et al. [19] used molecular markers to classify maize lines into similarity groups to eliminate less desirable lines and select those expressing traits desirable to breeding objectives. The use of DNA markers has facilitated early selection of maize highly yielding genotypes under stress conditions [20], [21]. Several molecular markers known as neutral genetic markers, have been used to characterize or analyze genetic diversity within maize populations [22], [23], [24]. Despite this diversity of markers, codominant markers, especially SNPs have become the preferred choice today due to their highly polymorphic, locus-specific and have potential for high throughput analysis as well as lower genotyping error rates [25], [26], [27]. The objectives of the study were to (i) assess the level of genetic variation within S_1 lines and the relationship with their parents using SNPs markers (ii) characterize the S_1 lines heterotic group for successfully breeding improved maize varieties based on agronomic potential.

II. Material And Methods

2.1. Plant materials

A total of 200 intermediate maturity group (105-110 days) maize S_1 lines derived from the cross (TZISTR1108 x 5057). The F_1 were originated from a cross between a resistant inbred line (TZISTR1108) and a susceptible inbred line (5057) to *S. hermonthica*. Subsequently, the F_1 was advanced to F_2 to develop F_2 bulk seeds. From the F_2 bulk seed, over 300 S_1 lines were, thereafter, developed. From these 300 lines, 200 S_1 lines were randomly selected alongside the two parents. The two parents plus two inbred lines with known resistance (9450) and tolerance (9030) reactions to *S. hermonthica* were included as benchmarks to assess the performance of the S_1 lines.

2.2. Phenotypic Evaluation

The S_1 lines were evaluated under Striga-infested conditions at Angaradebou (11 ° 20'N, 2 ° 43'E, 256 m at altitude) and Ina (09 °57 'N, 2 ° 43'E and 371 m) in Republic of Benin, and Abuja (9◦150 N, 7◦200'E; 490 m) and Mokwa (9◦210'N, 5◦100'E; 210 m) in Nigeria during the two years 2018 and 2019 growing seasons. The S_1 lines were arranged in a 51 × 4 lattice design with two replications and planted in strips. On each strip, each line has been planted on a row of 3 m length with 0.75 m inter-row spacing and 0.25 m intra-row spacing.

The field was treated with ethylene gas two weeks before planting to remove S. hermonthica seeds from the soil through suicidal germination. *S. hermonthica* seeds used for artificial infestation of the infested blocks were collected from farmers' sorghum fields in the previous planting year. Two maize seeds were planted in a 6 cm deep hole injected with 8.5 g of sand mixed with Striga seeds. The mixture contains approximately 3000 germinable Striga seeds. Two weeks after planting, all maize plants were thinned to one plant per hill to attain a population density of 53,333 plant ha^{-1}. Fertilizer was applied at the rate of 30 kg/ha of Nitrogen, 60

kg/ha each of phosphorus and potassium at planting, and an additional 30 kg/ha nitrogen was applied four weeks later. Weeds other than Striga were removed from plots manually throughout the planting season.

2.3. Trait Measurements

Data were recorded on the number of emerged Striga plants (STRCO) while host plant damage syndrome rating (STRRAT) was recorded at 10 weeks after planting on a scale of 1–9 where 1 = normal plant growth, no visible symptoms, and 9 = complete scorching of the leaves, resulting in premature death or collapse of host plant with no ear formation (Kim, 1991). Ear aspect was scored on a scale of 1 to 5, where 1 = clean, uniform, large and well-filled ears, and 5 = rotten, variable, poorly filled, and small ears. Grain yield (YIELD) (kg. ha^{-1}) was computed from grain weight of ears per plot and the moisture content was adjusted to 15% [28]. Moisture content at harvest was recorded for representative shelled kernels from each plot using a moisture meter.

2.4. Genotyping

DNA have been extracted from young leaves from parental line and of each progeny using a modified cetyltrimethylammonium bromide (CTAB) protocol [29]. Purified DNA was sent to Cornell University in the United States of America. The genotyping has been done by Genotyping-by-sequencing (GBS) method, as described in Elshire et al. [30]. Genomic DNA was digested with the restriction enzyme ApeK1, and genotyping-by-sequencing libraries were constructed in 96-plex and sequenced on Illumia HiSeq2500 [30]. Raw flow cell output was processed to genotype calls using the trait analysis by association, evolution and linkage (TASSEL)-GBS pipeline [31]. Reads and tags found in each sequencing result were aligned to the *Zea mays* L. genome reference, version AGPV4 (B73 RefGen v4 assembly).

2.5. Statistical analysis

2.5.1. Phenotypic Analysis

Analyses of variance combined across the eight environments (2 countries x 2 locations x 2 years) have been computed for the four traits measured based on mixed-model analysis with the restricted maximum likelihood procedure in SAS version 9.4 [32]. In this analysis, environments, replication (environment), and block (replication × environment) were considered as random effects. Boxplots based on pairwise test were performed for means comparison of S_1 lines groups using the ggplot2 package in R Software Version 4.0.3 [33].

2.5.2. Quality Control and Genotypic Analysis

The GBS generated 32,468 SNPs covering all the ten chromosomes of maize genome. The genotype data generated by GBS were filtered using TASSEL version: 4.1.12 based on two criteria: missing data and allele frequency. SNP loci, which had missing values and having minimum allele frequency Summary statistics, including gene diversity, expected heterozygosity, observed heterozygosity and polymorphism information content (PIC), were calculated using the Power Marker software [34]. The PIC value, described by Botstein et al. [35], was used to refer to the relative value of each marker with respect to the extent of polymorphism it revealed. Heterozygosity was calculated to quantify the genetic variation in the maize lines sampled. Genetic distance estimates were computed using the Roger's genetic distance [36] with the Power Marker software. A dendrogram was constructed from the Roger's genetic distance matrix using the UPGMA method (Unweighted Pair Group Method with Arithmetic) with the Power Marker and the resulting trees were visualized using MEGA version 5.2.2 [37].

III. RESULTS

3.1. Phenotypic variation of S_1 lines

From the combined analysis of variance (ANOVA), significant differences in mean squares of genotypes have been observed for all the traits, and environment had significantly affected all measured traits. Similarly, means squares for genotype × environment interaction (GEI) effects were significant for grain yield and Striga traits, except ear aspect (Table 3). However, grain yield ranged from 1, 328 kg ha^{-1} to 3, 000 kg ha^{-1} with an average of 1, 907 kg ha^{-1}, while the coefficient of variation was ranged from 11.37% for number of emerged Striga plants to 20.87% for ears aspect (Table 1).

3.2. Summary statistics of SNP markers and genetic characteristics

Among the 32,468 SNPs utilized for the GBS of the S_1 lines and parental lines in the present study, 19,718 SNP markers with call rate > 0.8 were informative. Thereafter, markers with minor allele frequency < 0.05 and monomorphic markers were eliminated, resulting in 8,883 high quality informative SNPs which were used for further analysis. Of these markers, a total of 1376, 1230, 897, 942, 1153, 613, 515, 704, 720 and 733 SNPs were mapped on chromosomes 1 to 10, respectively. Diversity indices statistics across the 8, 883 SNPs indicated an average minor allele frequency (MAF) of 0.386 and polymorphic information content (PIC) of 0.342 with a range of 0.074 to 0.500 and 0.086 to 0.375, respectively (Table 2). The mean expected heterozygosity (0.444) was slightly higher than the observed heterozygosity (0.412) value. Of the 8,883 SNP markers, 5 570 (63%) markers showed PIC values greater than 0.25 and were found to be highly informative.

The analysis of chromosome-wise informative SNP markers revealed that SNP markers varied from 515 on chromosome 7 to 1376 on chromosome 1 with an average of 888 markers per chromosome. The gene diversity (GD), PIC and heterozygosity values among chromosomes were consistent and displayed slight variations among chromosomes. The observed GD among the S_1 lines varied from 0.346 on chromosome 2 to 0.446 on chromosome 3, PIC varied from 0.330 on chromosome 2 to 0.3362 on chromosome 3 and heterozygosity ranged from 0.423 on chromosome 2 to 0.470 on chromosome 8 (Fig. 1). Minimum and maximum values of gene diversity, PIC and heterozygosity were observed on chromosome 2 and chromosome 3, respectively. The gene diversity for 45% of the S_1 lines ranged from 0.10 to 0.40, indicating the presence of moderate level of genetic diversity within this population. About 65% of the inbred lines showed heterozygosity values varying from 0.10 to 0.30, with only 5% of the S_1 lines showing heterozygosity of 0.5–1.0.

3.3. Genetic diversity and genetic similarity analysis

Genetic similarity matrix analysis revealed that the estimated genetic distance varied from 0.011 between SA_134 and SA_135, to 0.79 between the two parental lines (PAR_249 and PAR_250). The estimated genetic distances between the S_1 lines and the resistant parent (PAR_249) and susceptible parent (PAR_250) were 0.48 and 0.39, respectively (Table 3). In general, the estimated genetic distances between the progenies and their parents were smaller than that between the two parents, and the small distances were obtained with the susceptible parent (Table 3).

The genetic distance-based on UPGMA method cluster analysis separated the maize S_1 lines into two major groups (Fig. 2). The resulting marker-based grouping of the S_1 lines was consistent with grouping based on the genetic similarity information of the lines, indicating that progenies with a high genetic similarity (close to 0) were grouped on the same branch. Cluster I was different from group II at the 18% threshold, and consisted of 125 S_1 lines and the 02 parents (Figure 2). This group was composed of lines with high genetic similarity to the two parents. The genotypes of this group displayed an acceptable grain yield (1,802.43±227.47 Kg ha^{-1}), good ear aspect (2.27±0.29) with a moderate resistance to *Striga hermonthica* (STRCO:11.57±5.21 and STRRAT: 4.29±0.46). Cluster II, consisted of 52 S_1 lines, had lower genetic similarity to the parental lines. This group is characterized by lines which tolerate Striga infestation (STRCO: 32.12±5.41and STRRAT: 4.03±0.59), presenting a good grain yield (2156.91±283.52 Kg ha^{-1}) and acceptable ear aspect (3.21±0.36) (Fig. 3).

IV. Discussion

In a breeding program, genetic characterization of plants based on phenotypic and molecular analyses allow selection of desirable genotypes. However, molecular characterization provides additional information on diversity and genetic relationships among and within breeding germplasms [38], [39]. Genetic characterization is essential in describing breeding genetic material and also facilitate identification of materials with desirable traits for breeding purposes. The present study involved characterization of S_1 lines based on SNPs markers and agronomic traits.

Analysis of variance under Striga infestation showed that there was significant variation among the S_1 lines used in this study. The significant difference observed across environments and genotype × environment interaction for most of the traits measured could be due to seasonal factors [40], [41]. The coefficients of variation were high for the traits measured (CV>10%). This indicate that there is high genetic variability for resistance/tolerance to Striga among the S_1 lines. The observed significant among the S_1 lines for the measured traits in the present study have shown the potential for selection of improved grain yield and Striga traits. This could be attributed to the differences in the genetic backgrounds of the parental lines. Differential responses of

maize genotypes under Striga infestation have been reported by earlier researchers [28], [42]. Significant genotype × environment interaction observed for the traits excepted, indicated that these traits are highly affected by the G×E interaction, which could be attributed to variation in climatic conditions across the two years in each location. Significant G×E, have reported for grain yield and Striga traits in previous studies [43], [44].

DNA markers platforms have been successfully used to quantify diversity in cereals including maize [45], [46], [47]. The mean PIC for SNPs dataset in the present study and genetic diversity (GD) known as expected heterozygosity (He) values were 0.34 and 0.44, respectively, and were higher than PIC and genetic diversity values estimated for tropical extra-early and early maize populations by Badu-Apraku et al. [48] and Obeng-Bio et al. [49]. Using SNPs markers, Wu et al. [50] estimated PIC and GD values of 0.60 and 0.67 during an assessment of diversity of maize inbred lines derived from temperate and tropical germplasms. These differences in results related to this study could be due to the use of different genetic materials, sample size, number and type of markers used. This result indicates the existence of high allelic frequency and thus genetic diversity in this population, as indicated by the major allele frequency value of 0.66. The observed heterozygosity although lower than the expected heterozygosity, indicate that this population has moderate heterozygosity so most genetic loci are not fixed in a homozygous (Ho = 0) or heterozygous (Ho = 1) state [51]. The S_1 lines would be favorable for the efficient development of open-pollinated maize populations with various levels of resistance to Striga [27].

Genetic distance provides information on the degree of relatedness between individuals in a population [52]. The results obtained revealed moderate genetic diversity among S_1 lines with a low number of pairs of individuals with low genetic distances. Furthermore, the genetic distances of the different pairs of S_1 lines (0.34) were lower than which between the parental lines (0.79). Therefore, it is inferred that most of the progenies used are not unique, and could have the potential to contribute new alleles for maize breeding program. Based on the 8,883 SNPs and genetic distances, the UPGMA phylogeny using 1000 nonparametric bootstraps, revealed two groups for the 177 inbred lines. The S_1 lines from Cluster I, were highly related to the parental lines, and displayed an acceptable grain yield, good ear aspect, and showed moderate resistance to Striga. The appearance of progenies of Cluster II, would be due by the distortion of allele [53]. The S_1 lines of this group are unique and could provide new alleles for the improvement of resistance to Striga in maize.

V. Conclusion And Perspectives

This study highlights the genetic variation and relationships between and within S_1 lines, and defines a database for further breeding activities. Genetic characterization based on molecular markers allowed the identification of high quality polymorphic markers for further genomic studies. It also provided insight into the genetic relationships of the S_1 lines the parental lines, which would facilitate the effective exploitation of these information. The selected markers will require further study to identify the genetic regions (QTLs/genes) controlling phenotypic variation Striga resistance in maize.

Table 1: Means square, descriptive statistics of grain yield, ears aspect and Striga traits

Source of variation	DF	YIELD	EASP	STRCO	STRRAT
Environment (Env)	7	10366084.8^{***}	1274.46^{***}	34359.52^{***}	975.30^{***}
Blk(Env*Rep)	800	242109.4^{***}	885.38^{**}	328.34^{***}	1.53^{***}
Rep(Env)	8	3142550.4^{***}	646.8153^{***}	1939.68^{***}	12.04^{***}
Genotype (G)	203	593665.8^{***}	194.73^{**}	266.85^{***}	2.99^{***}
G × E	1421	164142.5^{***}	692.57^{ns}	156.72^{**}	1.04^{***}
Residuals	1624	121433.6	116.18	125.38	0.75
Minimum	-	1328	112.9	2.3	2.78
Maximum	-	3000	256.2	48.5	5.39
Mean	-	1907	143.3	17.1	4.22

CV (%)	12.37	20.87	11.39	12.35

***; ** and * significant p-value of 0.001, 0.01 and 0.5, respectively and ns: non-significant.

DF: degree of freedom, YIELD: Grain yield, EASP: Ears aspect, STRCO: number of emerged Striga plants, STRRAT: Striga damage rate and CV: coefficient of variation.

Table 2: Diversity indices statistics of 177 maize S_1 lines based on 8883 SNP markers

	Minor allele frequency (MAF)	Expected Heterozygosity (He)	Observed Heterozygosity (Ho)	Polymorphic information content (PIC)
Minimum	0.047	0.090	0.061	0.086
Median	0.463	0.497	0.457	0.373
Maximum	0.50	0.50	0.904	0.375
Mean	0.386	0.444	0.412	0.342

Table 3: Summary of genetic distances estimated among S_1 lines and the parents with SNPs markers.

	S_1 lines		
	Minimum	Maximum	Mean
S_1 lines	0,011	0,551	0,345
Parents	0,011	0,790	0,351
TZISTR118 (PAR_249)	0,278	0,650	0,485
5057 (PAR_250)	0,233	0,527	0,395

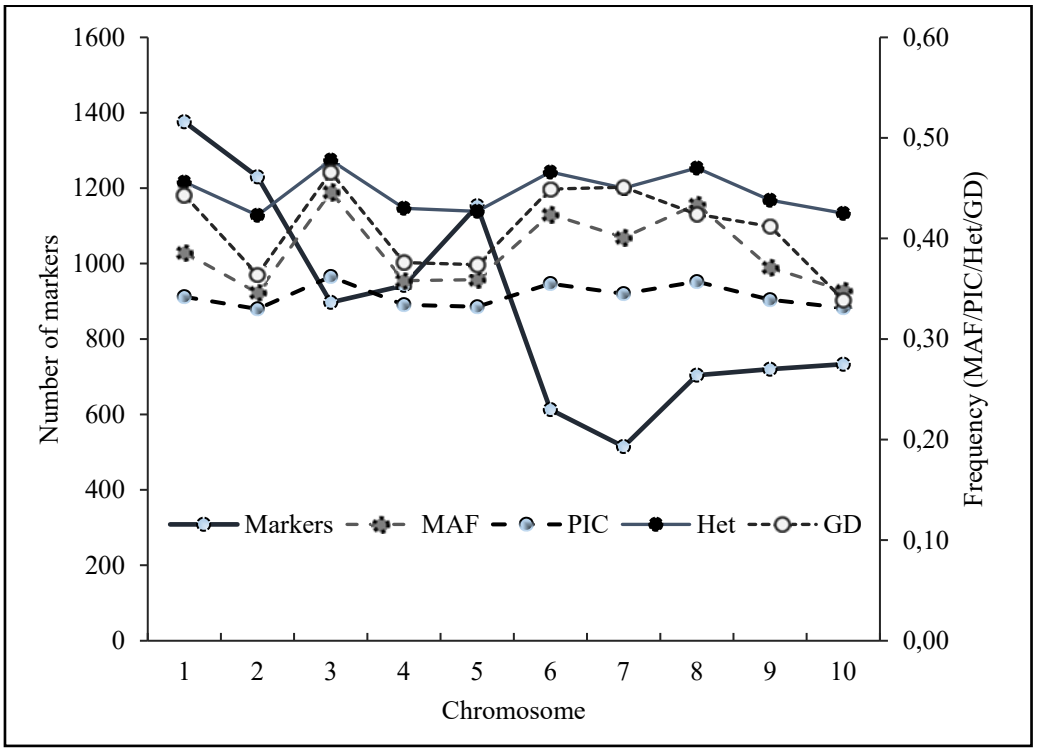

Fig.1. *Summary statistics of 8883 SNPs markers used for genotyping of 177 inbred lines Number of markers, mean polymorphism information content (PIC), gene diversity (GD) distribution and heterozygosity (Het) across chromosomes.*

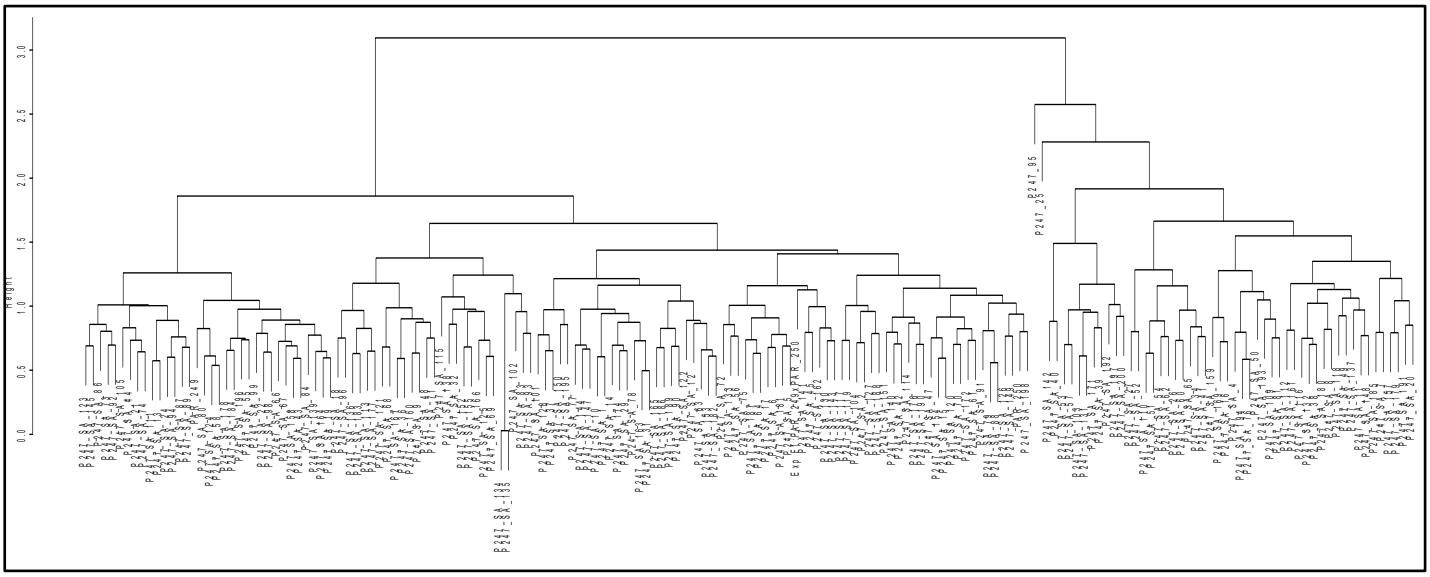

Fig. 2. Phylogenic tree for the 177 maize S_1 lines and the two parents based on the Roger's genetic distance.

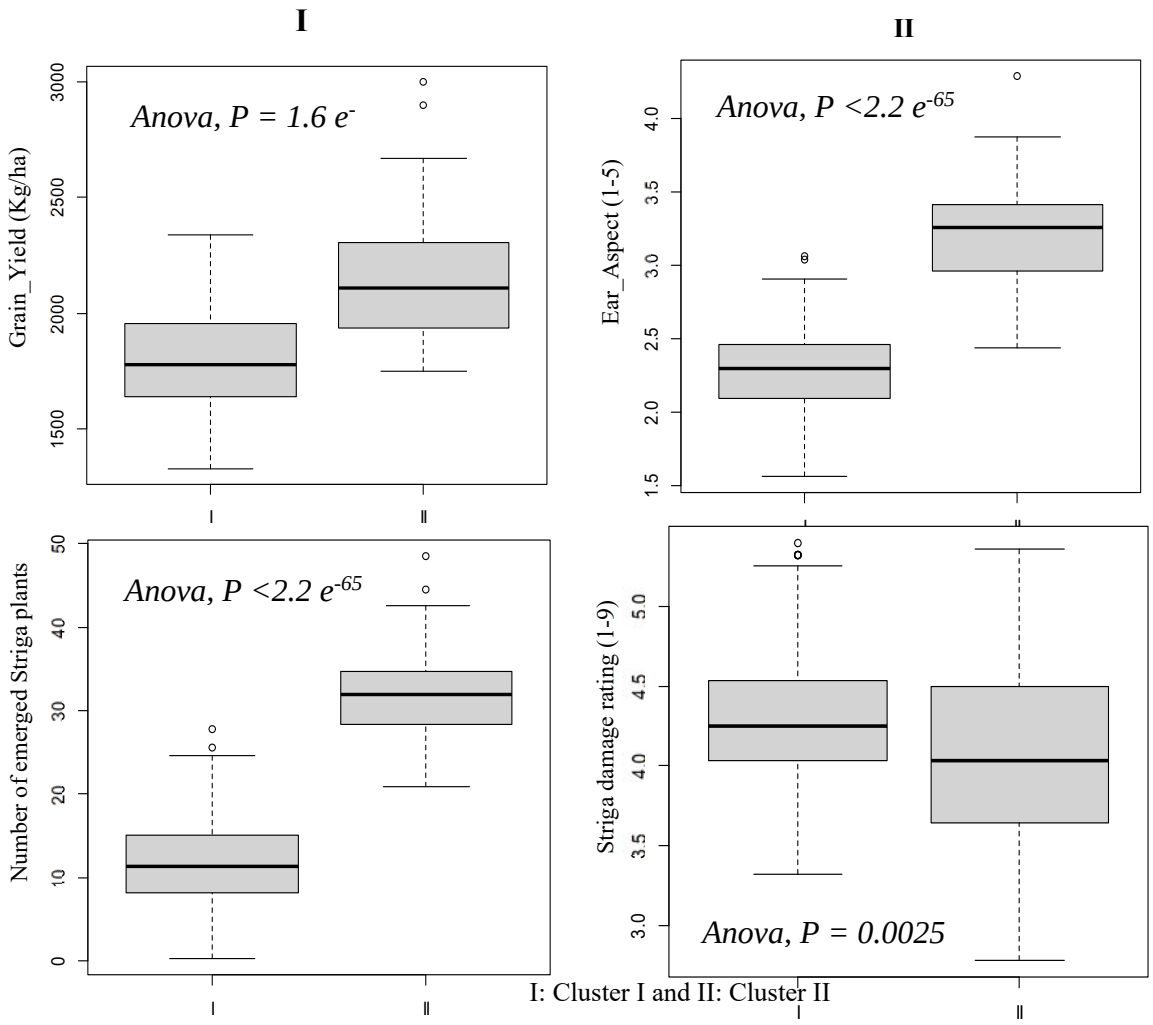

Fig.3. *Agronomic characteristic of the two clusters based on genetic similarity of the S_1 lines.*

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