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Application of marker-assisted backcrossing to improve cowpea (*Vignaunguiculata* L. Walp) for drought tolerance

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Molecular-assisted backcrossing (MABC) was used to introgress drought tolerance, Striga and root-knot nematode resistance QTLs into a farmer-preferred widely grown cowpea landrace adapted for intercropping in Burkina Faso. Two backcross populations were developed using two drought tolerant donor lines IT93K-503-1 (nematode resistant) and IT97K-499-35 (Striga resistant) and the drought sensitive landrace Moussa Local as the recurrent parent. A set of 184 genomewide EST-derived SNP markers spanning an average of 2-cM intervals and flanking known trait positions was employed for genotyping the backcross progenies using the cowpea KASP genotyping platform. BC_1F_1 individual plants that were heterozygous for SNPs associated with drought tolerance, Striga and/or nematode resistance (foreground SNPs) and carried as many recurrent-parent alleles as possible at other SNP loci (background SNPs) were selected for the next backcross cycle. This process was repeated to produce BC_3F_1 families of each donor population. The six best families from the two donors based on marker aided selection and preliminary yield performance under well-watered and water-restricted field trials and Striga resistance screening were selfed to increase seed (BC_3F_2) for further yield tests. This study demonstrated the high efficiency of using SNP markers in foreground and background marker selection in a MABC scheme to improve a widely grown cowpea variety by adding drought tolerance and biotic stress resistance traits.

Key words: Molecular-assisted backcrossing (MABC), drought tolerance, Striga, root-knot nematode, QTLs, EST-derived SNP markers, cowpea.

INTRODUCTION

Modern plant breeding based on the fundamental principles of inheritance has become an important component of agricultural science and technology. Conventional breeding methodologies have proved to be

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broadly successful in development of plant cultivars and germplasm. However, conventional breeding is still dependent to a considerable extent on subjective evaluation and empirical selection using often highly variable and time-consuming phenotyping assays. Recent technological advances in molecular marker-assisted breeding (MAB) have brought new opportunities and prospects for optimizing the accuracy and efficiency of breeding systems through indirect selection.

In backcross breeding, the main objective is the introgression of one or more genes from a donor into the background of an elite variety and to recover the recurrent parent genome as rapidly as possible (Semagn et al., 2006). In the past, this was usually achieved by conventional backcross methods, but in many ways the same objective is being pursued through transgenic breeding, bypassing recombination altogether but introducing a value-added trait. Recurrent backcrossing is thus a traditional breeding method commonly employed to transfer alleles at one or more loci from a donor to an elite variety (Allard, 1960). This method requires many generations over several years before recovering the background of the elite cultivar. According to Semagn et al. (2006), the recovery of 99.2% of the elite cultivar could take at least six generations when there is no deviation. In cases involving a deviation, Young and Tanksley (1989) for example found an introgressed segment as large as 4 centiMorgans (cM) in tomato cultivars developed after 20 backcrosses, and one cultivar developed after 11 backcrosses still contained the entire chromosome arm carrying the gene from the donor parent.

During the past two decades, development of genomic resources has facilitated gene transfer using molecular markers. The use of SNP markers in MAB programs has progressed rapidly together with development of technologies and platforms for the discovery and high-throughput (HTP) screening of SNPs in many crops (Mammadov et al., 2012). The MAB technology allows transfer of target genome regions coupled with extensive genetic mapping and QTL discovery for the development of molecular markers for use in marker-assisted back-crossing (MABC) and marker-assisted selection (MAS) (Semagn et al., 2006). Molecular markers are tools that can be used as chromosome landmarks to facilitate the introgression of genes associated with economically important traits. It is an approach that has been developed to avoid problems connected with conventional plant breeding by shifting the selection criteria from selection of phenotypes towards selection of genes that control traits of interest, either directly or indirectly. Unlike conventional backcross breeding, MAB can be viewed as a four-step selection process to quickly recover the recurrent parent genotype (Frisch et al., 1999). This includes: (1) selecting individuals carrying the targeted alleles, (2) selecting individuals homozygous for the recurrent parent genotype at loci flanking the target locus, (3) selecting individuals homozygous for recurrent parent genotype at remaining loci on the same chromosome comprising the targeted allele, and (4) selecting individuals that are homozygous for the recurrent parent genotype at most genotyped loci across the whole genome among those that remain.

The use of MAS for introgression of major quantitatively inherited trait loci for stress tolerance is increasingly being applied in crop improvement. However, the use of such technology has been slow in pulse breeding programmes (Kumar et al., 2011). Effort are made for the use of high-throughput genotyping platforms in pulses like chickpea, common beans and cowpea (Muchero et al., 2008; Muchero et al., 2009a; Muchero et al., 2009b).

In this study, the MABC method has been used to introgress drought-related QTLs (Muchero et al., 2009a; Muchero et al., 2009b), striga and nematode resistance genes from two IITA cowpea varieties into one local farmer-preferred variety.

MATERIALS AND METHODS

Leaf sampling and DNA extraction

A sampling kit (LGC Genomics, Teddington, UK) was used which is designed to facilitate both cutting of leaf discs and their transport and concomitant desiccation, for eventual DNA isolation. For each sample, 4 leaf discs of 6-mm-diameter were cut using the Harris Uni-Core leaf-cutting tool supported by the Harris self-healing cutting mat and placed into one well of a 96-well storage plate, whereupon the plate was sealed with a perforated (gas-permeable) heat seal by applying a medium hot household iron to the top of the seal for about 2 seconds. The sealed plate was then placed in a heavy-duty sealed bag in the presence of a desiccant to dehydrate and preserve the leaf tissue during transit at ambient temperature. Decontamination of the leaf-cutting tool between sampling different plants was achieved using 70% ethanol or 2% sodium hypochlorite. The leave samples were sent to LGC Genomics for DNA extraction and SNP genotyping using the KASP system.

Selection of markers

A set of 184 genome wide SNP markers spanning an average of 2-cM intervals was selected using the BreedIt© SNP Selector tool (http://breedit.org/) developed at University of California – Riverside (UCR). The SNP Selector provides an interface to generate customized lists of SNPs based on cM distance between markers genome-wide, and markers flanking known trait positions. All SNP markers were developed from EST of drought-stressed tissues, so there is a chance the markers are associated with drought tolerance candidate genes (Muchero et al., 2009b).

Plant materials and QTL introgression procedures

Two drought-tolerant lines from IITA (IT93K-503-1 and IT97K-499-35) that were found to be drought tolerant in Burkina Faso (Sawadogo, 2009) based on their yielding and staying green abilities under water stress conditions and in which drought-tolerant QTL have been discovered and mapped (Muchero et al., 2008; Muchero et al., 2009a; Muchero et al., 2009b; Muchero et al., 2010; Muchero et al., 2011) were used as donors of positive alleles of drought tolerance QTLs, and Striga and root-knot nematode resistance genes. 'Moussa Local', a local farmer-preferred purified variety from Burkina Faso, was used as the recurrent parent. The donor alleles for yield and nematode resistance were selected based on results from UCR/INERA collaborative on-going projects. The donor alleles for Striga resistance were selected based on synteny with the Striga resistance locus reported in Ouedraogo et al. (2002).

For the MABC scheme, IT93K-503-1 and IT97K-499-35 were crossed to Moussa Local to obtain F_1s. The F_1s were backcrossed to Moussa Local to obtain 95 BC_1F_1 seeds for each recurrent-donor combination. The BC_1F_1 seeds were planted in boxes in a greenhouse. Two weeks after planting leaf samples were collected from each plant and sent to LGC Genomics for genotyping with the 184 SNPs (supplemental Appendix 1). This allowed selection in each population of the BC_1F_1 individual plants that were heterozygous for SNPs associated with drought tolerance, Striga and/or nematode resistance (foreground SNPs) and carried as many recurrent-parent alleles as possible at other SNP loci (background SNPs) (supplemental Appendix 4).

The selected BC_1F_1 individual plants were backcrossed with Moussa Local to obtain 95 BC_2F_1 individuals that were SNP-genotyped. In the BC_2F_1 generation, the individual plants that were heterozygous for foreground SNPs and carried as many recurrent-parent alleles as possible at background SNPs were identified. Alleles A and B are designated for Moussa local and IT93K-503-1 or IT97K-499-35, respectively (supplemental, Appendix 2 and 3).

In the next cycle, each of the selected BC_2F_1 individual plants was backcrossed to Moussa Local to create BC_3F_1 lines. Four BC_3F_1 individuals from the cross Moussa Local/IT97K-499-35 and three BC_3F_1 individuals from the cross Moussa Local/IT93K-503-1 were selfed to obtain about forty BC_3F_2 seeds per line. Seed from BC_3F_2 were used for morphological characterization of the families and yield performance estimation. Ten entries (6 MABC lines and 4 controls) were planted using a randomized complete block design (RCBD) with two replications in two water regimes-water-stressed (WS) and well-watered (WW). The parents used in the introgression process (IT97K-499-35, IT93K-503-1, and Moussa Local) and one known drought tolerant variety (Gorom Local) were used as checks. The trial was conducted during the off-season in 2014 from April to June under a drip-irrigation system and striga naturally infested field at the Kamboinsé Research Station, near Ouagadougou, Burkina Faso.

RESULTS

QTLs introgression

Genotyping of the BC_1F_1 identified the plant named M503_BC1F1_31 carrying the donor IT93K-503-1 alleles for yield under drought, Striga and nematode resistance, and about 67% of recurrent parent Moussa Local alleles at background markers. In the cross from Moussa and IT97K499-35, the plant named M499_BC1F1_04 carried the donor IT97K-499-35 alleles for yield under drought and Striga resistance, and about 70% of Moussa Local alleles at background markers. In addition, some other BC_1F_1plants (M499_BC1F1_49, M499_BC1F1_48, M499_BC1F1_44, M503_BC1F1_54 and M503_BC1F1_92) carrying donor positive alleles but less Moussa Local background than M503_BC1F1_31 and M499_BC1F1_04 were also selected for backcrossing to Moussa Local. In total, 190 individuals were obtained from the BC_2 backcrosses. Genotyping of these BC_2F_1 plants identified 10 individuals carrying different combinations of donor IT93K-503-1 positive alleles and 80 – 97% of Moussa Local background. Three selected plants with highest Moussa Local background (M503_BC2F1_54P15, M503_BC2F1_54P8, and M503_BC1F2_92P27) were backcrossed to Moussa Local to generate the M503_BC3F1 families. Likewise, 21 plants were selected in the BC_2F_1 population; they carried different combinations of donor positive alleles for yield and Striga resistance and 69 – 93% of recurrent Moussa Local background. Five selected plants with highest Moussa Local background (M499_BC2F1_48P90, M499_BC2F1_44P19, M499_BC2F1_48P93, M499_BC2F1_48P85, and M499_BC2F1_4P67) were backcrossed to Moussa Local to generate the BC_3F_1 families. A total of six families derived from the two donors were retained and selfed (fiveM499_BC3F2s and oneM503_BC3F2s) to increase seed for further studies.

Morphological characterization of the MABC selected lines

Seed from BC_3F_2 were not enough to undertake a multi-location trial, so they were used for morphological characterization of the families in a single site yield trial. Table 1 shows the morphological characteristics of the selected families and the recurrent parent. Figure 1 also shows the plant type or growth habit of the lines and their dry pods form and color in comparison to Moussa Local.

Yield performance of the MABC selected lines

The yields of the ten lines per water regime are represented in Figure 2. The yields ranged between 287.2 and 1184.70 Kg ha^{-1} in the water-stressed environment, while in the well-watered environment the yields were higher, ranging from 272.3 to 1771.0 Kg ha^{-1}. Three BC3F2 families (M499_BC3F3_48P85, M499_BC3F3_4P67, and M499_BC3F3_48P90) yielded better than all parents and the local control Gorom Local. All BC3F2 families appeared to perform better than the recurrent parent Moussa Local under limited water conditions.

DISCUSSION

This study involving MABC methodology using SNPs breaks new ground for cowpea breeding, particularly in selection for drought tolerance. The methodology enabled rapid recovery of the recurrent-parent (Moussa Local) background (up to 97%) with only two backcross cycles (BC_2) by using ninety-five individuals for each set of backcrosses in the BC_1 generation. This expedient

Table 1. Morphological characteristics of MABC selected lines and their recurrent parent

Genotype	Flower Color	Green Pod color	Dry pod color	Plant growth habit	Striga presence
Moussa Local (RP)	**White**	**Purple**	**Purple**	**Spreading**	**1**
M499_BC3F3_44P19	White	Purple	Purple	Spreading	0
M499_BC3F3_4P67	White	Purple	Purple	Spreading	0
M499_BC3F3_48P90	White	Purple	Purple	Spreading	0
M499_BC3F3_48P85	White	Purple	Purple	Spreading	0
M503_BC3F3_92P27	White	Purple	Purple	Semi-erect	0
M499_BC3F3_48P93	White	Purple	Purple	Semi-erect	1

RP: recurrent parent.

Figure 1. A-Plant growth habit of selected MABC lines field-grown during the off-season with drip-irrigation; B- Dry pod shape and color of selected MABC lines compared to Moussa Local (recurrent parent).

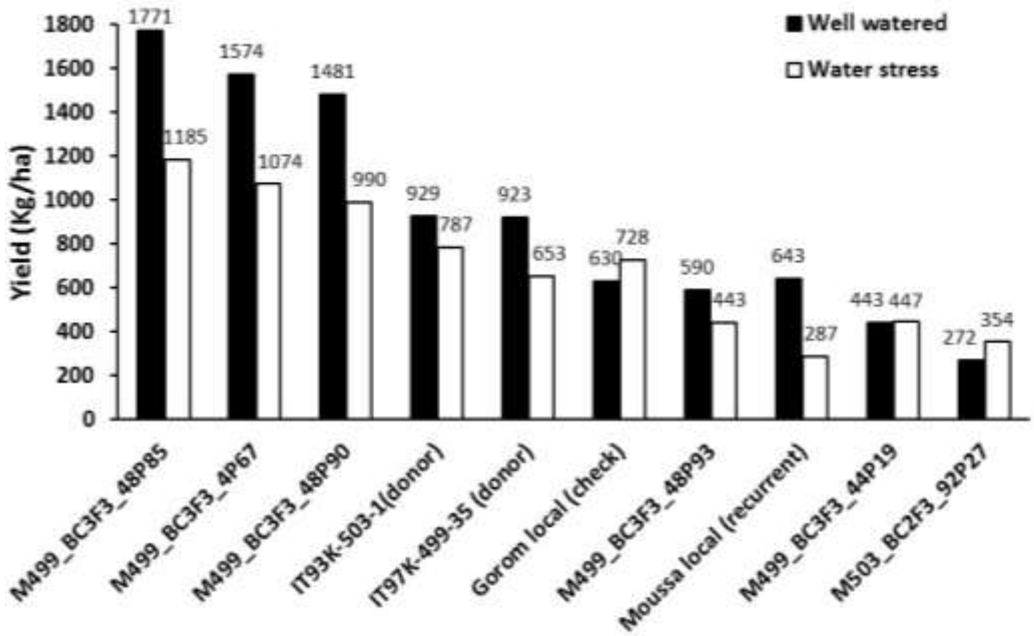

Figure 2. Yield performance of selected BC_3F_2 families, their parents and a local control under well-watered and limited-water conditions. Values are the yield mean (kg/ha) of two replications.

recovery of the recurrent parent background allowed early selection and helped to minimize population sizes at each generation, therefore reducing the required time and work load. The levels of recovery of the recurrent parent background confirmed the findings reported by Jiang (2013) that revealed a percentage of recovery of 98% at BC3 with a number of 100 individuals selected at BC_2.

In the present study, several donor loci (yield under drought, stay-green, Striga resistance and root-knot nematode resistance) were introgressed simultaneously. This decreases the chance to identify a line carrying all donor alleles and high Moussa background and therefore, limits the number of offspring to be selected in the subsequent generations. Sebolt et al. (2000) also reported that the rate of success decreases when large numbers of QTLs are targeted for introgression; by using MABC for two QTLs for seed protein content in soybean introgression, they eventually found that only one QTL was confirmed in BC3F4:5. Compared to MABC, conventional backcross breeding, however, requires a much larger backcross population of 500 or more plants to be produced to ensure that there are sufficient plants for background selection after the foreground and recombinant selection have been performed. During this process, unless breeders screen the material to identify those that are carrying the gene of interest, they may need to conduct 'blind crossing' to the recurrent parent. In such conditions conventional backcrossing can be extremely time-consuming and inefficient. By using a combination trait-flanking markers and evenly distributed markers across the recurrent parent genome, effective introgression can be done to avoid linkage drag and the use of large numbers of individuals.

In Burkina Faso, most of the cowpea landraces have a prostrate growth habit and are susceptible to Striga and are often grown with cereal crops like sorghum and millet. The prostrate growth habit allows soil conservation and maintenance of soil moisture by the cover of the vines. These morphological characteristics in the selected advanced BC lines are in accordance with the expectations of recovering the recurrent parent background (Figure 1). Moussa Local, a farmer-preferred landrace has some purple pods which remain purple even for dry pods. The six selected lines had the purple green and dry pod color character confirming that they recovered this character from the recurrent parent. The prostrate (spreading) growth habit of Moussa Local was also found in the selected MABC lines. These results, therefore, confirmed the molecular results that showed a high level of recovery of the Moussa Local recurrent parent plant type. The same trend was observed for Striga resistance. Only one line (M499_BC3F3_48P93) that had no Striga donor allele had Striga emergence in the well-watered environment. The Striga-resistant checks did not emerge Striga confirming their resistance while Moussa local had emerged Striga confirming its susceptibility to Striga. This result also confirms that the lines selected based on the presence of the Striga gene through the MABC introgression are effective in controlling Striga.

Yield is by far the most important criterion for varietal selection by African farmers (Tignegre, 2010; Some, 2012; Traore, 2013). Promising results from the preliminary yield performance trial indicated that three lines yielded better than the parents and the drought-tolerant control Gorom Local (Figure 2). In addition, the general performance of these lines reached the potential yield of the recently released varieties in Burkina Faso which is around 1.5 $t.ha^{-1}$ (Ouedraogo et al., 2012). The low yields of certain lines could be attributed to the fact that there were drought spells due to water shortages during the growing period at Kamboinsé. However, other authors have reported in maize three QTLs for two traits (earliness and yield) were introgressed between maize elite lines with MABC but the results were influenced by the function of other genes controlling the traits (Bouchez et al., 2002).

In the domain of molecular breeding, a lot of conventional breeding methods have been associated with markers to design a large number a marker-aided selection methods. Some examples are marker-assisted selection (MAS), marker-assisted recurrent selection (MARS), marker-assisted backcrossing (MABC), and ultimately genomic selection (GS). Among the molecular breeding methods, MABC has been the most widely and successfully used in plant breeding up to date. Marker-assisted backcrossing (MABC) is an effective method for developing improved versions of widely cultivated varieties, also referred to as Mega varieties (Neeraja et al., 2007). It has been applied to different types of traits (e.g. disease/pest resistance, drought tolerance and quality) in many species, e.g. rice, wheat, maize, barley, pear millet, soybean, tomato, etc. (Collard et al., 2005; Dwivedi et al., 2007; Xu, 2010).

Conclusion

The improvement of cowpea varieties by adding traits through MABC is now becoming a significant advancement in Africa, and work is continuing to transfer drought tolerance QTL, Striga resistance, aphid and nematode resistance among other traits into a range of economically important parental lines at INERA. The use of the marker technology tested in this study has allowed rapid recovery of the background of a farmer preferred widely grown cowpea landrace. The morphological characteristics of the improved lines developed in this study indicated that they could be good candidates for production under intercropping in farmer fields. Until now, no Striga resistant variety combining preferences has been proposed by INERA for intercropping. Since Moussa Local is used in intercropping, these improved lines hold much promise for performance under this production system. From this study three of the six MABC-improved lines are most promising based on good yield potential and their resistance to Striga. These three lines (M499_BC3F3_48P85, M499_BC3F3_4P67, and M499_BC3F3_48P90) are being seed-increased for multi-location trials to measure their performance and ability to withstand drought events and Striga attack.

Conflict of Interests

The authors have not declared any conflict of interests.

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Supplemental

Appendix 1: SNP ID and sequences used for MABC selection

SNPID	SNPNum	AlleleY	AlleleX	Sequence
1_0105	12650001	G	A	AAGTATGGCCAGACTTC[G/A]AATCTTGAGATCC
1_0709	12650016	G	A	AAGCCTGTCCGCAA[G/A]TTGTCTCTAGTCCAC
1_0917	12650017	G	A	ATAGCAAAGAAATG[G/A]TAAAAAGAAAGAAGG
1_0866	12650029	C	A	AACGCAAACTGTCGC[A/C]GGTTATATTTTCCT
1_1217	12650034	G	A	AAGCAGAGCCTGGA[G/A]TCGGACTCCGCCGGA
1_0594	12650038	C	A	ATTCTGTGCTGCCAC[A/C]TTAAGCAGGCTGTC
1_1370	12650039	G	A	TTCAATGCATTTCAC[A/G]TCTTCTGGCGGAAT
1_0706	12650043	G	A	TTTGTTGATGATTGT[A/G]TTCAAAGTGACATA
1_0754	12650049	G	A	GGACAGCACAAGTCT[A/G]ACTTCAGAAAAGCT
1_1413	12650048	C	A	ACTCCTCCTATGGC[C/A]GCAAAGGTCAAACCA
1_0256	12650056	G	A	GGCTCTTGGTAAGC[G/A]TATGCATAACGTTGT
1_0649	9030007	G	A	GTGAAAGTTGAAAAA[A/G]GTGAAACTGTCAAG
1_0262	12650063	T	A	AATCCCCGCCGCGTT[A/T]GCTCCACAGGGTCA
1_1103	12650070	G	C	AGCTTGCAGGATCAA[C/G]CCACCCTCCAGATT
1_1249	12650073	G	A	AAGTCATTGACGAT[G/A]TGAGGAATTTCATCG
1_0755	12650079	G	C	TGCTGCGGGGCATGT[C/G]AGAGAAGAATGTGA
1_0992	12650082	C	G	AGGGCAGAGATAAT[C/G]AATGAGGTAAAAAAT
1_0775	12650084	C	G	AGAAGAGTTCGAAA[C/G]AGATAAAATTATTTA
1_0392	12650095	G	A	CTGTTTCTTTGAGC[G/A]TCAAGTTGGGGTGGT
1_0370	12650098	G	A	TCGATGGACGATCC[G/A]GGAAGATTGGGCAGT
1_0126	12650104	G	A	ATTCGCATTTGGCG[G/A]GACTGAGGACCATCA
1_0757	12650116	C	A	TTATGAAGCTCTTGG[A/C]CTCACTTCCAAGCA
1_0081	12650121	G	A	AGAGCAAATATTTA[G/A]AACAAAATATCCCTC
1_0401	12650131	C	A	ATGCAAACTGAGAG[C/A]ATGCAAATACAAAAG
1_0432	12650136	C	A	CTTCGATTAAGTGCA[A/C]ACTCCTACTCTACC
1_0022	12650139	C	A	CCTCGTCTTCAAGTC[A/C]GGCATGGCCAAGTC
1_0307	12650150	G	A	ACACGTTTGTACATA[A/G]GAGTGTGTAAAGTT
1_0053	12650157	G	A	TTGCAGCAAGTACTC[A/G]TTTGACATGAGCTA
1_1360	12650159	T	A	TGGGTATGTAACTAA[A/T]GCCCTTAACCTTCA
1_0982	12650162	G	A	AAATTATTTTTGGTG[A/G]GCCTGAGGTTACAA
1_0993	12650174	G	A	TTGGGAAACACAAA[G/A]ATGTCACCTTTGTTA
1_0652	12650181	G	A	ACCTTAATTGGGGAC[A/G]TTGATCCAGTTCAA
1_0183	12650189	G	A	TCCGGAGAAACAGC[G/A]ACAGTGTTCACATAC
1_0052	12650197	C	A	TAGTTCTGGTGTGG[C/A]YTTGCAGGTACAGAA
1_1039	12650199	T	A	GATGAAACAGACTTA[A/T]GGGCTTATGATGTA
1_0033	12650200	G	A	CAAAAARATGTCCA[G/A]GCTAAAAAACAAAAG
1_0678	12650222	G	A	TGCTTCTTTTGATG[G/A]AAAATTTAGTTGTAC
1_0983	12650225	G	A	CAGAGTTCCTCCTC[G/A]ACGTCCCCGAACCTT
1_0670	12650228	G	A	AGCTCAACCATTCA[G/A]GCCTCAAAATTCAAA
1_0142	12650229	A	T	TTTGCAGTTCCACA[A/T]CCTATAGACAGCAAC
1_0139	12650234	C	A	GGCTACCATGAATC[C/A]GGAAAATTGATCGTG
1_0547	12650239	G	A	CATAAAACACTGTCG[A/G]AAACAAAAAAATGT
1_0703	12650262	G	A	AAGCATTCTATTGG[G/A]AAGTTCTCCAGGTTA
1_0082	12650269	G	A	TCTAAGGAAAGATGG[A/G]AAGAAGCCCAGTGC
1_0290	12650276	G	A	TCAAAAGGTAGTGGT[A/G]GTGCGGTGCGAAGA
1_0987	12650281	G	A	CAGAGGAACTGTGT[G/A]GTGGAAGTCCATCTG
1_1517	12650284	G	A	CTACTGATTGGATA[G/A]CAGGCCCAATATTGG
1_0565	12650286	C	G	CTAAAGCACCARTA[C/G]ACACTGCCAACAACA
1_1151	12650294	G	A	AGTGTATCTGTTAC[G/A]TGGGCAAAATAAAAG

Appendix 1: Contd.

1_0153	12650304	G	A	TATTATAAGAATGTG[A/G]GAATATGCAATGGC
1_1042	12650308	G	A	GATAGATGAGTCATC[A/G]CCTGCTAAATACCG
1_0732	12650314	G	A	TGAACTCCGTGGCC[G/A]AACGTGTAAACCTCC
1_0519	12650322	G	C	TCTCATCCATGCTTT[C/G]TGCTCCTTTGGATC
1_0679	12650323	G	A	GCTCCAACAATTTC[G/A]GTGGGTTCCTCTGCA
1_0127	12650329	G	A	AACCCAGAGAAAAC[G/A]AACTTACAAGACCTA
1_0823	12650331	C	A	TCCCACCTCGAAAA[C/A]GACGTTTGGGTTGGA
1_0322	12650336	C	A	ATCAAATGTTACGGT[A/C]AATTTGGAAGGACA
1_1189	12650339	G	A	CAGTCTCACTGCCA[G/A]CAACTACATCACGGG
1_0280	12650342	G	A	ATGACGCGATCTGC[G/A]ACCTCGGACTTGTCG
1_0567	12650348	C	G	GTCGCCGGTTCGGA[C/G]TGCGAGTCGGACAGC
1_0539	12650356	G	A	ACACAAAAATATTG[G/A]CATYAATCTCAAGTG
1_0242	12650357	C	A	ACAGGGGATTCACC[C/A]TGCGAACCCGTTGCA
1_0598	12650360	G	A	GTAGGGAAGAAARAG[A/G]GAGAGATAAAATAC
1_0171	12650366	G	A	AACTGTGAAAGATGG[A/G]AAACTATACATCTG
1_1072	9030019	G	A	CCTAGACAACCAGCA[A/G]AGTATGTTCAGATT
1_1021	12650373	G	C	ATGTCTAACCCTCCT[C/G]GGTCGTAGATTTCA
1_0136	12650380	G	A	CTCGCTGAATACCA[G/A]AGGGGGCTGGTGCTT
1_0377	12650390	G	A	GGGTCATCTCGACCC[A/G]GGGGCCATTAGTTT
1_1467	12650393	G	A	CAACATATGCAGTG[G/A]TAAATCCCTGAGGTT
1_0317	12650396	G	A	CAACAACATTTACAA[A/G]CGCAAGTATGAGGA
1_0531	12650417	C	G	CAGTGCCTATCCTC[C/G]GCAAGCTCAACAATA
1_0067	12650411	G	A	TGAATGGCGCAGAG[G/A]TTAGTGTCTTCAAAG
1_1333	12650420	C	A	ATTTTTTTTTTACTT[A/C]CAAAAAAAAATGTT
1_0436	12650421	C	G	CGCAGAAGAGATTT[C/G]GAAGCCAACCCATCT
1_0111	12650431	G	A	TTGGCTTCTTGCCAG[A/G]ATGGTGTTGCAAAT
1_0420	12650436	G	A	AGCTGAAGGWCTTGA[A/G]AATGGTCCCTCAGC

Appendix 1: Contd.

1_1214	12650443	C	G	AAGGCAAGCCAGAC[C/G]GCGGTGTTGCACTTG
1_0748	12650447	G	A	TCATTTTCATTCTGG[A/G]ACATGGGAAGATCG
1_0801	12650461	G	A	GGCCCTGAAAGTAGG[A/G]TTGTCCAGTCTGTT
1_1135	9030013	G	A	CCTCGCTTTAATCGT[A/G]CGCCACTGGGTTGA
1_1170	12650475	G	A	CAATGCGGCGACTA[G/A]CGTGAACACAACGGT
1_1431	12650476	G	A	TTCGAGCTCCAATA[G/A]ATTAGGTTGTTGCAA
1_0351	9030014	C	A	TTGCCTTAGTCTCAT[A/C]TCTCTGTTTTACGT
1_0752	12650483	G	C	GTTTCATGTGTATTT[C/G]ATGATTGCTATTGC
1_0937	12650516	G	C	GCCATACGACGTCGT[C/G]GCTGCGCTGCTCTG
1_1371	12650518	G	A	TCTGAACATATCTT[G/A]GCTTTCATTTCTTTA
1_0806	12650520	G	A	ATGCAGGAGTTACAT[A/G]TTAGAGGATGAGAA
1_1073	12650521	G	A	AGAGGAAAAGAAGGT[A/G]GAAGAGAAGAAGGA
1_0306	9030025	G	A	GCCACAGGAACCGGC[A/G]CCTGCTCCTTCAAC
1_0691	12650551	G	A	AACTCTTGAATTGGT[A/G]GCTATTGATGAGCC
1_1520	12650555	C	G	GAAACGACCCGATC[C/G]GTGATAACATCAATC
1_0157	12650562	G	A	GAAACCCTAGGTAAG[A/G]AAAAATGCCGGCTG
1_0807	12650566	C	G	CTAATCTGCGCTAC[C/G]GCAGAATTTAAAATC
1_1246	12650568	T	A	TCCGTCCGCTTCCTC[A/T]CCCGTCGGCGTTTC
1_0084	12650577	C	A	CGTTTTTCGTGATCG[A/C]ATGCCACGTTTGCA
1_0583	12650579	G	A	CTAGATCCCAAGACC[A/G]CCATAGATATCAAG

Appendix 1: Contd.

1_0794	12650583	G	A	TAGTCAATTTTAAC[G/A]GATCTTCAAAACTTG
1_1281	12650587	G	A	TGGTTTTGGCTCAAC[A/G]GAGTCTAAACAGGA
1_1157	12650589	G	A	ATTGAACAAGTGAA[G/A]AGAAAAATAGAAGGA
1_0060	12650602	C	A	TTATTTGTTGGTGGT[A/C]CCATTCATTCTGAT
1_0025	12650606	G	A	AATTTTCTTCCTTTC[A/G]GTTTCGTTAGCCAG
1_0123	12650616	G	A	AAAGGGAATTGGTAA[A/G]AGTGGAAAGCCTCT
1_0473	12650618	G	A	GCTCACGGATCTGGA[A/G]GAGGTTGAGGAGGT
1_0771	12650624	C	A	AACAGAAAATAATG[C/A]AACAGAGGAGGATCC
1_0388	12650635	T	A	GGCTACTTCCCACTT[A/T]CGCTTCACTTTAGT
1_0525	12650642	G	A	TGATGCTTTGATACA[A/G]AAAGTAAATGCTGA
1_0690	12650651	G	A	GGGCACCAGAGTCAG[A/G]GCACAAACCATGAA
1_1271	12650657	G	A	AATTACAAAATTCT[G/A]CGCATTACATCATCT
1_0330	12650662	A	T	TGGAGGCCAGGGTT[A/T]GCACTGCTGAAGATA
1_0438	12650667	G	A	CGTGAGTACCTCATC[A/G]CCAATTTTTAGCAG
1_1393	12650668	G	A	AAGAAAAAGAATGAA[A/G]TTAAAGAAGATTTT

Appendix 1: Contd.

1_0065	12650669	C	A	GTGGCAGTGGCATCA[A/C]CTACAATCCTAGGA
1_1087	12650674	G	A	GTTCATGTTCCATA[G/A]CTAACTTTTCTTCAG
1_0625	12650675	G	A	CAAGTATCATATGTA[A/G]AAGACTGCAGACAT
1_1007	12650677	G	A	GATATATATTCAGT[G/A]CCAATTATATGGCCA
1_1141	12650698	G	A	TTATATTAATGTTGC[A/G]AATCATTGCAACAA
1_0853	12650709	G	A	CGGCGGAGGACGCC[G/A]GAGATAATGCGGCTG
1_0056	12650712	G	A	TCCATGAGGAAAACA[A/G]CCTCTAAGTCTGTT
1_1129	12650713	G	A	ATGTTCATGGTATT[G/A]TAGTCATTTATCAAC
1_1096	12650718	G	A	TCACTTAATCACTCA[A/G]TCACTTTCATCTTC
1_0730	12650735	G	A	ATGGTTTTGGTTTC[G/A]GTCTGAAGAAGCTCG
1_1117	12650741	C	A	GTTTGTGTGCATTG[C/A]AGTCTGGGAGTTCTG
1_0514	12650751	G	A	GGAATCCTCTATCA[G/A]AGGCACCCAGTAAGA
1_0923	12650752	G	A	GCAAGCATTAACAGT[A/G]GCGGCTGCAGTTGG
1_0397	12650767	C	A	TGGTTCTCTTTGTGG[A/C]CCTGTTGTTGATCA
1_0222	12650773	G	A	AACCTTTGACTCCR[G/A]AGATTCTTGGTGAGT
1_1038	12650777	G	A	TGAGGAAGAGCGTA[G/A]CCCTCATAAATGGGG
1_0014	12650685	C	A	CCCTTTGCAGGTTT[C/A]GTCTGCACCAAAACA
1_1492	12650785	C	G	ACAATCTACCGTTT[C/G]TGAAACGCGTTACCT
1_1092	12650786	G	A	TGATACTACTGTCAA[A/G]ATTTACAATGGGAA
1_0449	12650788	G	A	TGAACATTAAAATG[G/A]GAAACATCTTATTAT
1_0058	12650793	G	A	GGAAACTGAGGAAAA[A/G]AAGGGGTTTCTTGA
1_0421	12650794	G	A	ACAGCACGCAATAT[G/A]TTTGCACCAGCGCCT
1_0529	12650804	G	A	TCATCCTGCTGTCAA[A/G]GGCCTTCTCCCAGA
1_0482	12650805	C	G	AAGAATTTGCACTT[C/G]AAGGATATCTTCCAA
1_0905	12650809	G	A	AGATCCAAGGACAGG[A/G]GAAGTGATTACGAA
1_0232	12650812	G	A	GAGGAATCGTGGTC[G/A]TGGATCTTCCCGGAA
1_0957	12650816	G	C	TAAAACTGCAAATGT[C/G]GGAACGAAGATATG
1_0510	12650817	G	A	GAGATCTGGAAGTTA[A/G]TTGTCATTTTGAAC
1_0657	12650821	G	A	CACTGACTTGGCCA[G/A]CACGGTGTAGTCCTC
1_0773	12650823	G	A	ACTGATGGAAGGAAC[A/G]CTGAAGAGAAGGGA
1_0451	12650833	C	G	CTGCCTCTTCTGGA[C/G]GATCACTCTGTGGAG
1_0062	12650864	G	C	AAGGAGGTAGGGCTA[C/G]CCAATGGGYTTTTA
1_0437	9030018	G	A	TAGTACCCCTCTTCT[A/G]ATATCTTTTATTTG
1_0605	12650911	C	A	GGATAACCGGACCGT[A/C]CTGGACGGGACCTT

Appendix 1: Contd.

1_1130	12650915	G	A	ATGATGTTGGCTTT[G/A]TGGACGGCGGTGACT
1_0319	12650924	G	A	GGAACCTGCTCAGC[G/A]CATGTAAGTAATTCA
1_0740	12650940	G	A	ATGAAGCTGCTTCT[G/A]TGTGGCTTCCTCTGG
1_0001	9030026	G	A	TTTAGAGATCTAAGG[A/G]ATGTGGTTTTTAAT
1_0107	12650955	G	C	CCGCCACAACCCCAA[C/G]CTCTCTTTCCTTCA
1_0178	12650964	C	A	TYTGGTTGGTGCACC[A/C]GGTGGCCTAAAAGC
1_0362	12650966	G	A	TGGGGTTCGATTCGC[A/G]GTTGAACCCGAACA
1_0718	12650968	G	A	GAGAAAAAATCGTTC[A/G]TTGTAACGTTTTCG
1_0425	12650971	G	A	AGATGCAAGTCCTTC[A/G]GGAAACGCTGCCGG
1_0246	12650978	G	C	ATTGGGCTCTYCTCT[C/G]CGCTATTAGTTTTC
1_1512	12651001	A	T	GCAATGATGAGCAT[A/T]CAGAGACCATTATTC
1_0834	12651004	G	A	AGTGCCGGCAGGGT[G/A]TTGCACAACTCCGGA
1_0699	12651008	C	G	CATGCAAGATACTT[C/G]GTAAACTGATCAATT
1_0663	12651013	G	A	GGATTCTGCTTCAA[G/A]TCGCCAAAAGACGGG
1_0878	12651014	G	A	TCCATTGAACCACA[G/A]GCAAGTCGTTTCCCA
1_0911	12651029	C	A	ACGGCTGAAACTGAG[A/C]AGAGGAGGATAGTC
1_0746	12651032	C	A	ATCATTTTCCTCAT[C/A]AATGTCGTCGTCGTC
1_0442	12651034	C	G	CGATTGATCGGCAT[C/G]GACGAGATGAAGAAC
1_0146	12651037	G	A	TTGACGACGAGGTT[G/A]GTGACGGAGTAGAGG
1_0876	12651065	T	A	TAGGATATTTTGACA[A/T]GTTATGTATCCGAT
1_0945	12651066	G	A	TTTCTCCTCACAGAA[A/G]CAGAGAATGCAGCG
1_1062	12651070	C	A	TTTAGTTAACAAAGC[A/C]TTGGTTCTCATAAC
1_0604	12651075	A	T	CAACCATCTATGAA[A/T]TGCCCTTTTGATGGA
1_0977	9030009	C	A	TGTAGTGGTCAATGG[A/C]TGTGCTCACATATA
1_0323	12651082	C	A	GAAACCAACTCTTA[C/A]CAAAAGGCGCAACAA
1_0128	12651083	G	C	GACCCTTCACCTTGT[C/G]CTCAGGCTTCGCGG
1_0588	12651090	G	A	TTTCGAGACTGTGTT[A/G]ATGGTTTAATGTAT
1_1367	12651092	G	A	TCAAAGATTAAACAT[A/G]CCTCTCATGTATCA
1_1121	12651101	G	A	CTGTGGGAGCTATGG[A/G]GATTATCCTGTGGA
1_0259	12651106	G	A	CTGCTGCACCGTTT[G/A]GAGTTATCCATTGCA
1_0889	12651110	C	G	TTTCAATACTGTTT[C/G]TTGTTAGTACTATCT
1_1255	12651114	T	A	ATCGATACAGTGTTG[A/T]GGAAGTGAAGAAAG
1_0238	12651129	G	A	CATCACCGATCTTAA[A/G]GGTGGCAAAGTCGG
1_0074	9030020	C	A	CTGGACACTTATGTG[A/C]GAGGAAATCTTGTG
1_0647	12651138	G	A	GAAAGAAGCTCAGG[G/A]AACTCTGTCTTCAAT
1_1037	12651147	G	A	ACAGACGAGATCAT[G/A]CATGACGATTTATAA

Appendix 2: Full genotyping results for selecting BC_2F_1 line carrying yield, stay green, and nematodes QTL in the cross Moussa local /IT93K-503-1//Moussa local

	Nematode resistance	**Yield, stay-green**				
Plant	**1_1170**	**1_0678**	**1_0128**	**1_0157**	**1_0992**	**Moussa background (%)**
M503_BC2F1_54P10	AA	AA	AB	AB	AB	97
M503_BC2F1_54P9	AA	--	AB	AB	AB	95
M503_BC2F1_82P21	AA	AA	AA	AA	AA	94
M503_BC2F1_54P15	AA	AB	AB	AB	AB	92
M503_BC2F1_54P7	AA	AB	AB	AB	AA	92
M503_BC2F1_54P17	AA	AA	AA	AA	AA	92
M503_BC2F1_54P8	AA	--	AB	AB	AB	89
M503_BC2F1_54P13	AA	AA	AA	AA	AA	89
M503_BC2F1_54P14	AA	AB	AB	AB	AB	88

Appendix 2: Contd.

M503_BC1F2_77P61	AA	AA	AA	AA	AA	88
M503_BC2F1_82P20	AA	AA	AA	AA	AA	87
M503_BC2F1_54P11	AA	AB	AB	AB	AB	87
M503_BC2F1_82P18	AA	AA	AA	AA	AA	87
M503_BC2F1_82P19	AA	AB	AB	--	AB	86
M503_BC1F2_92P27	AB	AA	AA	AA	AA	86
M503_BC1F2_83P34	AB	AA	AA	AA	--	86
M503_BC1F2_83P31	AA	AA	AA	AA	AB	86
M503_BC2F1_54P12	AA	AA	AA	AA	AA	86
M503_BC1F2_83P45	AA	AA	AB	AA	AA	82
M503_BC1F2_83P49	AA	AA	AA	AA	AA	82
M503_BC1F2_83P32	AA	AA	AA	AA	--	82
M503_BC1F2_83P39	AA	AA	AB	AA	AA	81
M503_BC1F2_77P64	AA	AA	AA	AA	AA	81
M503_BC2F1_54P16	AA	AB	AB	AB	AB	81
M503_BC1F2_92P24	AB	AA	AA	AA	AA	80
M503_BC1F2_77P55	AB	AA	AA	AA	AA	80

Appendix 3: Full genotyping results for selecting BC_2F_1 line carrying yield and striga QTL in the cross Moussa local /IT97K-499-35//Moussa local

	Yield			Striga	
Plant	**1_0022**	**1_1370**	**1_0567**	**1_0583**	**Moussa background (%)**
M499_BC2F1_47P2	AA	AA	AA	AA	97
M499_BC2F1_47P15	AA	AA	AA	AA	95
M499_BC2F1_47P13	AA	AA	AA	AA	95
M499_BC2F1_47P6	AA	AA	AA	AA	95
M499_BC2F1_47P11	AA	AA	AA	AA	95
M499_BC2F1_47P1	AA	AA	AA	AA	94
M499_BC2F1_48P94	AA	AA	AA	AA	94
M499_BC2F1_47P14	AA	AA	AA	AA	94
M499_BC2F1_47P9	AA	AA	AA	AA	93
M499_BC2F1_4P67	AA	AA	AA	AB	93
M499_BC2F1_29P64	AA	AA	AA	AA	93
M499_BC2F1_47P3	AA	AA	AA	AA	93
M499_BC2F1_49P28	AB	AB	AB	AA	93
M499_BC2F1_48P84	AA	AA	AA	AA	93
M499_BC2F1_48P90	AB	AB	AB	AA	93
M499_BC2F1_47P12	AA	AA	AA	AA	92
M499_BC2F1_49P33	AB	AA	AB	AA	92
M499_BC2F1_47P7	AA	AA	AA	AA	92
M499_BC2F1_44P17	AB	AB	AB	AA	92
M499_BC2F1_47P16	AA	AA	AA	AA	92
M499_BC2F1_47P5	AB	AA	AA	AA	92
M499_BC2F1_47P8	AA	AA	AA	AA	92
M499_BC2F1_48P81	AA	AB	AA	AA	92
M499_BC2F1_49P32	AB	AB	AB	AA	92
M499_BC2F1_27P4	AB	AA	AB	AA	91
M499_BC2F1_44P20	AA	AA	AA	AA	91
M499_BC2F1_44P21	AB	AA	AB	AA	91
M499_BC2F1_47P4	AA	AA	AA	AA	90

Appendix 3: Contd.

M499_BC2F1_44P19	--	AB	AB	AA	90
M499_BC2F1_31P55	AA	AA	AA	AA	90
M499_BC2F1_29P61	AA	AA	AA	AA	90
M499_BC2F1_47P10	AA	AA	AA	AA	90
M499_BC2F1_38P79	AB	AA	AB	AA	89
M499_BC2F1_48P83	AA	AA	AA	AA	89
M499_BC2F1_48P86	--	AA	AA	AA	89
M499_BC2F1_4P72	AA	AA	AA	AB	89
M499_BC2F1_49P31	AB	AB	AB	AB	88
M499_BC2F1_49P25	AA	AA	AA	AB	88
M499_BC2F1_27P2	AA	AA	AA	AB	88
M499_BC2F1_48P92	AA	AA	AA	AA	88
M499_BC2F1_31P48	--	AA	AA	AA	88
M499_BC2F1_48P85	AB	AB	AB	AA	88
M499_BC2F1_39P66	AA	AA	AA	AA	88
M499_BC2F1_31P44	AB	AA	AB	AA	87
M499_BC2F1_10P41	AA	AA	AA	AB	86
M499_BC2F1_27P1	AB	AA	AB	AA	86
M499_BC2F1_31P45	AA	AA	AA	AA	86
M499_BC2F1_31P49	AA	AA	AA	AB	86
M499_BC2F1_31P50	AA	AA	AA	AA	86
M499_BC2F1_39P65	AA	AA	AA	AA	86
M499_BC2F1_48P82	AB	AB	AB	AA	86
M499_BC2F1_10P40	AA	AA	AA	--	86
M499_BC2F1_48P88	AB	AB	AB	AA	86
M499_BC2F1_48P91	AB	AA	AB	AA	85
M499_BC2F1_29P58	AA	AA	AA	AB	85
M499_BC2F1_49P36	AB	AB	AB	AA	85
M499_BC2F1_49P22	AB	--	AB	AA	85
M499_BC2F1_27P3	AA	AA	AA	AB	85
M499_BC2F1_31P56	AB	AA	AB	AA	84
M499_BC2F1_48P93	AB	AB	AB	AA	84
M499_BC2F1_31P53	AA	AB	AA	AB	84
M499_BC2F1_48P87	AA	AA	AA	AA	84
M499_BC2F1_38P80	AA	AA	AA	AA	84
M499_BC2F1_44P18	AB	--	BB	AA	84
M499_BC2F1_29P59	AB	AA	AA	AB	84
M499_BC2F1_31P46	AB	AA	AB	AB	84
M499_BC2F1_10P39	AB	--	AB	AB	83
M499_BC2F1_49P26	AB	AB	AB	--	83
M499_BC2F1_49P35	AA	AA	AB	AB	83
M499_BC2F1_48P89	AA	AA	AB	AA	83
M499_BC2F1_31P47	AB	AB	AB	AA	83
M499_BC2F1_4P71	AA	AA	AA	AB	83
M499_BC2F1_31P52	AA	AA	AA	AA	81
M499_BC2F1_4P69	AA	--	AA	AB	81
M499_BC1F2_27P51	AA	AA	AA	AA	81
M499_BC1F2_67P80	AA	AA	AA	AA	80
M499_BC2F1_49P27	AA	AA	AA	AB	80
M499_BC2F1_49P37	AA	AA	AA	AB	80
M499_BC2F1_49P34	AA	AA	AA	AB	80
M499_BC2F1_31P54	AA	AA	AB	AA	79

Appendix 3: Contd.

M499_BC2F1_29P57	AB	AB	AA	AB	79
M499_BC2F1_27P5	AB	AA	AB	AA	79
M499_BC1F2_67P86	AB	AB	AB	AB	79
M499_BC2F1_4P68	AA	AA	AA	AB	79
M499_BC2F1_4P70	AA	AA	AA	AB	79
M499_BC2F1_49P23	AB	AB	AB	AB	79
M499_BC2F1_49P24	AA	AB	AA	AB	79
M499_BC1F2_67P85	AA	BB	AA	AB	78
M499_BC1F2_27P50	BB	AA	BB	AA	77
M499_BC2F1_31P51	AB	AB	AB	AB	77
M499_BC2F1_20P42	AB	AB	BB	AB	77
M499_BC2F1_20P43	AB	--	AB	AB	77
M499_BC2F1_38P78	AA	AA	AA	AB	77
M499_BC2F1_66P73	AB	AA	AB	AA	77
M499_BC1F2_27P73	AA	AA	AA	AB	76
M499_BC1F2_67P87	AB	AB	BB	BB	76
M499_BC2F1_70P38	AB	AB	AB	AA	76
M499_BC1F2_67P95	AA	AA	AA	AB	75
M499_BC2F1_66P74	AB	AA	AB	AA	73
M499_BC2F1_66P77	AB	AB	AB	AA	72
M499_BC1F2_67P91	AA	BB	AA	AB	71
M499_BC2F1_27P6	AB	AA	AB	AB	71
M499_BC1F2_27P77	AA	BB	AA	BB	71
M499_BC1F2_67P94	AA	AA	AA	AB	70
M499_BC1F2_27P68	AA	AA	AA	AA	70
M499_BC2F1_49P30	AB	AB	AB	BB	70
M499_BC1F2_67P89	AA	AA	AA	BB	70
M499_BC2F1_66P75	AB	AB	AB	AA	69
M499_BC2F1_29P63	AB	AB	AB	AB	69

Appendix 4. Position of trait-linked markers on cowpea consensus genetic map

Trait	Marker	LG	cM	Donor
Nematode	1_1170	3	28.568	IT93K-503-1
Yield, Stay green	1_0678	4	25.390	IT93K-503-1
Yield, Stay green	1_0128	4	27.408	IT93K-503-1
Yield, Stay green	1_0157	4	30.339	IT93K-503-1
Yield, Stay green	1_0992	4	33.146	IT93K-503-1
Yield	1_0022	8	7.935	IT97K-499-35
Yield	1_1370	8	9.173	IT97K-499-35
Yield	1_0567	8	19.501	IT97K-499-35
Striga	1_0583	10	50.534	IT97K-499-35