American Journal of Plant Sciences, **2012, 3, 1169-1180**
http://dx.doi.org/10.4236/ajps.2012.39142 Published Online September 2012 (http://www.SciRP.org/journal/ajps)

Scientific Research

Transferability of Sorghum Genic Microsatellite Markers to Peanut

Siddanna B. Savadi[1], Bashasab Fakrudin[1*], H. L. Nadaf[2], M. V. C. Gowda[2]

[1]Department of Biotechnology, University of Agricultural Sciences, Dharwad, India; [2]Department of Genetics and Plant Breeding, University of Agricultural Sciences, Dharwad, India.
Email: bfakrudin@gmail.com

Received April 14th, 2012; revised May 12th, 2012; accepted May 20th, 2012

ABSTRACT

Currently development of new marker types has shifted from anonymous DNA fragments to gene-based markers. Simple Sequence Repeats (SSRs) are useful DNA markers in plant genetic research including in peanut. However, *de novo* development of SSRs is expensive and time consuming. Gene-based DNA markers are transferable among related species owing to the conserved nature of genes. In this study transferability of sorghum EST-SSR (SbEST-SSR) markers to peanut was prospected. A set of 411 SbEST-SSR primer pairs were used to amplify peanut genomic DNA extracted from cultivated peanut where 39% of them successfully amplified. A comparison of amplification patterns between sorghum and peanut showed similar banding pattern with majority of transferable SbEST-SSRs. Among these transferable SSR markers, 14% have detected polymorphism among 4 resistant and 4 susceptible peanut lines for rust and late leaf spot diseases. These transferable markers will benefit peanut genome research by not only providing additional DNA markers for population genetic analyses, but also allowing comparative mapping to be possible between peanut and sorghum—a possible monocot-dicot comparison.

Keywords: Arachis Hypogea; Transferability; EST-SSR; Polymorphism

1. Introduction

Peanut (*Arachis hypogaea* L.) is an important oilseed crop and it has acquired prominence because of its economic importance as well as its nutritional value. It is the third major oilseed crop in the world next only to soybean and cotton. *A. hypogaea* is believed to have originated in the Southern Bolivia to Northern Argentina region of South America. The present day cultivated peanut is an allotetraploid (2n = 4x = 40) while most of wild relatives are diploid (2n = 2x = 20) in nature. The yield of the peanut crop has been very low due to biotic and abiotic stresses and the varietal improvement in peanut has been difficult due to the limited knowledge on the inheritance of important traits and lack of proper understanding of genetic diversity and population structure. Molecular markers have become an important tool in crop breeding programs for dissecting loci controlling complex traits: genetic diversity among accession and evolutionary conservation studies can be done. However, application of molecular markers in peanut crop improvement has been relatively lagging behind chiefly owing to limited knowledge of genome and seldom molecular variations revealed by RFLP [1], RAPD [2] and isozyme markers systems [3]. Indeed, there is an urgent need to focus efforts on a systematic and comprehensive examination of the germplasm accessions available in the peanut employing robust marker types such as SSRs to reveal polymorphism at the molecular level [4]. Simple Sequence Repeats (SSRs) or microsatellites have become one of the most widely preferred molecular marker systems for genetic analysis for their advantages compared to other molecular markers: high reproducibility, high polymorphism, being multi-allelic, co-dominant, higher relative abundance and extensive genome coverage are some of the advantages envisaged with SSRs [5]. Previous studies in peanut have shown that SSR markers could detect more polymorphism than other molecular markers like RFLPs [6], AFLPs [7] and RAPDs [8,9].

The *de novo* development of SSRs markers is a costly and time-consuming endeavor [5,10], as it involves approaches, such as genomic library construction, enrichment and screening which are laborious and time consuming: this reduces the general utility of this marker system [11] and also dramatically discounts the advantages [12]. The progress of development or discovery of new marker types has shifted from anonymous DNA fragments to gene-based markers, also called as functional markers. Gene based markers are more powerful

*Corresponding author.

Copyright © 2012 SciRes.

than others for breeding applications and allele discovery [13]. ESTs are presently used on a large scale for the systematic development of gene-based SSR and SNP markers. EST-SSR markers have been developed for a number of plant species, such as pigeon pea [14], grape [15], rice [16], durum wheat [17], rye [18], barley [19], ryegrass [20], wheat [21], peanut [22] and cotton [23]. EST-SSRs are advantageous over genomic SSRs, as they can be obtained from public EST databases.and transferable across taxonomic barriers [24]. A putative function can be deduced for the EST-SSRs as they represent ESTs, they serve as gene-tagged markers and can be directly associated with an expressed gene: this offers linking with putative qualitative or quantitative trait locus alleles. Thus, EST-SSR markers are superior and more informative compared to anonymous markers [25]. Comparative genetic analysis has shown that different plant species often share orthologous genes for very similar functions [26] and gene contents and gene orders among different plant species could be highly conserved [27,28].

As EST-SSR markers are derived from expressed genes, they are more conserved and have a higher level of transferability to related species. Study of transferability of markers has been attempted in several plant species across different taxa [14,15,19,29-32] as well as in peanut [12,33,34]. However, the conserved nature of EST-SSRs may also limit their degree of polymorphism. The feasibility of utilizing EST-SSRs from monocots in dicots has been investigated. Plant genes display significant conservation between the monocots and dicots, thus, theoretical possibility of transferability from monocots to dicots is a possibility [35]. Requiring more concerted efforts in using modern genomic tools, peanut genome research has made less progress [36,37]. Thus, one of the pressing needs in peanut genomic research is to take advantage of progress made in the well characterized other crops. About 25% SSRs [38] and 34% EST-SSRs [39] transferability from soybean to peanut has been reported [40]. A 20% transferability of EST-SSRs from *Medicago* to peanut has been reported. In this study, we focused on analyzing the utility of EST-SSR markers from sorghum (monocot) to peanut (dicot) experimentally. Sorghum is considered to be model grass genome where genetic study has been done at good pace [41] and its genome sequencing is completed [42], and hence it could be good source of transferable markers especially the gene-based markers.

2. Materials and Methods

Total DNA from sorghum cultivar E36-1 and a set of four resistant and four rust and leaf spot diseases susceptible peanut cultivars (**Table 1**) was isolated following CTAB protocol of Murry and Thompson (1980) [43] with suitable modifications. The genomic DNA was used as the template for all PCR amplifications. Sorghum EST-SSRs (SbEST-SSRs) developed at IABT, UAS, Dharwad and synthesized from Sigma-Aldrich pvt. Ltd, USA, were screened for amplification of peanut DNA using optimized PCR reaction mixture and touchdown PCR Profiles. PCR optimization was done using three different programs of "Touchdown" PCR [44] with base annealing temperature ranges of 55˚C - 50˚C, 60˚C - 55˚C, and 65˚C - 60˚C. The primers were classified into three groups based on annealing temperature range required by them to produce sharp bands without much of spurious products. In the initial annealing steps, the annealing temperature was decreased by one centigrade after two subsequent cycles for first 10 cycles. Products were thereafter amplified for 30 cycles at the appropriate optimum annealing temperature with a final extension of 20 min. Reaction mixtures of 10 μl containing 10 mM Tris-HCl (pH 8.8), 50 mM KCl, 10 mM $MgCl_2$, 2.5mM of each of dNTPs, 20 pM each of forward and reverse primers, 50 ηg of genomic DNA and 2.5U *Taq* DNA polymerase (Fermentas) was used for PCR amplification.

Transference is defined as the positive amplification of a PCR band of the expected size [45]. SbEST-SSR primers amplified during primer screening of peanut were also used for comparing amplification patterns (size and

Table 1. Transferable markers were checked for their ability to detect polymorphism in following eight groundnut cultivars.

Sl No	Genotypes	Rust	Late leaf spot	Phenotypes
1	LSVT-I-2003-1	2	2	R
2	ISK-I-2004-4	2	1	R
3	IVT-I-2005-5	2	1	R
4	GPBD-4	3	3	R
5	JL-24	7	8	S
6	TMV-2	7	8	S
7	TAG-24	7	8	S
8	TG-26	7	8	S

Cultivars with a 1 - 3 disease score were designated as resistant and a 4 - 9 score as susceptible according to Pande and Rao (2001).

Copyright © 2012 SciRes.

number of bands) to further confirm orthology or transferability by carrying out amplification in both sorghum and peanut DNA using optimized PCR reaction mixture and touchdown PCR profiles. The transferable SbEST-SSRs were then tested for their ability to detect polymorphism in a set of four resistant and four susceptible breeding lines/cultivars (**Table 1**) for rust and late leaf spot diseases of peanut and the ones showing polymerphic banding pattern on 4% PAGE gel were considered as polymorphic markers.

3. Results

3.1. Screening of SbEST-SSRs and Comparison of Amplification Pattern between Sorghum and Peanut and Their Polymorphism in Peanut Cultivars

Out of 411 sorghum EST-SSR primer pairs tested, 161 (39%) were amplifiable in peanut (**Table 1**) showing clear sharp bands but other primers gave smear with light bands or did not amplify under three Touch Down (TD)-PCR profile conditions (**Figure 1**). The remaining primer pairs either recorded no amplification products or produced a number of faint bands indicating non-specific amplifications. Out of 161 amplified primers 16 amplified at 65˚C - 60˚C, 95 at 60˚C - 55˚C and 61 at 55˚C - 50˚C TD-PCR temperature ranges (**Table 2**). These amplifiable markers implied that 39% of primer-binding sites were conserved between sorghum and peanut genomes. These primer pairs produced clear PCR bands and the majority of primers produced multiple bands. The number of bands amplified by each SbEST-SSR primer pairs varied from 1 to 16 on 4% polyacrylamide gel stained following silver staining procedure (**Figure 2**). Further, comparison of the kind of amplification pattern between sorghum and peanut crop species showed similar banding pattern for many of the SbEST-SSRs; however, it varied in some of the cases (**Figure 3**) and the difference was in number of bands amplified, which were more or less in either of the crops. Of the161 EST-SSRs 18% were found polymorphic on 4% polyacrylamide gel.

3.2. Structural Analysis and Annotations of SbEST-SSRs

Among these conserved SbEST-SSRs, 91 (56%) dinucleotide repeat motifs appeared to be the most abundant type, followed by 61 (37.2%) trinucleotides, 8 (6.3%) consisting of complex nucleotide repeats and then one tetranucleotide (0.5%) repeats (**Table 3**). Among these repeats TC/CT were 35, AG/GA were 28, AGC/CAG/GCA were 17 and AGC/CAG/GCA were 16. The composition of repeat motifs of transferred SbEST-SSRs is presented in **Table 4**. Of 161 SbEST-SSR markers, 24 (14%) detected polymorphism on 4% PAGE among 8 accessions of peanut consisting of equal number of resistant and susceptible accessions (**Figure 2**). Twenty four polymorphic SbEST-SSRs consisted of 17 (18.7%) dinucleotide repeats, 6 (9.8%) trinucleotide repeats and one (1.2%) consisted of complex repeat motifs. These results indicate that there is some kind of correlation between polymorphism and repeat number. The distribution of transferred SSRs' positions was found highest in 5'ESTs with 67, then 32 in 3'ESTs and 62 in other ESTs. Among these the SSRs from 3'ESTs were more polymorphic (21.9%) than 5'ESTs (13.4%) and others (12.9%) (**Table 4**).

Annotation for the common SbEST-SSRs was performed using the GenBank databases and BLASTX tool with an expectation value of 1e-5 or better. Eighty three (52%) of the common ESTs were annotated using BLASTX and are listed in **Table 5**. Most of annotated SbESTs were related to metabolism, photosynthesis, signal transduction, growth, and transportation across membranes, stress and defense. The remaining SbESTs when searched for putative functions resulted in no hits (2.5%),

Table 2. Screening results for 411 SbEST-SSR primers on groundnut genotypes: total number and the percentages of primer pairs amplified at three different Touch Down PCR profiles.

Sl. No	PCR	No of Primers	Rate	65˚C - 60˚C	60˚C - 55˚C	55˚C - 50˚C
1	Amplified	161	39%	19 (12%)	81 (50%)	61 (38%)
2	Unamplified	250	61%	-	-	-
	Total	**411**	**100%**	**-**	**-**	**-**

Table 3. Structural details of repeat motifs in transferable SbEST-SSRs and polymorphism revealed by them in groundnut.

Type	Dinucleotides	Trinucleotides	Tetranucleotide	Complex
Amplified	91	61	1	8
Per cent Amplification	56.5	37.9	0.6	5
Polymorphic	17	6	0	1
Per cent Polymorphic	18.7	9.8	0	1.2

Copyright © 2012 SciRes.

Table 4. Distribution of repeats location or SSRs positions in ESTs of transferable SbEST-SSRs.

Type	3'ESTs	5'ESTs	Others
Amplified	32	67	62
Per cent Amplification	19.9	41.6	38.5
Polymorphic	7	9	8
Per cent Polymorphic	21.9	13.4	12.9

Table 5. Description of SbEST-SSR markers transferred to groundnut: NCBI accession ID, repeat motifs, their ability to detect polymorphism among groundnut accessions and their functional annotation by BLASTX.

Sl No	GI No	PRIMER	PUTATIVE FUNCTION	E-value	Repeat Motif	P/M	Temp Condn
1	30936043	*iabtgs*83	Eukaryotic translation initiation factor 3	2.00E–15	(CCG)n (CGC)n (GCC)n	M	T-III
2	31332261	*iabtgs*144	Carbamoyl-phosphate synthase l atp-binding	3	(TCC)n (CTC)n (CCT)n	M	T-II
3	31331586	*iabtgs*145	Armadillo/beta-catenin repeat family protein	3.00E–12	(CCG)n (CGC)n (GCC)n	M	T-II
4	31330911	*iabtgs*146	Putative auxin response factor 10		(AGG)n (GAG)n (GGA)n	M	T-III
5	30976409	*iabtgs*148	Unknown protein	2.00E–06	(CTAG)n	M	T-III
6	30975909	*iabtgs*149	Membrane protein-like	3.00E–23	(AGC)n (CAG)n (GCA)n	M	T-II
7	30969042	*iabtgs*151	Hypothetical protein osj_010305	4.00E–10	(AGG)n (GAG)n (GGA)n	M	T-III
8	30966593	*iabtgs*152	Heat shock complementing factor1	1.00E–17	(AGC)n (CAG)n (GCA)n	M	T-II
9	30963637	*iabtgs*155	Dna methylase n-4/n-6 domain protein	3.8	(AGC)n (CAG)n (GCA)n	M	T-III
10	30946150	*iabtgs*157	Dnaj heat shock n-terminal domain-containing	7.00E–12	(AGG)n (GAG)n (GGA)n	M	T-III
11	18061855	*iabtgs*158	Hypothetical protein osi_023589	8.00E–29	(TCG)n (CGT)n (GTC)n	M	T-III
12	30935909	*iabtgs*162	Gdsl-motif lipase/hydrolase family protein	1.00E–56	(TCG)n (CGT)n (GTC)n	M	T-II
13	18061163	*iabtgs*164	Putative vascular plant one zinc finger protein	2.00E–27	(AGC)n (CAG)n (GCA)n	M	T-III
14	14593539	*iabtgs*165	Putative peroxidase	9.00E–48	(AGC)n (CAG)n (GCA)n	M	T-II
15	12616750	*iabtgs*170	Hypothetical protein	8.00E–70	(TCG)n (CGT)n (GTC)n	M	T-II
16	12776313	*iabtgs*173	Hypothetical protein	0.16	(TCC)n (CTC)n (CCT)n	M	T-III
17	12501226	*iabtgs*174	Hypothetical protein		(TCG)n (CGT)n (GTC)n	M	T-II
18	12498888	*iabtgs*175	Hypothetical protein	1.00E–32	(TGG)n (GTG)n (GGT)n	M	T-III
19	11996438	*iabtgs*177	Hypothetical protein	9.00E–08	(CGG)n (GCG)n (GGC)n	M	T-III
20	11920940	*iabtgs*178	No significant similarity found		(AGC)n (CAG)n (GCA)n	M	T-III
21	11679357	*iabtgs*179	Leucine-rich repeat transmembrane protein	5.00E–23	(TTC)n (TCT)n (CTT)n	M	T-III
22	11677886	*iabtgs*181	Hypothetical protein		(AGT)n (TAG)n (GTA)n	M	T-II
23	9850409	*iabtgs*188	CBS domain-containing protein	1.00E–65	(TCC)n (CTC)n (CCT)n	M	T-III
24	9299930	*iabtgs*190	Di-haem cytochrome c peroxidase	3.2	(AGC)n (CAG)n (GCA)n	M	T-II
25	9299634	*iabtgs*191	Putative fatty acid elongase	9.00E–25	(GCG)n (GGC)n	M	T-II
26	7553794	*iabtgs*196	hypothetical protein	2.00E–06	(ACG)n (CGA)n (GAC)n	M	T-III
27	7551234	*iabtgs*198	AT hook-containing DNA-binding protein	2.00E–10	(AGT)n (TAG)n (GTA)n	M	T-II
28	7535116	*iabtgs*199	No significant similarity found		(TCG)n (CGT)n (GTC)n	M	T-II
29	7553718	*iabtgs*200	ripening regulated protein	5.00E–08	(TTC)n (TCT)n (CTT)n	M	T-III
30	30161896	*iabtgs*201	NADPH-thioredoxin reductase	3.00E–87	(CCG)n (CGC)n (GCC)n	P	T-II
31	31347554	*iabtgs*202	unnamed protein product	6.00E–42	(CCG)n (CGC)n (GCC)n	M	T-II
32	34446671	*iabtgs*203	CYP77B1 (cytochrome P450, family 77	4.00E–54	(CCG)n (CGC)n (GAC)n (GCC)n	M	T-III

Copyright © 2012 SciRes.

Continued

33	30977482	*iabtgs*205	transporter-related	1.00E–35	(AGG)n (GAG)n (GGA)n	M	T-II
34	34512855	*iabtgs*207	hypothetical protein OsI_000407	6.00E–75	(TTG)n (TGT)n (GTT)n	M	T-III
35	7536147	*iabtgs*208	putative xyloglucan endotransglycosylase	9.00E–46	(CGG)n (GCG)n (GGC)n	M	T-II
36	30950922	*iabtgs*209	3-ketoacyl-CoA synthase	1.00E–51	(AGC)n (CAG)n (GCA)n	M	T-III
37	45949294	*iabtgs*210	ETHYLENE-INSENSITIVE3-like 1 protein	3.00E–45	(AGC)n (CAG)n (GCA)n	M	T-II
38	30968428	*iabtgs*220	putative copper chaperone	5.00E–27	(TC)n (CT)n /(TGC)n (CTG)n (GCT)n	M	T-II
39	57812929	*iabtgs*221	ACR4 (ACT REPEAT 4)	2.00E–26	(ACT)n (TAC)n (CTA)n/(TC)n (CT)n	P	T-II
40	37753412	*iabtgs*222	harpin inducing protein	1.00E–09	(TGC)n (CTG)n (GCT)n	P	T-II
41	9296509	*iabtgs*223	dehydration responsive element binding	2.00E–22	(ACC)n (CAC)n (CCA)n	M	T-III
42	61099098	*iabtgs*225	hypothetical protein OsJ_024137	2.00E–17	(CGG)n (GCG)n (GGC)n	M	T-II
43	45993487	*iabtgs*226	glycoside hydrolase family 28 protein	1.00E–56	(TGC)n (CTG)n (GCT)n	P	T-I
44	9305233	*iabtgs*227	unknown protein		(AGC)n (CAG)n (GCA)n	M	T-I
45	34510491	*iabtgs*228	Hypothetical protein		(CGG)n (GCG)n (GGC)n	P	T-II
46	9303289	*iabtgs*229	Nitrate induced NOI protein	1.00E–40	(CCG)n (CGC)n (GCC)n /(AG)n (GA)n	M	T-II
47	18052712	*iabtgs*230	ZIM motif-containing protein	0.12	(AGC)n (CAG)n (GCA)n	M	T-I
48	33110457	*iabtgs*231	hypothetical protein	1.00E–19	(TCG)n (CGT)n (GTC)n	P	T-I
49	45957262	*iabtgs*234	leaf senescence related protein-like	9.00E–78	(AGC)n (CAG)n (GCA)n	M	T-II
50	14089228	*iabtgs*235	Ethylene receptor	3.00E–61	(CCG)n (CGC)n (GCC)n	M	T-I
51	57808251	*iabtgs*237	CHCH	2.00E–12	(CCG)n (CGC)n (GCC)n	M	T-III
52	45965553	*iabtgs*242	Cytochrome P450 71E1	3.00E–85	**(ACG)n (CGA)n (GAC)n**	M	T-II
53	30163708	*iabtgs*244	phosphoglycerate mutase-like protein	8.00E–52	(AGC)n (CAG)n (GCA)n	M	T-II
54	34443079	*iabtgs*250	No significant similarity found.		(AGG)n (GAG)n (GGA)n	M	T-III
55	30968371	*iabtgs*251	putative copper chaperone	5.00E–27	(AGC)n (CAG)n (GCA)n	M	T-II
56	34443334	*iabtgs*259	No significant similarity found		(AAG)n (AGA)n (GAA)n	M	T-II
57	45990071	*iabtgs*260	Diadenosine5',5'''-P1 tetraP hydrolase	1.00E–88	(CCG)n (CGC)n (GCC)n	M	T-III
58	34517721	*iabtgs*263	putative inositol-3-phosphate synthase	3.00E–30	(AGC)n (CAG)n (GCA)n	M	T-III
59	30945913	*iabtgs*264	chloroplast O2-evolving enhancer protein 1	8.00E–68	**(ACG)n (CGA)n (GAC)n**	M	T-III
60	45968979	*iabtgs*269	EREBP transcription factor	9.00E–46	(AGG)n (GAG)n (GGA)n	M	T-II
61	9303947	*iabtgs*274	hypothetical protein	3.00E–25	(ACC)n (CAC)n (CCA)n	M	T-II
62	33108470	*iabtgs*280	PyroP-dependent phosphofructokinase	2.00E–22	(AAG)n (AGA)n (GAA)n/(AG)n (GA)n	M	T-II
63	34440910	*iabtgs*281	putative 4,5-DOPA dioxygenase extradiol	1.00E–37	(AGG)n (GAG)n (GGA)n	M	T-II
64	45975671	*iabtgs*282	Plastocyanin precursor	7.00E–19	(TCC)n (CTC)n (CCT)n	M	T-II
65	31332334	*iabtgs*287	No significant similarity found.		(TC)n (CT)n	M	T-II
66	37711087	*iabtgs*289	Cyclic beta 1-2 glucan synthetase	3	(AG)n (GA)n	M	T-II
67	18062597	*iabtgs*301	No significant similarity found		(AT)n (TA)n	M	T-I
68	30946136	*iabtgs*304	unknown protein	8.00E–09	(TC)n (CT)n	M	T-III
69	30164571	*iabtgs*305	No significant similarity found		(TC)n (CT)n	M	T-III
70	7535895	*iabtgs*307	Mitogen activated protein kinase 6	2.00E–06	(TC)n (CT)n	P	T-II
71	11921286	*iabtgs*309	AMP-binding protein	1.00E–35	(AG)n (GA)n	M	T-III
72	9851429	*iabtgs*310	unknown		(AC)n (CA)n	P	T-III
73	30939660	*iabtgs*314	putative polyprotein	9.00E–14	(TG)n (GT)n	P	T-III
74	30964510	*iabtgs*322	hypothetical protein	3.00E–15	(AG)n (TC)n	M	T-II
75	11922775	*iabtgs*323	hypothetical protein	2.00E–26	(AT)n (TA)n	M	T-II

Continued

76	31329611	*iabtgs*324	hypothetical protein OsJ_018717	8.00E–58	(AG)n (GA)n	M	T-II
77	18052335	*iabtgs*327	proline-rich spliceosome-associated factor	8.00E–37	(AT)n (TA)n	M	T-II
78	31383744	*iabtgs*340	cytochrome P450 monooxygenase CYP77B5	7.00E–52	(AG)n (GA)n	P	T-II
79	57821918	*iabtgs*341	Chloroplast phytoene synthase 1	9.00E–38	(AG)n (TG)n (GA)n (GT)n	M	T-III
80	57807306	*iabtgs*342	Serine/threonine-protein kinase MHK	3.00E–07	(AC)n (CA)n	M	T-III
81	45993419	*iabtgs*343	APX4_SOLLC L-ascorbate peroxidase	8.00E–33	(AG)n (GA)n	P	T-II
82	37759348	*iabtgs*345	hypothetical protein HEAR0860	8.7	(AG)n (GA)n	M	T-III
83	45969660	*iabtgs*346	E3 ubiquitin ligase	9.00E–06	(TC)n (CT)n	M	T-III
84	34509854	*iabtgs*349	LHT2 (LYSINE HISTIDINE TRANSPORTER 2)		(TG)n (GT)n	P	T-II
85	34517131	*iabtgs*350	auxin efflux carrier family protein	2.00E–40	(TC)n (CT)n	M	T-III
86	37753820	*iabtgs*352	Urease accessory protein UreD	2.3	(AT)n (TA)n	P	T-II
87	37711166	*iabtgs*353	COG0583: Transcriptional regulator	3.2	(TC)n (CT)n	M	T-II
88	37706443	*iabtgs*355	isoprenylcysteine carboxyl methyltransferase	9.3	(TG)n (GT)n	M	T-II
89	37705823	*iabtgs*356	No significant similarity found		(TC)n (CT)n	M	T-III
90	37705032	*iabtgs*357	SJCHGC01974 protein	2.1	(AC)n (CA)n	M	T-III
91	34509495	*iabtgs*359	hypothetical protein	3.00E–05	(AG)n (GA)n	M	T-II
92	34442668	*iabtgs*360	hydroxycinnamoyl transferase	3.00E–31	(AG)n (GA)n	M	T-II
93	33110250	*iabtgs*362	NADH-ubiquinone oxidoreductase-protein	3.00E–27	(AG)n (GA)n	M	T-III
94	34443764	*iabtgs*363	No significant similarity found		(AC)n (CA)n	P	T-I
95	34440481	*iabtgs*364	hypothetical protein	2.00E–09	(AC)n (CA)n	M	T-III
96	33110768	*iabtgs*366	putative shrunken seed protein	0.78	(TC)n (CT)n	M	T-II
97	33107765	*iabtgs*367	No significant similarity found		(AT)n (TA)n	M	T-III
98	31330527	*iabtgs*369	No significant similarity found		(TC)n (CT)n	P	T-II
99	30979643	*iabtgs*371	cation efflux system protein ,RNA helicase	4.4	(TG)n (GT)n	M	T-II
100	30976566	*iabtgs*375	hypothetical protein		(AT)n (TA)n	M	T-III
101	30974675	*iabtgs*377	Plant viral-response family protein	5.00E–06	(CG)n	M	T-III
102	30974381	*iabtgs*378	putative Myb-like DNA-binding protein	1.00E–07	(AG)n (GA)n	M	T-II
103	30947756	*iabtgs*381	hypothetical protein	1.6	(AG)n (GA)n	M	T-II
104	30964406	*iabtgs*382	Glycyl-tRNA synthetase	0.59	(AG)n (GA)n	M	T-III
105	30966299	*iabtgs*383	GHMP kinase family protein	7.00E–39	(AG)n (GA)n	M	T-II
106	30937801	*iabtgs*384	putative membrane protein	0.9	(TC)n (CT)n	M	T-II
107	18052318	*iabtgs*385	hypothetical protein Os01g0260800	0.002	(AT)n (TA)n	P	T-I
108	30936750	*iabtgs*386	heat shock protein	2.00E–33	(TG)n (GT)n	M	T-II
109	18065756	*iabtgs*387	No significant similarity found		(TG)n (GT)n	M	T-II
110	18060131	*iabtgs*397	hypothetical protein		(AG)n (GA)n	P	T-II
111	18051780	*iabtgs*399	No significant similarity found		(TC)n (CT)n	M	T-II
112	14513091	*iabtgs*407	hypothetical protein	1.00E–27	(TG)n (GT)n	M	T-II
113	13587956	*iabtgs*408	hypothetical protein	4.00E–05	(TC)n (CT)n	M	T-III
114	13469439	*iabtgs*410	No significant similarity found		(AG)n (GA)n	M	T-II
115	11678719	*iabtgs*411	putative laccase	3.00E–67	(TC)n (CT)n	M	T-II
116	12776058	*iabtgs*416	hypothetical protein	2.00E–44	(AT)n (TA)n	M	T-III
117	11409368	*iabtgs*421	hypothetical protein	2.2	(AC)n (CA)n	M	T-II
118	9850860	*iabtgs*425	WRKY transcription factor 16	2.2	(AT)n (TA)n	M	T-II

Copyright © 2012 SciRes.

Continued

119	9305649	*iabtgs*429	No significant similarity found		(AT)n (TA)n	M	T-III
120	7536347	*iabtgs*430	calcium channel	8.5	(TC)n (CT)n	M	T-III
121	30973885	*iabtgs*439	Adenosine 5'-phpsphosulfate reductase 6	2.00E–76	(TC)n (CT)n	M	T-III
122	57815251	*iabtgs*440	Endoxyloglucan transferase		(TG)n (GT)n	P	T-II
123	30160959	*iabtgs*441	shaggy-like kinase etha (OSKetha)	9.00E–50	(CCG)n (CGC)n	M	T-I
124	31332570	*iabtgs*445	para-hydroxybenzoate-polyprenyltransferase	3.6	(TC)n (CT)n	M	T-III
125	33109119	*iabtgs*447	NADP dependent maleic enzyme	1.00E–14	(AG)n (GA)n	P	T-III
126	7659319	*iabtgs*449	No significant similarity found		(TG)n (GT)n	M	T-III
127	11922518	*iabtgs*450	transcription factor MybS3	3.00E–53	(TC)n (CT)n	P	T-III
128	30974889	*iabtgs*455	Nucellin like aspartic protease	9.00E–43	(AG)n (GA)n	M	T-II
129	33109611	*iabtgs*456	unknown protein	8.00E–06	(TC)n (CT)n	M	T-I
130	17886525	*iabtgs*457	hypothetical protein		(AG)n (GA)n	M	T-II
131	61099192	*iabtgs*458	No significant similarity found		(AC)n (CA)n	M	T-I
132	33108498	*iabtgs*460	hypothetical protein	0.65	(AG)n (GA)n	M	T-III
133	33108027	*iabtgs*464	hypothetical protein		(TC)n (CT)n	M	T-III
134	34442937	*iabtgs*470	ATTIC21/CIA5/PIC1/(chloropast Import)	3.00E–20	(TG)n (GT)n	P	T-II
135	30937618	*iabtgs*472	No significant similarity found		(TGC)n (CTG)n (GCT)n /(TG)n (GT)n	M	T-I
136	30942400	*iabtgs*473	ribosomal-protein-alanine acetyltransferase	3.2	(TC)n (CT)n	M	T-II
137	18070466	*iabtgs*478	VHS2 protein	1.00E–04	(AT)n (TA)n	M	T-I
138	30939303	*iabtgs*484	Alpha tubulin	9.00E–08	(TC)n (CT)n	M	T-I
139	12497850	*iabtgs*487	hypothetical protein	5.1	(AT)n (AC)n /(TA)n (TC)n (CA)n (CT)n	M	T-II
140	31385392	*iabtgs*488	Protease inhibitor/seed storage/LTP family	1.00E–15	(AG)n (GA)n	M	T-II
141	30975546	*iabtgs*491	quinone-oxidoreductase QR1	6.00E–07	(TC)n (CT)n	M	T-II
142	45960926	*iabtgs*492	No significant similarity found		(TC)n (CT)n	M	T-II
143	30952757	*iabtgs*493	hypothetical protein	3.00E–07	(TG)n (GT)n	P	T-III
144	12618782	*iabtgs*496	putative receptor-like kinase	7.00E–10	(TC)n (CT)n	M	T-III
145	9304578	*iabtgs*499	retrotransposon protein	0.2	(AC)n (CA)n	M	T-II
146	18066228	*iabtgs*500	Serine/threonine-protein kinase SSN3	8.5	(TG)n (GT)n	M	T-I
147	12775763	*iabtgs*502	putative membrane protein	4.00E–07	(TC)n (CT)n	M	T-III
148	61115436	*iabtgs*504	No significant similarity found		(AT)n (TA)n	M	T-I
149	8088843	*iabtgs*505	peptidase C14, caspase catalytic subunit p20	8.7	(AG)n (GA)n	M	T-III
150	45961441	*iabtgs*507	No significant similarity found		(AG)n (GA)n	M	T-III
151	33109939	*iabtgs*510	prolylcarboxypeptidase-like protein	0.089	(TC)n (CT)n	P	T-I
152	11678708	*iabtgs*512	hypothetical protein	0.002	(AGC)n (CAG)n (GCA)n/(AG)n (GA)n	M	T-II
153	5043542	*iabtgs*514	hypothetical protein		(AT)n	M	T-II
154	34445779	*iabtgs*516	Chitin-inducible gibberllin-responsive protein	4.00E–40	(AG)n (GA)n	M	T-II
155	33108255	*iabtgs*517	No significant similarity found		(TC)n (CT)n	M	T-I
156	30973999	*iabtgs*518	hypothetical protein	2.00E–15	(TC)n (CT)n	M	T-III
157	57821918	*iabtgs*520	No significant similarity found			M	T-II
158	34515133	*iabtgs*528	Ubiquitin-conjugating enzyme like	3.00E–12	(TC)n (CT)n	M	T-II
159	33108946	*iabtgs*529	hypothetical protein		(TC)n (CT)n	P	T-II
160	9852958	*iabtgs*530	No significant similarity found		(AT)n (TA)n	M	T-I
161	45988690	*iabtgs*531	RISBZ4		(CGG)n (GCG)n (GGC)n/(AG)n (GA)n	M	T-I

-T-I, T-II and T-III represent the three Touchdown PCR thermal profiles *viz.*60˚C - 55˚C, 55˚C - 50˚C and 50˚C - 45˚C respectively.

Copyright © 2012 SciRes.

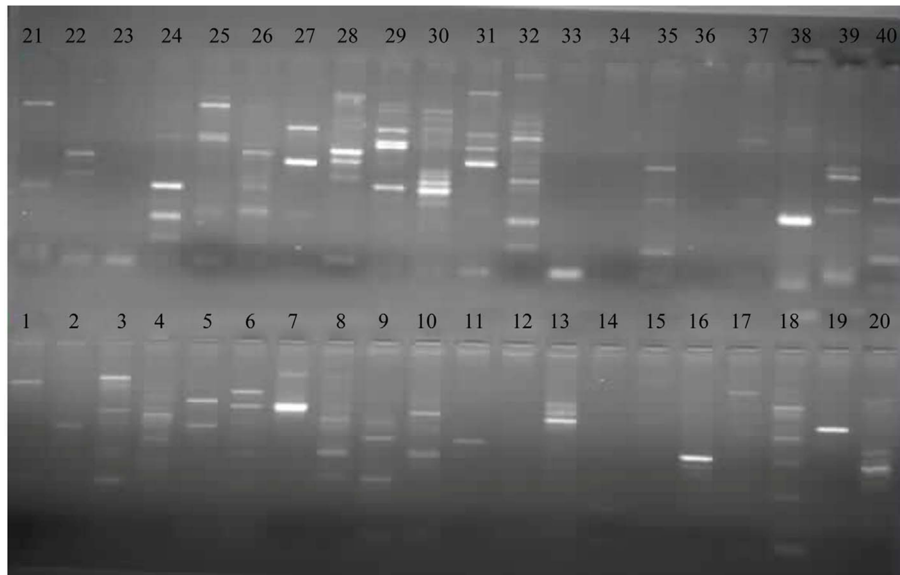

Figure 1. Amplification pattern generated by sorghum EST-SSR (SbEST-SSRs) during primer screening in groundnut using genomic DNA. 1-40: Amplification products of SbEST-SSR primers in groundnut during primer screening.

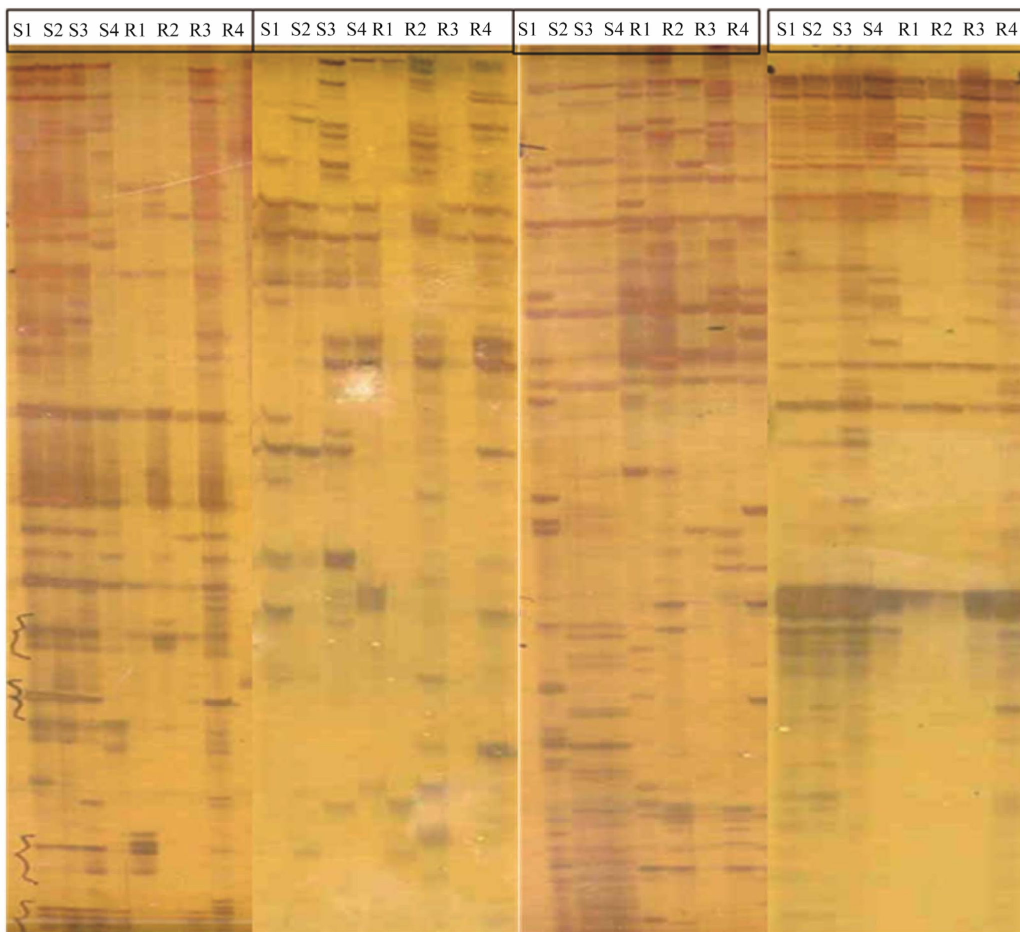

Figure 2. The figure is a composite of multiple polyacrylamide gels which illustrates the multiple and polymorphic bands generated using the SbEST-SSR primers in eight groundnut accessions. S1 - S4: Four rust and leafspot susceptible genotypes JL-24, TMV-2, TAG-24 and TG-26; R1-R4: Four rust and leafspot susceptible genotypes LSVT-I-2003-1, ISK-I-2004-4, IVT-I-2005-5and GPBD-4.

Copyright © 2012 SciRes.

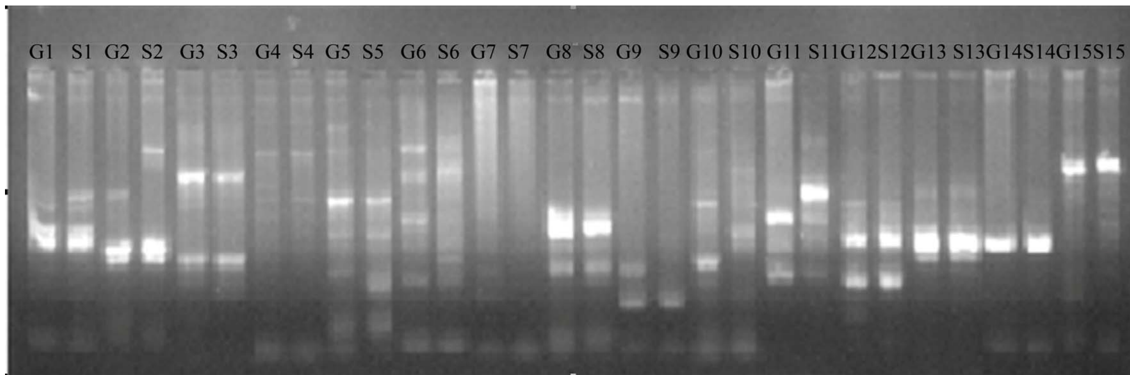

Figure 3. Comparison of amplification patterns in both groundnut and sorghum using SbEST-SSR markers. G1 S1 - G15 S15: Comparison of PCR amplification in groundnut (G) and sorghum (S) with SbEST-SSRs.

no significant homology (9.3%), or hypothetical proteins (37%) (**Table 1**). In major cases of ESTs putative functions matched both monocots (rice, maize) and dicots (*Arabidopsis*, soybean) indicating their common sharing.

4. Discussion

Despite the tremendous diversity, plant geneticists have found that plants exhibit extensive conservation of both gene content and gene order [27]. Sequence similarity of the barley ESTs with 379,944 ESTs of the two model dicot species, *Arabidopsis* and *Medicago* suggested theoretical transferability of barley markers into dicot species although at low frequency [24] and EST-SSR by virtue of the sequence conservation of the transcribed regions of the genome are more likely to function in distantly related species than SSR primer pair derived from genomic libraries.

In the present study 161 out of 411 SbEST-SSRs amplified in peanut. So, about 39% transferability or conservation of SSR motifs and flanking sequences found between sorghum (monocot) and peanut (dicot). The orthology was further confirmed by comparing amplifycation pattern (number of amplification products and size) in both sorghum and peanut genomes. Majority of them had similar amplification pattern but a few showed extra bands with common ones either in sorghum or peanut which may be due to duplications, insertions or deletion mutations during course of evolution which diverged 150 million years ago [46]. Similar bands amplified regardless of phylogenetic distances are an important feature of EST-SSR markers which are transferred across species or even genera [29]. But one of the concerns is alleles of identical size with different numbers of repeats within the SSR (size homoplasy)) observed in most of studies, suggesting a need for caution when interpreting alleles of identical size found using cross-amplified SSRs based on band migration in the absence of DNA sequences. So knowledge of DNA sequence is essential before SSR loci can be meaningfully used to address applied and evolutionary questions. Majority of SbEST-SSRs produced multiple bands (range 1 - 16) which is a common feature reported in most of the studies involving transferability of EST-SSRs.

Transferability of SbEST-SSRs in the present study is more (39%) compared to study of He *et al.* [47] using soybean genomic SSRs (25%) in peanut. But the polymorphism detection rate in this study (18%) is less compared to latter study (28%). This illustrate that EST-SSRs are more transferable across species or distant taxa and are less efficient in polymorphism detection than genomic SSRs as they are derived from transcribed regions of genome which are conserved across species. However, transferability (39%) in this study is less compared to the study of Gao *et al.* (2003) [12]. In which 69% transferability from wheat (monocot system) to soybean (dicot system) was observed, but percent transferability or conserved EST-SSRs from wheat to rice, maize and soybean in the same study 43%. With regard to developing microsatellite markers, 3'-sequences yielded more polymorphic markers (22.9%) than 5'-ESTs (13.4%) did. This result is not unexpected as during the process of cDNA generation (poly T priming) there is a preferential selection of untranslated regions (UTR) within 3'-ESTs, therefore are more variable than 5'-ESTs. In the distribution of SSR motifs, dinucleotides were found more common than tri, tetra or complex nucleotide repeats in transferred SbEST-SSRs or new gene based markers accounting for 56%, 37.2%, 0.5% and 5% respectively and also SbEST-SSRs with dinucleotide repeats detected more polymerphism (18.7%) than tri (9.8%,) and complex nucleotide (1.2%) repeat motifs. Dinucletotides were also found to detect higher polymorphism than others, which was observed in some the previous studies [48]. This shows that there seems to be some correlation between repeat number and polymorphism.

The transferable SbEST-SSRs subjected to BlastX with an e-value more than or equal to 1E-5 as a significant homology, could annotate putative functions for

Copyright © 2012 SciRes.

52% of the common ESTs (**Table 3**). Most of annotated SbESTs were related to basic functions of plant cells such as metabolism, photosynthesis, signal transduction, transcription, growth, and transportation across membranes, stress and defense. The remaining SbESTs search for putative functions resulted in poor hits (2.5%) no significant homology (9.3%) or hypothetical proteins (37%). These may represent transcriptomes which are yet to be characterized for their putative functions. In major cases of ESTs putative functions matched both monocots (rice, maize) and dicots (*Arabidopsis*, soybean) suggesting that these are highly conserved across plant species mainly encoding for basic functions. Thus annotations of transferred SbEST-SSRs help to explore the potential utility of the EST-SSR loci for comparative mapping in peanut. Functional EST-SSRs exhibiting sequence similarity to genes with a range of functions could be used directly in determining putative traits. For example, EST-sequences of *iabtgs*366 and *iabtgs*269 showed a strong homology to putative shrunken seed protein and EREBP transcription factor, which is involved in stress tolerance respectively. This potential will make them a valuable source of new genic SSR markers so called "perfect" genetic markers.

Thus, by using transferability technique it was possible to develop a set of new gene based markers for peanut crop using genomic resources of sorghum that will be useful for different genetic studies in peanut. In this study, we could demonstrate the feasibility of utilizing EST-SSRs from monocots in dicots as plant genes display significant conservation even after the long period of independent evolution.

5. Acknowledgements

Authors are thankful to the Indo-US Agricultural Knowledge Initiative and Department of Biotechnology (DBT) of Government of India for supporting research in authors' (BF) laboratory.

REFERENCES

[1] T. Halward, H. T. Stalker and G. Kochert, "Development of an RFLP Linkage Map in Peanut Species," *Theoretical and Applied Genetics*, Vol. 87, No. 3, 1993, pp. 379-384. doi:10.1007/BF01184927

[2] M. D. Burrow, C. E. Simpson, A. H. Paterson and J. L. Starr, "Identification of Peanut (*Arachis hypogaea* L.) RAPD Markers Diagnostic of Root-Knot Nematode (*Meloidigyne arenaria* (Neal) Chitwood) Resistance," *Molecular Breeding*, Vol. 2, No. 4, 1996, pp. 368-379. doi:10.1007/BF00437915

[3] G. Lacks and H. Stalker, "Isozyme Analyses of *Arachis* Species and Interspecific Hybrids," *Peanut Science*, Vol. 20, No. 2, 1993, pp. 76-81. doi:10.3146/i0095-3679-20-2-3

[4] A. K. Singh, J. Smartt, C. E. Simpson and S. N. Raina, "DNA Markers in Cultivated Peanut (*Arachis hypogaea* L.)," *BMC Plant Biology*, Vol. 3, 1998, pp. 3-10.

[5] L. Zane, L. Bargelloni and T. Patarnello, "Strategies for Microsatellite Isolation: A Review," *Molecular Ecology*, Vol. 11, No. 1, 2002, pp. 1-16. doi:10.1046/j.0962-1083.2001.01418.x

[6] M. A. Gimenes, A. A. Hoshino, A. V. G. Barbosa, D. A. Palmieri and C. R. Lope, "Characterization and Transferability of Microsatellite Markers of the Cultivated Peanut (*A. hypogaea*)," *BMC Plant Biology*, Vol. 7, 2007, p. 9. http://www.biomedcentral.com/bmcplantbiol. doi:10.1186/1471-2229-7-9

[7] G. He and C. Prakash, "Evaluation of Genetic Relationship among Botanical Varieties of Cultivated Peanut (*Arachis hypogaea* L.) Using AFLP Markers," *Genetic Resources and Crop Evolution*, Vol. 48, No. 4, 2001, pp. 347-352. doi:10.1023/A:1012019600318

[8] S. L. Dwivedi, S. Gurtu, S. Chandra, W. Yuejin and S. N. Nigam, "Assessment of Genetic Diversity among Selected Groundnut Germplasm. I: RAPD Analysis," *Plant Breeding*, Vol. 120, No. 4, 2001, pp. 345-349. doi:10.1046/j.1439-0523.2001.00613.x

[9] V. Subramanian, S. Gurtu, R. C. Nageswara Rao and S. N. Nigam, "Identification of DNA Polymorphism in Cultivated Groundnut Using Random Amplified Polymorphic DNA (RAPD) Assay," *Genome*, Vol. 43, No. 4, 2000, pp. 656-660. doi:10.1139/g00-034

[10] J. Squirrell, P. M. Hollingsworth, M. Woodhead, J. Russell, A. J. Lowe and M. Gibby, "How Much Effort Is Required to Isolate Nuclear Microsatellites from Plants?" *Molecular Ecology*, Vol. 12, 2003, pp. 1339-1348. doi:10.1046/j.1365-294X.2003.01825.x

[11] J. R. Ellies and J. M. Burke, "EST-SSRs as a Resource for Population Genetic Analyses," *Heredity*, Vol. 99, No. 2, 2007, pp. 125-132. doi:10.1038/sj.hdy.6801001

[12] L. Gao, J. Tang, H. Li and J. Jia, "Analysis of Microsatellites in Major Crops Assessed by Computational and Experimental Approaches," *Molecular Breeding*, Vol. 12, No. 3, 2003, pp. 245-261.

[13] Jia and Bonierbale, "Validation of Conserved Orthologous Markers, A Proposal for Extension of Commissioned Research in Subprogram 2 'Comparative Genomics' Cluster 2: Marker Development Generation Challenge Program," 2005.

[14] N. L. Raju, B. N. Gnanesh and R. Varshney, "The First Set of EST Resource for Gene Discovery and Marker Development in Pigeonpea (*Cajanus cajan* L.)," *BMC Plant Biology*, Vol. 10, 2010, pp. 45-67. doi:10.1186/1471-2229-10-45

[15] G. M. Cordeiro, R. Casu, C. L. Mcintyre, J. M. Manners and R. J. Henry, "Microsatellite Markers from Sugarcane (*Saccharum spp.*) ESTs cross Transferable to Erianthus and Sorghum," *Plant Science*, Vol. 160, No. 6, 2001, pp. 1115-1123. doi:10.1016/S0168-9452(01)00365-X

[16] S. Temnykh, G. Declerck, A. Lukashova, L. Lipovich, S. Cartinhour and S. Mccouch, "Computational and Experimental Analysis of Microsatellites in rice (*O. sativa* L.): Frequency, Length Variation, Transposon Associa-

Copyright © 2012 SciRes.

tions, and Genetic Marker Potential," *Genome Research*, Vol. 11, No. 8, 2001, pp. 1441-1452. doi:10.1101/gr.184001

[17] I. Eujayl, M. E. Sorrells, P. Wolters, M. Baum and W. Powell, "Isolation of EST-Derived Microsatellite Markers for Genotyping the A and B Genomes of Wheat," *Theoretical and Applied Genetics*, Vol. 104, No. 2-3, 2002, pp. 399-407. doi:10.1007/s001220100738

[18] B. Hackauf and P. Wehling, "Identification of Microsatellite Polymorphisms in an Expressed Portion of the Rye Genome," *Plant Breeding*, Vol. 121, No. 1, 2002, pp. 17-25. doi:10.1046/j.1439-0523.2002.00649.x

[19] T. Thiel, W. Michalek, K. Varsheny and A. Graner, "Exploiting EST Databases for the Development of cDNA Derived Microsatellite Markers in Barley (*Hordeum vulgare* L.)," *Theoretical and Applied Genetics*, Vol. 106, No. 3, 2003, pp. 411-422.

[20] M. J. Faville, A. C. Vecchies, M. Schreiber, M. C. Drayton, L. J. Hughes, E. S. Jones, K. M. Guthridge, K. F. Smith, T. Sawbridge, G. C. Spangenberg, G. T. Bryan and J. W. Forster, "Functionally Associated Molecular Genetic Marker Map Construction in Perennial Ryegrass (*Lolium perenne* L.)," *Theoretical and Applied Genetics*, Vol. 110, No. 1, 2004, pp. 12-32. doi:10.1007/s00122-004-1785-7

[21] J. H. Peng and N. L. Lapitan, "Characterization of EST-Derived Microsatellites in the Wheat Genome and Development of eSSR Markers," *Functional & Integrative Genomics*, Vol. 5, No. 2, 2005, pp. 80-96. doi:10.1007/s10142-004-0128-8

[22] G. Q. Song, M. J. Li, H. Xiao, X. J. Wang, R. H. Tang, H. Xia, *et al.*, "EST Sequencing and SSR Marker Development from Cultivated Peanut (*Arachis hypogaea* L.)," *Electronic Journal of Biotechnology*, Vol. 13, No. 3, 2010, pp. 1-9. doi:10.2225/vol13

[23] Z. Han, C. Wang, X. Song, W. Guo, J. Gou, C. Li, X. Chen and T. Zhang, "Characteristics, Development and Mapping of *Gossypium hirsutum* Derived EST-SSRs in Allotetraploid Cotton," *Theoretical and Applied Genetics*, Vol. 112, No. 3, 2006, pp. 430-439. doi:10.1007/s00122-005-0142-9

[24] R. K. Varshney, R. Sigmund, A. Borner, V. Korzun, N. Stein and M. E. Sorrells, "Interspecific Transferability and Comparative Mapping of Barley EST-SSR Markers in Wheat, Rye and Rice," *Plant Science*, Vol. 168, No. 1, 2005, pp. 195-202. doi:10.1016/j.plantsci.2004.08.001

[25] J. R. Andersen and T. Lubberstedt, "Functional Markers in Plants," *Trends in Plant Science*, Vol. 8, No. 11, 2003, pp. 554-560. doi:10.1016/j.tplants.2003.09.010

[26] C. A. Fatokun, D. I. Menacio-Hautea, D. Danesh and N. D. Young, "Evidence for Orthologous Seed Weight Genes in Cowpea and Mung Bean Based upon RFLP Mapping," *Genetics*, Vol. 132, 1992, pp. 841-846.

[27] J. L. Bennetzen and M. Freeling, "Grasses as a Single Genetic System: Genome Composition, Colinearity and Compatibility," *Trends in Genetics*, Vol. 9, No. 8, 1993, pp. 259-261. doi:10.1016/0168-9525(93)90001-X

[28] M. D. Gale and K. M. Devos, "Comparative Genetics in the Grasses," *Proceedings of the National Academy of Sciences of the USA*, Vol. 95, 1998, pp. 1971-1974.

[29] V. Decroocq, M. G. Fave, L. Hagen, L. Bordenave and S. Decroocq, "Development and Transferability of Apricot and Grape EST Microsatellite Markers across Taxa," *Theoretical and Applied Genetics*, Vol. 106, No. 5, 2003, pp. 912-922.

[30] M. Woodhead, J. Russell, J. Squirrell, P. M. Hollingsworth, L. Cardle, L. Ramsay, M. Gibby and W. Powell, "Development of EST-SSRs from the Alpine Lady-Fern, Athyrium Distentifolium," *Molecular Ecology Notes*, Vol. 3, No. 2, 2003, pp. 287-290. doi:10.1046/j.1471-8286.2003.00427.x

[31] M. V. Gutierrez, M. C. Vaz Patto and T. Huguet, "Cross-Species Amplification of Medicago Truncatula Microsatellites across Three Major Pulse Crops," *Theoretical and Applied Genetics*, Vol. 110, No. 7, 2005, pp. 1210-1217. doi:10.1007/s00122-005-1951-6

[32] X. Jia, Y. Shi, Y. Song, G. Wang, T. Wang and Y. Li, "Development of EST-SSR in Foxtail Millet (*Setaria italica*)," *Genetic Resources and Crop Evolution*, Vol. 54, No. 2, 2007, pp. 233-236. doi:10.1007/s10722-006-9139-8

[33] X. Liang, X. Chen, Y. Hong, H. Liu, G. Zhou, S. Li and B. Guo, "Utility of EST-Derived SSR in Cultivated Peanut (*Arachis hypogaea* L.) and *Arachis* Wild Species," *BMC Plant Biology*, Vol. 9, 2009, p. 35. doi:10.1186/1471-2229-9-35

[34] B. Gautami, K. Ravi, M. L. Narasu, D. A. Hoisington and R. K. Varshney, "Novel Set of Peanut SSR Markers for Germplasm Analysis and Interspecific Transferability," *International Journal of Integrative Biology*, Vol. 7, No. 2, 2009, pp. 100-106.

[35] R. K. Varshney, A. Graner and M. E. Sorrells, "Genic Microsatellite Markers in Plants: Features and Applications," *Trends in Biotechnology*, Vol. 23, No. 1, 2005, pp. 48-54. doi:10.1016/j.tibtech.2004.11.005

[36] M. Luo, P. Dang, B. Z. Guo, G. He, C. C. Holbrook, M. G. Bausher and R. D. Lee, "Generation of Expressed Sequence Tags (ESTs) for Gene Discovery and Marker Development in Cultivated Peanut," *Crop Science*, Vol. 45, No. 1, 2005, pp. 346-353. doi:10.2135/cropsci2005.0346

[37] M. Luo, P. Dang, M. G. Bausher, C. C. Holbrook, R. D. Lee, R. E. Lynch and B. Z. Guo, "Identification of Transcripts Involved in Resistance Response to Leaf Spot Disease Caused by *Cercosporidium personatum* in Peanut (*Arachis hypogaea*)," *Phytopathology*, Vol. 95, No. 4, 2005, pp. 381-387. doi:10.1094/PHYTO-95-0381

[38] G. He, R. H. Meng, H. Gao, B. Guo, G. Gao, M. Newman, R. N. Pittman and C. S. Prakash, "Simple Sequence Repeat Markers for Botanical Varieties of Cultivated Peanut (*Arachis hypogaea* L.)," *Euphytical*, Vol. 142, No. 1, 2005, pp. 131-136. doi:10.1007/s10681-005-1043-3

[39] G. H. He, R. H. Meng, M. Newman, G. Q. Gao, R. N. Pittman and C. S. Prakash, "Microsatellites as DNA Markers in Cultivated Peanut (*Arachis hypogaea* L.)," *BMC Plant Biology*, Vol. 3, 2003, p. 3. doi:10.1186/1471-2229-3-3

[40] M. L. Wang, N. L. Barkley, R. Dean, C. Holbrook and R.

Copyright © 2012 SciRes.

N. Pittman, "Transfer of *Medicago* EST-SSRs to Peanut for Germplasm Evaluation and Cross-Species Cloning," *Annual American Peanut Research & Education Society Proceedings*, Vol. 7, 2004, pp. 6-12.

[41] J. E. Mullet, R. R. Klein and P. E. Klein, "*Sorghum bicolor*—An Important Species for Comparative Grass Genomics and a Source of Beneficial Genes for Agriculture," *Current Opinion in Plant Biology*, Vol. 5, No. 2, 2002, pp. 118-121. doi:10.1016/S1369-5266(02)00232-7

[42] A. H. Paterson, *et al.*, "The *Sorghum bicolor* Genome and the Diversification of Grasses," *Nature*, Vol. 457, No. 7229, 2009, pp. 551-556. doi:10.1038/nature07723

[43] M. G. Murray and W. F. Thompson, "Rapid Isolation of High Molecular Weight Plant DNA," *Nucleic Acids Research*, Vol. 8, 1980, pp. 4321-4325. doi:10.1093/nar/8.19.4321

[44] R. H. Don, P. T. Cox, B. J. Wainwright, K. Baker and J. S. Mattick, "Touchdown PCR to Circumvent Spurious Priming during Gene Amplification," *Nucleic Acids Research*, Vol. 19, 1991, p. 4008. doi:10.1093/nar/19.14.4008

[45] S. C. Gonza´lez-Marti´nez, N. C. Wheeler Elhan Ersoz, D. C. Nelson and D. B. Neal, "Association Genetics in *Pinus taeda* L. I. Wood Property Traits," *Genetics*, Vol. 175, 2007, pp. 399-409.

[46] W. L. Crepet and G. D. Feldman, "The Earliest Remains of Grasses in the Fossil Record," *American Journal of Botany*, Vol. 78, No. 7, 1991, pp. 1010-1014. doi:10.2307/2445181

[47] G. H. He, F. E. Woullard, I. Marong and B. Z. Guo, "Transferability of Soybean SSR Markers in Peanut (*Arachis hypogaea* L.)," *Peanut Science*, Vol. 33, No. 1, 2006, pp. 22-28. doi:10.3146/0095-3679(2006)33[22:TOSSMI]2.0.CO;2

[48] J. K. Yu, A. E. Strand and B. G. Milligan, "EST-Derived SSR Markers for Comparative Mapping in Wheat and Rice," *Molecular Genetics and Genomics*, Vol. 271, No. 6, 2004, pp. 742-751. doi:10.1007/s00438-004-1027-3

Copyright © 2012 SciRes.