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Marker Assisted Foreground Selection for Identification of Striga Resistant Backcross Lines in Sorghum bicolor

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Abstract: Striga is a major constraint to sorghum production causing high yield loss due to increasing infestation. Locally-adapted cultivars with resistant genes/QTLs could be an effective control strategy for Striga. Marker-Assisted Foreground Selection was used to select backcross lines possessing Striga resistance QTLs from N13. Marker polymorphism was conducted for the donor parent N13 and 10 recurrent parents using 10 Simple Sequence Repeat (SSR) markers. Recurrent parents with SSR alleles, polymorphic to the donor parent allele were selected. F1 lines were developed by making a cross between the selected recurrent parent and the donor. The F1 were confirmed for heterozygosity using SSR markers. Selected heterozygote F1s were backcrossed to their recurrent parent to develop backcross populations (BC1F1 and BC2F1). BC1F1 and BC2F1 populations were genotyped using SSR markers flanking the Striga resistant QTLs in N13. Forty two DANYANA-N13 BC2F1 lines (with 4 QTLs in 3 lines, 3 QTLs in 10 lines and other 28 lines having 1 to 2 QTLs) were selected for the presence of N13 QTLs. Forty three SAMSORG39-N13 BC2F1 lines (with 3 QTLs in 2 lines while 41 lines had 1 to 2 QTLs) were also selected for the presence of N13 QTLs. Although, selected lines will be genotyped for the recovery of recurrent parent background and evaluated to identify elite genotypes for possible release as varieties, the successful introgression of Striga resistance QTLs using Marker Assisted Selection suggests that in developing superior sorghum varieties, breeders could make use of molecular marker technologies to speed up breeding programmes.

Keywords: Backcross, Marker Assisted Selection, Striga, Sorghum, Simple Sequence Repeats (SSR).

1. Introduction

Sorghum is grown in the savanna and the semi-arid regions of Nigeria and it is daily used as staple food by millions of people in this region and also in other parts of the nation [1]. Foods produced from sorghum grains possesses high nutritive value and are also high in energy as such, they are recommended for infants, pregnant and lactating mothers, the aged and sick individuals [2]. Thus, the production of sorghum in Nigeria has a significant benefit to Nigerian farmers and also important for national food security. However, the production of sorghum is hampered with lots of biotic and abiotic constraints. A major biotic constraint with high economic importance is Striga hermonthica. This weed which attaches itself and penetrates the root of its host for water and nutrient is known to cause up to a 100% yield loss [3] under highly endemic situation and affects the majority of farmers in the northern part of the country. The issue of Striga is becoming more prevalent mostly in field where wild sorghum is found and where continuous mono-cropping is practiced. It is also getting more serious as a result of erratic rainfall pattern, low soil fertility in regions where farming is on the high side and agricultural input is low thereby affecting resource-poor subsistence farmers [4, 5].

Due to the intrinsic host-parasite interaction, the huge amount of seed produce and the length of seed viability in soil, Striga problem have become more complex over the years [6]. However, there are various measures that have been put in place to combat the scourge of Striga. Despite all efforts, these measures in most cases have not been economically possible or successful [7]. It has thus been accepted that a most durable and accessible technology would be an integrated management strategies with host plant resistance as the focal point. Though Striga resistant sorghum varieties could contribute to the reduction in reproductive activity of Striga and to the number of seeds in the soil, these varieties are not always adapted to the local environment and are also not superior varieties. Thus, an important aspect on the integrated management strategy is the use of resistant, farmer-preferred varieties [8]. To fight the scourge of Striga through integrated strategy of using resistant farmer preferred varieties with no environmental influence on selection, molecular marker based selection technique must be employed.

According to Collard et al., [9], marker assisted or marker based selection (MAS), involves the use of molecular markers to select individual plants possessing genomic regions which influence the expression of certain traits of interest. With MAS, the major goal of crop improvement can be enhanced when dealing with important traits that are difficult to study due to environmental influences [10]. As compared with conventional plant breeding methods, same breeding progress that will be achieved over a long period will be achieved within a short period of time when MAS is employed [11]. Thus, at early stage of a plant, without the loss of identity, traits can be transferred into preferred plant varieties, gene pyramiding can also be achieved and the efficiency of breeding can be increased [12, 13]. Significant headway has been made in the development of molecular markers and in the use of these makers in detecting QTLs [8] and tagging of genes [14] influencing Striga resistance in sorghum. Consequently, those molecular markers that are tightly linked with Striga resistance are thus being used to increase the value of conventional breeding through indirect selection of plants which possess the desirable gene in segregating populations [15].

The transfer of these QTLs/genes into elite sorghum background through marker assisted backcrossing (MABC) will provide a solid foundation to improve Striga resistance in farmers preferred lines. Allard

[16], describes back crossing (BC) as a breeding technique which is used to introduce one or a few genes into an adapted variety. With the use of marker assisted back crossing (MABC) tools, lines which will be used as parents in next generation are selected [17]. This is made possible with the aid of molecular markers that are closely linked or flanking to already detected and validated QTLs.

The objective of this research activity therefore is to (i) develop backcross populations from crosses involving recurrent and donor parent (ii) conduct foreground selection for presence of Striga resistant QTLs using reported SSR markers.

II. Materials and Methods

A. Plant materials for generation of backcrosses

N13 sorghum line with 5 Striga resistance QTLs [8] obtained from ICRISAT, Mali, was used as donor parent, for introgression of resistance QTLs into farmers' sorghum varieties. Farmers' varieties, which included SAMSORG17, SAMSORG40, SAMSORG43, SAMSORG14, SAMSORG39, SAMSORG41, DANYANA, CRS-01 and CRS-02, were obtained from the Institute for Agricultural Research (IAR), Samaru, Nigeria. All these were used at the initial stage for the development of F1s. Due to the polymorphism result; this was then narrowed down to SAMSORG39 and DANYANA as recurrent parents for the transfer of N13 alleles.

B. Development of Backcross population

All the accessed genotypes were selfed to ensure the use of pure lines in the crossing program. The selfed seeds were planted out on the crossing block at IAR, Samaru and N13 was used as pollen parent on all the farmers' varieties to produce F1 seeds. The F1 seeds were sown under irrigated environment at the National Center for Genetic Resources and Biotechnology (NACGRAB). DNA was extracted from individual F1 plants at the molecular biology laboratory of NACGRAB, Nigeria and was shipped to ICRISAT, India. The DNA samples were screened using SSR markers for F1 hybridity confirmation. Selected F1 were then used as pollen parents on their respective recurrent parent for development of backcross populations (BC1F1 and BC2F1).

C. Genotyping of backcross (BC1F1 and BC2F1) population

At every backcross stage, DNA was extracted from the population using CTAB protocol and samples were shipped to ICRISAT, India for genotyping. Twenty five SSR markers for N13 QTLs were selected and used for genotyping and only those markers which revealed polymorphism between the donor parent (N13) and the respective recurrent parents were used in selecting lines with the N13 Striga resistant QTLs.

Polymerase Chain Reaction (PCR) for amplification of regions of interest was done on the Gene Amp PCR systems 9600 (PE-Applied Biosystems). After PCR reaction process, a few of the amplicons from each SSR marker were randomly selected to confirm proper amplification and product concentration. After confirming the primers amplification on agarose gel electrophoresis, primers with good amplification profile were selected for subsequent PCR analysis on the entire population (BC1F1 and BC2F1). Samples with good amplification were subjected to capillary electrophoresis to determine their sizes via fragment analysis by using the ABI 3730xl DNA analyzer which is a fluorescent fragment detection system. Sizing of the PCR fragments and allele scoring was carried out by using the Gene-Mapper version 4.0 software.

Results

Confirmation of F1 generations

Eight different crosses were made using N13 as donor with 8 farmers preferred

varieties (SAMSORG17, SAMSORG40, SAMSORG43, SAMSORG14, SAMSORG39, SAMSORG41, CRS-01, CRS-02 and DANYANA) obtained from IAR, Zaria. For each cross, 10 F1s lines were produced and were genotyped to confirm heterozygosity with 10 SSRs marker of which 6 were polymorphic between the parental lines. Out of the 80 F1 lines screened, 6 were selected and only two were advanced to the next generation (Table 1) to reduce the amount of crosses and for easy handling and tracking.

Foreground selection of BC1F1 progenies

Foreground selection was carried out to identify the BC1F1 lines having the Striga QTLs from N13 using polymorphic markers that were tightly linked to the respective QTL.

Out of the 49 BC1F1 plants were genotyped for the N13 Striga resistant QTLs, only 9 were found to be having one to two QTLs introgressed. These 9 were heterozygous for the introgressed QTLs on chromosomes 1, 2 and 5. Four lines had only one QTL introgressed. The other five were heterozygous for two QTLs each from chromosomes 2 and 5. Out of the 9 selected lines, only 3 were advanced to BC2F1 (Table 2).

Foreground selection of BC2F1 progenies

A total of 143 lines were genotyped of which 71 were for the cross between N13 and SAMSORG39 while 73 were for the cross between N13 and DANYANA. A total of 43 BC2F1 plants were selected with about 1 to 3 QTLs being introgressed for the cross between N13 and SAMSORG39. While for the cross between N13 and DANYANA, 42 BC2F1 plants were selected with about 1 to 4 QTLs being introgressed. Result of marker assisted foreground selection is summarized in Table 2.

Table 1: List of F1 advanced to BC1F1

Crosses	F1 Genotypec	Hybrid	Hybrids advanced t BC1F1
Samsorg39 x N13	10	4	1
Danyana x N13	10	2	1

TABLE 2: Summary of Marker assisted foreground selection

Crosses	F1 hybrid advanced t BC1F1	BC1F1 plants genotyped	BC1F1 advanced to BC2F1	BC2F1 selected
SAMSORG39 : N13	1	35	1	43
DANYANA : N13	1	14	2	42

III Discussion

Backcross breeding method is basically used to transfer favorable alleles from a donor genotype, which has mostly poor agronomic properties, into a recipient elite genotype [16].

There is the possibility of accelerating the process of QTLs or genes introgression and also recovering of the recurrent parent genome when selection is made via the use of markers flanking or linked to the QTLs and also uniformly spaced markers from

other chromosomes of the recurrent parent [9].

In this research work foreground selection was made, and such kind of selection is made only for the marker alleles of the donor parent mainly at the target locus so as to ensure that the target locus is in heterozygous state till the last backcross is done. At the end of the backcross program, the selected plants are then selfed and those with homozygous donor parent alleles for the selected markers are then harvested for further evaluation and release.

In N13, the target regions used for foreground selection were basically 5 stable QTLs for the area under Striga number progress curve (AUSNPC) which was common to 2 sets of recombinant inbred line populations derived from N13 [8]. These 5 QTLs were located on linkage group SBI-01, SBI-02, SBI-05(two QTLs) and SBI-06. The selected QTLs explain approximately 11- 45% of the phenotypic variation. Thus SSRs markers flanking these QTLs were used for foreground selection at each backcross generation. The use of tightly linked flanking markers ensures an effective foreground selection. As such, there is a high selection reliability when flanking markers are used. Also, there is the reduction of linkage drag when markers that flank a target gene is used [18].

Using the marker assisted backcrossing foreground selection method, we were able to introgress genomic regions from the popular Striga resistant donor parent N13 into the genetic backgrounds of elite farmers' varieties SAMSORG39 and DANYANA. According to Morris et al., [19], marker assisted selection has the possibility of tremendously reducing the time needed for selecting desirable genotypes possessing traits of interest.

At BC1F1, only nine plants were selected with the N13 QTLs alleles of which two were from SAMSORG39 with just one QTL introgressed (SBI-01-1(QTL1)) and the other 7 were from the recurrent parent DANYANA with 2 QTLs on linkage group SBI-02-1 and SBI-05-2 introgressed into 5 of the lines. The selection of very few lines with only one to two QTLs introgressed could be attributed to the extremely low DNA quality due to degradation in transit as a result of long distance between Nigeria and India. This resulted in some of the samples being completely lost and most of the selected SSRs markers not being able to amplify the samples. Although for a PCR analysis with SSR markers, the DNA requirement in terms of quantity is small and in the terms of quality is medium [17] but in this case it was not applicable. With good quality DNA of high absorbance ratio from 1.8 to 2.0%, [20] genotyped and reported 12 BC1F1 lines with one to three Striga resistant QTLs introgressed.

Taking a look at the number of lines with the introgressed Striga resistant QTLs, for the cross between N13 and DANYANA, only 14 BC1F1 plants were genotyped out of which 7 were selected with 5 having 2 QTLs introgressed and two of the selected lines having one QTL introgressed. This result is somewhat really encouraging because if the population size would have been increased to the required, then there is the possibility that more QTLs would have been captured. This is almost similar to what Mehtre, [21] got in his research. Out of thirty BC1Fl plants in a backcross population, he reported about 14 heterozygous plants detected by 11 SSR primer pairs that flanked four shoot fly QTLs. Also in another BC1F1 backcross population, out of thirty plants screened at seedling stage with 11 SSR marker loci linked with shoot fly resistance traits, Nineteen BC1F1 plants that were heterozygous for one or more targeted QTLs were identified [21].

A number of authors have reported varying population sizes ranging from hundreds to thousands that are ideal for a 99%

probability of introgressing about 1 to 5 QTLs at any backcross generation [22, 23, 24]. Which was actually not visible in the case of the cross between N13 and DANYANA due to less seed production and also poor germination. Out the 159 BC1F1 lines genotyped with 2 markers used in the monitoring of each QTL in the research of Niyibigira et al.,[20], 12 lines were selected as having the Striga resistant QTLs introgressed, one of the selected 12 was seen to be heterozygous for 3 QTLs, 8 were heterozygous for 2 QTLs while the other 3 were heterozygous for one QTL. In the case of the cross between N13 and DANYANA with only 14 BC1F1 lines genotyped, about 4 or more markers were used to monitor each of the QTLs. Although most of the markers did not amplify the DNA samples due to the level of degradation. However, for the QTL on SBI-02-1, 4 of the markers used had a successful amplification which also aided the selection of the 5 lines with 2 introgressed QTLs. Looking at the cross between N13 and SAMSORG39, out of 35 BC1F1 lines that were genotyped, only 1 was advanced with just one QTL from SBI-01. According to Collard et al., [9], a single QTL selected for, through marker assisted selection is very useful in plant breeding as long as such QTL is stable over varying environments and accounts for the largest quota of phenotypic variance for the trait.

At BC2F1, 42 lines of DAYANA had 1 to 4 N13 Striga resistant QTLs introgressed with 4 QTLs being introgressed into 3 lines, 3 QTLs being introgressed into 10 lines and others with 1 to 2 QTLs. While 43 lines of SAMSORG39 had 1 to 3 QTLs introgressed at BC2F1 also with 3 QTLs being introgressed into 2 lines and others with 1 to 2 QTLs being introgressed. Similar result has been reported by Mehtre [21] in his research in which he genotyped 224 BC2Fl plants obtained from five backcross populations with 10 SSR marker loci associated to QTLs influencing shoot fly resistance traits in four linkage groups. Out of the 224, about 100 plants were heterozygous for one or more targeted QTL. In a computer simulation in which tomato was used as a model, Tanksley et al [25], explained that it is very possible to recover the genome of the recurrent parent in two generations for every best plant selected out of the total of 30 plants per generation. Thus, if a background selection is made, there is the possibility that out of the selected BC2F1 plants, there are those with high percentage of the recurrent parent genome.

IV. Conclusion

The results of this study has proven the possibility of using a popular donor germplasm to improve locally adapted lines via marker-assisted backcrossing.

One up to four Striga resistances QTL were introgressed successfully in the SAMSORG39 and DANYANA sorghum variety grown in Nigeria. Nonetheless, it is very important to conduct a background selection for the recovery of the recurrent parent genome. Also, more advanced backcross, if possible up to BC3F1, is needed in order to stabilize the Striga resistance QTL. The introgressed lines are expected to be useful for improving sorghum productivity in the Striga prone areas of Nigeria if the introgressed resistance QTLs are actually effective against S. hermonthica populations in those regions.

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