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Efficiency of molecular markers to select for *Striga gesnerioides* resistance in cowpea [*Vigna unguiculata* (L.)Walp]

L. O. OMOIGUI[1], A.Y. KAMARA[2], M. P. TIMKO[3], H. AJEIGBE[4] D.F. AYENI[1] & B.N. MOTAGI[4]*

[1]Department of Plant Breeding and Seed Science,
University of Agriculture, Makurdi. PMB 2373, Makurdi, Nigeria
[2]International Institute of Tropical Agriculture (IITA), Kano Station, Nigeria
[3]Department of Biology, University of Virgina, USA.
[4]International Crops Research Institute for Semi-Arid Tropics (ICRISAT), Kano, Nigeria
*Corresponding author (luckyomoigui@gmail.com)

Abstract - *Striga gesnerioides* (Willd) Vatke is a major biological constraint to cowpea productivity in the dry savannas of sub-Saharan Africa. Over the last two decades, the use of molecular markers in crop improvement has gained prominence owing to its ability to shorten the breeding cycle. The available molecular marker techniques are being improved upon and continuously tested for higher precision, shorter duration of application and better cost effectiveness. In the present study, a total of four molecular markers developed for selecting *Striga resistant* cowpea were used to genotype F_2 population derived from a cross between Borno Brown and IT97K-499-35 to identify markers more closely linked to *S. gesnerioides* resistance. SSR1 and 2 were found to be tightly linked to *Striga gesnerioides* with a genetic distance of 1 and 2cM.The selection efficiency of SSR-1 and SSR-2 were 99 and 98 % and was better than that of C42-B 85.5%.

Key words: *Striga gesnerioides*, resistance, molecular markers, cowpea

Introduction

The root parasitic weed, *Striga gesnerioides* causes extensive damage in the Sudano-sahelian belt of West and Central Africa (Parker, 2009; Matsura *et al.*, 2008) with yield loss due to this parasite could be as high as 83% and total crop loss of susceptible cultivars have been reported (Emechebe *et al.* 1991; Muleba *et al.* 1997; Alonge *et al.*, 2005). The major irreversible damage is done before the parasite emerges from the soil thereby making weeding an ineffective option in its control. It also produces thousands of seeds, which remain viable in the soil up to 20 years (Ouedraogo *et al.*, 2012).

For decades, breeding efforts have been put in place to develop elite cultivars resistant to the *S. gesnerioides* races per region in Africa. However, the predominant approach has been conventional. Although this approach has been extensively utilised, there are limitations to its applications which include the time frame to develop improved lines and escapes due to environmental effects. Due to the differential virulence of *S. gesnerioides* races (Singh 2002; Parker and Polniaszek (1990)) and with the information of the single dominance nature of the gene controlling *Striga* resistance (Atokple *et al.*, 1993), it is essential to employ new tools that could fast track breeding processes and enable selection with high precision.

Molecular markers have been reported to be of high utility in plant breeding especially marker assisted selection (MAS) and genetic engineering; its use however, needs to be maximized (Huang *et al.*2002).The primary aim of plant breeding is the selection of specific plants with desirable traits. Selection therefore requires evaluating a breeding population for one or more traits in order to assemble more desirable combinations of genes in new varieties (Collard and Mackhill, 2008). The selection efficiency is therefore required in deciding which marker to use for selection to ensure that no desirable trait is lost.

Marker assisted selection has been successfully employed in breeding for *Striga* resistance. However, more lines need to be improved for *Striga* resistance owing to the different adaptability and dynamic end-users preference. To enhance breeding for *Striga* resistance using MAS, several DNA based markers have been identified to be tightly linked to varied *Striga* resistance genes (Ouédraogo *et al.* 2001 and 2002; Omoigui *et al.*, 2009; Boukar *et al.*, 2004; Omoigui *et al.*, 2012).Some authors have reported different marker selection efficiency across different population or genotype collections (Omoigui *et al.*, 2015). Although the *Striga* races prsevalent in Ghana is yet to be established, Asare *et al.* (2013) reported selection efficiency of 92.6% and 85.7% for SSR1 and C42B respectively for a recombinant inbred line of cowpea in Ghana. Prior to this report, there is no reference to the selection efficiency of SSR2 marker which is also tightly linked to *Rsg* 3. The selection efficiency and precision of this and other *Striga* resistance linked markers require being established.

Materials and Methods

Two cowpea cultivars of parallel reaction to *S. gesnerioides* were selected for this study. This includes a *Striga* resistant genotype IT99K-499-35 and a *Striga* susceptible cultivar, Borno Brown. These cultivars were crossed using the susceptible genotype as the maternal background. The resultant F_1 lines were selfed to obtain the F_2 population. Experimental pots were prepared with a sharp sand-topsoil ratio of 1:1 and infested with *Striga* seeds. The *Striga* seeds were conditioned for 7 days prior to sowing of the cowpea seeds. A hundred F_2 seeds were subsequently planted out in *Striga* infested pots alongside 8 F_1 lines and the both parent genotypes in a screenhouse *Striga*-screening experiment. Two seeds were sown per pot and thinned to single plant stand per pot at 7 days after planting.Pot were maintained and watered to field capacity. At 14 DAP, DNA samples were collected from individual plants on FTA plant Saver card and processed for PCR following the methods of Omoigui *et al.*, (2011).

Two SSR Markers (SSR1 and SSR2) and a SCAR marker (C42B) were used to amplify regions flanking the *Striga* resistance gene in cowpea. These markers were used independently and in a multiplex. PCR reaction mixture contains 16ul Accupower PCR premix in Molecular Biology Grade water, 1ul of working solution mixture of Forward and Reverse primers. The PCR products were resolved on a 2% agarose gel. The bands of each F_2 and F_1 line were compared with those of the parents for scoring.

Thereafter, the genetic similarity of each primer, and the multiplex to the phenotypic score was calculated using the Nei's genetic similarity index (Nei and Li, 1979).Genetic

similarity (GS) was estimated for all primers using the equation:

$$S = \frac{2N_{xy}}{N_x + N_y}$$

Where N_{xy} is the sharing bands of two individuals, x and y; N_x and N_y represent the individual bands of x and y, respectively. The mean S in a breed reflects the similarity or different degree of DNA fingerprinting.

Results and Discussion

The screen house pot experiment showed the F_2 segregation ratio of 75R:25S perfectly fitting into the expected ratio of 3:1 for a single dominance gene-controlled trait. The DNA-based markers gave segregating ratios close to that of the phenotypic scores. SSR1 gave the ratio of 74R:26S while all others gave a ratio of 73R:27S highlighting SSR1 as more efficient in identifying *Striga* resistant genotypes.

A sharp contrast was observed in the phenotypic expressions of the *Striga* resistant and *Striga* susceptible lines. The susceptible cowpea lines experienced severe attacks owing to successful *Striga* parasitism attached to the roots. As early as 23 DAP, *Striga* seedlings emergedo n the surface of the soil in pots planted with susceptible cowpea lines. As anticipated, varied symptoms due to*Striga*-parasitic stress were expressed by the susceptible lines. These include leaf necrosis, stunted growth, chlorosis, stunted growth, leaf necrosis, chlorosis, and early senescence. Contrary to what was observed in *Striga* resistant lines, there was little to none pod formation. The resistant lines exhibited normal growth and healthy root developments, completely free of *Striga* attachment/emergence.

The individual marker analysis revealed the different discriminating power of the markers used even though all the markers co-segregated with the *Striga* resistance gene Rsg 3 (Table 2). A similar finding was observed by Omoigui *et al.* (2009) and Asare *et al.* (2013). The SSR1 marker analysis characterised the resistant lines with a single 176bp band which is absent in the susceptible lines. SSR2 single resistant band pattern is similar with SSR1 but at ~480bp which is also absent in the susceptible lines. C42B amplified a sharp ~280bp band in the resistant lines with faint bands of the same size and an additional ~500bp in the susceptible lines.

In the multiplex, SSR1 marker region were also amplified at an unusual and higher annealing temperature of 60^0C showing clear bands. SSR2 was not amplified in the multiplex contrary to the amplification of SSR1 region in the multiplex. This is suggestively due to common amplification region of both SSR1 and SSR2 markers of the higher annealing temperature. It was observed that the characteristic susceptible bands of C42B wer e not also amplified in the mutiplex.

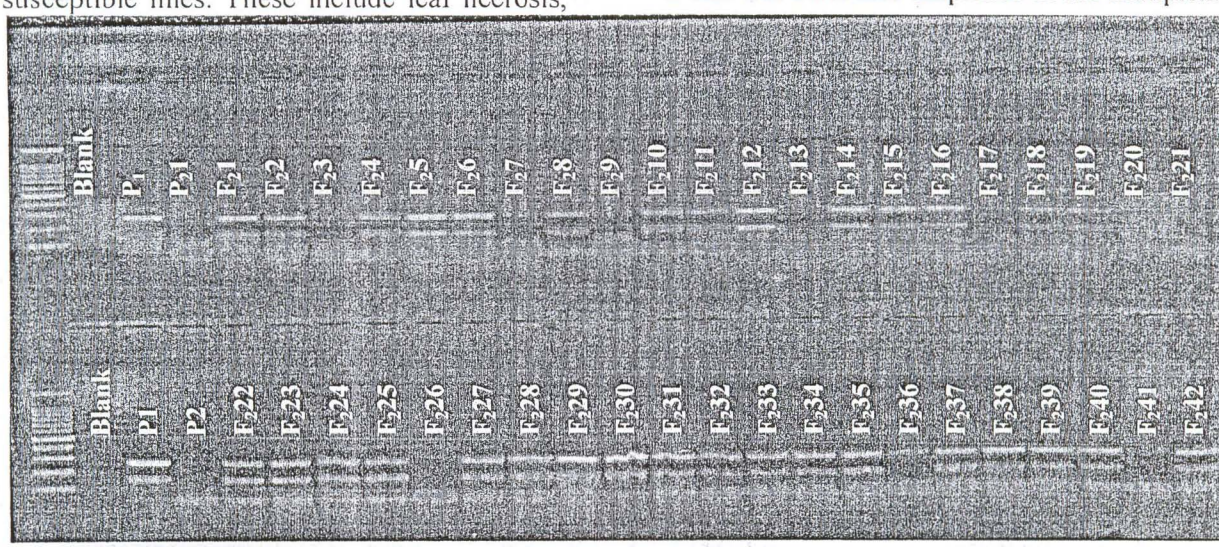

Figure 1: Gel image showing DNA bands from Multiplex PCR amplification products of SSR-1, SSR2 and C42B for F2 populations of the cross Borno Brown x IT97K-499-35. The PCR products were resolved using 2 % Agarose gel stained with ethidium bromide. The presence of a 176 bp from SSR1 and ~ 260bp from C42B indicates the presence of the resistance gene marker while the absence of these bands indicates susceptibility.

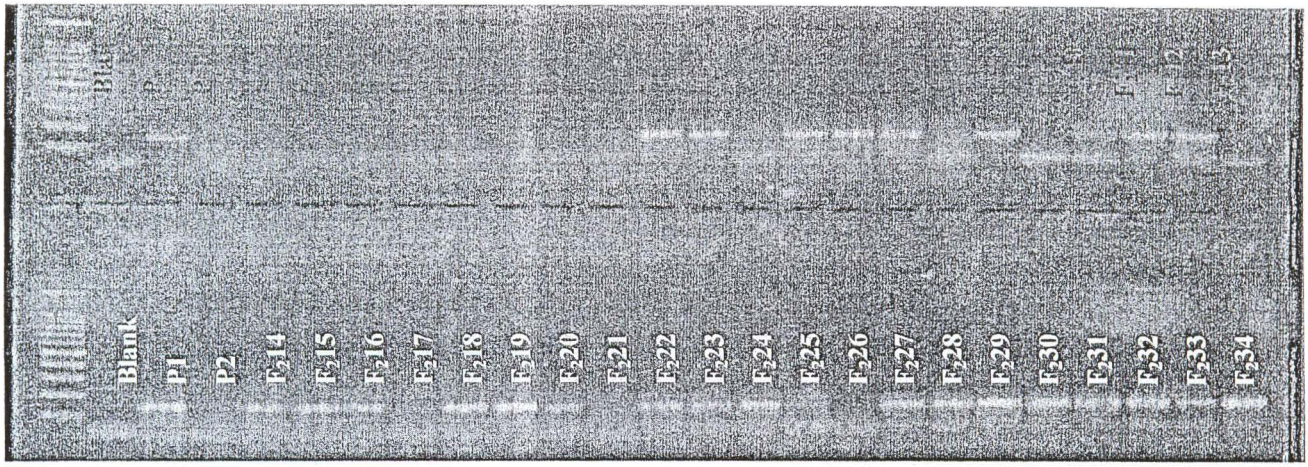

Figure 1: Gel image showing DNA bands from amplification products of SSR-1 for F_2 populations of the cross Borno Brown x IT97K-499-35. The PCR products were resolved using 2 % Agarose gel stained with ethidium bromide. The presence of a 176 bp indicates the presence of the resistance gene marker while the absence indicates susceptibility.

Conclusion

In cowpea breeding targeting selection for *Striga gesnerioides* resistance, application of tightly linked marker markers will be efficient in identifying resistant and susceptible genotypes. Based on the findings of this study, SSR1 marker was found to be tightly linked to the *Striga* resistance gene and is recommended for characterizing segregating populations for *Striga* resistance.

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