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Identification of QTLs Controlling Resistance/Tolerance to *Striga hermonthica* in an Extra-Early Maturing Yellow Maize Population

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Abstract: *Striga hermonthica* parasitism is a major constraint to maize production in sub-Saharan Africa with yield losses reaching 100% under severe infestation. The application of marker-assisted selection is highly promising for accelerating breeding for *Striga* resistance/tolerance in maize but requires the identification of quantitative trait loci (QTLs) linked to *Striga* resistance/tolerance traits. In the present study, 194 $F_{2:3}$ families of TZEEI 79 × TZdEEI 11 were screened at two *Striga*-endemic locations in Nigeria, to identify QTLs associated with *S. hermonthica* resistance/tolerance and underlying putative candidate genes. A genetic map was constructed using 1139 filtered DArTseq markers distributed across the 10 maize chromosomes, covering 2016 cM, with mean genetic distance of 1.70 cM. Twelve minor and major QTLs were identified for four *Striga* resistance/tolerance adaptive traits, explaining 19.4%, 34.9%, 14.2% and 3.2% of observed phenotypic variation for grain yield, ears per plant, *Striga* damage and emerged *Striga* plants, respectively. The QTLs were found to be linked to candidate genes which may be associated with plant defense mechanisms in *S. hermonthica* infested environments. The results of this study provide insights into the genetic architecture of *S. hermonthica* resistance/tolerance indicator traits which could be employed for marker-assisted selection to accelerate efficient transfer host plant resistance genes to susceptible genotypes.

Keywords: maize (*Zea mays* L.); *Striga* resistance/tolerance; QTL mapping; $F_{2:3}$ biparental mapping; Marker-assisted selection

1. Introduction

Maize (*Zea mays* L.) is the most widely grown staple food crop in sub-Saharan Africa (SSA), and accounts for a large proportion of carbohydrates, proteins, lipids and vitamins for millions of people in the sub-region [1,2]. The root hemi-parasitic plant *Striga hermonthica* is an important biotic constraint limiting maize production in SSA. The *S. hermonthica* problem in SSA is the result of the shift from traditional cereal based farming system which facilitated longer fallow periods which ensured that the soil *Striga* seed bank was maintained at levels that plants could tolerate [1]. However, the pressure on agricultural land has necessitated land use intensification, cereal mono-cropping and reduced fallow periods resulting in increased *Striga* seed bank and infestation levels that threaten the livelihood of millions of farmers [3,4]. De Groote et al. [5] reported that over six million hectares of agricultural land in Western, Eastern and Southern Africa are seriously affected by *Striga*. Reduction in grain yield due to *S. hermonthica* parasitism may be up to 100% under severe infestation and unfavorable environmental conditions such as low soil fertility, erratic rainfall patterns and low-input conditions [6–8]. The subsistence farmers are usually the most severely affected. The extent to which *S. hermonthica* affects the growth of its host varies tremendously, depending

on the level of host plant resistance/tolerance, extent of infestation, and the prevailing environmental conditions [9,10]. Resistance to *Striga* denotes the capability of the host plant to induce the germination of *Striga* seeds but prevents the parasite from attaching to the roots of the maize plants or kills the attached parasitic plants. Under *S. hermonthica* infestation, the resistant genotype supports considerably fewer *Striga* plants and produces a greater yield than the susceptible genotype [11–13]. Contrarily, a *Striga* tolerant genotype supports as many *Striga* plants as the sensitive or susceptible genotype [14] but produces more dry matter and shows fewer damage symptoms [15]. *Striga* damage in maize is used as the indicator of tolerance while emerged *Striga* plants is the indicator of resistance. The identification of maize genotypes that combine outstanding levels of resistance and tolerance is a promising breeding strategy and has been recommended for *Striga* resistance breeding in several studies [12–14,16,17]. In selecting for tolerance or resistance and high grain yield under *Striga* infestation, the primary traits of interest are the *Striga* damage and number of emerged *Striga* plants. Presently, maize genotypes with combined resistance and tolerance to *S. hermonthica* (possessing both low *Striga* damage and few *Striga* emergence counts) as well as high grain yield have been identified in the International Institute of Tropical Agriculture - Maize Improvement Program (IITA-MIP). *Striga* damage rating score is positively associated with *Striga* emergence counts, and the two traits are negatively associated with yield under *S. hermonthica* infested conditions. Similarly, large positive additive genetic correlation was recorded between grain yield and ears per plant as well as moderately large negative genetic correlations between grain yield and flowering traits [18]. Comparable results were reported by earlier workers [19,20]. Nevertheless, the genotypic correlation between *S. hermonthica* damage rating and *S. hermonthica* emergence counts have been found to be low, implying that different genes control the inheritance of the two traits [18,21].

Striga infestation is dependent on *Striga* seedbank in the soil resulting from continuous cropping of host plants, leading to the accumulation of *Striga* seeds which can remain dormant in the soil for more than a decade [22]. The germination of *Striga* seed is induced by the production of plant hormones called strigolactones produced by the maize plant in the roots. The hormones are released when the plant is under stress [23]. For the germinated *Striga* seed to survive as an obligate parasite, it must produce haustoria that attach to the roots of the maize plant through which it draws water and photosynthates [24]. Even though *Striga* possesses chlorophyll for photosynthesis, it still depends on its host for survival [25], as a result of its inability to accumulate enough photosynthates for autotrophic growth. Therefore, *Striga* establishes direct xylem links with the root system of the host [26] to obtain nutrients from its xylem sap [27]. Furthermore, a higher rate of transpiration in *Striga* compared to the host plant creates a water potential gradient from the host to the *Striga* plant [28]. *Striga* phytotoxic effects on the maize plant direct the partitioning of assimilates into the roots rather than the shoots for grain filling, thereby resulting in plant biomass and yield reduction [29].

Presently, management strategies of *S. hermonthica* include cultural, chemical, and biological approaches, which are non-economical and/or knowledge-intensive for subsistence farmers [6]. Planting of *Striga*-resistant maize varieties is presently considered the best control strategy and easy to adopt or deploy, particularly when combined with other management practices [30–33]. Resistance to *S. hermonthica* parasitism is mainly attributed to low production of *Striga* germination stimulants by the host plant, attachment of few *Striga* plants to the roots of the host plant as well as fewer *Striga* emergence [13,34]. When breeding for *S. hermonthica* resistance in maize, a combination of these resistance mechanisms is desirable in achieving effective and durable resistance [33]. The slow rate of development and deployment of *Striga* resistant genotypes is largely attributable to the complex genetics of resistance as well as limited knowledge of the specific mechanisms associated with resistance to *Striga* [35]. The resistance to *S. hermonthica* in maize is regulated by many genes or quantitative trait loci (QTL) with small additive effects and it is significantly influenced by the environment [13,36]. Therefore, breeding for *Striga*-resistant cultivars using conventional approaches by selecting maize cultivars with enhanced resistance which requires evaluation in multi-locations and years, has been less effective and time-consuming [27].

Marker-assisted breeding makes use of genotypic data in the identification of genotypes possessing desirable alleles, using linked genetic markers. Breeders employ marker-assisted selection (MAS) when an important trait that is difficult to assess phenotypically, is tightly linked to a molecular DNA marker that can be scored quickly and precisely [37]. QTL mapping approaches are important genomic tools employed in dissecting the genetic architecture of complex traits [38,39] as well as identification of genetic linkage through wide genotyping of a panel of germplasm displaying contrasting phenotypes across different environments [40]. For QTL identification, the development of next-generation sequencing technology has become a practicable technique to rapidly identify large number of single nucleotide polymorphisms (SNPs) throughout the genome [41]. Unlike the use of second-generation molecular markers which result in low-quality mapping, SNP markers provide new insights to rapidly identify QTL of interest.

Information on map positions of genes and linked markers on a chromosome are crucial for efficient determination of genetic architecture of polygenic traits in crop plants [42]. Several QTLs and candidate genes controlling resistance to *Striga* have been reported in cereals. For instance, Swarbrick et al. [32] identified three *S. hermonthica*-resistant QTLs in Kasalath–Koshihikari rice backcross inbreds, two of these QTLs originated from the Kasalath allele and one from the Koshihkari allele. The largest-effect QTL (Kasalath-derived allele) explained 16% phenotypic variance in the mapping population and was located on linkage group 4. Haussmann et al. [43] detected molecular markers associated with *S. hermonthica*-resistant QTLs, with the most significant QTL corresponding to the major-gene locus low germination stimulant (LGS) in linkage group I. Five genomic regions (QTLs) linked to stable *S. hermonthica*-resistant alleles from resistant variety N13 were detected through evaluation across a large number of field trials in Mali and Kenya. However, limited reports are available on the QTLs and genes controlling *Striga* resistance in maize. In a recent study by Adewale et al. [44] to identify molecular markers linked to *S. hermonthica* resistance in maize, 24 SNPs significantly associated with *S. hermonthica* resistance indicator traits under artificial *S. hermonthica* infestation were detected. The authors also identified four candidate genes on chromosomes 3, 5, 9 and 10, with functions closely associated with maize plant defense mechanisms against *S. hermonthica* parasitism. Identification of QTLs linked to *S. hermonthica* resistance followed by gene introgression into elite genetic backgrounds, has the potential to reduce yield losses due to *Striga* and will ultimately provide solid foundation for improving *Striga* resistance [45].

The objectives of this study were to identify QTL and underlying candidate genes conferring resistance/tolerance to *S. hermonthica* in maize using $F_{2:3}$ biparental mapping population derived from a cross between a *Striga* resistant inbred line, TZEEI 79 and *Striga* susceptible inbred TZdEEI 11.

2. Materials and Methods

2.1. Germplasm and Phenotyping

Based on the reports of previous studies, two extra-early maturing yellow inbred lines, TZEEI 79 (*Striga* resistant/tolerant) and TZdEEI 11 (*Striga* susceptible) were selected as parents to generate the $F_{2:3}$ progenies used in the present study [1]. TZEEI 79 is an outstanding *S. hermonthica* resistant/tolerant, drought and low-soil N tolerant inbred line developed in the IITA-MIP from the broad-based *S. hermonthica* resistant/tolerant as well as drought and low-soil N tolerant population, TZEE-Y Pop STR C_0. TZEEI 79 has significant positive GCA (general combining ability) for grain yield as well as significant negative GCA effects for *Striga* damage and *Striga* emergence counts under *Striga* infestation and has been extensively used in the IITA hybrid program as an important resource for developing high-yielding, multiple stress tolerant hybrids as well as an efficient tester for classifying other inbreds into heterotic groups [46]. Crosses were made between TZEEI 79 and TZdEEI 11 designated as P_1 and P_2 respectively, to generate 220 F_1 progenies. The F_1 progenies and the parental lines were planted, and leaf samples were collected at 3 weeks after planting. Verification of the parental type alleles (quality control analysis) was carried out on the F_1 progenies prior to advancement to F_2.

The F_1 progenies were screened using two SSR primers (bnlg 182 and umc 1568) which were found to be polymorphic between the two parents. The analysis identified 170 true-to-type F_1 hybrids which were advanced to F_2. The 170 true-to-type F_2 ears were planted ear-to-row and 194 F_2 individuals which were randomly selected and selfed were used in the present study.

The $F_{2:3}$ progenies and the two parental lines were screened under artificial *S. hermonthica* infestation at Mokwa (9°18′ N, 5°4′ E, 210 m above sea level, 1100 mm yearly rainfall, luvisol soil) and Abuja (9°16′ N, 7°20′ E, 445 m above sea level, 1500 mm yearly rainfall, ferric-luvisol soil) in the Southern Guinea savanna of Nigeria in 2018. At each experimental site, the trial was laid out using randomized incomplete block design (14 × 14 lattice) with two replicates. The experimental units were 3 m long single-row plots, with an inter-row spacing of 0.75 m and within-row spacing of 0.4 m, to achieve a target population density of 66,666 plants/ha. The fields for artificial *S. hermonthica* infestation at Mokwa and Abuja were treated with ethylene gas at 2 weeks before planting to eliminate any potential *Striga* seeds present in the soil. The *S. hermonthica* seeds used for the experiment were obtained from sorghum farms around the test locations at Abuja and Mokwa in 2017. The artificial *S. hermonthica* infestation was carried out as proposed by the IITA Maize Program [16]. Briefly, about a week before inoculation, the *S. hermonthica* seeds were carefully mixed with finely sieved sand at the ratio 1:99 by weight to ensure rapid and uniform infestation. A standard scoop calibrated to deliver approximately 5000 germinable seeds per hill was utilized for the artificial infestation. Three maize seeds were planted per infested hill and the seedlings were later thinned to two plants per stand at 2 weeks after emergence. Fertilizer application on the maize plots was delayed till about 30 days after planting, in order to subject the maize plants to stress, a condition that was expected to enhance strigolactone production. This ensured good germination of *Striga* seeds and attachment of *Striga* plants to the roots of host plants. At this plant growth stage, 20–30 kg Nha^{-1}, 30 kg each of P and K were applied as NPK 15-15-15, taking into consideration the fertility status of the soil. Reduction in the fertilizer application rate was important because *Striga* emergence decreases at high N rate [16]. At 10 weeks after planting, typical symptoms of *Striga* infestation on the host plants were observed, such as chlorosis, leaf scorching (firing) and blotching, stunting, decrease in ear and tassel size, brown necrotic spots, leaf wilting and rolling, stalk lodging, open-tip of ears at late growing stage, and premature death of host plants. Host plant *Striga* damage severity was scored using a scale of 1 to 9. Rating scales 1–5 indicated resistance while 6–9 indicated susceptibility, where 1 = normal plant growth, no obvious symptoms, and 9 = all leaves completely scorched, collapse of host plants and no ear formation [16]. In addition, data were collected on *Striga* emergence count at 10 WAP as the number of *Striga* plants thriving on the maize root system as well as ears per plant (EPP) by dividing the total number of ears harvested per plot by the number of plants in a plot at harvest. Grain moisture was determined using Kett moisture tester PM-450 and grain yield (kg/ha) was calculated using the field weight of harvested ears per plot, adopting a shelling percentage of 80, adjusted to 15% moisture content [47].

2.2. DArTseq Genotyping and SNP Data Filtering

Young and healthy leaf samples from single plants of the F_2 individuals and bulk samples from the parental lines were collected and frozen immediately after harvesting using liquid nitrogen and thereafter stored at −80 °C. Genomic DNA extraction was carried out following the DArT protocol (www.diversityarrays.com/files/DArT_DNA_isolation/). The extracted DNA was assessed for quality by visualization on agarose gel (2% w/v) and the quantity was estimated on NanoDrop-1000 spectrophotometer (NanoDrop, Wilmington, DE, USA) using the absorbance ratio A_{260}/A_{280} to determine the concentration (ng/μL) and purity level of the DNA.

Genotyping of the 194 F_2 individuals plus the two parents was carried out using DArTseq technology [48,49]. Genome complexity reduction which involved the use of a combination of two restriction enzymes (*Pst*I–*Mse*I) was used to create a genome representation of the analyzed samples. All fragments generated were amplified and sequenced to identify the single nucleotide polymorphisms (SNPs) using a proprietary analytical pipeline developed by DArT P/L. After a strict quality control process, which included parameters such as call rate, data reproducibility (~20% of samples replicated), and rate of monomorphism to eliminate monomorphic markers, 9951 SNPs were extracted from the evaluated germplasm. The 9951 SNPs were filtered for unmapped markers, duplicate markers and markers segregating between the *Striga* resistant and *Striga* susceptible parents. A total of 1139 high-quality DArTseq markers distributed across the 10 maize chromosomes were retained for the construction of genetic linkage map as well as the QTL mapping.

2.3. Data Analysis

The data recorded on emerged *Striga* counts and *Striga* damage severity scores were subjected to natural logarithm transformation. Thereafter, data collected on grain yield, ears per plant, *Striga* damage as well as *Striga* emergence counts were tested for normality using Shapiro–Wilk's (W) test [39,50] before analysis of variance. Box plots were made to visualize the distributions of grain yield and other traits under each research environment using ggplot2 library [51]. Analysis of variance was conducted across research environments using the general linear model procedure (PROC GLM) implemented in the Statistical Analytical System (SAS), version 9.3 [52]. In the analysis, environment, replications (environments), blocks (replications × environments) were considered as random and the $F_{2:3}$ families (genotypes) as fixed effects. Estimates of broad sense heritability of the traits (H^2) across research environments were computed on a family-mean basis as proposed by Holland et al. [53], using the following formula:

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_{ge}^2}{e} + \frac{\sigma_e}{re}}$$

where σ_g^2 = variance component due to the genotypes, σ_{ge}^2 = genotype × environment variance, σ_e = experimental error variance; e = number of environments, and r = number of replications within environment.

Correlation coefficients were estimated among the traits with the adjusted means of the $F_{2:3}$ families using the Ggally function implemented in GGally package [54]. Furthermore, the mixed linear model (MLM) established in META-R software [55] was used to compute the best linear unbiased estimates (BLUEs) for each genotype in each and across environments which were for the QTL analysis. The R/qtl was used to construct a linkage map [56]. Markers that were identical across all genotypes were identified and eliminated as duplicates. Furthermore, χ^2-test for goodness-of-fit ($p \leq 0.0001$) was used to identify markers with distorted segregation patterns [50,57,58]. Markers with significant deviation from the expected Mendelian segregation ratio (1:2:1) for $F_{2:3}$ population were excluded from the analysis, resulting in a total of 1139 SNP markers used for the genetic map construction and QTL analysis.

2.4. QTL Analysis and Candidate Gene Identification

Quantitative trait loci (QTL) mapping for each and across environments was carried out for four *Striga* resistance/tolerance adaptive traits (grain yield, *Striga* damage at 10 WAP, emerged *Striga* counts at 10 WAP and ears per plant (EPP)), using R/qtl package with the composite interval mapping (CIM) algorithms as proposed by Wang et al. [50]. The statistical significance of the QTL was assessed using permutation tests (1000 replications) for all traits. A logarithm of odds (LOD) of 3.0 was set through the permutation test to identify significant QTLs for the traits [59]. The additive effects and proportion of phenotypic variance explained (PVE) by each QTL were estimated using the "fitqtl" function of R version 3.3.4. The sign of the effect of each QTL was used to identify the origin

of the favorable alleles [60]. The potential locations of the QTLs were described according to their LOD peaks and their surrounding regions. Identified QTLs were named based on conventions method described by Bo et al. [61]. For example, *qepp-2* represented the QTL identified for number of ears per plant on chromosome 2. Putative candidate genes were searched within a 2.0 Mb interval downstream and upstream of the significant associated SNPs using the MaizeGDB database version (RefGen_v4).

3. Results

3.1. Phenotypic Analysis of Grain Yield and Other Striga Resistance Adaptive Traits

The 194 $F_{2:3}$ families and the two parental lines were evaluated under artificial *Striga* infestation to assess variation in their level of resistance. The distributions of grain yield, *Striga* damage, number of emerged *Striga* plants as well as ears per plant in the $F_{2:3}$ population are displayed in Figure 1. Significant variation was detected among the genotypes under each and across research environments (Figure 1, Table S1). The performance of the genotypes ($F_{2:3}$ families and the parental lines) for *Striga* emergence count and ears per plant were not significantly influenced by the environment whereas grain yield and *Striga* damage displayed significant genotype × environment interactions. The two parental lines TZEEI 79 and TZdEEI 11 differed significantly and consistently in their performance under artificial *Striga* infestation, and phenotypic values for each trait of segregating population displayed wide ranges (Table 1). Transgressive segregation was observed for all traits in that some of the $F_{2:3}$ families showed higher and lower levels of grain yield, *Striga* damage, number of emerged *Striga* plants and ears per plant compared to the parental lines (Table 1). The *Striga* resistant inbred line TZEEI 79 exhibited high grain yield and ears per plant as well as reduced *Striga* damage and *Striga* emergence count whereas the *Striga* susceptible line TZdEEI 11 showed significantly lower grain yield and ears per plant as well as increased *Striga* damage and emergence count. Grain yield (kg/ha) across the $F_{2:3}$ population varied from 1070.1 to 4113.9, with a mean of 2439.2 (Table 1). In addition, individual means varied from 0.5 to 1.4, 2.4 to 7.5, 0.4 to 3.7 for ears per plant, *Striga* damage and *Striga* emergence count, respectively. Broad-sense heritability estimates of the traits derived from the variance components varied from 0.47 for *Striga* damage to 0.70 for ears per plant. The normality tests by Shapiro–Wilk (*W*) revealed that the distributions of grain yield and *Striga* damage phenotypic data were normally distributed while those of *Striga* emergence counts and ears per plant were not (Table S2). High *W*-test values were obtained for all studied traits ranging from 0.97–0.99 (Table S2). Correlation analysis revealed significant and positive correlations between number of ears per plant and grain yield whereas negative and significant correlations were observed between the ears per plant and *Striga* damage as well as between *Striga* damage and grain yield (Figure 2).

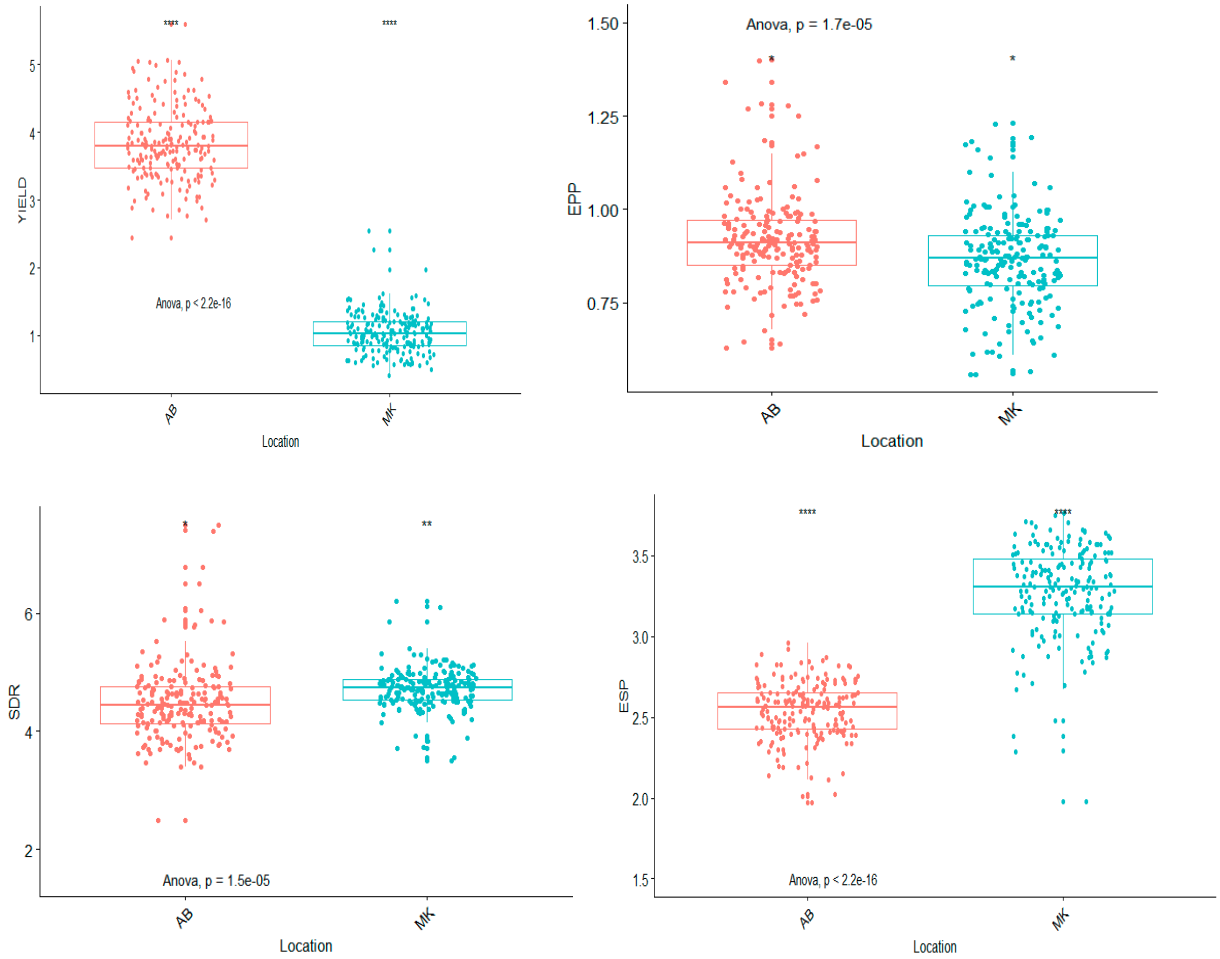

Figure 1. Box plots showing the distribution of (**A**) grain yield (YIELD, t/ha), (**B**) emerged *Striga* plants (ESP), (**C**) *Striga* damage rating (SDR) and (**D**) number of ears per plant (EPP) under artificial *Striga* infestation at Mokwa (MK) and Abuja (AB) in 2018. The points represent the $F_{2:3}$ families and the parental genotypes.

Table 1. Descriptive statistics of *Striga* resistance indicator traits of parents and $F_{2:3}$ population derived from the cross between TZEEI 79 × TZdEEI 11 across *Striga*-infested environments.

	Grain Yield, kg/ha	**Ears per Plant**	***Striga* Damage**	***Striga* Count**
Parents				
TZEEI 79	2517.5	0.9	2.5	0.5
TZdEEI 11	1485.9	0.8	7.0	3.2
$F_{2:3}$ population				
Range	1070.1–4113.9	0.5–1.4	2.4–7.5	0.4–3.7
Mean ± SE	2439.2 ± 578.9	0.9 ± 0.14	4.6 ± 0.7	2.5 ± 0.5
H^2	0.49	0.70	0.47	0.48
CV (%)	25.2	21.3	18.2	25.4

H^2—broad sense heritability, CV—coefficient of variation.

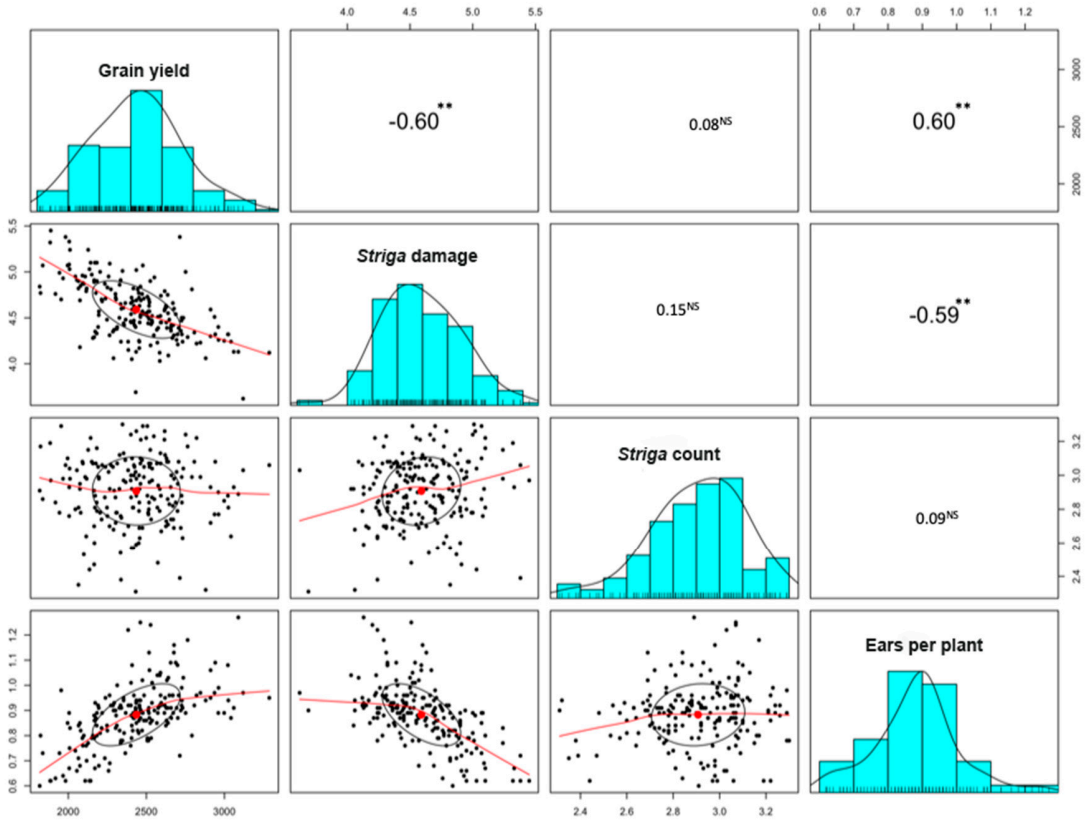

Figure 2. Correlation between grain yield and other *Striga* resistance indicator traits in an $F_{2:3}$ mapping population derived from TZEEI 79 × TZdEEI 11 under artificial *Striga* infestation. The axis displayed the range of values obtained for each trait; black circles represent the most predominant values for each trait among the genotypes while the red lines represent the direction of the relationship between two traits.

3.2. Linkage Map

A genetic linkage map containing 1139 SNPs mapped on the 10 maize chromosomes was constructed (Figure S1, Table 2). The resulting map spanned a total genetic distance of 2016 cM, with mean interlocus distance of 1.70 cM. The average genetic distances between successive markers ranged from 0.86 cM to 11.86 cM for chromosomes 1 and 7 respectively.

Table 2. Summary statistics of the linkage map.

Linkage Group	Number of Markers	Genetic Length (cM)	Average Marker Interval (cM)
1	349	304.3	0.86
2	188	229.3	1.22
3	148	224.5	1.52
4	73	243.0	3.36
5	167	221.7	1.30
6	15	167.4	7.69
7	16	178.4	11.86
8	46	160.1	3.37
9	28	139.2	4.74
10	109	148.1	1.36
Total	1139	2016.0	1.70

3.3. QTL Detection and Identification of Potential Candidate Genes

Through the QTL analysis, a total of 12 QTLs with significant LOD score ≥ 3.0, were identified for the four *Striga* resistance indicator traits using the integrated genetic map and mean phenotypic data across research environments (Table 3). The 12 QTLs identified included three for grain yield, five for ears per plant, three for *Striga* damage and one for *Striga* emergence counts. The proportion of phenotypic variation explained by the QTLs varied from 2.0% for *qepp-2.1* to 13.5% for *qepp-1*. Three QTLs *qgy-1.1*, *qgy-2.1* and *qgy-7* detected for grain yield explained 5.6, 10.3 and 2.3% phenotypic variation, respectively. Furthermore, five QTLs *qepp-1*, *qepp-2.1*, *qepp-3*, *qepp-7*, and *qepp-8.1* were identified for ears per plant, explaining phenotypic variation ranging from 2.0 to 13.5%. Similarly, QTLs *qsd-2*, *qsd-5.1* and *qsd-7* detected for *Striga* damage displayed phenotypic variance of 8.0, 3.0 and 3.2 respectively. The only QTL (*qsc*-3.1) identified for *Striga* emergence count had PVE of 3.1. The QTL *qsd-7* and *qepp-7* were detected at the same position for *Striga* damage and ears per plant. Similarly, *qgy-2.1* and *qsd-2* detected for grain yield and number of emerged *Striga* plants were consistently identified at the same position in each of the two locations. Three major QTL genomic regions were detected on chromosomes 1, 2, and 8 with flanking marker intervals 216–226 cM, 134–156 cM and 35–38 cM, respectively (Figure S2). In all cases, favorable alleles for *Striga* resistance/tolerance were contributed by the *Striga* resistant inbred line TZEEI 79.

A total of 116 protein coding genes were identified within 2.0 Mb interval downstream and upstream of the significantly associated SNPs (Table S3). Of the 116 candidate genes, 17 key candidate genes associated with the identified QTL for *Striga* resistance/tolerance indicator traits under artificial *Striga* infested environments are presented in Table 4. For grain yield, the *qgy-1.1* was found associated with GRMZM2G408305 which encodes ARM repeat superfamily protein as well as GRMZM2G072376 which encodes bHLH-transcription factor 56. The QTL *qgy-7*, *qepp-7* and *qsd-7* were linked to GRMZM6G199466 (hsp3—heat shock protein3), GRMZM2G008234 (ereb114—AP2-EREBP-transcription factor 114) as well as GRMZM2G044194 (phytosulfokine peptide precursor1). Similarly, for ears per plant, *qepp-1* was found associated with GRMZM2G324999 which encodes the WRKY-transcription factor 25; *qepp-2.1* was associated with GRMZM2G174784 (EREB197—putative AP2-EREBP transcription factor superfamily protein), GRMZM2G174917 (ereb47—AP2-EREBP-transcription factor 47) and GRMZM2G131961 (bzip27—bZIP-transcription factor 27); *qepp-3* was linked to Zma-MIR167g which promotes lateral root development in plants. On chromosome 8, QTL *qepp-8.1* detected for ears per plant was linked to the genes GRMZM2G051528 which encodes myb transcription factor95 and GRMZM2G053503 which encodes ethylene-responsive factor-like protein. For *Striga* damage, QTL *qsd-5.1* was associated with genes GRMZM2G059851 which encodes the heat shock factor protein as well as GRMZM2G099334 which encodes myb3—WD40 repeat protein. The QTL *qsc-3.1* detected for *Striga* emergence count was found associated with the genes GRMZM2G054050 which encodes multicopper oxidase protein, GRMZM2G162709 (MYB-transcription factor 137), and GRMZM2G340342 which encodes the ARM repeat superfamily protein.

Table 3. Summary of quantitative trait loci (QTLs) mapped in the $F_{2:3}$ population derived from TZEEI 79 × TZdEEI 11 under artificial *Striga* infestation.

Trait	Location	QTL	Chr	Position (cM)	Flanking Markers	LOD	Add	Dom	PVE (%)
Grain yield	Across	*qgy-1.1*	1	37.6	S1_21989679, S1_39100143,	4.4	88.34	30.58	5.6
		qgy-2.1	2	141.0	S2_133867986, S2_15644237	5.5	116.80	0.49	10.3
		qgy-7	7	28.4	S7_28123354, S7_39973949	3.8	36.60	14.29	2.3
	AB	*qgy-1.2*	1	23.0	S1_19567556, S1_26003993	3.2	154.78	−52.26	0.8
		qgy-2.1	2	141.0	S2_133867986, S2_15644237	4.8	196.49	−43.08	1.0
	MK	*qgy-2.2*	2	141.0	S2_133867986, S2_149550704	4.5	2.80	−1.94	6.4
		qgy-7	7	28.4	S7_2840233, S7_39973949	3.8	1.73	−0.49	4.1
		qgy-8	8	93.6	S8_89593967, S8_96110467	4.5	1.93	4.43	9.6
Ears per plant	Across	*qepp-1*	1	219.2	S1_216043878, S1_225511262	5.8	0.03	0.03	13.5
		qepp-2.1	2	62.7	S2_5979544, S2_63035407	5.8	0.04	0.02	2.0
		qepp-3	3	122.0	S3_121468077, S3_122362644	4.3	−0.04	0.03	6.0
		qepp-7	7	28.4	S7_28123354, S7_39973949	6.1	0.04	−0.01	3.4
		qepp-8.1	8	37.5	S8_34609716, S8_37717543	4.5	0.01	0.01	10.0
	AB	*qepp-2.2*	2	151.9	S2_147705224, S2_154942934	3.8	0.04	0.02	0.3
	MK	*qepp-2.1*	2	62.7	S2_57731037, S2_68592918	3.4	3.63	0.61	1.2
		qepp-7	7	28.4	S7_28123354, S7_39973949	3.2	1.75	−1.31	3.2
		qepp-8.2	8	34.6	S8_30333465, S8_37493871	3.5	2.07	−2.31	5.5
Striga damage	Across	*qsd-2*	2	141.0	S2_133867986, S2_147705224	5.0	−0.13	0.05	8.0
		qsd-5.1	5	172.0	S5_171268215, S5_188880765	4.3	0.11	−0.03	3.0
		qsd-7	7	28.4	S7_28123354, S7_39973949	3.0	−0.04	0.04	3.2
	AB	*qsd-2*	2	141.0	S2_140980014, S2_149550704	3.0	−0.18	0.08	0.3
		qsd-5.2	5	63.5	S5_5997392, S5_67117772	4.4	−0.03	−0.04	3.0
	MK	*qsd-2*	2	141.0	S2_133867986, S2_149550704	3.0	0.23	0.11	5.2
Striga emergence count	Across	*qsc-3.1*	3	23.9	S3_21323318, S3_28235766	5.8	−0.09	0.03	3.2
	AB	*qsc-3.2*	3	156.7	S3_152189313, S3_158981913	3.0	−0.02	0.03	2.0

AB—Abuja, MK—Mokwa, Across—across the two *Striga* infested environments; Add—Additive effect, LOD—Logarithm of odds, PVE—proportion of phenotypic variance explained by single QTL. Grain yield (kg/ha), ears per plant (number of ears per plant), *Striga* damage (based on rating scale 1–9) and *Striga* emergence count (number of emerged *Striga* plants).

Table 4. Key putative candidate genes associated with the identified QTLs for key *Striga* resistance/tolerance indicator traits under artificial *Striga* infested environments.

Trait	QTL	LG: Start–End Position *	Gene_ID	Sequence Description
Grain yield	*qgy-1.1*	1:35591728–39591728	GRMZM2G408305	ARM repeat superfamily protein
			GRMZM2G072376	bHLH—transcription factor 56
Ears per plant	*qepp-1*	1:217230073–221230073	GRMZM2G324999	wrky25—WRKY-transcription factor 25
	qepp-2	2:4272353–8272353	GRMZM2G174784	EREB197—putative AP2- EREBP transcription factor superfamily protein
			GRMZM2G174917	ereb47—AP2-EREBP-transcription factor 47
			GRMZM2G131961	bzip27—bZIP-transcription factor 27
	qepp-3	3:119393084–123393084	Zma-MIR167 g	miR167—lateral root development
	qepp-8.1	8:35493871–39493871	GRMZM2G053503	ERF1—ethylene-responsive factor-like protein
			GRMZM2G051528	myb95—myb transcription factor95
Striga damage	*qsd-5.1*	5:170001287–174001287	GRMZM2G059851	HSF-6—Heat shock factor protein
			GRMZM2G099334	myb3—WD40 repeat protein
Grain yield, ears per plant and *Striga* damage	*qgy-7*, *qepp-7*, *qsd-7*	7:840233–4840233	GRMZM6G199466	hsp3—Heat shock protein3
			GRMZM2G044194	psk1—phytosulfokine peptide precursor1
			GRMZM2G008234	ereb114—AP2-EREBP-transcription factor 114
Striga emergence count	*qsc-3.1*	3:21951408–25951408	GRMZM2G054050	Multicopper oxidase
			GRMZM2G340342	ARM repeat superfamily protein
			GRMZM2G162709	myb137—MYB-transcription factor 137

* Linkage group start and end positions within 2.0 Mb interval downstream and upstream of the significant associated SNPs.

4. Discussion

Marker-assisted selection (MAS) is an efficient approach for increasing the accuracy and efficiency of selection using markers tightly linked to genes or QTLs of interest, to complement phenotypic selection [40,62]. The identification of QTLs associated with *Striga* resistance/tolerance would facilitate rapid development of *Striga* resistant/tolerant maize genotypes using MAS, due to the polygenic nature of host–parasite relationship and its interaction with environmental factors [63]. The normal distribution observed for grain yield and *Striga* damage in the present study is a result of the highly diverse genotypes segregating in the mapping population [64]. The selection of parental lines with varying levels of resistance to *Striga* allowed sufficient segregation of the traits in the population. The distribution of measured traits in the $F_{2:3}$ population indicated the existence of transgressive segregation (i.e., progenies performing outside the range of the parental genotypes). Transgressive segregation has been observed in populations screened under low N [65] and *Striga* infestation [66,67]. This phenomenon results from the accumulation of favorable and unfavorable alleles resulting from both parents. Moderate-to-high broad sense heritability estimates (0.47–0.70) observed for grain yield and other *Striga* resistance/tolerance indicator traits confirmed that high-quality phenotypic data were used for the genetic analysis. The moderate-to-high heritability estimates obtained in the present study implied that the observed genetic variation among the genotypes was strongly influenced by genetic factors, and that the *Striga* resistance indicator traits could be effectively improved in *Striga* resistance breeding programs. Previous studies reported moderate-to-high heritability values, ranging from 0.53–0.84 for *Striga* resistance/tolerance adaptive traits [68,69].

Linkage map density and resolution largely depend on population size and type, marker density, as well as the accuracy of genotyping [70,71]. The $F_{2:3}$ mapping population, developed from the cross between inbred TZEEI 79 (*Striga* resistant) and inbred TZdEEI 11 (*Striga* susceptible) was used to investigate the inheritance of *Striga* resistance/tolerance. The QTL mapping in the TZEEI 79 x TZdEEI 11 $F_{2:3}$ mapping population identified twelve QTLs for the *Striga* resistance indicator traits across the two research environments. These QTLs explained moderate variation of the phenotype, with values ranging from 2.0% for *qepp-2* to 13.5% for *qepp-1*. This finding confirmed the complexity of the genetic basis of *S. hermonthica* resistance [13,44]. The 12 QTLs identified included three for grain yield, five for ears per plant, three for *Striga* damage as well as one for *Striga* emergence counts. The identified QTLs were located on chromosomes 1, 2, 3, 5, 7 and 8. Similarly, Adewale et al. [44] identified markers linked to *Striga* resistance indicator traits in maize on chromosomes 1, 3, 5, 7 and 8. Samayoa et al. [72] found QTLs associated with Mediterranean corn borer resistance in maize on chromosomes 1, 5 and 6 using Recombinant Inbred Lines (RIL) population obtained from the cross B73 × CML103. In a study by Haussmann et al. [43], five genomic regions (QTLs) linked to *Striga* resistance in sorghum were reported on chromosomes 1, 2, 5 and 6. Generally, the QTLs mapped in the present study provided more information on the genetic basis of *Striga* resistance/tolerance in maize, indicating that the resistance/tolerance to *Striga* is quantitatively inherited. The additive effects of the identified QTLs indicated that favorable alleles for each QTL were contributed by either the resistant or susceptible parent, depending on the signs of the QTL additive effects. The resistant parental inbred TZEEI 79 contributed favorable alleles for resistance/tolerance to *Striga* for most of the identified QTLs. Estimates of genetic effects of the QTL indicated that additive gene action was preponderant in most cases for *Striga* resistance indicator traits.

The QTL analysis in the $F_{2:3}$ mapping population identified three major QTL (*qepp-1*, *qgy-2.1* and *qepp-8.1*) genomic regions on chromosomes 1, 2, and 8 with flanking marker intervals of 216–226 cM, –156 cM and 35–38 cM, respectively. Interestingly, the QTLs detected on chromosome 2 were found to be consistent across environments for grain yield and *Striga* damage. The QTL *qgy-2.1* identified for grain yield on chromosome 2 at 141.0 cM was found to be pleiotropic with QTL *qsd*-2 detected for *Striga* damage. Similarly, QTL *qgy-7* at 28.4 cM detected for grain yield on chromosome 7 was pleiotropic with the QTL for *Striga* damage and number of ears per plant. The co-localization of QTLs for these traits may reflect the high correlation coefficients observed among the different *Striga*

resistance indicator traits. These two QTLs would be invaluable genomic resources for fine mapping and candidate gene discovery. The validation of a common QTL region in different environments and/or genetic backgrounds is important for application in MAS to improve breeding efficiency [73]. The QTL *qgy-2.1* and *qsd*-2 from TZEEI 79 located on chromosome 2 (133.9–156.4) and identified in the two test locations in this study have not been previously reported. This QTL could be a hot spot for genes for genetic improvement of *Striga* resistance in maize. Overlapping regions of QTL on chromosome 7 (28.1–39.9) for *Striga* damage and ears per plant were identified in the present study. This common region could also provide better prospects for breeders to enhance resistance to *Striga* parasitism in maize using MAS.

Putative candidate genes associated with some of the identified QTLs for *Striga* resistance indicator traits are presented in Table 4. The gene model GRMZM2G054050 (*qsc-3.1*), associated with *Striga* emergence count encodes a multicopper oxidase Lpr-2 (low phosphate root 2) protein, whose homologous gene, Lpr-1 has been found [74,75] to regulate primary roots length under Pi (inorganic phosphate) deficient conditions. Lpr-1 and Lpr-2 play important roles in Pi sensing at root tips [75]. Similarly, the gene model GRMZM2G044194 linked to QTLs for grain yield (*qgy-7*), ears per plant (*qepp-7*), and *Striga* damage (*qsd-7*) were associated with the psk1 (phytosulfokine peptide precursor1) gene. PSK genes have been reported to promote cell growth especially in the quiescent centre cells of the root apical meristem [76]. Similarly, the gene model GRMZM2G408305 (*qgy-1.1*) associated with grain yield encodes the ARM family proteins which promote lateral root growth in plants [77]. QTL *qepp-3* located on chromosome 3 was found to be associated with MIR167g. In Arabidopsis, soybean and maize, miR167 has been reported to play important roles in lateral root growth and architecture [78]. Under plant nutrient deprivation conditions such as *Striga* parasitism, plants alter their root systems to discover heterogeneous soil regions for nutrients. The branching of secondary roots from primary roots in plants is one of the processes through which plants efficiently obtain nutrients from the soil. The QTL *qsd-5.1* was associated with the gene GRMZM2G059851 encoding the heat-shock factor protein, HSF 6. Heat-shock proteins are ubiquitous proteins responsible for protein folding, assembly, translocation as well as degradation in response to biotic stresses, depending on the nature of the causal organisms and plant genotypes (either susceptible or resistant), as well as plant's growth stage [64,79]. In addition, Ng et al. [80] identified AP2/ERF, MYB, bHLH, WRKY as well as bZIP as major transcription factor families involved in plant defense signalling. In a recent study, Adewale et al. [44] identified four putative candidate genes GRMZM2G060216, GRMZM2G103085, GRMZM2G057243 and GRMZM2G164743 located on chromosomes 3, 5, 9 and 10, having functions related to plant defense mechanisms under *Striga* infested conditions. The identified candidate genes in the present study differ from those earlier reported. The candidate genes identified from the dissection of *qsc-3.1*, *qgy-1.1*, *qepp-3*, and *qsd-5.1* are suggestive of *Striga* resistance response mechanisms. The QTL identified in the present study would be validated in different genetic backgrounds and in different environments to verify the reproducibility for effective use in MAS breeding for resistance to *Striga*. Overall, the QTL/markers with significant association to *S. hermonthica*-resistant adaptive traits would be useful as potential candidate loci for the enhancement of *Striga* resistance in maize. The application of these markers for selection would lead to the elimination of the bulk of *Striga* susceptible genotypes, which in turn may significantly reduce the number and cost of screening required to improve maize for *Striga* resistance. Based on our results, we initiated a program aimed at developing extra-early mapping populations from different genetic backgrounds so that putative markers identified in our studies could be validated and deployed in maize breeding programs through MAS.

5. Conclusions

A total of 12 QTLs associated with *S. hermonthica* resistance/tolerance traits in maize were identified across *Striga* infested environments in the present study. The identified QTLs displayed varying contributions to phenotypic expression and are in regions that play roles which may be associated

with plant defense response under *Striga* infestation in maize. The co-localization of QTL for grain yield and other traits indicated strong associations between the traits. The QTLs mapped in this study could be candidates for marker-assisted introgression of *Striga* resistance/tolerance genes in maize, after validation in different genetic backgrounds and in different environments.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/10/8/1168/s1, Table S1: Mean squares of $F_{2:3}$ mapping population evaluated under artificial *Striga* infestation at both Abuja and Mokwa in 2018 growing season. Table S2: Shapiro-Wilk's normality tests for *Striga* resistance/tolerance indicator traits for $F_{2:3}$ population derived from the cross between TZEEI 79 (*Striga* resistant) and TZdEEI 11 (*Striga* susceptible). Table S3: Candidate genes associated with the identified QTL for key Striga resistance/tolerance indicator traits under artificial *Striga* infestation. Figure S1: Linkage map of $F_{2:3}$ mapping population based on 1139 DArTseq markers. Left bar of the linkage map indicates cM distance while right bar of linkage map displayed the marker names. Red bars and letters indicate QTL identified across *Striga* infested environments. Figure S2. Major QTL identified for *Striga* resistance in the extra-early yellow mapping population. A likelihood of odds (LOD) scan showing the QTL identified on chromosomes 1, 2, and 8 explaining ≥ 10% phenotypic variation.

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Data Availability: The DArTseq datasets used/analyzed in the manuscript have been deposited at the IITA-CKAN repository. doi:10.25502/aabs-rc02/d; doi:10.25502/8dkd-0h42/d.

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