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SSR markers as tools for screening genotypes of maize (*Zea mays* L.) for tolerance to drought and *Striga hermonthica* (Del.) Benth in the Northern Guinea Savanna Zone of Ghana

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Resistant or tolerant crop cultivars provide the most economical, practical and sustainable method of control of Striga and drought stresses. However, the development of resistant/tolerant maize cultivars is hampered by the complexity of the environment, the host/parasite interactions, and lack of reliable screening method. The invention of molecular markers to tag gene(s) that confer important traits offer new hope for Striga and drought control. A study was conducted at the Biotechnology laboratory of the Savanna Agricultural Research Institute in Nyankpala, Northern Ghana, to screen six parent and thirty F_1 progenies of maize for tolerance to drought and *Striga hermonthica* using 17 microsatellites. Genomic DNA was extracted with the CTAB method and PCR was performed based on the common method for microsatellite markers. PCR products were separated using 6% polyacrylamide denaturing gel. Frequencies were run to determine the genotypes that produce bands across all or most microsatellite primers. The results indicated that the F_1 hybrids IWD x TAIS03, IWD x GUMA03, DT x KOBN03, TAIS03 x IWD, TAIS03 x DT, TAIS03 x SISF03, SISF03 x TAIS03, SISF03 x GUMA03, GUMA03 x IWD, GUMA03 x DT and GUMA03 x KOBN03 contained the quantitative traits responsible for both drought and *Striga hermonthica* tolerance. The parental lines DT-STR-W-C2, TAIS03 and IDW-C3-SYN-F2, and F_1 hybrid populations IWD x TAIS03, IWD x GUMA03, DT x KOBN03, TAIS03 x IWD, TAIS03 x DT, TAIS03 x SISF03, TAIS03 x KOBN03, SISF03 x DT, SISF03 x TAIS03, SISF03 x GUMA03, GUMA03 x IWD, GUMA03 x DT, GUMA03 x SISF03, GUMA03 x KOBN03 and KOBN03 x IWD contained the quantitative traits responsible for *Striga hermonthica* tolerance. The study revealed that the parent populations IDW-C3-SYN-F2, TAIS03, GUMA03-OB and KOBN03-OB, and F_1 hybrid populations IWD x TAIS03, IWD x GUMA03, IWD x KOBN03, DT x TAIS03, DT x SISF03, DT x KOBN03, TAIS03 x IWD, TAIS03 x DT, TAIS03 x SISF03, SISF03 x TAIS03, SISF03 x GUMA03, SISF03 x KOBN03, GUMA03 x IWD, GUMA03 x DT and GUMA03 x KOBN03 showed bands across nine drought-linked SSR markers, and therefore, contain the quantitative traits responsible for drought tolerance. The study led to identification of informative SSR markers, such as Phi074, Nc005, Phi015, Phi061 and Phi055, which significantly contributed to the differentiation of Striga tolerant and susceptible genotypes. Identification of informative SSR markers (P-umc1542, P-bnlg1429, P-umc1566, P-umc2189, P-umc2225, P-umc2226, P-

bnlg1014, P-bnlg1124 and P-umc1292) which significantly contributed to the differentiation of drought tolerant and susceptible genotypes was also made.

Keywords: Maize; drought and striga tolerance; simple sequence repeat markers; quantitative trait loci; guinea savanna; Ghana

The most important limiting factors to maize production in Africa are drought, *Striga* infestation and low soil fertility (Edmeades *et al*. 1995). To achieve the growing need for maize in Africa it is necessary to boost productivity through reducing yield losses caused by various stress factors such as diseases, pests, parasitic weeds and drought (Dagne *et al*., 2004). The development of drought and *Striga* tolerant varieties in maize may improve the performance of the crop even under water stressed and/or *Striga* infested conditions, and hence increase yield to meet the food requirements of the people of Ghana in particular, and the sub-Saharan Africa in general.

Breeding for resistance or tolerance in maize is recognized as the most feasible method for *Striga* and drought control (*Ejeta et al*., 1992). However, developing crop cultivars with durable Striga or drought resistance is slowed for lack of a reliable screening method. Conventional breeding for Striga and drought tolerance is slow and greatly constrained by lack of reliable screening methods, as well as lack of knowledge of the genetics of Striga and drought resistance (Haussmann *et al*., 2000a). Hess and Ejeta (1992) attributed the lack of progress on inheritance studies and breeding for resistance to rarity of genotypes that exhibit stable résistance across geographical regions, problems of having uniform field infestations, and the difficulty encountered in evaluating resistance. There is also a lack of alternative and reliable screening assays that can adequately predict the field performance of a given genotype (Omanya *et al*., 2001). Because of the difficulties encountered in screening plant materials for biotic and abiotic stresses using conventional breeding methods, breeders now resort to the use of molecular approaches to improve on the efficiency of breeding programmes. In this era of biological sciences, efforts should be made to track important gene(s) that govern important traits using SSR molecular markers. These markers will not only ease the screening for Striga or drought tolerance, but also help tracking important Striga or drought resistance genes.

For the purpose of this study the simple sequence repeat (SSR) markers (microsatellites) were used to screen for drought and *Striga hermonthica* tolerance. In this study, six superior parent genotypes of maize were crossed in a complete diallel manner to produce 30 F_1 hybrids. These hybrids and their parents were evaluated using simple sequence repeat (SSR) markers. The objective of this study was therefore to evaluate six parent genotypes of maize and their 30 F_1 hybrids for tolerance to drought and/or *Striga hermonthica* using SSR markers. The study also sought to identify simple sequence repeat (SSR) markers that are effectively linked to the quantitative traits responsible for drought and/ or *Striga hermonthica* tolerance among the parents and hybrids.

Materials and methods

Experimental design and location

The study was conducted at the biotechnology laboratory of Savanna Agricultural Research Institute (SARI). A total of 36 genotypes of maize, comprising 6 parents and 30 F_1 hybrids, were raised under irrigated conditions at SARI Technology Park in February 2015. Youngest leaves from the maize accessions were collected for genomic

deoxyribo nucleic acid (DNA) extraction for the study. A total of 17 simple sequence repeat (SSR) primers from the 'phi', 'nc', 'pumc' and 'pbnlg' series, which are widely distributed across the maize genome (Gemenet *et al*., 2010), were used to screen the genomic DNAs of the 6 parents and 30 F_1 progenies of maize for tolerance to drought and/ or *Striga hermonthica.* The primers were procured from Metabion International AG (Germany). Out of the 17 SSR primers, 10 of them (P-umc1566, P-umc1292, P-bnlg1124, P-bnlg1179, P-bnlg1014, P-umc2225, P-bnlg1429, P-umc2226, P-umc1542 and P-umc2189) were used to investigate the genetic diversity among the parents and F_1 progenies for drought tolerance; whilst 7 primers, including Phi055, Phi074, Phi076, Phi015, Phi061, Phi021 and Nc005, were used to screen the parents and F_1 progenies for quantitative trait loci (QTL) responsible for Striga tolerance.

The six parent genotypes used in this study were developed by the International Institute for Tropical Agriculture (IITA) and obtained from the Savannah Agricultural Research Institute (SARI) of the Council for Scientific and Industrial Research (CSIR). The parent genotypes include KOBN03-OB, GUMA03-OB, DT-STR-W-C2, SISF03-0B, TAIS03 and IWD-C3-SYN-F2. The parent populations were then crossed in a complete diallel fashion to develop 30 F_1 hybrids, which include: IWD x DT, IWD x TAIS03, IWD x SISF03, IWD x GUMA03, IWD x KOBN03, DT x IWD, DT x TAIS03, DT x SISF03, DT x GUMA03, DT x KOBN03, TAIS03 x IWD, TAIS03 x DT, TAIS03 x SISF03, TAIS03 x GUMA03, TAIS03 x KOBN03, SISF03 x IWD, SISF03 x DT, SISF03 x TAIS03, SISF03 x GUMA03, SISF03 x KOBN03, GUMA03 x IWD, GUMA03 x DT, GUMA03 x TAIS03, GUMA03 x SISF03, GUMA03 x KOBN03, KOBN03 x IWD, KOBN03 x DT, KOBN03 x TAIS03, KOBN03 x SISF03 and KOBN03 x GUMA03. Completely randomized design (CRD) was used for this experiment.

DNA extraction, PCR amplification and gel electrophoresis

The youngest leaves from two-week old maize plants were collected for genomic DNA extraction. Genomic DNA was isolated from all the 6 parents and 30 F_1 hybrids of maize, including reciprocals. The extraction of the DNA from leaves of the maize plants was carried out following the protocol of Gawel and Jarret, (1991).

The DNAs were extracted from the leaves of the two-week old maize plants using the CTAB method with little modification. About 200 mg of the young leave were harvested from the seven plants in each of the 36 genotypes and put into properly labeled separate 2 ml Eppendorf tubes containing two ball bearings each to aid proper lysis of the cells. The tubes after each harvest were placed in liquid nitrogen. The leaf samples were ground to fine powder using a tissue lyser. This was done three times with the tubes being returned into the liquid nitrogen in-between grinding to increase the efficiency of the process. An amount of 1 ml of pre-warmed CTAB buffer (2% CTAB, 20 mM EDTA, 100 mM Tris-HCl and 1.4M NaCl) with 0.2% mercapto-ethanol (2µl/1ml of CTAB buffer) was added to each eppendorf tube and placed in a water bath at 60 °C for 1 hour. During this time the content of the tubes were mixed gently by inverting for 6 - 7 times. Then 200 µl of potassium acetate was added and put on ice for 20 min. Later, 700 µl of chloroform: Isoamyl alcohol (24: 1) was added to each tube and mixed gently by inverting, and this was left undisturbed for 5 min.

The tubes were then centrifuged at 10000 rpm for 15 min and the middle aqueous layer transferred into properly labeled new 2 ml tubes using 1000 µl tips. The same volume of chloroform: Isoamyl alcohol as in new tubes was added, mixed

gently and left undisturbed for 5 min. This was centrifuged at 10 000 rpm for 10 min and the supernatant transferred into new properly labeled 1.5 ml tubes. An amount of 500 µl of ice cold isopropanol was added to get a white precipitate. For maximum precipitation of the DNA, the tubes were left over night in -20 °C. The tubes were centrifuged for 5 min at 1000 rpm and the supernatant removed carefully leaving pellets of DNA. The pellets were washed twice with about 300 ml of 75 % ice cold ethanol depending on pellet size. Each followed by centrifugation at 15000 rpm for 5 min and allowed to air dry. About 300 ml of double distilled water was added and kept in 4 °C for dissolution of the pellet. An amount of 2µl of RNAse was added to each tube to get rid of RNA in the DNA and put in an incubator at 3 °C for an hour.

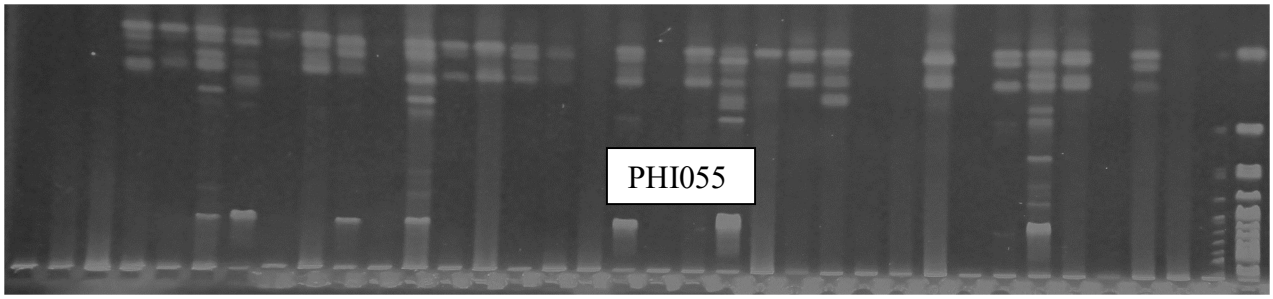
DNA quality check was done using agarose gel electrophoresis following the protocol of Sambrook *et al*. (1989). In determining the purity of the NDA extract, 1.0% agarose gel with (3 µl) 0.003% ethidium bromide gel red solution was prepared. An amount of 5 µl sample DNA was pippetted and 1µl loading buffer (X6 bromophenol blue) added. The sample was then loaded in the wells on gel submerged in 1X TAE buffer. The sample was then run at 90 volts for 45 min. Photograph under UV light was then taken.

Polymerase Chain Reaction (PCR) amplification of the extracted DNAs were then carried out using the following conditions: denaturation at 94 °C for 30 seconds, annealing at 56 °C for 30 seconds and extension at 72 °C for 30 seconds, in a reaction volume of 10 µl (6.5 µl of ddH_2O, 5 ng/µl of DNA, 1 mM of primer and 1.5 µl of premix). After PCR reaction, the PCR products were dissolved on a 6% horizontal polyacrylamide gel at a voltage of 150, running time of three hours and stained with ethidium bromide. From the gel pictures, number of bands produced was counted and frequencies were run using SPSS to determine the levels of polymorphism and band distribution among parent and F_1 hybrid populations.

Results and discussion

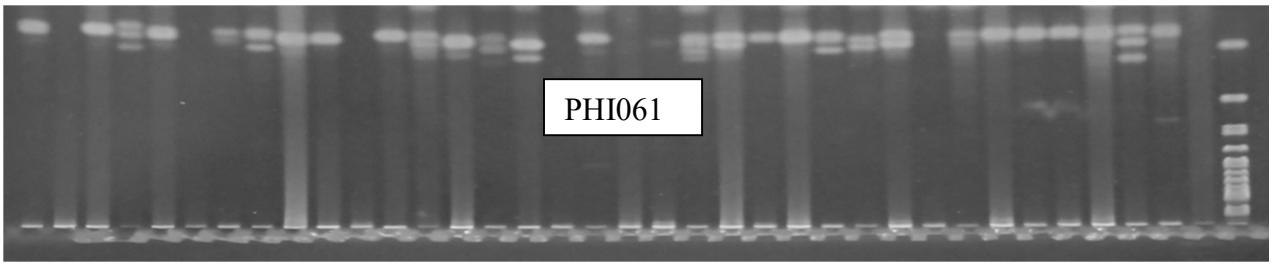
Screening for drought and *Striga hermonthica* tolerance

Results from the gel pictures of Striga-linked SSR amplification products showed that the parent genotype, IWD-C3-SYN-F2 in lane 1 did not show any band (Figure 1). Among the F_1 hybrids, IWD x TAIS03 (lane 3), IWD x GUMA03 (lane 5) and IWD x KOBN03 (lane 6) revealed presence of bands. There were also bands for TAIS03 (lane 13), TAIS03 x IWD (lane 14), TAIS03 x DT (lane 15), TAIS03 x SISF03 (lane 16), and TAIS03 x KOBN03 (lane 18), whilst TAIS03 x GUMA03 in lane 17 was empty. The occurrence of empty lanes in the gel picture is probably because the marker might have represented a null allele. The null allele thus appears as an empty lane because of failure of amplification of target DNA. This confirms the observation made by Callen *et al.* (1993), who reported that mutations in the binding region of one or both of the microsatellite primers may inhibit annealing that may result in the reduction or loss of a PCR product. The results of Phi074 amplification indicated that the marker amplified bands in lanes 13, 14, 15, 16, 17 and 18 (Figure 3). The presence of bands was also shown in the lanes of genotypes KOBN03 (lane 31) and KOBN03 x IWD (lane 32), while KOBN03 x DT (lane 33), KOBN03 x TAIS03 (lane 34), KOBN03 x SISF03 (lane 35) and KOBN03 x GUMA03 (lane 36) were empty. The presence of bands in lanes is probably due to the occurrence of similar base pairs within the target DNA and the primer thus, resulting in amplification of target DNA.



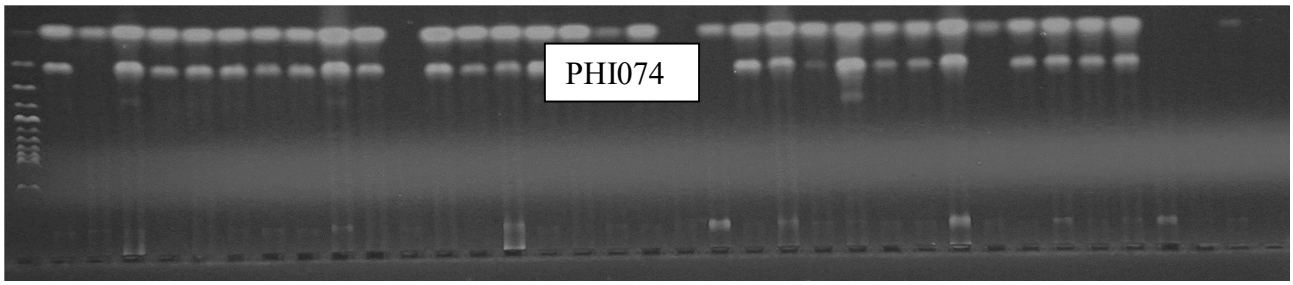
35 34 33 32 31 30 29 28 27 26 25 24 23 22 21 20 19 18 17 16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 L

Figure 1: Gel picture of SSR marker (PHI055) amplification product
Lanes with numbers 1, 7, 13, 19, 25 and 31contain the parent populations IWD-C3-SYN-F2, DT-STR-W-C2, TAIS03, SISF03-OB, GUMA03-OB and KOBN03-OB respectively; the rest of the numbered lanes contain F_1 progenies derived from diallel crossing of parent populations
Lane 2= IWD x DT, Lane 3= IWD x TAIS03, Lane 4= IWD x SISF03, Lane 5= IWD x GUMA03, Lane 6= IWD x KOBN03, Lane 8= DT x IWD, Lane 9= DT x TAIS03, Lane 10= DT x SISF03, Lane 11= DT x GUMA03, Lane 12= DT x KOBN03, Lane 14= TAIS03 x IWD, Lane 15= TAIS03 x DT, Lane 16= TAIS03 x SISF03, Lane 17= TAIS03 x GUMA03, Lane 18= TAIS03 x KOBN03, Lane 20= SISF03 x IWD, Lane 21= SISF03 x DT, Lane 22= SISF03 x TAIS03, Lane 23= SISF03 x GUMA03, Lane 24= SISF03 x KOBN03, Lane 26= GUMA03 x IWD, Lane 27= GUMA03 x DT, Lane 28= GUMA03 x TAIS03, Lane 29= GUMA03 x SISF03, Lane 30= GUMA03 x KOBN03, Lane 32= KOBN03 x IWD, Lane 33= KOBN03 x DT, Lane 34= KOBN03 x TAIS03, Lane 35= KOBN03 x SISF03 and Lane 36= KOBN03 x GUMA03; L represents the DNA ladder



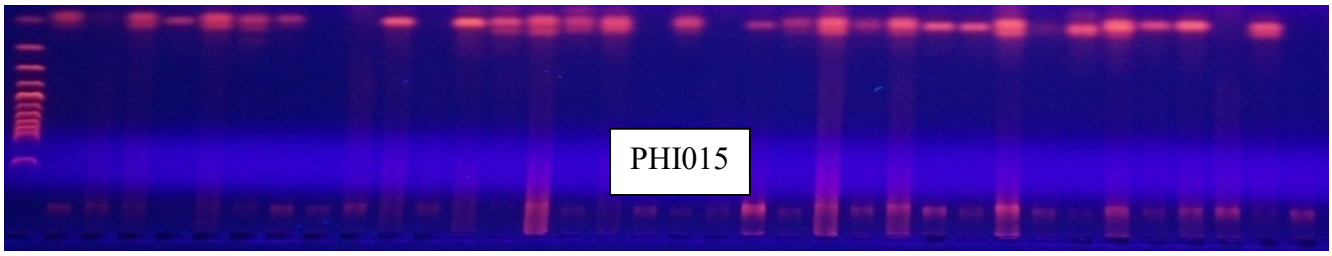
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 L

Figure 2: Gel picture of SSR marker (PHI061) amplification product
L represents the DNA ladder; Lanes 1-36 represent the maize genotypes as defined in figure 1

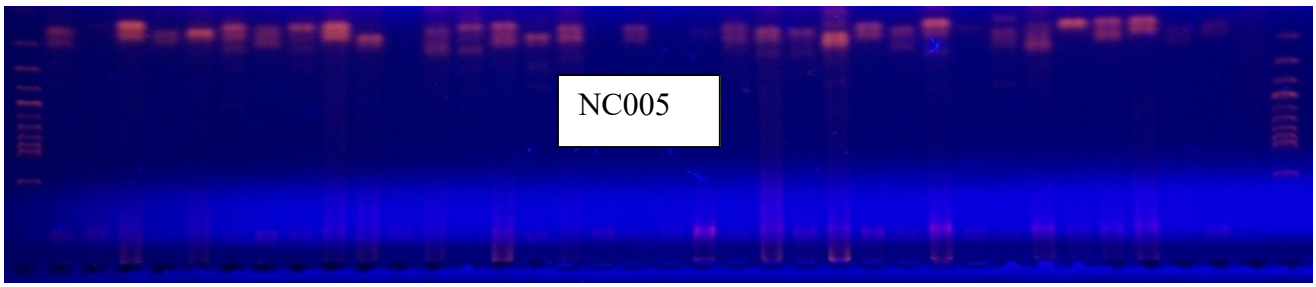


L 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36

Figure 3: Gel picture of SSR marker (PHI074) amplification product
L represents the DNA ladder; Lanes 1-36 represent the maize genotypes as defined in figure 1



L 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36
Figure 4: Gel picture of SSR marker (PHI015) amplification product
L represents the DNA ladder; Lanes 1-36 represent the maize genotypes as defined in figure 1



L 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36
Figure 5: Gel picture of SSR marker (NC005) amplification product
L represents the DNA ladder; Lanes 1-36 represent the maize genotypes as defined in figure 1

Results from the gel picture produced by drought-linked SSR marker (P-umc1542) revealed that polymorphic bands were amplified by the SSR marker in lanes 1, 7, 13, 25 and 31 of the parent genotypes IWD-C3-SYN-F2, DT-STR-W-C2, TAIS03, GUMA03 and KOBN03, respectively (Figure 6). Among the F_1 hybrids, IWD x DT (lane 2), DT x GUMA03 (lane 11), TAIS03 x SISF03 (lane 16), TAIS03 x GUMA03 (lane 17), SISF03 x IWD (lane 20), GUMA03 x TAIS03 (lane 28), GUMA03 x SISF03 (lane 29), KOBN03 x IWD (lane 32) and KOBN03 x GUMA03 (lane 36) recorded empty lanes, while the rest of the hybrids showed bands in their lanes. The empty lanes might have occurred as a result of the presence of null alleles. The null alleles appear as an empty lane because of failure of amplification of target DNAs. The maize SSR marker P-bnlg1429 was used to amplify the PCR products in Figure 7. The results of the PCR products indicated that lanes 1, 7, 13, 25 and 31 of parental genotypes IWD-C3-SYN-F2, DT-STR-W-C2, TAIS03, GUMA03-OB and KOBN03-OB respectively, revealed presence of bands, while the lane of SISF03-OB (lane 19) was empty. For the F_1 hybrids, the gel picture revealed absence of bands for IWD x DT (lane 2), DT x GUMA03 (lane 11), TAIS03 x GUMA03 (lane 17), GUMA03 x TAIS03 (lane 28) and KOBN03 x GUMA03 (lane 36). However, all the remaining hybrids recorded bands in their lanes. From the study, the gel picture of P-umc2225 revealed that Lanes 2, 6, 11, 17, 19, 20, 28 and 36 were empty, whilst the rest of the lanes recorded bands (Figure 10). Some lanes of Figure 10 were empty probably because they might represent null alleles. A null allele appears as an empty lane because of failure of amplification of target DNA. The study further revealed that some of the SSR markers, including P-umc2225, amplified double bands within the genomic regions of some maize genotypes. The amplification of similar sequences in different genomic regions due to duplications could have been the possible reason for the occurrence of double-band phenotypes.

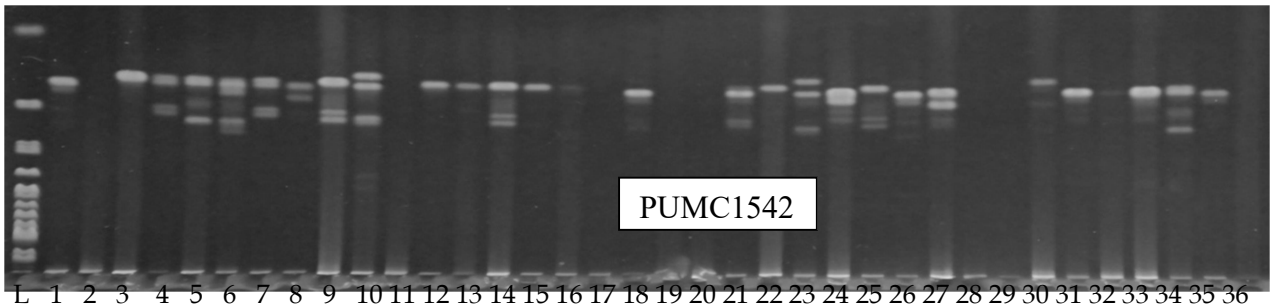


Figure 6: Detection of polymorphism using *P-umc1542* SSR marker

Lanes with numbers 1, 7, 13, 19, 25 and 31 contain the parent populations IWD-C3-SYN-F2, DT-STR-W-C2, TAIS03, SISF03-OB, GUMA03-OB and KOBN03-OB respectively; the rest of the numbered lanes contain F_1 progenies derived from diallel crossing of parent populations

Lane 2= IWD x DT, Lane 3= IWD x TAIS03, Lane 4= IWD x SISF03, Lane 5= IWD x GUMA03, Lane 6= IWD x KOBN03, Lane 8= DT x IWD, Lane 9= DT x TAIS03, Lane 10= DT x SISF03, Lane 11= DT x GUMA03, Lane 12= DT x KOBN03, Lane 14= TAIS03 x IWD, Lane 15= TAIS03 x DT, Lane 16= TAIS03 x SISF03, Lane 17= TAIS03 x GUMA03, Lane 18= TAIS03 x KOBN03, Lane 20= SISF03 x IWD, Lane 21= SISF03 x DT, Lane 22= SISF03 x TAIS03, Lane 23= SISF03 x GUMA03, Lane 24= SISF03 x KOBN03, Lane 26= GUMA03 x IWD, Lane 27= GUMA03 x DT, Lane 28= GUMA03 x TAIS03, Lane 29= GUMA03 x SISF03, Lane 30= GUMA03 x KOBN03, Lane 32= KOBN03 x IWD, Lane 33= KOBN03 x DT, Lane 34= KOBN03 x TAIS03, Lane 35= KOBN03 x SISF03 and Lane 36= KOBN03 x GUMA03; L represents the DNA ladder

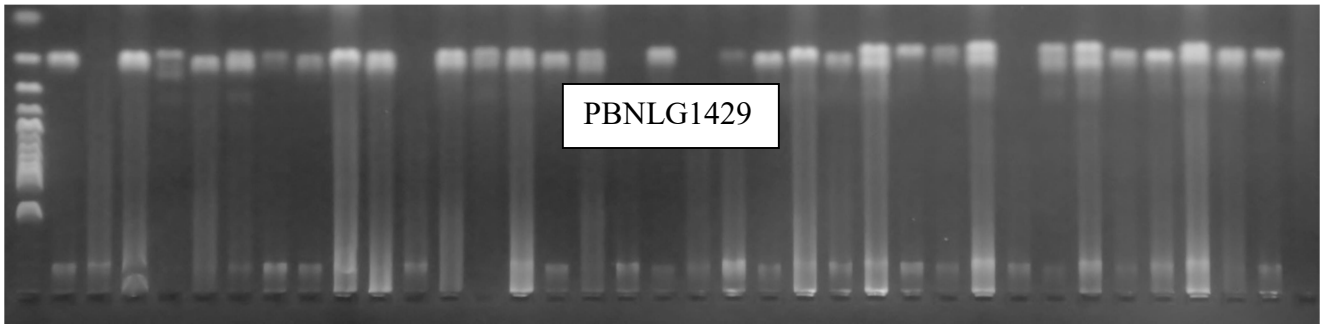


Figure 7: Detection of polymorphism using *P-bnlg1429* SSR marker

L represents the DNA ladder; Lanes 1-36 represent the maize genotypes as defined in figure 6

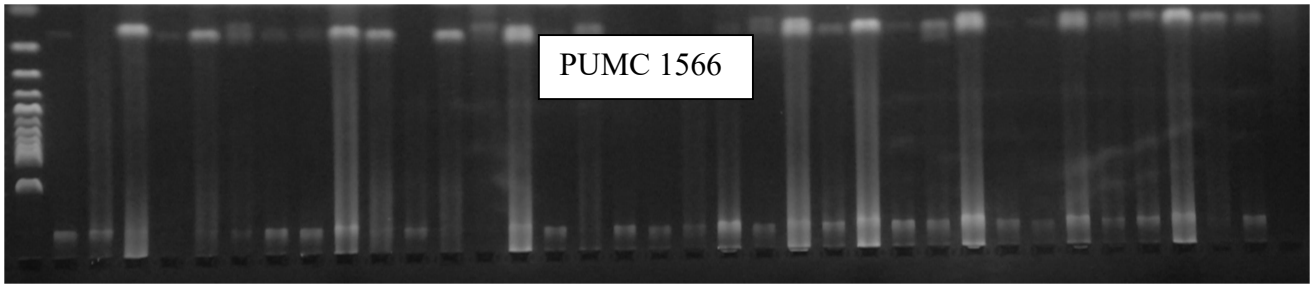
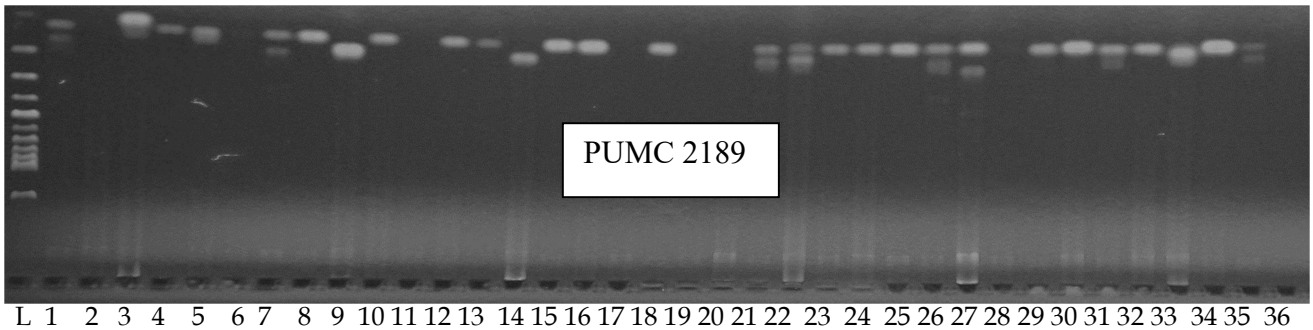


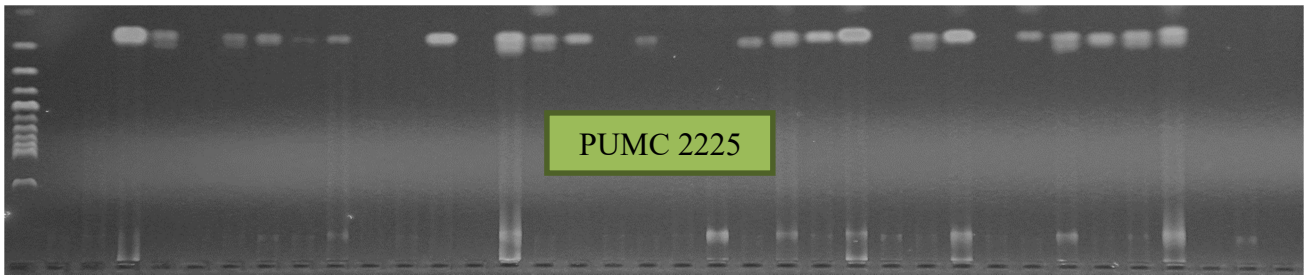
Figure 8: Detection of polymorphism using *P-umc1566* SSR marker

L represents the DNA ladder; Lanes 1-36 represent the maize genotypes as defined in figure 6



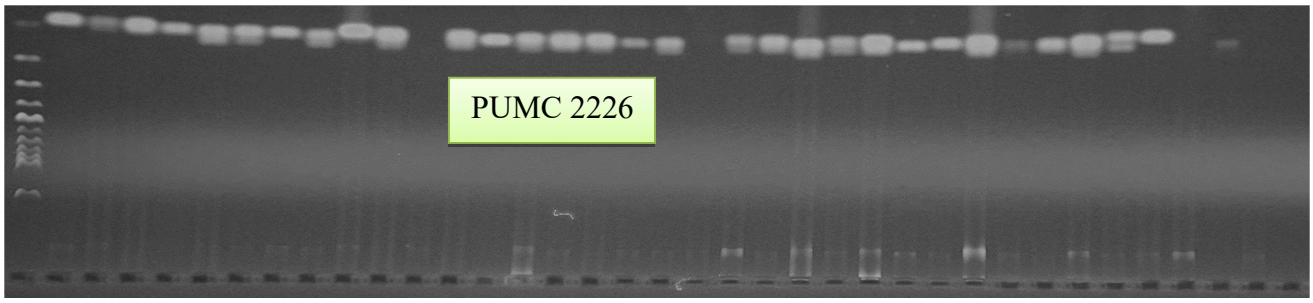
L 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36

Figure 9: Detection of polymorphism using *P-umc2189* SSR marker
L represents the DNA ladder; Lanes 1-36 represent the maize genotypes as defined in figure 6



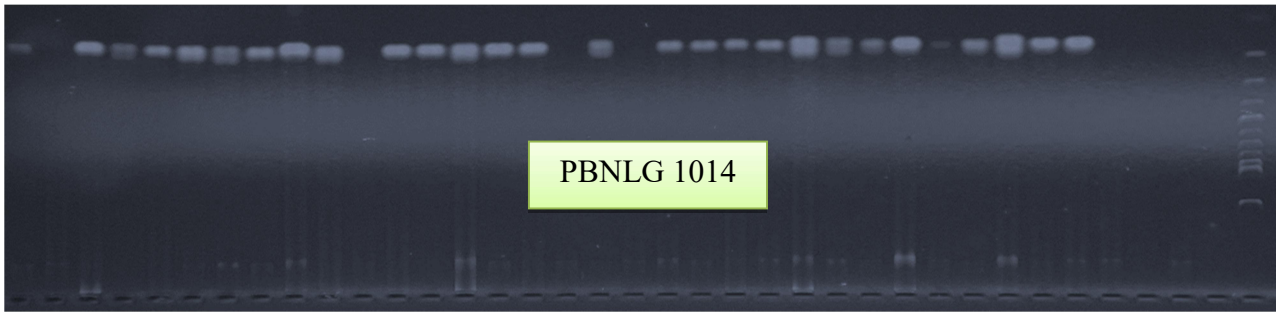
L 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36

Figure 10: Detection of polymorphism using *P-umc2225* SSR marker
L represents the DNA ladder; Lanes 1-36 represent the maize genotypes as defined in figure 6



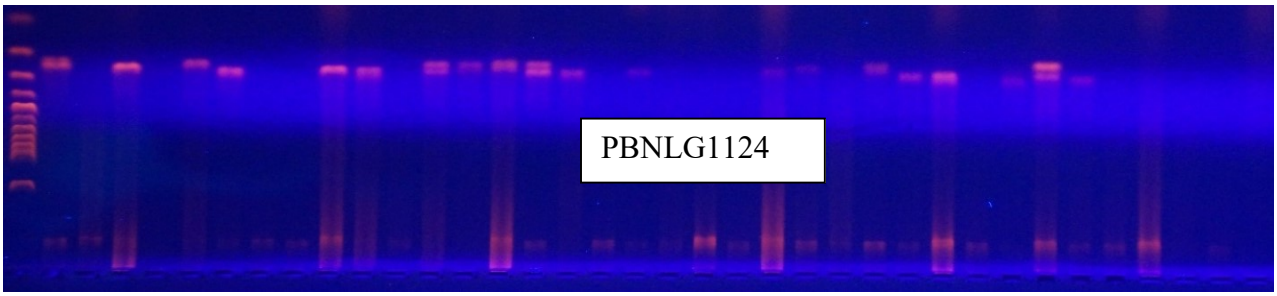
L 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36

Figure 11: Detection of polymorphism using *P-umc2226* SSR marker
L represents the DNA ladder; Lanes 1-36 represent the maize genotypes as defined in figure 6

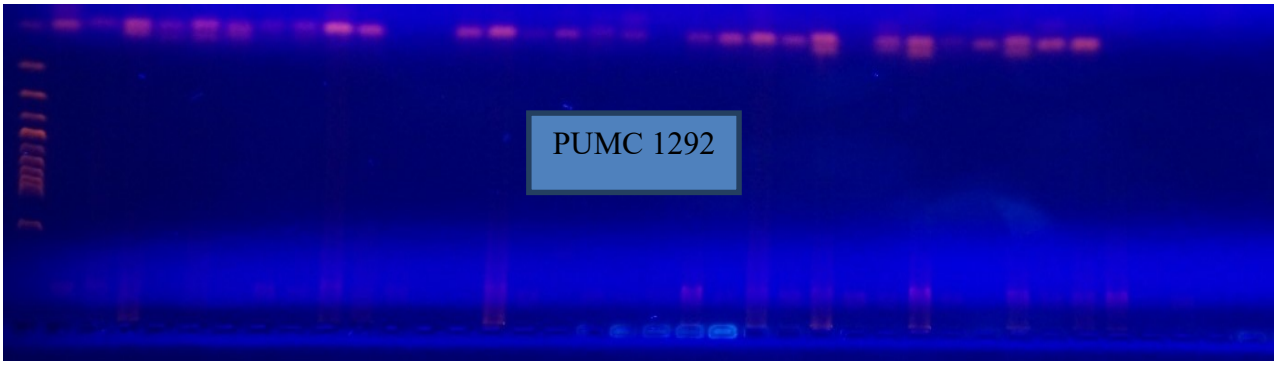


1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 L

Figure 12: Detection of polymorphism using *P-bnlg1014* SSR marker
L represents the DNA ladder; Lanes 1-36 represent the maize genotypes as defined in figure 6



L 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36
Figure 13: Detection of polymorphism using *P-bnlg1124* SSR marker
L represents the DNA ladder; Lanes 1-36 represent the maize genotypes as defined in figure 6



L 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36
Figure 14: Detection of polymorphism using *P-umc1292* SSR marker
L represents the DNA ladder; Lanes 1-36 represent the maize genotypes as defined in figure 6

The results of screening of parents and F_1 hybrids for polymorphism established that the hybrids IWD x TAIS03, IWD x GUMA03, DT x KOBN03, TAIS03 x IWD, TAIS03 x DT, TAIS03 x SISF03, TAIS03 x KOBN03, SISF03 x DT, SISF03 x TAIS03, SISF03 x GUMA03, SISF03 x KOBN03, GUMA03 x IWD, GUMA03 x DT, GUMA03 x SISF03, GUMA03 x KOBN03 and KOBN03 x IWD amplified bands in all the five Striga-linked microsatellites used in the study. However, 3 F_1 hybrids including DT x GUMA03, GUMA03 x TAIS03 and KOBN03 x GUMA03 could not amplify bands in any of the five SSR markers used in the study. For the parental lines, DT-STR-W-C2, TAIS03, GUMA03-OB and KOBN03-OB amplified bands in all the five markers, while SISF03-OB failed to amplify in any of the five microsatellite markers (Figure 15).

The results of screening for polymorphism further established that the F_1 hybrids (IWD x TAIS03, DT x TAIS03, TAIS03 x IWD and GUMA03 x DT) were polymorphic and amplified bands in all the nine drought-linked microsatellite markers used for the study. Six F_1 hybrids including, IWD x GUMA03, DT x SISF03, SISF03 x TAIS03, SISF03 x KOBN03, GUMA03 x IWD and GUMA03 x KOBN03 were able to amplify in eight SSR markers, whilst KOBN03 x GUMA03 failed to amplify in all the nine SSR markers (Figure 16). Among the parent populations, SISF03-OB could not amplify even a single band among all the nine markers. However, the parents TAIS03, GUMA03-OB and KOBN03-OB were polymorphic in seven SSR markers (Figure 16).

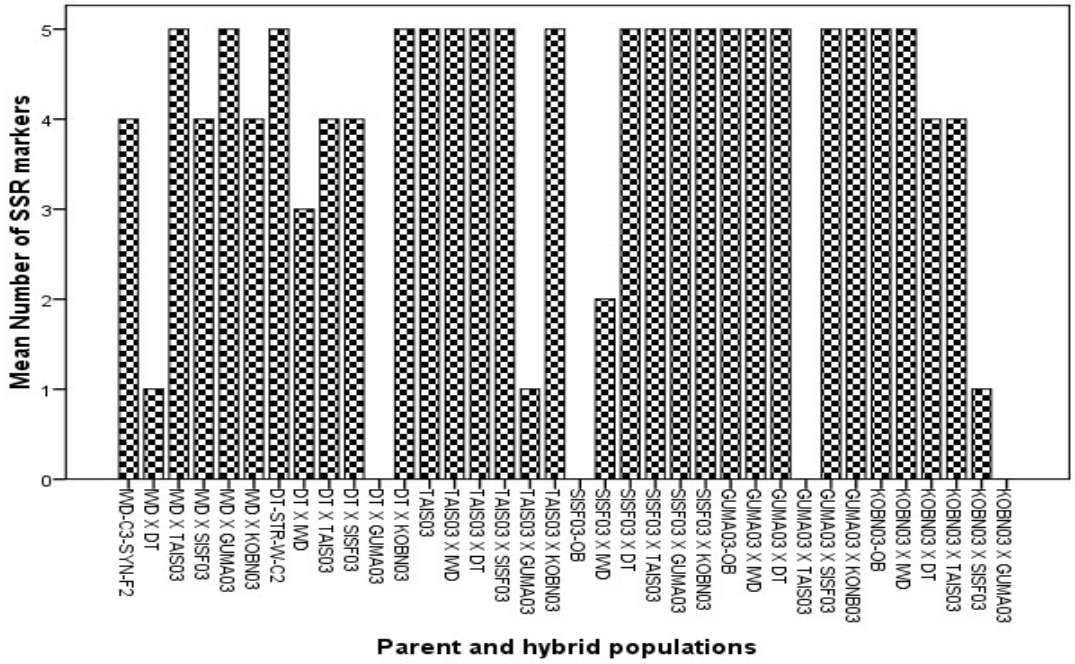


Figure 15: Trend of band amplification of 6 parent and 30 F_1 hybrid populations from gel pictures of five SSR markers during laboratory screening in 2015

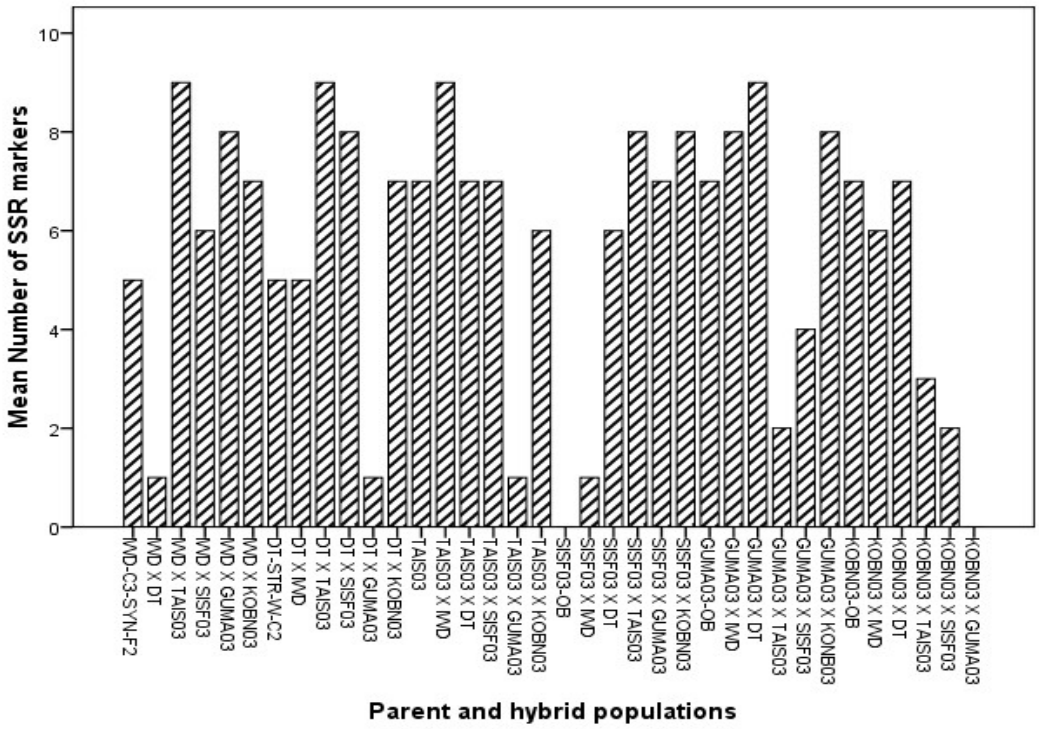


Figure 16: Trend of band amplification of 6 parents and 30 F_1 hybrid populations from gel pictures of nine drought-linked SSR markers during laboratory screening in 2015

The results of screening for polymorphism showed that the F_1 hybrids IWD x DT, TAIS03 x IWD and GUMA03 x DT were highly polymorphic among all the drought and Striga-linked microsatellite markers. The three hybrids amplified bands in nine drought-linked as well as the five Striga-linked microsatellite markers. Six F_1 hybrids including, IWD x GUMA03, DT x SISF03, SISF03 x TAIS03, SISF03 x KOBN03, GUMA03 x IWD and GUMA03 x KOBN03 were able to amplify bands in all the five Striga-linked SSR markers, but could however amplify in eight of the nine drought-linked SSR markers. The study however established that the parent SISF03-OB failed to amplify in all the Striga and drought-linked SSR markers (Figure 17).

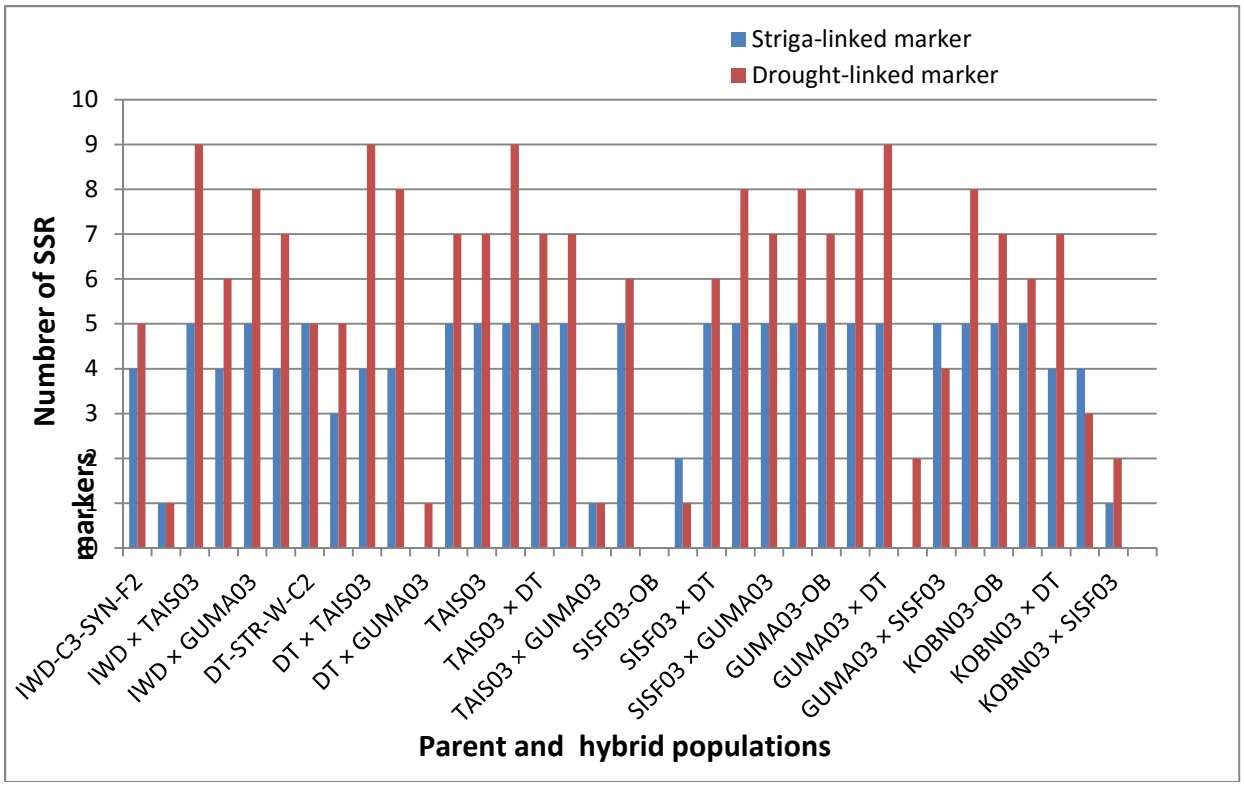


Figure 17: Trend of band amplification of 6 parents and 30 F_1 hybrids from gel pictures of five Striga-linked and nine drought-linked SSR markers during laboratory screening in 2015

Screening SSR markers for polymorphism

Seventeen Simple Sequence Repeat (SSR) markers were screened for polymorphism among the six parents and the thirty F_1 hybrids of maize. The small quantity of extracted DNA in each of the 36 maize lines was enough to run all the seventeen PCR reactions using all the seventeen markers, as one PCR reaction required 0.03 µg. This is in consonance with the observations made by Semagn *et al*. (2006a), Semagn *et al*. (2006b) and Korzum (2003) that SSR markers require very little DNA unlike other types of molecular markers. After the PCR amplification and gel electrophoresis, five out of seven Striga-linked SSR markers were amplified, while two failed to amplify (Table 1a). For the drought-linked SSR markers, nine were polymorphic, while one failed to amplify (Table 1b).

Table 1a: SSR markers used in screening for Striga QTL polymorphism among parents and F_1 progenies

SSR marker	Sequence of forward primer (F 5¹-3¹)	Sequence of reverse primer (R 5¹-3¹)
Phi055*	GAGATCGTGTGCCCGCACC	TTCCTCCTGCTCCTCAGACGA
Phi074*	CCCAATTGCAACAACAATCCTTGGCA	GTGGCTCAGTGATGGCAGAAACT
Phi076	TTCTTCCGCGGCTTCAATTTGACC	GCATCAGGACCCGCAGAGTC
Phi015*	GCAACGTACCGTACCTTTCCGA	ACGCTGCATTCAATTACCGGGAAG
Phi061*	GACGTAAGCCTAGCTCTGCCAT	AAACAAGAACGGCGGTGCTGATTC
Phi021	TTCCATTCTCGTGTTCTTGGAGTGGTCCA	CTTGATCACCTTTCCTGCTGTCGCCA
Nc005*	CCTCTACTCGCCAGTCGC	TTTGGTCAGATTTGAGCACG

*SSR markers are polymorphic; QTL, quantitative trait loci

Table 1b: SSR markers used in screening for drought QTL polymorphism among parents and F_1 progenies

SSR marker	Sequences of forward primer (F 5¹-3¹)	Sequences of reverse primer (R 5¹-3¹)
P-umc1566*	ATCTCGTCTACCTAACCCACCCTC	CAGGTGAAGAATCTGGTGAGGTC
P-umc1292*	GAAGTGAATGTGGGAACATGGTTC	TCACGGTTCAGACAGATACAGCTC
P-bnlg1124*	TCTTCATCTCTCTCTATCAAACTGACA	TGGCACATCCACAAGAACAT
P-bnlg1179	GCGATTCAGTCCGCAGTAGT	GTACTGAACAAACCGGTGGGC
P-bnlg1014*	CACGCTGTTTCAGACAGGAA	CGCCTGTGATTGCACTACAC
P-umc2225*	TCGGCTGACATAATAAAACCATAGC	ATGCGAATTTTACCGGGTTTTT
P-bnlg1429*	CTCCTCGCAAGGATCTTCAC	AGCACCGTTTCTCGTGAGAT
P-umc2226*	TGCTGTGCAGTTCTTGCTTCTTAC	AGCTTCACGCTCTTCTAGACCAAA
P-umc1542*	TAAAGCTATGATGGCACTTGCAGA	CATATTTGCCTTTGCCCTTTTGTA
P-umc2189*	CGTAAGTACAGTACACCAATGGGC	ACACCGACTACAAGCCTCTCAACT

*SSR markers are polymorphic; QTL, quantitative trait loci

It was therefore established that about 71% of the screened *Striga*-markers (Table 1a) and 90% of the screened drought-markers (Table 1b) were polymorphic. Therefore, majority of the SSR markers used in this study were effective in detecting polymorphism, and could be used to select the Striga or drought tolerant QTL. This finding is in consonance with the observation made by Collard *et al*. (2005) who reported that in conducting parental foreground selection, one should select those polymorphic markers that are tightly linked to the Striga resistance/tolerance QTL and that polymorphism should be determined by the differences in allele sizes among the parents after allele scoring.

Conclusions

The study was conducted to screen 36 accessions of maize populations for drought and/or Striga tolerance, using seventeen simple sequence repeat (SSR) markers. For the parent populations, DT-STR-W-C2, TAIS03 and IDW-C3-SYN-F2 showed bands across five of the seven amplification products produced by the striga-linked SSR markers. Therefore, the three parents contain the quantitative traits responsible for *Striga hermonthica* tolerance. Also IDW-C3-SYN-F2, TAIS03, GUMA03-OB and KOBN03-OB showed bands across nine of the ten amplification products produced by the drought-linked SSR markers. Therefore, the four parents contain the quantitative traits responsible for drought tolerance. The parent SISF03-OB showed empty lanes across almost all the gel pictures produced by both the

Striga linked and drought-liked SSR markers. The parent therefore, does not contain the quantitative traits responsible for drought and *Striga hermonthica* tolerance.

For the F_1 hybrids, IWD x TAIS03, IWD x GUMA03, DT x KOBN03, TAIS03 x IWD, TAIS03 x DT, TAIS03 x SISF03, TAIS03 x KOBN03, SISF03 x DT, SISF03 x TAIS03, SISF03 x GUMA03, GUMA03 x IWD, GUMA03 x DT, GUMA03 x SISF03, GUMA03 x KOBN03 and KOBN03 x IWD showed bands in their lanes across all the gel pictures produced by five Striga linked SSR markers. Therefore the fifteen F_1 hybrid populations contain the genomic DNA responsible for *Striga hermonthica* tolerance. The F_1 hybrids, IWD x TAIS03, IWD x GUMA03, IWD x KOBN03, DT x TAIS03, DT x SISF03, DT x KOBN03, TAIS03 x IWD, TAIS03 x DT, TAIS03 x SISF03, SISF03 x TAIS03, SISF03 x GUMA03, SISF03 x KOBN03, GUMA03 x IWD, GUMA03 x DT and GUMA03 x KOBN03 showed bands in their lanes across all the gel pictures produced by the drought-linked SSR markers. Therefore, the fifteen hybrid populations contain the quantitative traits responsible for drought tolerance. The F_1 hybrids, IWD x TAIS03, IWD x GUMA03, DT x KOBN03, TAIS03 x IWD, TAIS03 x DT, TAIS03 x SISF03, SISF03 x TAIS03, SISF03 x GUMA03, GUMA03 x IWD, GUMA03 x DT and GUMA03 x KOBN03 revealed presence of bands across all gel pictures produced by both drought-linked and Striga-linked SSR markers. Therefore, these hybrids contain the quantitative traits responsible for both drought and *Striga hermonthica* tolerance.

Among the seven Striga-linked SSR markers used in this study, five of them (Phi074, Nc005, Phi015, Phi061 and Phi055) are identified to be highly informative, and can significantly contribute to the differentiation of the Striga tolerant and susceptible genotypes. Among the ten drought-linked SSR markers used in the study, nine of them (P-umc1542, P-bnlg1429, P-umc1566, P-umc2189, P-umc2225, P-umc2226, P-bnlg1014, P-bnlg1124 and P-umc1292) are identified to be highly informative, and can significantly contribute to the differentiation of the drought tolerant and susceptible genotypes.

The cultivation of the hybrid populations that revealed presence of bands across all gel pictures produced by both drought-linked and Striga-linked SSR markers (IWD x TAIS03, IWD x GUMA03, DT x KOBN03, TAIS03 x IWD, TAIS03 x DT, TAIS03 x SISF03, SISF03 x TAIS03, SISF03 x GUMA03, GUMA03 x IWD, GUMA03 x DT and GUMA03 x KOBN03) on Striga-infested and/ or drought prone areas will result in increased yield. The five Striga-linked SSR markers that generated polymorphisms (Phi074, Nc005, Phi015, Phi061 and Phi055) are highly informative and can significantly contribute to the differentiation of the Striga tolerant and susceptible genotypes of maize. The nine drought-linked SSR markers that generated polymorphisms (P-umc1542, P-bnlg1429, P-umc1566, P-umc2189, P-umc2225, P-umc2226, P-bnlg1014, P-bnlg1124 and P-umc1292) are highly informative and can significantly contribute to the differentiation of the drought tolerant and susceptible genotypes of maize.

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