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RESEARCH ARTICLE

Assessment of Genetic Diversity in Sesame (*Sesamum indicum* L.) Genotypes, Using EST-Derived SSR Markers

Vijay Yepuri[1,*], Malathi Surapaneni[1], Vijaya Sudhakar Rao Kola[1], Vemireddy LR[1], Jyothi B[2], Dineshkumar V[3], Anuradha G[1], Siddiq EA[1]

[1]*Institute of Biotechnology, Acharya NG Ranga Agricultural University, Rajendranagar, Hyderabad, 500030, India*
[2]*Directorate of rice research Rajendranagar, Hyderabad, 500030, India*
[3]*Directorate of oil seed research Rajendranagar, Hyderabad, 500030, India*

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Abstract

A total of 16,619 ESTs sequences (SSRs) of sesame (*Sesamum indicum* L.) were mined from Genbank. From sequences, 156 primer pairs were designed and characterized to determine the diversity among 49 sesame accessions. Twenty SSRs were found to be polymorphic and the number of alleles ranged from two to five per locus. The allele size varied from 101 to 399 bp. The average PIC value of the 20 SSR loci was 0.72 ranging from 0.49 (SEM-12-68) to 0.90 (SEM-12-27). Dendrogram analysis grouped the 49 genotypes into five separate clusters exhibiting a genetic similarity coefficient from 0.59 to 1.0. Hence, these EST-derived SSRs markers could be useful in assessing the diversity of sesame accessions and could also help in identifying diverse parents for sesame improvement programs.

Key words: diversity, ESTs, *Sesamum indicum* L., SSR markers

Introduction

Sesame (*Sesamum indicum* L.), considered the queen of oil seeds because of its oil quality and the presence of antioxidants like sesamin and sesamolin. Sesame belongs to the *Pedaliaceae* family, containing 60 species organized into 16 genera (Ashri 1998). The genus Sesamum comprises of 36 species (Kobayashi 1981). *Sesamum indicum* L. is the most commonly cultivated species (Nayar and Mehra 1970). Occasionally, the wild sesame species like *S. angusti folium* and *S. radiatum* are cultivated in Africa. Recent reports on molecular phylogeny analyses confirm that *Sesamum indicum* L. and *Sesamum orientale* var. *malabaricum* are the most closely related species (Bedigian 2010). These results provide unassailable evidence of domestication of sesame.

Bedigian (1981) reported that the chromosome number of the *Sesamum indicum* L. was 26 (2n) and the wild forms share the same diploid chromosome number (Hiremath 2008). Diversity in the Indian sesame collection (3,129 accessions) representing all eco-geographical regions, for a range of morphological and agronomic characters was studied by Bisht et al. in 1998. Sesame is generally considered a self-pollinated crop despite varying degrees of natural crosses ranging from less than 5 to over 50% (Pathirana 1994; Rheenen 1980).

India and China are world's largest sesame producers, followed by Myanmar, Sudan, Uganda, Ethiopia, Nigeria, Tanzania, Pakistan, and Paraguay. Among these, sesame India is the largest seasame growing country with 1.94 M ha accounting for about 25% of the global seasame cultivacted area. However, the yield of sesame (330.53 kg ha^{-1}) is considerably lower than the average world yield (442.73 kg ha^{-1}) (FAO:http://faostat.fao.org/ 2009).

Genetic diversity in different crop species can be determined using morphological and agronomic characteristics, isozyme analysis, and molecular marker analysis (Liu et al.1997). However, the use of morphological and agronomic characteristics is associated with a strong influence from environmental factors and is therefore dependent on the conditions during cultivation. Molecular markers overcome this

Vijay Yepuri (✉)
E-mail: vijayyepurister@gmail.com
Tel: +91- 8886280999 / Fax: +9140-24012695

limitation as they are not influenced by the environment. Recently, molecular marker technologies have become the encouraging method to identify plant genetic diversity. In sesame till date, the molecular markers like randomly amplified polymorphic (RAPD) (Adbellatef et al. 2008; Bhat et al. 1999; Ercan et al. 2004; Fazal Akbar et al. 2011; Kumar et al. 2009; Pham et al. 2011; Zhang et al. 2004), amplified fragment length polymorphism (AFLP) (Ali et al. 2007; Laurentin and Karlovsky 2006, 2007; Sun et al. 2007), inter-simple sequence repeats (ISSR) (Kim et al. 2002; Parsaeian et al. 2011; Kumar et al. 2012), simple sequence repeat (SSR) (Dixit et al. 2005; Gebremichael et al. 2010; Kumar et al. 2012; Nweke et al. 2012; Spandana et al. 2012;), and sequence-related amplified polymorphism (SRAP) (Li et al. 2007; Li and Quiros et al. 2001; Zhang et al. 2011) have been used to analyze the genetic variation analysis.

SSRs are powerful tool for the analysis of genetic diversity because they are often co-dominant, highly reproducible, most frequent, and reveal high allelic diversity. Sesame specific SSRs developed by hybridization method have been reported by Dixit et al. (2005), Jyothi et al. (2009), and Spandana et al. (2012). But only 146 numbers of genomic SSRs have been developed in sesame and only these have used in diversity analysis. Unfortunately, the de-novo development of SSRs is a costly and time-consuming endeavor (Squirrell et al. 2003; Zane et al. 2002). The rapid and inexpensive development of SSRs from expressed sequence tag (EST) databases has been shown to be a feasible option for obtaining high-quality nuclear markers (Bhat et al. 2005; Gupta et al. 2003). Characterization of the germplasm by using EST derived SSRs was reported by Bin et al. (2008) and Jyothi et al. (2009). Recently, EST-SSRs were developed by using sesame transcriptomes from different tissues of the plant. A total of 7,702 unigenes were converted into EST-SSRs by Wei et al. (2011). A total of 2,164 SSR primer pairs were identified in the 4,440 EST sequences (Zhang et al. 2012) with the objective of the generation of additional EST-SSRs and validation in a set of sesame genotypes.

Materials and Methods

Plant material

49 sesame genotypes were collected from diverse places of India. Approximately 25 seeds of each sesame accession were germinated in the greenhouse and leaves from 20-day-old sesame seedlings raised in a greenhouse were used for DNA isolation. The details of accessions are presented in Table 1.

DNA extraction

DNA extraction was carried out as per the protocol developed by Rao et al. (2010). DNA quality and concentration was measured using Nanodrop® ND-1000 spectrophotometer (Saveen Werner, Sweden).

Table 1. Accessions used for studying the properties of newly developed EST-SSRs

S.No	Genotype	State	Pedigree
1	CO1	Tamilnadu	(Tmv-3 X SI 1878) X SI 1878
2	CHANDANA	Andhrapradesh	T-85X 5107
3	GIYT2	Gujarat	NA
4	GOURI	Andhrapradesh	Selection from Kokkirapalli local A.P
5	GUJARATT3	Gujarat	NA
6	HIMA	Andhrapradesh	5039 X AT-1
7	HT1	Haryana	NA
8	IC110315	NA	NA
9	IC199439	NA	NA
10	IC208179	NA	NA
11	IC208612	NA	NA
12	IC295957	NA	NA
13	KAS0697	NA	NA
14	KKS98049	NA	NA
15	MADHAVI	Andhrapradesh	Selection from local A.P
16	MKN2	NA	NA
17	MKN7	NA	NA
18	MKN22	NA	NA
19	JLT26	Maharastra	NA
20	NSKMS12	NA	NA
21	NSKMS20	NA	NA
22	NSKMS115	NA	NA
23	NSKMS126	NA	NA
24	NSKMS129	NA	NA
25	NSKMS260	NA	NA
26	NSKMS261	NA	NA
27	NSKMS267	NA	NA
28	OSC362002	Orissa	NA
29	PAIYUR11	Tamilnadu	NA
30	PBT11	NA	NA
31	PHULETIL	Maharastra	NA
32	RAJESHWARI	Andhrapradesh	Selection from 62-39 of Chhatarpur local
33	RT54	Rajasthan	(M.P)
34	SHEKAR	Uttarpradesh	NA
35	SWETHA	Andhrapradesh	NA
36	TC25	Rajasthan	E-8 X IS-13
37	TMV4	Tamil Nadu	NA
38	TMV6	Tamil Nadu	Selection From Sattur Variety.
39	TMV3	Tamil Nadu	
40	TKG22	Madhyapradesh	local X Malbar Wild
41	T13	Uttarpradesh	NA
42	T13B	Uttarpradesh	NA
43	UMA	Orissa	NA
44	VRI1	Tamilnadu	NA
45	VRISV1	Tamilnadu	NA
46	YLM17	Andhrapradesh	Selection from Kokkirapalli local A.P
47	YLM11	Andhrapradesh	Selection from Kokkirapalli local A.P
48	YLM66	Andhrapradesh	YLM 17 X P.S.201
49	*S.mulayanum*	NA	NA

PCR and Electrophoresis

DNA amplification was performed in a reaction volume of 10 µL containing 50 ng DNA template (2 µL), 1 x PCR reaction buffer (15 mM Tris-HCl) (1 µL), 2 mM dNTPs (1 µL), 1U Taq DNA polymerase (Jonaki, CCMB, Hyderabad), 10 µM of forward and reverse primers 0.5 µL and sterile distilled water (4.8 µL). PCR was carried out in a *Veriti*™, *96-Well Thermal Cycler* (Applied Biosystems, CA, USA). The PCR conditions were 94°C for 5 min, followed by 94°C for 45 s, (Ta°C) for 45 s, 72°C for 1 min, then a final extension of 72°C for 10 min. The PCR products were fractionated on a 3% metaphor agarose (Lonza) gel for screening genotypes

with 0.05 μg μL^{-1} ethidium bromide. Samples were loaded with a reference 50 bp DNA ladder (NEB, U.S.A). Gels were electrophoresed at 120V. After separation, gels were documented using the Molecular Imager Gel Doc (BIO-RAD).

Development of SSR Markers for Sesame

Mining of ESTs database and primer design

A total of 16,619 sesame EST sequences were used from the NCBI's Expressed Sequence Tags (ESTs) database. WebSat, web software for microsatellite marker development was used to screen each sequence for the presence of microsatellites (www. http://wsmartins.net/websat). A criterion for the minimum number of repeat motifs was six for di, tri, tetra, penta, and hexanucleotides. A total of 143 SSR containing primer sequences were obtained, these 143 sequences selected for designing the primer pairs. (Supplementary Table)

Data scoring and analysis

The SSRs band profiles were scored as '1' for 'presence' and '0' for the 'absence' of band for each locus across the 49 genotypes. The molecular size of each fragment was determined by comparing with the molecular size markers. Cluster analyses were done using NTSYSpc vers. 2.1 program (Rohlf 2000).

Results

We performed mining for about 16,619 EST sequences; only 156 EST sequences were found to have SSR repeats with sufficient flanking regions for designing primers. Of the 156 primer pairs, a representative set of 50 which yielded clear scorable alleles were chosen for assessing genetic diversity analysis of 49 sesame accession (48 cultivated accessions, one wild). Of the 50 primers used, 20 were found to be polymorphic. The number of alleles per SSR marker ranged from 2 to 5 per locus with an average of 3.0. A wide range of fragment sizes was observed from 101 to 399 bp.

Genetic relationships between the 49 genotypes were assessed by UPGMA cluster analysis. Two major groups were identified at the similarity coefficient ranging from 0.79 to 1.00. Group I included 23 genotypes with a similarity coefficient of 0.86 to 1.00. Group I was further subdivided into four subgroups having varying degrees of similarity. Subgroup I included Co-1 and Giy-t-2 with a similarity coefficient of 0.88. In subgroup II, Gujarat-T-3, Hima, Chandana, and Gowri exhibited 100% genetic similarity. Subgroup III included 11 genotypes of which IC-208179, Kas-06197, and Madhavi showed 100% similarity. The remaining varieties showed 94 to 99% similarity among themselves. Subgroup IV included NSKMS-126, NSKMS-12, and JLT-26 showing 88% similarity. Group II included 22 genotypes divided into three subgroups with a similarity coefficient ranging from 81 to 100% genetic similarity. Subgroup III was the major cluster having 19 genotypes with a similarity coefficient ranging

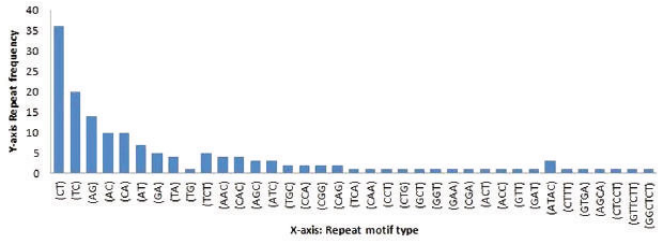

Fig. 2. Frequency of repeat motifs in156 EST-SSR primers in sesame.

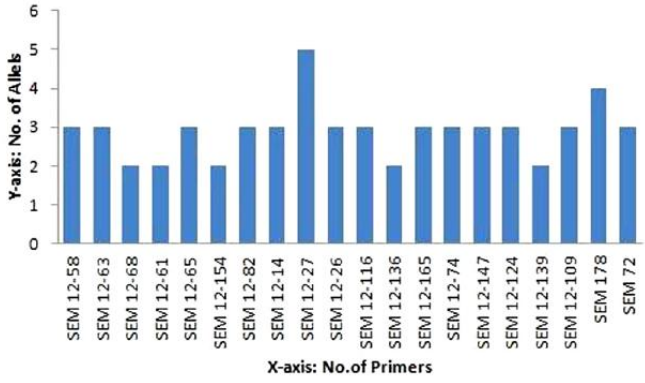

Fig. 3. Distribution of allele number.

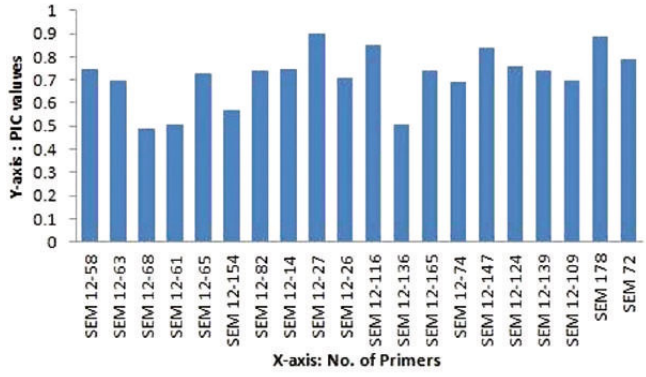

Fig. 4. Distribution of PIC values.

from 0.87 to 1.00. Most of the cultivated varieties like Swetha, Rajeswari, TMV-3, and RT-54 fell into this group. The only one wild species used in the study *S. mulayanum* was derived as a single cluster in this group.

YLM-66 and NSKMS-261 branched out as separate clusters with a similarity coefficient of 0.75 and 0.71. A small group containing cultivars like NSKMS-267 and OSC-36-2002 was found to be related to the rest of the varieties with a similarity coefficient of 0.59 (Fig. 1).

Discussion

EST-SSR markers were used in this study to evaluate the levels of genetic variation among different sesame genotypes. The choice of the EST-derived SSR markers was motivated by the fact that there was a very limited number of SSR markers available in this crop for the study of diversity

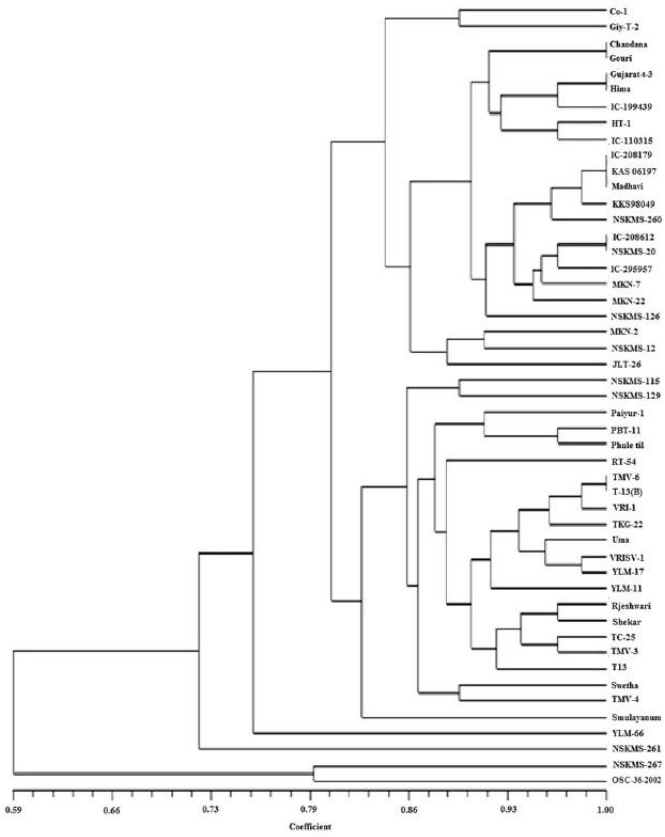

Fig. 1. Dendrogram showing the phylogenetic relationship between 49 sesame accessions cultivars

analysis and to construct a linkage map in sesame. The present study was carried out to evaluate diversity of 49 sesame accessions collected from different geographical regions of India. The genetic relationships among accessions were determined by using SSR markers. Dixit et al. (2005) first analyzed the genetic diversity by using the SSR markers in sesame. Bhat et al. (1999) studied the genetic diversity of 36 germplasm using 24 RAPD primers and found that the genetic similarity coefficients were between 0.19 and 0.89. Zhang et al. (2010) analyzed the genetic diversity of sesame germplasm using sequence-related amplified polymorphism (SRAP) and EST-SSR markers and reported that the genetic distance of foreign accessions (0.23) was significantly higher than domestic accessions (0.16). The PIC value demonstrates the informativeness of each SSR marker. Values of PIC ranging from 0 to 1 and loci having PIC values closer to 1 are more desirable (Mateescu et al. 2005). The average PIC value for all the 20 SSR loci was 0.718, with a range of 0.49 (SEM-12-68) to 0.90 (SEM-12-27).

Frequency and distribution of repeat motif types

Five different types of repeat motifs were observed, at frequencies of 68.6% (dinucleotides), 25% (trinucleotides), 3.84% (tetranucleotides), 0.64% (pentanucleotides), and 1.28% (hexanucleotides). Dinucleotide repeats were the most frequent SSR motif type observed. Among the dinucleotide repeats, AG/CT (32.05%) was the most frequent motif in our dataset, as earlier reported by Wei et al. (2011), approximately 46.29% of AG/CT repeat motifs. However, TA/TG motifs were very rare (3.20%). Among the trinucleotide repeats, the TCT/AAC motif was common (5.76%) among the microsatellites, respectively. The combined data set of amplified bands obtained for all genotypes was analyzed using the UPGMA method. The resulting dendrogram showed that cultivars were divided into four groups at a genetic similarity of 0.59%. The group I was the largest consisting of 23 cultivars. The maximum genetic similarity (100%) was observed with Chadana, Gowri, Gujarathi-T-3, Hima, IC-208179, KAS-06197, and Madhavi, while the lowest genetic similarity of 88.3% was observed with MKN-2, NSKMS-112, and JLT-26. Tmv-6 and T-13 (B) formed Group–II with 100% genetic similarity and the lowest genetic similarity was observed in S. mulayanum (82.6%). The highest yielding variety YLM-66 (75.3%) formed Group III and was distinctly different from the other cultivars. YLM-66 is known for its characteristic brown-colored seed with 51% oil content. Group IV included the cultivar NSKMS -261. Among the 49 cultivars tested, the NSKMS-267 and OSC-36-2002 were distinct from all the captives indicating the maximum percentage of diversity. This might be because of the pedigree which needs to be further analyzed.

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Supplementary Table. Properties of the isolated EST derived SSRs

S.No	Primer ID	Sequence	No. of Bases	Expected size (bp)	Repeat motifs	Genebank ID	Annealing temperature Ta°C
1	SEM12-01F	GGGACTCACTCACTCACTCACA	22	235	(CT)8	GI|372265999	46
	SEM12-01R	GAAGATCAGCAAACGGAAGAGT	22				
2	SEM12-02F	AGGGTCAAGGGTAGAAGAGGTC	22	223	(AG)11	GI|372265860	48
	SEM12-02R	GCCGAGAATGACTGATAGACAA	22				
3	SEM12-03F	AAAAGGTTTCTGCATCGTCTTC	22	392	(AT)7	GI|372265650|	55
	SEM12-03R	GAATTGGAAAGTTATTCAGCCG	22				
4	SEM12-04F	AGCACATCTACAAGTCAAGCCA	22	168	(CT)6	GI|372265539	50
	SEM12-04R	AGGGAGAGGAAAAGAATCCAAG	22				
5	SEM-12-05F	CTACTCTTCACTCCTTTCCCCA	22	117	(TC)6	GI|372265374	48
	SEM-12-05R	GGATGGCAGACTTTTGTTGAG	21				
6	SEM-12-06F	CCCCTTTTCTTCTCTTCTGCTT	22	194	(TCT)6	GI|372265252	47
	SEM-12-06R	GGACCGTTGTAGTATCCTTGTTG	23				
7	SEM-12-07F	TACTTAAACACCCGTCATTCCC	22	239	(AC)8	GI|372265045	45
	SEM-12-07R	TTGAGGTAGAAGCTCTTAGCGG	22				
8	SEM-12-08F	ATCACTACTGTCTGGCCTGGAT	22	281	(CAG)6	GI|372265088	50
	SEM-12-08R	TGGTTGTATTGGTATTGCTGCT	22				
9	SEM-12-09F	CACTTCAGTAGACGAGCGAGAA	22	346	(AG)6	GI|372264884	52
	SEM-12-09R	GACAACATCCATAAAACCAGCA	22				
10	SEM-12-11F	CTTTCTCCATTCTCATCCCATC	22	378	CA)6	GI|372264832	50
	SEM-12-11R	TCGATCCAGATGTACTCAGCC	21				
11	SEM-12-12F	AAAGCCTCAAACCCACAAAA	20	277	(TC)9	GI|372264830	50
	SEM-12-12R	ACCGTATGTATCCCAAGCTGAT	22				
12	SEM-12-13F	CATTAACCTCGCCACATCCT	20	200	(TGC)6	GI|372262867	50
	SEM-12-13R	AGCAGAAGAGGGGATCAGTTTT	22				
13	SEM-12-14F	ATAAGACTGCGAAAACCCTCAA	22	274	(AT)7	GI|372262856	55
	SEM-12-14R	ATGCTGATAATGGCAGACAGAA	22				
14	SEM-12-15F	GACAGAGAGCCGCAAACTAACT	22	299	(CT)7	GI|372262834	50
	SEM-12-15R	GGGAATAGATGAATGGATTGGA	22				
15	SEM-12-16F	CAAGAAGCTCATGGACTACCCT	22	163	(CCA)6	GI|372262827	55
	SEM-12-16R	GTAGACATTTGGAGGTGGAGGA	22				
16	SEM-12-17F	AAGTTGTGGTTCCTTGAGCATT	22	282	(CT)7	GI|372262701	55
	SEM-12-17R	CTTCCAGAGAGAGCAAGTCTCC	22				
17	SEM-12-18F	GATGATTTTCTCGGTCGCAT	20	379	(ATAC)6	GI|372262638	50
	SEM-12-18R	GCATGTATTCTGGTCATGGTGT	22				
18	SEM-12-19F	CTGTGGAGAGAGAGAGAGAGGG	22	345	(AG)17	GI|372262405	48
	SEM-12-19R	AGGACAGCTAAACACCCCAGTA	22				
19	SEM-12-20F	CCTGAGTCCCCTCTCGCT	18	299	(AG)7	GI|372262349	50
	SEM-12-20R	ATCCTCATTAGCTCCTCCTTCC	22				
20	SEM-12-21F	CACTTCAGTAGACGAGCGAGAA	22	303	(AG)6	GI|372262269	50
	SEM-12-21R	ACTTTCCCATCAACAAGGACAT	22				
21	SEM-12-22F	GGCCTTCTCTTGACCTTTTGT	21	299	(TC)6	GI|372262208	50
	SEM-12-22R	GAACTTGGAACGCATGTTGATA	22				
22	SEM-12-23F	GGCCTAGAAGGAAGACTGACTG	22	101	(GTGA)6	GI|372262021	46
	SEM-12-23R	ATTCCAACAAACCTGAAGGCTA	22				
23	SEM-12-24F	TTCGTCTTAATTTCTGCGACTG	22	135	(AG)7	GI|372261976	55
	SEM-12-24R	ACTTCTCGACCACTCCTATGCT	22				
24	SEM-12-25F	GTTCATCCTCCTTTCCACCAC	21	190	(TC)6	GI|372261877	50
	SEM-12-25R	CCGATAGTTGCTGCGATAAGAT	22				
25	SEM-12-26F	GGGGCTCTACTCTGCTACCACT	22	155	(CCA)6	GI|372261779	55
	SEM-12-26R	GAGCTGGATTATGGTGTTGAGG	22				
26	SEM-12-27F	GAATCCTCGATAACCAAACTGC	22	215	(CA)7	GI|372261756	55
	SEM-12-27R	AAGGGACCTCAACCATAACCTT	22				
27	SEM-12-28F	CATCAGTCCGTCTTTCCCTC	20	192	(CT)7	GI|372261724	50
	SEM-12-28R	TAGCAACCTCCAACAAAGTGTC	22				
28	SEM-12-29F	GGGGAACTATCCAAATTCATCA	22	384	(AT)9	GI|372261404	50
	SEM-12-29R	CCATAGACAGAGGGGTAAACGA	22				
29	SEM-12-30F	TCCCAACTCCATCCATCTTTAC	22	212	(TC)11	GI|372261365	48
	SEM-12-30R	GGAATGTGCTGGTACTTCAACA	22				
30	SEM-12-31F	TTCGTTTACACATTTGACCTGC	22	125	(AC)7	GI|372261278	46
	SEM-12-31R	GTGAAGCATCTGGTTTGATTGA	22				
31	SEM-12-32F	GACATCTTCGCTCCTTCTCTGT	22	164	(CT)10	GI|372261251	50
	SEM-12-32R	GCATGGTGATGAGAGCTGAGTA	22				
32	SEM-12-33F	AGAACTCACCCACTCTTTGTCC	22	149	(CT)8	GI|372261174	49
	SEM-12-33R	GATTGCCACTTCACTTCCTCTT	22				

S.No	Primer ID	Sequence	No. of Bases	Expected size (bp)	Repeat motifs	Genebank ID	Annealing temperature Ta°C
33	SEM-12-34F	TTCTGGTTCTGGAAATGTGAGA	22	399	(TA)7	GI|372261080	46
	SEM-12-34R	GAAAATTGTGCTAGTTCAGGCA	22				
34	SEM-12-35F	CCTTCTCTTGACCTTTTGTCGT	22	297	(TC)6	GI|372261037	50
	SEM-12-35R	GAACTTGGAACGCATGTTGATA	22				
35	SEM-12-36F	TTCCTTTTGATACCTTACCCCA	22	337	(AC)8	GI|372260985	48
	SEM-12-36R	GAGGGTGTTACGACAGGTCTTC	22				
36	SEM-12-38F	GCTTCAAATACGGAGAATACGG	22	131	(CTTT)7	GI|372260828	45
	SEM-12-38R	AGTAGTAATGGGATGGGGACG	21				
37	SEM-12-39F	CCCGCTGTCTGGAATTAGTATC	22	332	(CT)7	GI|372260731	55
	SEM-12-39R	GGGAAAATTATGCAAAGTCTGG	22				
38	SEM-12-40F	TCAGCTCTTCTCTTCTGCGTCT	22	360	(CT)11	GI|372260564	50
	SEM-12-40R	GATTTTGTTCTTCTGCCTTTGG	22				
39	SEM-12-41F	GATTCTCAGGCATTCCCATTC	21	282	(GTTCTT)6	GI|372260388	50
	SEM-12-41R	GGACTTTAACAAGCAGGGATCA	22				
40	SEM-12-42F	GGTGGTGCTATTATCTTCGCTC	22	237	(CT)7	GI|372260334	47
	SEM-12-42R	GAAAACTAGGCGGAAGTGTGTG	22				
41	SEM-12-43F	GCATTGGATTTTGTTGCGT	19	363	(ATAC)6	GI|372260219	51
	SEM-12-43R	GCATGTATTCTGGTCATGGTGT	22				
42	SEM-12-44F	GAATCTTCCTGTCGTGACTCGT	22	272	(TCA)6	GI|372260057	49
	SEM-12-44R	GTGGCGGTAGAGGAGTACCTG	21				
43	SEM-12-45F	GGGGCTCATAATTCTCTTTTCA	22	391	(CT)11	GI|372259918	50
	SEM-12-45R	ACATCCCTCATCTCATCCAAAC	22				
44	SEM-12-46F	TACTTAAACACCCGTCATTCCC	22	243	(AC)10	GI|372259876	46
	SEM-12-46R	TTGAGGTAGAAGCTCTTAGCGG	22				
45	SEM-12-47F	AAAAGAGAACTTGTGCCCGGAG	22	132	(AGC)6	GI|372259827	55
	SEM-12-47R	CTGAGCGGCATCGCCTTT	18				
46	SEM-12-48F	GAGTTGCTCCAATTAGCGTTCT	22	140	(AC)7	GI|372259779	51
	SEM-12-48R	AGCTTTGTTTCAGCCACCAT	20				
47	SEM-12-49F	CAGAAGTCATTCCTTGAAACCC	22	370	(CAC)6	GI|372259709	47
	SEM-12-49R	CGAAGGTGGAGGTGTGATG	19				
48	SEM-12-50F	TGTTTGCCTGTGAGGAAGAAG	21	311	(GA)6	GI|372259611	51
	SEM-12-50R	AGTGACCAGGACGGTTACATTT	22				
49	SEM-12-51F	CCTCAAAACACCCTCACACTTC	22	101	(CT)6	GI|372259594	51
	SEM-12-51R	GATACTCAACTTCGTTCGTCCC	22				
50	SEM-12-52F	CTTTGTTGAGGTGTGATCCAAG	22	206	(AG)19	GI|372259570	55
	SEM-12-52R	AGCAGAATACTTGAGAGCGTCC	22				
51	SEM-12-53F	ACGAAGCAGGTGAGACACG	19	188	(AG)11	GI|372259442	46.5
	SEM-12-53R	GCCGAGAATGACTGATAGACAA	22				
52	SEM-12-54F	TTCTCATCCCATCATCTCCTCT	22	274	(CT)6	GI|372259399	54.5
	SEM-12-54R	TTGTGAATGTGTGTGTGGTT	22				
53	SEM-12-55F	GTCCCTTTTATCCTGCACACTC	22	176	(TCT)6	GI|372259348	45.2
	SEM-12-55R	CGGAAGTAAGACAGAGAAAGCG	22				
54	SEM-12-56F	GTCCCTTTTATCCTGCACACTC	22	231	(TC)12	GI|372259348	45.2
	SEM-12-56R	TGTTGAGACATGGTGACAAGAA	22				
55	SEM-12-57F	GGAGTTGATGTTGGAGTGTCTG	22	224	(TA)6	GI|372259255	56.5
	SEM-12-57R	AGGTTTCTTGTGCTTGGGAATA	22				
56	SEM-12-58F	CACTCAACTGGGCTTCTTTCTT	22	194	(TA)7	GI|372259255	55
	SEM-12-58R	GGGGAACTTGTTGCTGGTATAA	22				
57	SEM-12-59F	CAGAAGTCATTCCTTGAAACCC	22	370	(CAC)6	GI|372259060	55
	SEM-12-59R	CGAAGGTGGAGGTGTGATG	19				
58	SEM-12-60F	GGGGAACAACAACCATATCAAT	22	288	(CT)8	GI|372259003	53.5
	SEM-12-60R	GTTTAGGAGTGGCCTTGTCTTG	22				
59	SEM-12-61F	GAAGAAGAAGGTGAGTGGGAGA	22	150	(GA)7	GI|372258991	55
	SEM-12-61R	GTAACTGATGAAGCTGGCTGAA	22				
60	SEM-12-62F	TCCCTCCTCTAGCTTCAAAGTG	22	394	(CAG)8	GI|372258615	51.5
	SEM-12-62R	CCAATTCTTTTGTGTCTTTCCG	22				
61	SEM-12-63F	GTGGATTTTCATTTCACCTCG	21	270	(TC)6	GI|372258591	55
	SEM-12-63R	GCTTTCCTTTTCTCAACCTCAA	22				
62	SEM-12-64F	CGTCTTTCTCACTCTACCACCC	22	393	(CT)8	GI|372258591	55
	SEM-12-64R	GCCAGGATTCACATGATAACAA	22				
63	SEM-12-65F	CCCCTCTCTCTCTCTCTGTG	22	157	(AC)7	GI|372258591	55
	SEM-12-65R	GTGAAAAGCCATAAGGTTGAGG	22				
64	SEM-12-66F	ACCTCTCCCATCTTACCTCCTC	22	276	(TC)10	GI|372258495	48
	SEM-12-66R	TGTTCTCGACCATCTCTTCGTA	22				

S.No	Primer ID	Sequence	No. of Bases	Expected size (bp)	Repeat motifs	Genebank ID	Annealing temperature Ta°C
65	SEM-12-67F	GATCCATCTTTACGTTTGGCTA	22	187	(TC)10	GI|372258411	44.2
	SEM-12-67R	TACTTCAACAGTCTCGCATGG	21				
66	SEM-12-68F	TGGTGAACTGTGTTAAAGGGTC	22	170	(CT)7	GI|372258388	55
	SEM-12-68R	TTCAAGAAAATAGGACCAGGGA	22				
67	SEM-12-69F	ATGTTCACTATCTTCCCCAAGC	22	222	(CA)8	GI|372258386	45.5
	SEM-12-69R	GCCTGAGTGGTTCAGTCGATA	21				
68	SEM-12-70F	ATCTTCCCCTTCTTCTTCTCC	22	235	(TCT)9	GI|372258316	54.5
	SEM-12-70R	TCTAGTGGTTGAGAATGAGCGA	22				
69	SEM-12-72F	TATTGCGCTGTTACTCTTTCCA	22	394	(TCT)13	GI|372258255	54.5
	SEM-12-72R	TCAACCAGTGCTAAAGTCAAGG	22				
70	SEM-12-73F	ACTTTATCATCTCTCCCTCCCC	22	163	(CT)6	GI|372258127	53.5
	SEM-12-73R	AGCTCGTAGACTTGGGTTGTTC	22				
71	SEM-12-74F	GTGCTGAACAAGACAAGGGAAT	22	354	(CTCCT)6	GI|372258051	52.4
	SEM-12-74R	GTTGAGGGTCGTAAGAATCACC	22				
72	SEM-12-75F	GATAGAGCCATTCCCTTTTCCT	22	383	(CT)14	GI|372258035	55
	SEM-12-75R	ACCAATCTTCACCTTCAGCTTC	22				
73	SEM-12-76F	ATCACCGTTCATCCTCCTTTC	21	383	(CT)6	GI|372257901	55
	SEM-12-76R	TATCCGACTTCTCGACCTTTTC	22				
74	SEM-12-77F	GCCTCATATCTACTCCCCTCCT	22	175	(CA)7	GI|372257874	54.5
	SEM-12-77R	CTCAAACTCAGGCATCCTTTCT	22				
75	SEM-12-78F	GATGTCAACCTGCATGAGAAAA	22	370	(AC)10	GI|372257114	45.2
	SEM-12-78R	TGGGTGTATGTGTGTTTGTGTG	22				
76	SEM-12-79F	AAATACACACACACACACCC	22	126	(AC)9	GI|372257114	51.7
	SEM-12-79R	GTTTCGCCACATTTTATGCT	20				
77	SEM-12-80F	CGTTGGAGCAGAAGATAAAAGC	22	315	(AC)6	GI|372257114	48.5
	SEM-12-80R	TGTGTGTGTGTGTGTTTGTGTG	22				
78	SEM-12-82F	TCGCTATCTTCTCCAGATTCAT	22	205	(CT)11	GI|372257506	55
	SEM-12-82R	GCACAAGCTATACTGCTCCG	20				
79	SEM-12-83F	GGGACACACACTCTCTCTCG	20	247	(CT)8	GI|372257410	45.5
	SEM-12-83R	CGAATCTCAGCCATCTTTATTG	22				
80	SEM-12-84F	AGTACGCAGAAGATCGAGGAAC	22	201	(CAA)12	GI|372257193	50.2
	SEM-12-84R	GTAGTCGGAGGTGAAGGAGGA	21				
81	SEM-12-86F	TGATTGTCCTGTTTGTGTCGTT	22	272	(CA)6	GI|372257159	58.8
	SEM-12-86R	GAAGGGGTCGGGGTAGATAG	20				
82	SEM-12-87F	GTCCAATTCATCCAACTCATCC	22	166	(AG)11	GI|372257152	52.8
	SEM-12-87R	GGAGAAGACGAAAGAGGTGCT	21				
83	SEM-12-88F	CTTTCTCCATTCTCATCCCATC	22	277	(CT)6	GI|372257114	50.2
	SEM-12-88R	ATGTGTGTGTGTGGTTGTGTGT	22				
84	SEM-12-89F	GATGTCAACCTGCATGAGAAAA	22	215	(AC)6	GI|372257114	51.7
	SEM-12-89R	ATTTACGGGTGTGTGTGTGT	22				
85	SEM-12-91F	ACTCGCATACCCGGAAAAG	19	238	(AG)13	GI|372256796	49.5
	SEM-12-91R	AATCAATAGCAATGGTGGGAAC	22				
86	SEM-12-93F	TATTCGTGCTCGTCAAACTCAC	22	268	(CCT)6	GI|372256682	49.5
	SEM-12-93R	AAGGGAAGGAAAAGACTGAAGC	22				
87	SEM-12-95F	CTCCTCCTCTCTCGTTCCTGTA	22	281	(CA)10	GI|372256462	45.2
	SEM-12-95R	CTTCGTGTCCTTCTTCTGCTTT	22				
88	SEM-12-97F	AGGGTCAAGGGTAGAAGAGGTC	22	126	(AG)12	GI|372256287	50.5
	SEM-12-97R	GGACGTTTGCACGCTCTC	18				
89	SEM-12-98F	CACTTTGACCTCACTTCTGCC	21	202	(CTG)7	GI|372256285	55
	SEM-12-98R	ACAACTGCAAGAACAACTGCAT	22				
90	SEM-12-99F	GGCACGCCACCTTAGTTC	18	188	(TC)8	GI|372256066	49.5
	SEM-12-99R	CCTCTGGCATTCATCTTCTTCT	22				
91	SEM-12-101F	GGGGCCTCGCCTCCATTC	18	244	(CT)8	GI|372255855	46.5
	SEM-12-101R	TGTGAACATGCCCATTTGCTGA	22				
92	SEM-12-102F	ACCGATCAACAGGAAGTTTAGC	22	365	(CGG)6	GI|372255771	50.5
	SEM-12-102R	GCATACGGATTGCTACTTGGTT	22				
93	SEM-12-103F	TGCCTCTCTCTCTTTCTTTCCA	22	185	(AG)7	GI|372255755	49.5
	SEM-12-103R	TGTTCTCCCTCTCTTTCCATGT	22				
94	SEM-12-104F	GATTTACTCGCTGGACGAAGAG	22	347	(ATC)10	GI|372255750	56.5
	SEM-12-104R	AACATGACGGAGACAGATTTCA	22				
95	SEM-12-106F	ACCTTTTGTCATCACCGTTCA	21	222	(TC)6	GI|372255595	44.5
	SEM-12-106R	TAGAGATCGAAGGGAACCGATA	22				
96	SEM-12-107F	AACCATTTTCTGCTCTAACCCA	22	383	(CT)7	GI|372255580	44.5
	SEM-12-107R	CGAGGATCTCTGTCCATTTCTT	22				

S.No	Primer ID	Sequence	No. of Bases	Expected size (bp)	Repeat motifs	Genebank ID	Annealing temperature Ta°C
97	SEM-12-109F	ATGGTCATGCTATTTACCTGGC	22	343	(GCT)8	GI|372255169	55
	SEM-12-109R	CCTGAAAATCGACCCAACTTAC	22				
98	SEM-12-111F	GACTAAAGCGAGAAGACGGAAA	22	376	(AT)7	GI|372255061	53.5
	SEM-12-111R	AAAGCTAAAGAAATCGACCGTG	22				
99	SEM-12-112F	AACGGTCCATGCCTGATAAC	20	156	(GGT)6	GI|372255058	50.5
	SEM-12-112R	ACTTGGTCTCTTTTCCGATGTC	22				
100	SEM-12-113F	CACGAGGAAAACGGGATG	18	155	(GAA)7	GI|372254995	55.5
	SEM-12-113R	ATATGATTTGGAGGTGGAGACG	22				
101	SEM-12-114F	CCCCAAAGAAGAAGACGACC	20	297	(CGA)7	GI|372254908	46.5
	SEM-12-114R	CTTCATAAACCCAACGAGATGC	22				
102	SEM-12-115F	GCTTGCTTAATTCTCATTCCCA	22	180	(ACT)6	GI|372254849	45.5
	SEM-12-115R	CAAACTAGACGAACCAAAAGGG	22				
103	SEM-12-116F	GGGAGCCTTATTTCTGTCTTCA	22	186	(TCT)11	GI|372254618	45.5
	SEM-12-116R	TAGCTCAATACCAGGAGCAAAA	22				
104	SEM-12-117F	GGAAGTCGGTTGTGTCTGAAAT	22	322	(AT)6	GI|372254594	47.5
	SEM-12-117R	CCTGTTGATCCTAATCGTCTGC	22				
105	SEM-12-120F	TGAATGTCGTCGTGCTCAGAT	22	213	(ACC)8	GI|372254472	51.7
	SEM-12-120R	AGGCCGTGGTGAAGGAAG	18				
106	SEM-12-122F	TTCTCATCCCATCATCTCCTCT	22	262	(CT)6	GI|372254094	46.5
	SEM-12-122R	GTGTGTGGTTGTGTGTGTGAAA	22				
107	SEM-12-124F	CCCCACACTCCTTTTCTCATTA	22	195	(CT)6	GI|372253914	55
	SEM-12-124R	CTCATTCCCATCAATCTCATCA	22				
108	SEM-12-125F	TCCTGTTTTCGGATAGCTTCAT	22	125	(TC)6	GI|372253903	44.5
	SEM-12-125R	GCGGTGGACTAGGAGTAGGTAA	22				
109	SEM-12-126F	TCCTCATCATCTCACTGTTCGT	22	272	(GTT)6	GI|372253895	56.5
	SEM-12-126R	TAAGCGTCGTAGGAGAAGGAAG	22				
110	SEM-12-127F	GCGTATTTGTTGTCCAAAAGGT	22	285	(GA)7	GI|372253833	47.5
	SEM-12-127R	TGATCCGCATTACTAGACCTGA	22				
111	SEM-12-128F	AATCAAAATCCATACCTCAGCG	22	324	(AGC)	GI|372253818	46.5
	SEM-12-128R	GAAAGTTCCAAGCAATAATCGG	22				
112	SEM-12-129F	GTTGATGTTGGAGTGTCTGTCG	22	221	(TA)6	GI|372253785	49.5
	SEM-12-129R	AGGTTTCTTGTGCTTGGGAATA	22				
113	SEM-12-130F	CCTTCACCTTTACTTGCATATCG	23	202	(TC)7	GI|372253730	44.5
	SEM-12-130R	AAGCATCTCAAAGACTGTTCGC	22				
114	SEM-12-131F	TTGAGGTTTTGAGGTTTTAGGG	22	304	(TC)12	GI|372253706	58.5
	SEM-12-131R	GCCTTCATTCCTAGCTTTAGCA	22				
115	SEM-12-132F	GAATCAAGACGAGAGTGGATCA	22	255	(AAC)7	GI|372253682	58.7
	SEM-12-132R	TGAGCTGGGTTAGATTGCTGTA	22				
116	SEM-12-133F	GGGGATAATGATGCTGTGTTTT	22	290	(TGC)7	GI|372253682	57.9
	SEM-12-133R	CTTGATTGTTGATGCTTGCCT	21				
117	SEM-12-134F	CTAAGCCTCCATCATCCGAATA	22	231	(GAT)7	GI|372253478	51.7
	SEM-12-134R	TCTCTTCCTCAGTCTCCTCACC	22				
118	SEM-12-135F	CGCTAATTCCAAGTCCAGACA	21	254	(CT)6	GI|372253305	44.5
	SEM-12-135R	ACGAAGAAATGTCCACTCCATT	22				
119	SEM-12-136F	CAACCTCAAACACTCCTAAAAG	22	270	(ATC)9	GI|372253276	44.5
	SEM-12-136R	GGTATGACGCAAAGGATAGATA	22				
120	SEM-12-137F	GGGTAGTGGTTCTTTCTTTTCCT	23	390	(GA)6	GI|372253231	48.5
	SEM-12-137R	TTCTCTCTCACACACGCTTCTC	22				
121	SEM-12-138F	GATTATATGCCCCTCCTTCCTC	22	348	(AAC)7	GI|372253127	55.
	SEM-12-138R	CGTCTGAATTATATCCTGCGTG	22				
122	SEM-12-139F	ATAAGATGGTTCCTGCTTGCAT	22	387	(AT)6	GI|372253066	55
	SEM-12-139R	GCCAAATCGAGGTAGAAGACAC	22				
123	SEM-12-140F	CACTTCAGTAGACGAGCGAGAA	22	345	(GA)6	GI|372252928	50
	SEM-12-140R	GACAACATCCATAAAACCAGCA	22				
124	SEM-12-141F	CCCCAAAGAAGAAGACGACC	20	297	(CGA)7	GI|372252681	48.5
	SEM-12-141R	CTTCATAAACCCAACGAGATGC	22				
125	SEM-12-142F	GGATTTTCTCGGTCGCATT	19	257	(ATAC)6	GI|372252416	44.5
	SEM-12-142R	AGGAAGCGATTGTAATGGATGT	22				
126	SEM-12-143F	CTCTCGTCTCCTAGAGGGTGTG	22	198	(CGG)6	GI|372252360	50
	SEM-12-143R	CGGTAAGAATCACGCTGGTAG	21				
127	SEM-12-144F	AAAGCCTCAAACCCACAAAA	20	358	(CT)10	GI|372252353	45.2
	SEM-12-144R	CAAGCAGGTCCAGAGAGAAAAT	22				
128	SEM-12-145F	GATTTACTCGCTGGACGAAGAG	22	208	(ATC)10	GI|372252245	55
	SEM-12-145R	TAAGTAGAAAAGGGTTCGGGGT	22				

S.No	Primer ID	Sequence	No. of Bases	Expected size (bp)	Repeat motifs	Genebank ID	Annealing temperature Ta°C
129	SEM-12-146F	TATCGTCATCGTCAATTCTTCG	22	242	(AT)8	GI|372252014	55
	SEM-12-146R	GTTGTAGAAAGGGAAGCTGGAA	22				
130	SEM-12-147F	GCGATTTTATCCCCTTTCACTT	22	330	(TC)7	GI|372251976	55
	SEM-12-147R	ACTTGAGCTTCTCCCTGAGCTT	22				
131	SEM-12-148F	CCTTTTGTCCTTTCACCACAC	21	305	(CAC)9	GI|372251853	45.2
	SEM-12-148R	ATTTGACATCCTCCTCCTCATC	22				
132	SEM-12-149F	TCAGCTCTTCTCTTCTGCGTCT	22	360	(CT)11	GI|372251696	44.2
	SEM-12-149R	GATTTTGTTCTTCTGCCTTTGG	22				
133	SEM-12-150F	TTCTCATCCCATCATCTCCTCT	22	274	(CT)6	GI|372251677	44.2
	SEM-12-150R	TTGTGAATGTGTGTGTGTGGTT	22				
134	SEM-12-151F	ACAACCCAAGTCTACGAGCTTC	22	199	(CA)6	GI|372251677	44.2
	SEM-12-151R	TCGATCCAGATGTACTCAGCC	21				
135	SEM-12-153F	CTTTCTCCATTCTCATCCCATC	22	380	(CA)6	GI|372251648	45.2
	SEM-12-153R	TCGATCCAGATGTACTCAGCC	21				
136	SEM-12-154F	GGGGCCTTTTATTTGCAC	18	240	(TC)8	GI|372251613	55
	SEM-12-154R	CTGATTACTGTCTACATCGGGG	22				
137	SEM-12-155F	TCTTCATTTCTGGGGTTCTTGT	22	230	(AG)8	GI|372251531	44.2
	SEM-12-155R	GTTCCGCCAATGCTGTTACTAC	22				
138	SEM-12-156F	CACCAGTCCGTCTTTCCCT	19	243	(CT)7	GI|372251423	44.5
	SEM-12-156R	AATGGGCTGATAGTGGTCGTAG	22				
139	SEM-12-159F	TCCCAACTCCATCCATCTTTAC	22	202	(TC)11	GI|372251246	46.5
	SEM-12-159R	CTGGTACTCCAACAGTCTCGC	21				
140	SEM-12-163F	AGCTGAACCGAAAATAAGACGA	22	291	(TC)6	GI|372250987	50.5
	SEM-12-163R	CTTTCTTGCTCACACACACACA	22				
141	SEM-12-164F	CAGACATAGCTGAACCGAAAAT	22	333	(TG)6	GI|372250987	52.5
	SEM-12-164R	GGTAGTGAAAATCTAGTCGTACTCTTC	27				
142	SEM-12-165F	TGACAATTCTTACACGCACCA	21	138	(CA)8	GI|372250881	55
	SEM-12-165R	AGCCTTCCCATTCTTCCATT	20				
143	SEM-12-166F	TTCTATCCTTGCCTCTACTCGC	22	196	(AAC)6	GI|372250733	50.5
	SEM-12-166R	GCAGGACTTCCATTGTTCATCT	22				
144	SEM-12-167F	CAGAAGTCATTCCTTGAAACCC	22	369	(CAC)6	GI|372250555	46.5
	SEM-12-167R	CGAAGGTGGAGGTGTGATG	19				
145	SEM-12-169F	ACATACACACACCAGGAGGGAT	22	257	(GCC)	GI|372250363	50.5
	SEM-12-169R	TGCTTCTTCACAGTTGCTTGAT	22				
146	SEM-12-170F	CTCTTTCTCCCACCCTCTACCT	22	269	(AG)13	GI|372250293	50.5
	SEM-12-170R	GTATCCTGCTTGTTCTTCACCC	22				
147	SEM-12-171F	CCTGACTTGTCTGTCCCTTTCT	22	302	(CTCA)6	GI|372250277	53.5
	SEM-12-171R	GCGAATCTCAGCCATCTTTAT	21				
148	SEM-12-172F	ACTCACCCACTCACTCACACAC	22	232	(CT)8	GI|372250277	47.5
	SEM-12-172R	GAAGATCAGCAAACGGAAGAGT	22				
149	SEM-12-174F	AACAACTCCCAACCAGCACTAT	22	104	(AGC)6	GI|372249801	56.5
	SEM-12-174R	GACGTGAAGTTTGTCGAGAGGT	22				
150	SEM-12-175F	ACTTTATCATCTCTCCCTCCCC	22	245	(CT)6	GI|372249768	49.5
	SEM-12-175R	TTGTGAATGTGTGTGTGTGGTT	22				
151	SEM-12-176F	ACAACCCAAGTCTACGAGCTTC	22	199	(CA)6	GI|372249768	52.5
	SEM-12-176R	TCGATCCAGATGTACTCAGCC	21				
152	SEM-12-177F	CTACTCTTCACTCCTTTCCCCA	22	117	(TC)6	GI|372249651	50.5
	SEM-12-177R	GGATGGCAGACTTTTGTTGAG	21				
153	SEM-12-178F	GCCCACCCATAGAAAGAAAA	20	235	(CT)9	GI|372249620	55
	SEM-12-178R	TTCTGCCCTAACCTCTCAACTC	22				
154	SEM-12-179F	CTCTGGCTCCGGCTCTATC	19	245	(GGCTCT)23	GI|372249526	46.5
	SEM-12-179R	TCTCACAAGGGGTGGTCG	18				
155	SEM-12-180F	CTTGTTCCTCTTTCCATCCTTG	22	207	(AAC)6	GI|372249458	44.5
	SEM-12-180R	GCAGGACTTCCATTGTTCATCT	22				
156	SEM-12-181F	GATTGACCTGATTGAGCTGGAC	22	149	(AGCA)6	GI|372249433	44.5
	SEM-12-181R	GGAAGGTGAGTGGAGAAAACTG	22				