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Hybridity Testing of Eggplant F_1 Progenies Derived from Parents with Varying Response to Drought Using SSR Markers

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Eggplant (*Solanum melongena* L.) production is highly affected by drought stress, with effects including reduction in plant height, dry matter and fruit yield. However, some eggplant varieties were found to have tolerance to drought and can be used to confer drought tolerance to other varieties. Commercial eggplant varieties Mara and Mistisa were crossed with drought-tolerant eggplant accessions PHL 2789 and PHL 4841, respectively. To confirm that the F_1 progenies indeed came from the cross made between the two selected parents, analysis was done at the molecular level using simple sequence repeat (SSR) markers. Out of 65 SSR markers screened for polymorphism, six markers (EM141, eme05B09, EM133, emh11O01, emf21I02 and EM117) were able to discriminate between Mistisa and PHL 4841 and four markers (CSM20, eme09E09, EM131 and EES063) were able to distinguish Mara from PHL 2789. These markers were used to determine the hybridity of the 30 progenies from each cross. Based on marker data, all progenies except for progeny number 13 were identified as hybrids for the cross Mistisa x PHL 4841 while all the 30 progenies from the cross Mara x PHL 2789 were confirmed as hybrids.

Key words: drought, eggplant, hybridity testing, hybrids, SSRs

INTRODUCTION

Eggplant (*Solanum melongena* L.), also referred to as aubergine and brinjal (Ali et al. 2011), is one of the most common vegetable crops grown in different parts of the world (Bletsos et al. 2003). It is a good source of vitamins and minerals, with nutritional value comparable to tomatoes (Kalloo 1993). In the Philippines, total production volume of eggplant is around 226,000 metric tons valued at Php 4.1 billion with Ilocos Region, Cagayan Valley and Central Luzon as the top eggplant-producing regions of the country (PSA 2015).

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Eggplant is susceptible to fruit quality decrease and yield loss when subjected to water stress (de la Peña & Hughes 2007). Although eggplant has some degree of tolerance to drought (Chen & Li 1996), it can experience decrease in plant height, dry matter and fruit yield when exposed to long periods of moisture stress (Kirnak et al. 2007). Under field conditions, drought caused reduction in transpiration rate, stomatal conductance and photosynthetic activity in eggplant (Delfin et al. 2015). Some eggplant accessions were found to have tolerance to drought and can be used as sources of drought resistance to improve commercial eggplant varieties (Delfin et al. 2015).

The present study aims to confirm the hybridity of F_1 progenies from crosses of *S. melongena* and commercial varieties that were selected in a previous study for their differing response to drought (Delfin et al. 2015). The use of DNA markers for characterization and identification of genotypes is essential for rapid and early verification of true hybrids at seedling stage. Among the DNA markers, simple sequence repeats (SSRs) or microsatellites are the most suitable for hybridity testing due to its co-dominance detecting the presence of DNA sequences corresponding to distinct alleles contributed by both parents in a specific cross (Cordiero et al. 2000). SSR markers have been proven effective in confirming hybridity in various crops such as sugarcane (Manigbas & Villegas 2004) and corn (Sudharani et al. 2014).

SSR markers were utilized in eggplant for genetic diversity analyses and genetic characterization and linkage map construction (Barchi et al. 2011; Barchi et al. 2012; Cericola et al. 2014; Portis et al. 2015; Saracanlao et al. 2016; Toppino et al. 2016). This present study is the first report in eggplant where SSR markers are used to confirm true hybrids derived from parents with differing drought response directed towards eggplant improvement for moisture stress tolerance.

The study identified polymorphic SSR markers differentiating male and female parental genotypes and assessed the hybridity of the progenies. True hybrid plants were then identified from the cross between drought-tolerant and moderately tolerant eggplant parental genotypes. These hybrids can be used in producing drought tolerant eggplant populations.

MATERIALS AND METHODS

Plant Materials

Four eggplant (*S. melongena* L.) genotypes from the Philippines were utilized in this study. IPB-bred eggplant varieties Mara and Mistisa were used as female parents in the crosses. Mara is an open-pollinated variety with shiny, purple-skinned fruits measuring 15.91 cm long and 3.03 cm in diameter. The variety can be planted throughout the year with average yield of 14.71 tons/ha. Mistisa is characterized by its striped light violet and cream fruits that are 16-20 cm long and 3-4 cm in diameter. The average yield of Mistisa is 30 tons/ha during dry season.

S. melongena accessions, PHL 2789 and PHL 4841 were selected from among 29 eggplant selections evaluated in the field for drought tolerance (Delfin et al. 2015). These accessions were selected based on their absolute and relative fruit yield as well as biomass production during drought and recovery period. For instance, PHL 4841 had an average yield reduction of 15.5% whereas Mistisa showed 37% yield reduction. Biomass production data also showed higher reduction of 28% for Mistisa and 9% for PHL 4841. PHL 2789 on the other hand, produced the highest absolute yield during drought and recovery period with an average of 33% higher yield than variety Mara. Biomass production and total leaf area were observed to have increased during drought for PHL 2789 whereas reductions of 39 and 37% for both parameters were observed for Mara.

Mara and Mistisa (moderately tolerant to drought) were crossed to the drought-tolerant eggplant accessions, PHL 2789 and PHL 4841, respectively to generate eggplant hybrid progenies (Table 1).

DNA Extraction and Quantification, PCR Amplification, and Gel Electrophoresis

Young, fully expanded and damage-free leaves of eggplant were collected from the parental genotypes and progenies from each cross. Genomic DNA was extracted following the CIMMYT protocol (CIMMYT 2005) with modifications. Approximately 1 g fresh leaf samples of at least one-month old eggplant seedlings were ground into a fine powder with liquid nitrogen using mortar and pestle. DNA quality and yield were determined by agarose gel electrophoresis. Electrophoresis was carried out in 1% UltraPure agarose (Invitrogen Corp., Carlsbad, California, USA) in 0.5X Tris-acetate EDTA (TAE) running buffer at 100 V for approximately 30 min. The agarose gel was stained with 0.1x SYBR™ Safe stain (Invitrogen Corp., Carlsbad, California, USA) and detected under UV light (Bio-Rad Gel Doc™ XR+ Imaging System, Bio-Rad Laboratories Inc., Hercules, California, USA). DNA concentration was estimated by visual comparison of the intensity of fluorescence of sample DNA aliquots with the four known concentrations of lambda (λ) DNA (Invitrogen Corp., Carlsbad, California, USA) standards. Working stock for each DNA sample was prepared and stored at 4° C. Stock DNA was stored at -20° C.

Table 1. Eggplant accessions and putative hybrids used.

Entry No.	Genotype	Origin	Drought Response	No. of Plants
1	Mistisa	Philippines	Moderately tolerant	1
2	PHL 4841	Philippines	Tolerant	1
3	(Mistisa x PHL 4841) F_1	Philippines	-	30
4	Mara	Philippines	Moderately tolerant	1
5	PHL 2789	Philippines	Tolerant	1
6	(Mara x PHL 2789) F_1	Philippines	-	30

Polymerase chain reaction (PCR) was performed using the optimized SSR amplification conditions for eggplant. Each 10 µL PCR reaction consisted of 10 ng genomic DNA, 1X PCR buffer with 1.5 mM $MgCl_2$ (KAPA Biosystems, Boston, Massachusetts, USA), 0.2 mM dNTPs (Invitrogen Corp., Carlsbad, California, USA), 0.2 µM each of forward and reverse primer and 0.5 U Taq DNA polymerase (KAPA Biosystems, Boston, Massachusetts, USA). Amplifications were carried out in a Bio-Rad T100™ Thermal Cycler (Bio-Rad Laboratories Inc., Hercules, California, USA) with the amplification conditions of initial denaturation at 94º C (30 s), 30 cycles of 94º C (30 s) denaturation, 55-68º C annealing (1 min) and 72º C (1 min) extension, followed by one cycle at 72º C (5 min) final extension.

Polyacrylamide gel electrophoresis (PAGE) was used to separate the amplified DNA fragments. A laboratory-optimized protocol on PAGE was adopted using C.B.S. Scientific Triple Wide Mini Vertical System™ (C.B.S. Scientific Company Inc., Del Mar, California, USA). PCR amplification products were electrophoresed in 8% polyacrylamide gel using 1X Tris-borate EDTA (TBE) running buffer. Electrophoresis was carried out for 90 minutes at 100 V. The polyacrylamide gel was stained with 0.1X SYBR™ Safe stain (Invitrogen Corp., Carlsbad, California, USA) and detected under UV light using the Bio-Rad Gel Doc™ XR+ Imaging System (Bio-Rad Laboratories Inc., Hercules, California, USA).

SSR DNA Marker Analysis

The four parental genotypes: Mara, Mistisa, PHL 4841 and PHL 2789 were screened for polymorphism using 65 SSR primer pairs obtained from published literatures (Nunome et al. 2003; Nunome et al. 2009; Vilanova et al. 2012; Ge et al. 2013). The eggplant SSR primers were selected based on previous SSR primer screening conducted in the laboratory. Polymorphic SSR markers identified in each parental cross were utilized in the eventual hybridity testing of progenies generated per cross. Marker data were analyzed and scored, checking for the presence of both alleles from the parents of a certain cross. Progenies were selected as true F_1 hybrids if they show both alleles from their respective parents.

RESULTS AND DISCUSSION

Polymorphic SSR DNA Markers

Of the 65 SSR primers screened for polymorphism, 10 SSR markers were found to be polymorphic in the two crosses (Table 2). A total of six SSR markers (EM141, eme05B09, EM133, emh11O01, emf21I02 and EM117) were polymorphic between Mistisa and PHL 4841 cross. On the other hand, four polymorphic SSR markers (CSM20, eme09E09, EM131 and EES063) were identified between Mara and PHL 2789 eggplant parental genotypes. Figure 1 shows the SSR polymorphism screening among the eggplant parental genotypes.

Very low SSR polymorphism among the parental genotypes screened was primarily due to the self-pollinating nature of eggplant. Although the four parental genotypes utilized have contrasting drought response, all came from a single species, *S. melongena*. Furthermore, the polymorphic SSR markers identified were not distributed across the 12 chromosomes of the eggplant genome.

Putative F_1 Hybrids based on Polymorphic SSR Markers

(Mistisa x PHL 4841) F_1 Progenies

Of the six polymorphic SSR markers identified in this cross and utilized in the hybridity testing, only four SSR markers (EM117, emh11O01, emf21I02 and EM141) distinguished true F_1 hybrids. At least one polymorphic SSR marker confirmed the F_1 progenies as true hybrids in this specific cross except for progeny 13 wherein the SSR marker banding pattern obtained is either of maternal

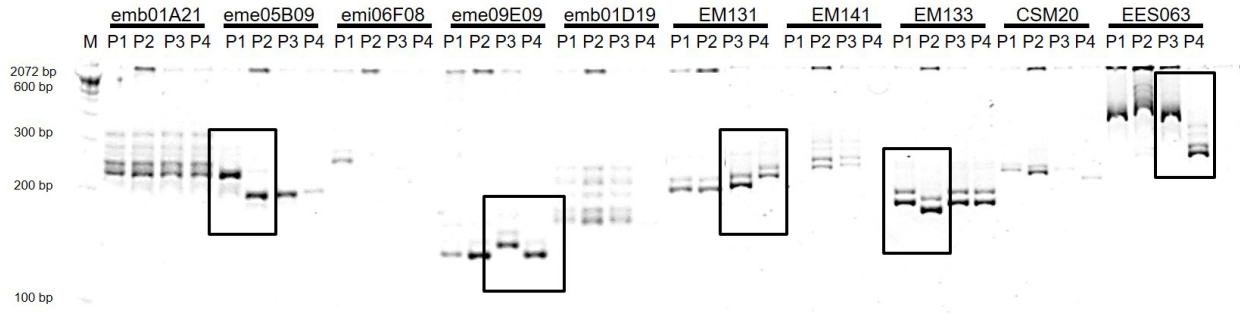

Figure 1. SSR marker polymorphisms between the Mara and Mistisa (moderately drought susceptible) and PHL 4841 and PHL 2789 (drought tolerant) parental genotypes of eggplant (*S. melongena* L.). Lanes M = 100 bp DNA ladder (Invitrogen); P1 = Mistisa; P2 = PHL 4841; P3 = Mara and P4 = PHL 2789.

Table 2. List of SSR primers used in the polymorphism screening including forward and reverse sequences, expected amplification product and polymorphism.

Entry No.	Primer Name	Forward Sequence	Reverse Sequence	Linkage Group	Expected Product Size (bp)
1	eme01D03	ACAAGAATCGGTCCTCTTTGCATTGT	GTTTGCTTTTCACCTCTCCGCTATCTC	1	275
2	ecm001	ACCTTACGCAATTTACACTTCCCC	GTTTCAATGGCGTCACCTCTCTCTCT	3	229
3	emf11D18	AGAGACAGGGAGAGTGCATTCTATG	GTTTGCAGTTCATAAGGTTGCATCAATAC	6	289
4	emg11D22	AGGCCCATGTTTGGCATTTAT	GTTTATGGATATCTCAATGGACCTGA	6	291
5	emi06F08	ATAATGAACCAAAGCGAGAGCAAC	GTTTCAGGTCCATAGGGGTGGATCTATG	11	261
6	emb01H20	TCTTGTTCCCAGTCTATCGCTAATCA	ATCCGAATTTAGTCGGGCTTCAAT	9	351
7	emb01G19	AATTAAGGCTGAGAGGGGAAGACG	AAAGGAGGAAAGGGAAAGGGAAAG	1	322
8	emf21I02	AGTGCATTTCTCAAATCAAAAGGG	GTTTCAATTTCACAGGCTCCTGCATTA	7	204
9	emf01G17	ATGGCAACTGATAATGCAGACGTG	GTTTCTCACTCTTACATGTGGCTGGC	8	289
10	emh21J12	ACAGAACAATTCACCAGCAGTCAA	GTTTAGGAACAGGGAAAATCGTATCGGT	3	303
11	eme25D01	AGTCCCAACCAAAATCGTAGAGGC	GTTTCACTGAAGGATGTGGAGTGTGA	6	299
12	emg11A06	AGTGCTAATATGCAAGGGGAATGG	GTTTACGGTGATCTTTCCGTATTCCAAA	7	257
13	emg01B17	ACAAGGCTCAAAGTCACAAGTCAA	GTTTGGCTCTGCCCCTAACATCTACAAA	4	250
14	emf11H23	ATTCTGAAAACAAGAGCAGCCCTC	GTTTCTCAACACCTCTGTGTCTGGCAT	6	260
15	emg11I03	ATTAGGCACAAGTGCCACCTGAAT	GTTTCAGCCGGGAGTCTGATAGGTAAAA	10	212
16	emf11L21	ATAGCCTAGGTAACGTACCCCTCG	GTTTGGCTCTATTTCCTGGGCTTTTCAT	6	298
17	emd12B05	ACGGAGTAGGCTCGGAGCGTGATATT	GTTTGAAAGGGCAAAAAGTCCAAACAAC	8	277
18	emd05F05	ACGGGGGTGTCTCATTACACTACTGG	GTTTACCCGTTCCTCAGCTTATAGACCC	3	334
19	emf01O01	AGGAATTGGATTTCCACTCATACG	GTTTGGAAGATGAGATTCCTTTCTTGA	1	296
20	emf21K08	ATCAATGACACCCAAAACCCATTT	GTTTGAAAACCCAATACAAATCCGA	1	228
21	emb01F16	AAAACAGAAGCAAAGTCGGCAGTC	GTCCACCAACACCTTACCATCCTC	2	204
22	emh11G21	ATGTGTGAACTCAAATGGAAGGGA	GTTTCGAATTGCTTTTTGGTGCATGTAG	3	251
23	emf21P02	ATGAAGCAGATCTTTCGACTGCAC	GTTTAGGCCAAGGATGTCAAACTGGT	3	294
24	emh05B02	ATACCAAAGACACGTTGGGATCAT	GTTTCTAGGAGAGCATCTCCCTCCCT	3	240
25	emd15D09	ATAATGGGCAAAGGGTCCATTAAC	GTTTGGAACCATGCAGTACCAGACATGA	4	296
26	emj04E17	ACACGCTGCTGAAATAGTTTCTTAG	GTTTCGAGTTATGCTGAGAGCAGTGTGA	4	217
27	emh11N11	ATTCAGTTCTTCGCTTTGGAGCTT	GTTTCCAAACCCGACCCATCCTAAATAA	5	287
28	emj01G23	ATTAACTGGCCATGAACACCTGTC	GTTTGACCTCAATAAAGGGGGTTTGCAT	6	290
29	emd18B04	ATTTCTGAGGTTTAACATCGCCGT	GTTTCGGAGGAGAGCAAGTTCTGCTTTA	6	283
30	emb01A21	TCATGGTAGGTGGAGACAGAACCA	GTTTGGATTAGCATGTGGAGGACTGAA	7	239
31	eme05B09	ATGAAAACTCCACTCTACTCTACTCCAC	GTTTGCTAACGTACGCCTCAATTGCTCT	7	237
32	emh11O01	ATTGTGTCGATGAGATTTTGGTCA	GTTTAGCTACGTTGGTTTGGTGCTGAA	10	213
33	eme09E09	ACGGTATCGAAGAGAGTGAATGCCT	GTTTCCCCATTTCATCTGAAAAATCCAC	11	144
34	emb01O01	TTAACATCGCCGTTGGCTTCTTAG	GTTTCGATAACCAAAAGGGGTTTCAACA	11	213
35	emb01D19	CGACCCCAGATCCAGAAATAAAGA	CCCAAGAGTTGTACTCGTCAACCA	12	163
36	EES019	TTGTCTCATTGTTGGTATGGA	GCCCATTGTTGAGGTGATTA	-	265
37	EES021	AAAAATCCCCAAATCCATCT	ACGCTCTCTCACAACAACAA	-	352
38	EES022	CAAAGTACCTTCCATTTATCCAG	CAGGTGCAGGTATCATCGTA	-	219
39	EES026	GATGGAATTCAACAGTTACACAA	GGTCAATCCTGGTAAAGGTG	-	305
40	EES028	ACCGTTCTCGTCTCTTTGTC	CAACAACAGTTCAACCCAAA	-	266
41	EES030	CATTCTACCGTCTCCAAACC	AAACAGCCGCTCTACCTCTA	-	289
42	EES031	AGAGGAGAAAGCGCTAGACA	TGATCAATCTTTGCATCCAC	-	226
43	EES043	AATGCCAGGACATCTGAAAT	AAACGGAAACGATGAAGAAG	-	271

Actual Annealing Temp. (°C)	Polymorphism	
	Mistisa x PHL 4841	Mara x PHL 2789
65	M	M
65	M	M
65	M	M
65	M	M
63	M	M
65	M	M
65	M	M
65	P	M
68	M	M
65	M	M
65	M	M
65	M	M
65	M	M
65	M	M
65	M	M
65	M	M
68	M	M
65	M	M
65	M	M
65	M	M
65	M	M
65	M	M
65	M	M
65	M	M
65	M	M
65	M	M
65	M	M
65	M	M
65	M	M
65	M	M
65	M	M
65	P	M
68	P	M
65	M	P
65	M	M
65	M	M
65	M	M
55	M	M
55	M	M
55	M	M
63	M	M
55	M	M
55	M	M
65	M	M

table 2 continues next page . . . .

or paternal origin only. Figure 2 shows the SSR gel on hybridity testing of (Mistisa x PHL 4841) F_1 progenies using SSR marker emh11O01. Table 3 summarizes the results of hybridity testing in (Mistisa x PHL 4841) F_1 progenies. Two SSR markers, EM117 and emh11O01 confirmed the 29 F_1 progenies as true hybrids. The effectiveness of these two SSR markers in detecting true hybrids was 97%. The markers EM133 and eme05B09 showed almost all maternal alleles with a single paternal allele detected in progeny 13 using the SSR marker eme05B09. These SSR markers may not be appropriate for hybridity testing of eggplant progenies from the specific crosses made. SSRs emf21I02 and EM141 detected true eggplant hybrids at 37% and 40% efficiency, respectively. Therefore, except for Progeny 13, the other 29 (Mistisa x PHL 4841) F_1 progenies were considered as true hybrids.

(Mara x PHL 2789) F_1 Progenies

Only four SSR markers were polymorphic in this particular cross. Two SSR markers (CSM20 and EM131) showed 100% efficiency of detecting true hybridity wherein all the 30 F_1 progenies were confirmed as true hybrids (Figure 3). SSR markers EES063 and eme09E09 showed only maternal alleles in all the F_1 progenies tested (Table 4).

Any organism may be considered a hybrid due to introgression of genes or gene segments into certain parts of its genome that came from its paternal parent, and still have chromosomal segments which are of maternal origin. This is exhibited by all the progenies selected as hybrids. Progenies 3, 4, 8, 9, 10, 14, 15, 16, 18, 19, 21, 23, 24, 25, 26, 27, 28, 29 and 30 from the cross Mistisa x PHL 4841 were heterozygous when screened using SSR marker emf21I02 and were of maternal type when screened using SSR marker eme05B09 although these two markers from Nunome et al. (2009) are found to be located on the same linkage groups. This is possible since the SSR markers are found in different regions of the chromosome of the eggplant genome. The same case was found in the progenies from the cross Mara x PHL 2789. In regions detected by markers CSM20 and EM131, all the progenies are heterozygous. This means that the crossing of the two eggplant accessions were successful, hence the presence of both alleles from the female and male parents.

To have a successful hybridity testing, it is vital that the SSR markers selected for the screening will be able to clearly distinguish the heterozygotes. In this study, some of the markers that that did not show heterozygosity were not used in the final selection of hybrids.

In eggplant, one fertilization event leads to the formation of a single seed (Chen 1996). Therefore, it can be presumed that a progeny is indeed a product of cross-pollination given that in certain part of its genome, it

table 2 continuation

44	EES050	CTCCAGAATCTGCTCCTGTT	CCACCACCCATATCAAGAAT	-	166
45	EES051	CATCCACAATTTCAAAACAAA	TGAAAGCCATGAGATGCTAA	-	365
46	EES063	AGCAAACATTACAAAAGCAGTT	TCAGGCATCAGTATCACCAC	-	258
47	EES064	CAGCCGAAGTGATAAAGGTG	CCGAGATTAAACGAAAATGC	-	205
48	EES065	CATCAGACATATTCGGAGCA	AAGAGAGATGCAGAACCCTG	-	382
49	EES067	GGCCCTGCTTTGTTATATTT	CTCACAGTGCTGATCGTAGG	-	375
50	EES071	ACACAAACTGGCAACTTCAA	ATGCTTCGAGGACTTTTGTC	-	184
51	EES075	TTAATTTCGTCTGGACGTTG	TTCAAGCAAGCGACTGATTA	-	232
52	EES080	GCATCTGATATCCTTGACCC	CCAAACCAAATGGTAGGTTC	-	217
53	CSM4	GCGTACCAATTCTAACCACAAG	GTAATCCGCTTCCCATTTCTC	-	213
54	CSM12	CAATGGTATGTCTCCACTCGTC	AAGCTAAACATGAGATGCCGAT	-	210
55	CSM20	TTAGTGCCAGCAAAAATTGG	TTTTAAGCTTTAGCGCTCTCC	-	212
56	CSM31	CAACCGATATGCTCAGATGC	GCCCTATGGTCATGTTTTGC	-	259
57	CSM33	CTCCTCTTGGTGGAGCTCAG	TTTAGAGGGCGTTTGGATTG	-	231
58	CSM36	CCTCAATGGCAGTAGGTCAGA	GTTCTTTGAGCCTCCAGTGC	-	344
59	EM107	GGCCCCTAGACTGAGCTGAAATGTT	TGCTACAACCAACACAACCCTCAA	-	214
60	EM114	AGCCTAAACTTGGTTGGTTTTTGC	GAAGCTTTAAGAGCCTTCTATGCAG	-	221
61	EM117	GATCATCACTGGTTTGGGCTACAA	AGGGGAGAGGAAACTTGATTGGAC	-	160
62	EM126	GCATAGCTTATGAGTCAGGTGGCTTT	GCTCATCAAACCATCACATTCAAG	-	210
63	EM131	TCTGGGACACCAAGTGAAAAATCA	TGCGTTTTTGGCTCCTCTATGAAT	-	213
64	EM133	GCGGATCACCTGCAGTTACATTAC	TCCTTTGACCTATAGTGGCACGTAGT	-	177
65	EM141	TCTGCATCGAATGTCTACACCAAA	AAAAGCGCTTGCACTACACTGAAT	-	228

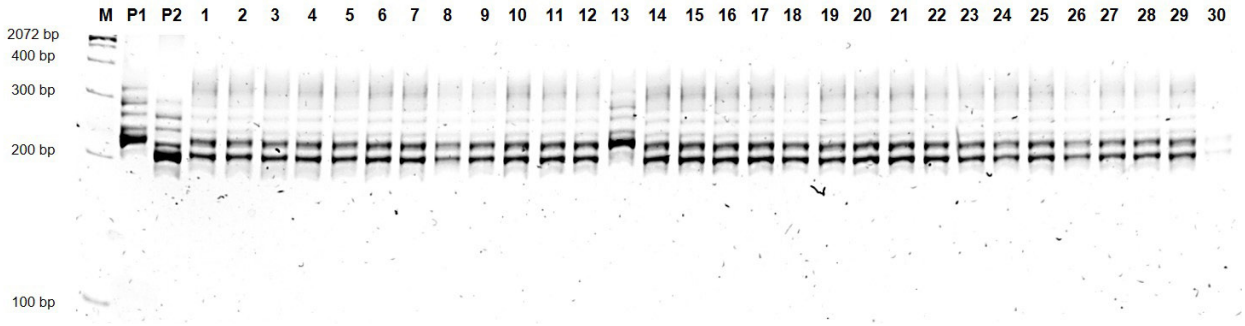

Figure 2. Hybridity testing of (Mistisa x PHL 4841) F_1 progenies using SSR marker emh11O01. Lanes M = 100 bp DNA ladder (Invitrogen); P1 = Mistisa; P2 = PHL 4841 and 1-30 F_1 progenies.

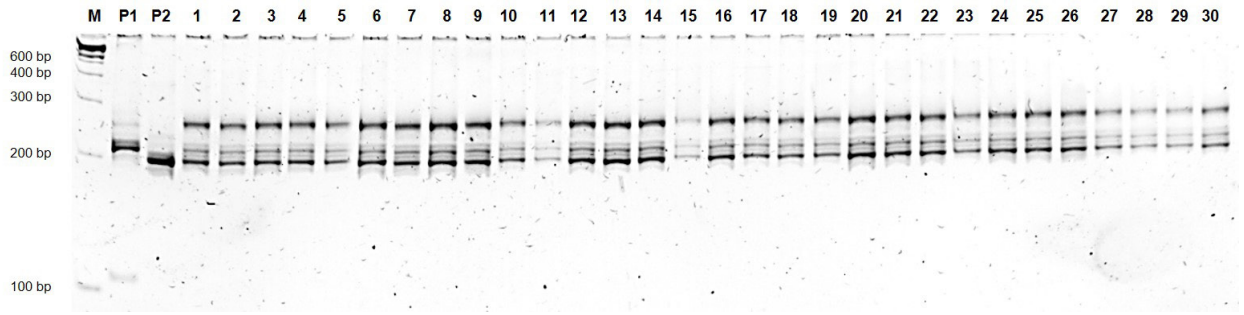

Figure 3. Hybridity testing of (Mara x PHL 2789) F_1 progenies using SSR marker CSM20. Lanes M = 100 bp DNA ladder (Invitrogen); P1 = Mara; P2 = PHL 2789 and 1-30 F_1 progenies.

60	M	M
60	M	M
57	M	P
58	M	M
55	M	M
53	M	M
53	M	M
53	M	M
53	M	M
58	M	M
58	M	M
57	M	P
65	M	M
60	M	M
68	M	M
68	M	M
65	M	M
60	P	M
65	M	M
65	M	P
65	P	M
65	P	M

contains segments that are inherited from its male parent. If it were an off-type, for example, a product of self-pollination, it should have had only the maternal allele exhibited in all the markers used in hybridity screening. The selected hybrids, although of maternal type in some of the SSR markers, exhibited heterozygosity in most of the SSR markers. This means that there was an introduction of paternal chromosome segments into the genome of the progeny and cross-pollination was successful.

CONCLUSION

This study shows that SSR markers can be used to successfully detect hybrids in eggplants. Screening SSR markers for polymorphism showed that different eggplant genotypes can be represented by different alleles which is helpful in identifying true hybrids among progenies. The use of SSR markers provided an early detection method to select and screen out plants even at an early stage of development.

Table 3. Summary of hybridity testing in (Mistisa x PHL 4841) F_1 progenies using six SSR markers.

Progeny No.	SSR Marker					
	EM133	**eme05B09**	**EM117**	**emh11O01**	**emf21I02**	**EM141**
1	maternal	maternal	hybrid	hybrid	maternal	hybrid
2	maternal	maternal	hybrid	hybrid	maternal	hybrid
3	maternal	maternal	hybrid	hybrid	hybrid	hybrid
4	maternal	maternal	hybrid	hybrid	hybrid	maternal
5	maternal	maternal	hybrid	hybrid	maternal	hybrid
6	maternal	maternal	hybrid	hybrid	maternal	maternal
7	maternal	maternal	hybrid	hybrid	maternal	hybrid
8	maternal	maternal	hybrid	hybrid	hybrid	maternal
9	maternal	maternal	hybrid	hybrid	hybrid	maternal
10	maternal	maternal	hybrid	hybrid	hybrid	hybrid
11	maternal	maternal	hybrid	hybrid	maternal	hybrid
12	maternal	maternal	hybrid	hybrid	maternal	maternal
13	maternal	paternal	paternal	maternal	maternal	paternal
14	maternal	maternal	hybrid	hybrid	hybrid	hybrid
15	maternal	maternal	hybrid	hybrid	hybrid	hybrid
16	maternal	maternal	hybrid	hybrid	hybrid	maternal
17	maternal	maternal	hybrid	hybrid	maternal	maternal

Table 3 continues next page . . . .

Table 3 continuation

18	maternal	maternal	hybrid	hybrid	hybrid	maternal
19	maternal	maternal	hybrid	hybrid	hybrid	maternal
20	maternal	maternal	hybrid	hybrid	maternal	hybrid
21	maternal	maternal	hybrid	hybrid	hybrid	maternal
22	maternal	maternal	hybrid	hybrid	maternal	hybrid
23	maternal	maternal	hybrid	hybrid	hybrid	hybrid
24	maternal	maternal	hybrid	hybrid	hybrid	hybrid
25	maternal	maternal	hybrid	hybrid	hybrid	hybrid
26	maternal	maternal	hybrid	hybrid	hybrid	hybrid
27	maternal	maternal	hybrid	hybrid	hybrid	hybrid
28	maternal	maternal	hybrid	hybrid	hybrid	hybrid
29	maternal	maternal	hybrid	hybrid	hybrid	hybrid
30	maternal	maternal	hybrid	hybrid	hybrid	maternal
Efficiency of SSR Markers in Hybrid Identification (%)	0	0	96.67	96.67	36.67	40

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Table 4. Summary of hybridity testing in (Mara x PHL 2789) F_1 progenies using four SSR markers.

Progeny No.	SSR Marker			
	CSM20	EM131	EES063	eme09E09
1	hybrid	hybrid	maternal	maternal
2	hybrid	hybrid	maternal	maternal
3	hybrid	hybrid	maternal	maternal
4	hybrid	hybrid	maternal	maternal
5	hybrid	hybrid	maternal	maternal
6	hybrid	hybrid	maternal	maternal
7	hybrid	hybrid	maternal	maternal
8	hybrid	hybrid	maternal	maternal
9	hybrid	hybrid	maternal	maternal
10	hybrid	hybrid	maternal	maternal
11	hybrid	hybrid	maternal	maternal
12	hybrid	hybrid	maternal	maternal
13	hybrid	hybrid	maternal	maternal
14	hybrid	hybrid	maternal	maternal
15	hybrid	hybrid	maternal	maternal
16	hybrid	hybrid	maternal	maternal
17	hybrid	hybrid	maternal	maternal
18	hybrid	hybrid	maternal	maternal
19	hybrid	hybrid	maternal	maternal
20	hybrid	hybrid	maternal	maternal
21	hybrid	hybrid	maternal	maternal
22	hybrid	hybrid	maternal	maternal
23	hybrid	hybrid	maternal	maternal
24	hybrid	hybrid	maternal	maternal
25	hybrid	hybrid	maternal	maternal
26	hybrid	hybrid	maternal	maternal
27	hybrid	hybrid	maternal	maternal
28	hybrid	hybrid	maternal	maternal
29	hybrid	hybrid	maternal	maternal
30	hybrid	hybrid	maternal	maternal
Efficiency of SSR Markers in Hybrid Identification (%)	100	100	0	0

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