

## EST sequencing and SSR marker development from cultivated peanut (*Arachis hypogaea* L.)

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**Keywords:** *Arachis hypogaea* L., cultivars, EST-SSR, polymorphism, wild-type peanut.

**Abbreviations:** BLAST: Basic Local Alignment Search Tool

DAP: days after pegging  
EST: expressed sequence tags  
MAS: marker assisted selection  
PCR: polymerase chain reaction  
SSR: simple sequence repeat

**Making use of the gene resources of wild type peanuts is a way to increase the genetic diversity of the cultivars. Marker assisted selection (MAS) could shorten the process of inter-specific hybridization and provide a possible way to remove the undesirable traits. However, the limited number of molecular markers available in peanut retarded its MAS process. We started a peanut ESTs (Expressed Sequence Tags) project aiming at cloning genes with agronomic importance and developing molecular markers. In this study we found 610 ESTs that contained one or more SSRs from 12,000 peanut ESTs. The most abundant SSRs in peanut are trinucleotides (66.3%) SSRs and followed by dinucleotide (28.8%) SSRs. AG/TC (10.7%) repeat was the most abundant and followed by CT/GA (9.0%), CTT/GAA (7.4%), and AAG/TTC (7.3%) repeats. Ninety-four SSR containing ESTs were randomly selected for primer design and synthesis, of which 33 pairs could generate good amplification and were used for polymorphism assessment. Results showed that polymorphism was very low in cultivars, while high level of polymorphism was revealed in wild type peanuts.**

Peanut is one of the most important oil seed crops in the world, cultivated mainly in tropical, subtropical and warm temperate climates (Proite et al. 2007). The cultivated peanut is an allotetraploid (AABB,  $2n = 4x = 40$ ), originated from chromosome duplication of hybrid between AA and BB wild type species, which happened about 3500 years ago. Polyploidy and self-pollination nature of this species blocked the genetic exchange between the cultivated and the wild type species which lead to limited genetic diversity of the major cultivars. Using of only very few central germplasms in breeding programs further limited the addition of genes with agronomic value. Due to its limited genetic variation peanuts are vulnerable to a wide variety of pathogens and abiotic stresses. For example, fungal foliar diseases of peanut such as rust, web blotch, and leaf spot cause severe loss of yield worldwide (Leal-Bertioli et al. 2009).

Wild type peanuts are genetically very diverse and selectively accumulated biotic and abiotic stress resistant

genes during their adaptation to different harsh environments (Holbrook and Stalker, 2003). However, hybrids between the diploid wild type and the allotetraploid cultivated peanut are sterile (Tallury et al. 2005). In order to obtain tetraploid fertile offspring of the diploid wild type and the allotetraploid cultivated peanut, complicated breeding programs have to be designed. It normally takes many years to get a hybrid with agronomical value (Simpson et al. 1993; Stalker and Beute, 1993; Simpson and Starr, 2001). Marker assisted selection could increase the efficiency and predictability during the process of wild gene pool utilization. However, it was retarded by the small number of peanut molecular markers and the unavailability of high density linkage map (Burow et al. 2001; Moretzsohn et al. 2005). The attempt to develop more peanut molecular markers for construction of high density linkage map is of great importance. Microsatellites (SSRs) as DNA markers are highly polymorphic, highly abundant, show co-dominant inheritance, analytically simple and readily transferable between different plant species (He et al. 2003). Previous studies showed that SSR markers could detect more polymorphism in peanut than other molecular markers like RFLPs (Gimenes et al. 2007), AFLPs (He and Prakash, 2001; Gimenes et al. 2002) and RAPDs (Dwivedi et al. 2001; Subramanian et al. 2000).

Beside sequencing SSR enriched genomic library for SSR marker development (He et al. 2003; Han et al. 2006), searching SSRs using peanut EST information is an alternative way for marker discovery (Wang et al. 2006; Proite et al. 2007; Liang et al. 2009). We started an EST project using immature peanut seeds cDNA library of a Chinese cultivar to generate more sequence information for gene cloning (Li et al. 2009, Li et al. 2010) and marker development. The objectives of the present study were to develop more SSR markers from peanut using these EST sequences, and to detect the polymorphisms of the newly identified SSR markers in a collection of cultivated and wild type peanut accessions. The resulted SSR markers especially those showed polymorphism in different peanuts accessions would be valuable for peanut marker linkage map construction and marker assisted selection in the future.

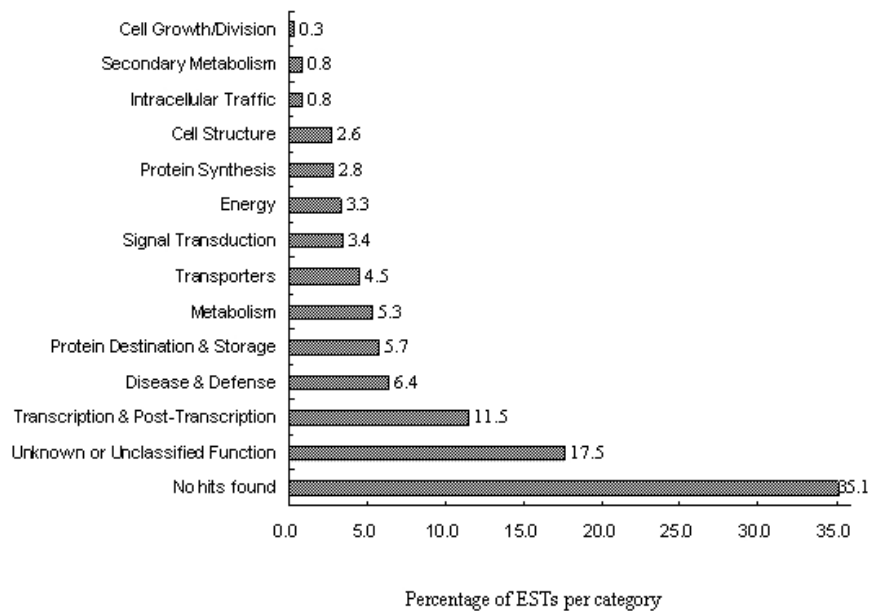


Figure 1. Percentage and functional categories of SSR containing ESTs.

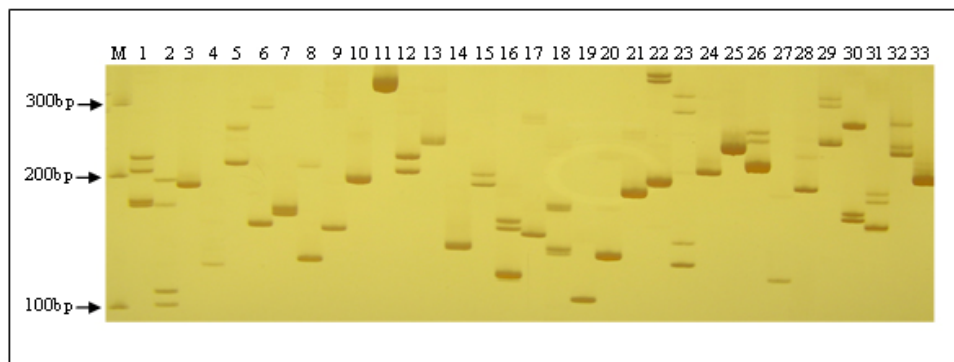


Figure 2. The amplification results of 33 pairs of primer using cultivated peanut Luhua-14. M, DNA marker; lanes 1 to 33, primer pairs of F2, F3, F9, F13; PD10, PD11, PD15, PD17, PD18, PD19, PD20, PD23, PD28, PD33, PD35, PD36, PD37, PD38, PD40, PD49, PD52, PD54, PD59; H2, H4, H6, H7, H8, H10, H11, H13, H14, H15.

## MATERIALS AND METHODS

### Plant material

Luhua-14 peanut used for cDNA library construction was obtained from Shandong Academy of Agricultural Sciences. A total of 98 accessions of wild type and cultivated peanuts were examined in this study. Twenty-five accessions belonging to section *Arachis* or *Heterantheae* which come from Nanning branch of the national wild peanut germplasm resource were used for polymorphism assessment. Seventy-three accessions belonging to cultivated species were used for genetic diversity analysis. Of these, fifty-six were collected from different provinces and the other 17 were from Wuhan national peanut germplasm resource belonging to *var.*

*fastigiata*, *var. hirsuta*, *var. hypogaea* and *var. vulgaris* (Table 1 and Table 2).

### Peanut cDNA library construction

The peanut seeds of different growing stages ranging from 20 to 60 DAP (days after pegging) were collected and placed into liquid N<sub>2</sub> immediately and stored in -80°C freezer. RNA was extracted using RNeasy kit (Promega) according to the manufacturer's instructions. The quantity and quality of RNA was evaluated by spectrophotometry and agarose gel electrophoresis. cDNA synthesis and library construction followed the protocol of Stratagene's pBluescript II cDNA library construction kit.

### Sequencing and ESTs analysis

Plasmid DNA was isolated from the randomly selected colonies and preserved in 96-well plate. Plasmid DNA was used as template for PCR amplification using T3 and T7 universal primers. PCR products were purified using AxyPrep™ PCR Cleanup Kit (AXYGEN), and sequenced using BigDye Terminator Cycle Sequencing Kit. Sequencing was performed on 3730XL Sequencer (ABI) using T3 or T7 primers. Sequences were edited by the software SEQENCHER and vector sequences were

trimmed manually.

### Analysis of microsatellites and primer design

EST sequences were searched for SSRs using SSR tool software (<http://www.gramene.org/db/searches/ssrtool>) to identify di- and trinucleotide and tetranucleotide SSR motifs. SSR motifs repeated more than five times in dinucleotide, four times in trinucleotide and tetranucleotide

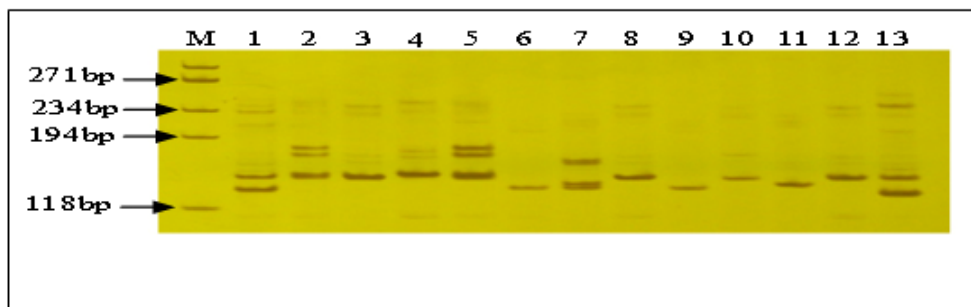
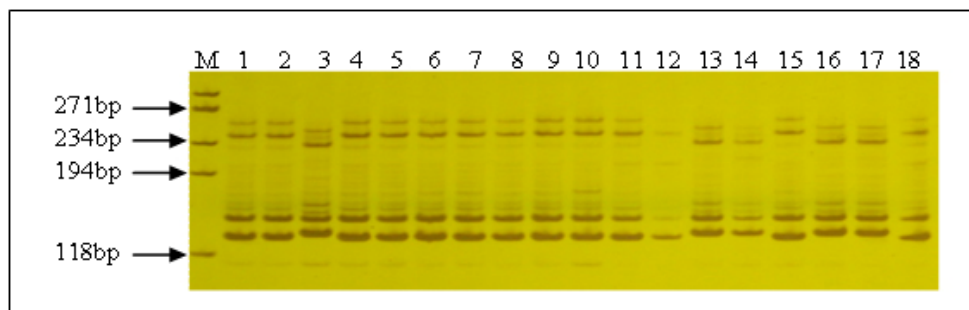


Figure 3. Polymorphism revealed by PD59 SSR maker in wild type peanuts. M, DNA Marker; lanes 1 to 12, wild type peanuts; lane 13, cultivated peanut Luhua-14.

Table 1. The accessions used for DNA polymorphism detection which came from Wuhan national peanut germplasm resource.

No.	Type	Cultivar	Origin
1	<i>var. fastigiata</i>	PI393531	America
2		Qiongsan Huasheng	China
3		Liaoning Silihong	China
4	<i>var. hirsuta</i>	Daye Pingtianzi	Jiang xi
5		Yingde Jidouzai	China
6		Jintang Shenwozi	Si chuan
7		Tuokexun Xiaohuasheng	China
8	<i>var. hypogaea</i>	Yangjiang Pudizhan	China
9		Xihua Goudou	China
10		Padou	China
11		Zhuzaidou	China
12		Yingde Zhusidou	China
13	<i>var. vulgaris</i>	Dedou	China
14		Bairizai	China
15		Guangliu	China
16		Sanyuening	China
17		Dingzi Xili	China



**Figure 4. Polymorphism revealed by PD59 SSR maker in peanut cultivars.** M, DNA Marker; lanes 1 to 18, cultivars PI393531, Qiongsan Huasheng, Liaoning Silihong, Daye Pingtianzi, Yingde Jidouzai, Jintang Shenwozi, Tuokexun Xiaohuasheng, Yangjiang Pudizhan, Xihua Goudou, Padou, Zhuzaidou, Yingde Zhusidou, Dedou, Bairizai, Guangliu, Sanyuening, Dingzi Xili, and Luhua-14.

**Table 2. The accessions used for DNA polymorphism detection which collected from different provinces.**

NO.	Cultivar	NO.	Cultivar	NO.	Cultivar
1	Huayu 16	20	Jinhua 1012	39	Yueyou 92
2	Huayu 17	21	Guihua 17	40	Trifumer
3	Huayu 19	22	Heyou 4	41	Hua 27
4	Huayu 21	23	Yueyou 551	42	Xuxi 1
5	Huayu 22	24	Quanhua 327	43	Xuhua 3
6	Huayu 23	25	Luhua 9	44	FT001
7	Luhua 10	26	Hua 17	45	Luhua 2
8	Luhua 11	27	AR-2	46	Luhua 12
9	Fenghua 1	28	Dayingzui	47	FT002
10	Fenghua 5	29	FT004	48	616
11	Zhufeng 1	30	Furonghuasheng	49	FT005
12	Qinglan 2	31	Yuhua 15	50	Haihua 1
13	Weihua 6	32	Quanhua 646	51	FT003
14	Shuangji 22	33	AR-3	52	Yueyou 116
15	Heihuasheng	34	GFA-1	53	FN006
16	Baisha 1016	35	Quanhua 10	54	Jihua 2
17	Minhua 5	36	Yuhua 14	55	Yuhua 10
18	Minhua 6	37	HS002	56	Luhua-14
19	Minhua 10	38	GFA-2		

were counted. Primers were designed using Primer 5 software.

#### DNA extraction and PCR amplification

Seventy-three cultivated peanuts and 25 wild type peanuts were used for polymorphism study. Genomic DNA was extracted from young leaf tissue using CTAB method (Murray and Thompson, 1980). DNA concentration was determined by spectrophotometer. PCR mixture consisted



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polymorphism was very limited. Only one primer pair (PD59) could amplify polymorphic bands. In order to further evaluate the polymorphism of these 33 SSRs, seventeen cultivated peanut (from Wuhan national cultivated peanut germplasm resources) including *var. fastigiata*, *var. hirsuta*, *var. hypogaea* and *var. vulgaris*

Table 3. Peanut microsatellite markers with good amplification quality in Luhua- 14.

Primer pair	Forward primer (5'-3')	Reverse primer (5'-3')	Motif	Annealing		Expected Size (bp)
				X	Y	
F2	GGATTCATTCGAGGGTGG	AAGGCATCTTTGTCGTTG	(CTT)6	58	56	168
F3	CAGCAACTTTCTTCAACC	CTCTGAATCTCGCACCT	(AGA)6	58	56	108
F9	TTCGAGCTTGAGGAAGAG	AACGGAATTTGAGATGATAG	(AATC)6	56	54	191
F13	ATCCTACGATTTCTTACTG	TCGATGGAGGGATGATTT	(CT)25	50	48	152
H2	TCCAGAACAACAATCAC	AGAAGAATACGAGCACCA	(TCT)5	54	52	215
H4	TCTTGATCTTATCTCCCTG	CAAATGTGGTGTGCTGA	(TCA)5	56	54	244
H6	CCAACCTCGACTCGGCTTTC	TTGCTCTCTAACAACCTTCT	(TC)7	56	54	221
H7	CCTCACCAACCAGGATAC	TTTCGTCTTTGCAGTTTC	(CTT)4	54	52	122
H8	ATCAAGCCATAATATGTTCC	CAACCCAAGCACCTCTAC	(TA)6	54	52	193
H10	AGAGTAAGGGTGCTTCTG	ATGCTTCTCCATTCCA	(GAT)5	54	52	142
H11	CCGAACTCAACCCAAACA	AGCTCCGGCAAGAAAGAA	(CAC)4	56	54	178
H13	GCTTTTATTTACATACATCAC	GGTAGGGCACTGTTCACTT	(CTT)7	54	52	165
H14	ACGAAGCCTAAGAACGAAAG	ATGCCACCAGCAAAGGAG	(AC)5	56	54	258
H15	CGGTCGTAGTAGTTGTGG	GAGGCTTAAAGGAAGTGG	(CT)6	56	54	216
PD10	ACTTGCTTTGGATGCTTG	CTTTTCTTTCCCGACT	(TTA)7	50	48	208
PD11	CTCTTTTATTATTGGGTTTG	AGATTGTGCAGCCTCATC	(TAA)7	54	52	152
PD15	ACTAACACCATTTCCGACAG	TGGGAGCCTATGAAACCT	(TTC)5	58	56	163
PD17	CTTTCCCATATTCCTTCGT	TGTTCTCTCCTGCTTT	(TCT)6	56	54	129
PD18	ATCATCATCATCATCGTC	AGTTTCTGGTACTGTGCG	(TCA)6	54	52	152
PD19	CGTGGAGAACGTGCTTAG	CCATAGATCGAAATAGGTAG	(TTA)5	54	52	210
PD20	GAAGAAAATAGCAGGACT	CGTAGTATCTTGATAACCC	(CTT)6	56	54	254
PD23	TACAATGTTGCCTTTTAC	CAACTCCTCACTACTCCC	(TAA)6	56	54	205
PD28	ATTAAGATGGCTTTGGAG	AGAGTAGTGGCAGTGACCT	(AAG)6	54	52	240
PD33	GGGGAACATGGTCTCATC	GGGCTTTCTCATAGGTGG	(TCT)5	60	58	139
PD35	GAACCTTAGAGCTTGTGG	GACGAGTAATTCTCCTTT	(TCA)5	50	48	194
PD36	GCATCTACCATTGGAGTG	ACAAAGCAAAGCGGATAC	(TAC)5	56	54	122
PD37	GTGAGAATGGAGACTTTG	TCACCATCACTTTATCAG	(TAA)5	54	52	149
PD38	GGGAGTGAAGTTAAAGAA	TGAATCCAACACCGTATC	(GCT)5	54	52	140
PD40	CTAGTACTTTTCTAAATTGGAC	TAATGGTTTTGGGTCACA	(GAA)5	54	52	108
PD49	CAACTGGTCCCAATAATCC	GCTATGCCTCCTTCTACAAAA	(ACA)5	58	56	135
PD52	CAGTTGAAATGCAAGGGTC	TGGAGTATCCAGGTGAGG	(AAC)5	58	56	189
PD54	CTCTTCTTCTGCTCTGC	AGTAGCAACAACCCTCAT	(TCAA)5	56	54	202
PD59	ACCCTTTCACCCTCTTCA	AATGTGGCTGCTCTGTTA	(TCT)8	56	54	130



were analyzed (Table 1). Similarly, low polymorphism was revealed by these SSR markers and only 4 (12.1%) primer pairs, F9, F13, PD20 and PD59, could amplify polymorphic bands (Figure 4).

For the 73 cultivated peanuts, primer pair PD59 could always amplify either one or both of two bands between 120 and 150 bp (Figure 4). The upper band showed same size within cultivated peanut accessions tested. However, the lower band displayed varied size in different cultivars (Figure 4). The lower band of four cultivated peanuts, PI393531, Liaoning Silihong, Jinhua-1012 and Luhua-14 were recovered from the agarose gel for sequence analysis. The sequencing results were shown in Figure 5. Cultivars PI393531 from America and Luhua-14 had one TCT less than Liaoning Silihong which belonging to *var. fastigiata* and Jinhua-1012 which belongs to *var. hypogaea*.

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## REFERENCES

BUROW, Mark D.; SIMPSON, Charles E.; STARR, James L. and PATERSON, Andrew H. Transmission genetics of chromatin from a synthetic amphidiploid to cultivated peanut (*Arachis hypogaea* L.): Broadening the gene pool of a monophyletic polyploid species. *Genetics*, October 2001, vol. 159, no. 2, p. 823-837.

CUC, Luu M.; MACE, Emma S.; CROUCH, Jonathan H.; QUANG, Vu D.; LONG, Trand D. and VARSHNEY, Rajeev K. Isolation and characterization of novel microsatellite markers and their application for diversity assessment in cultivated groundnut (*Arachis hypogaea*). *BMC Plant Biology*, 2008, vol. 8, no. 55.

DWIVEDI, S.L.; GURTU, S.; CHANDRA, S.; YUEJIN, W. and NIGAM, S.N. Assessment of genetic diversity among selected groundnut gemplasm. I: RAPD analysis. *Plant Breeding*, August 2001, vol. 120, no. 4, p. 345-349.

FERGUSON, M.E.; BUROW, M.D.; SCHULZE, S.R.; BRAMEL, P.J.; PATERSON, A.H.; KRESOVICH, S. and MITCHELL, S. Microsatellite identification and characterization in peanut (*A. hypogaea* L.). *Theoretical and Applied Genetics*, April 2004, vol. 108, no. 6, p. 1064-1070.

GAO, Dong; DU, Fei and ZHU, You-Yong. Low-background and high-resolution contracted silver-stained method in polyacrylamide gels electrophoresis. *Hereditas (Beijing)*, June 2009, vol. 31, no. 6, p. 668-673.

GIMENES, M.A.; LOPES, C.R. and VALLS, J.F. M. Genetic relationship among *Arachis* species based on

AFLP. *Genetics and Molecular Biology*, 2002, vol. 25, no. 3, p. 349-353.

GIMENES, Marcos A.; HOSHINO, Andrea A.; BARBOSA, Andrea V.; PALMIERI, Dario A. and LOPES, Catalina R. Characterization and transferability of microsatellite markers of the cultivated peanut (*Arachis hypogaea*). *BMC Plant Biology*, 2007, vol. 7, no. 9.

HAN, Zhiguo; WANG, Changbiao; SONG, Xianliang; GUO, Wangzhen; GOU, Jinying; LI, Chunhong; CHEN, Xiaoya and ZHANG, Tianzhen. Characteristics, development and mapping of *Gossypium hirsutum* derived EST-SSRs in allotetraploid cotton. *TAG Theoretical and Applied Genetics*, February 2006, vol. 112, no. 3, p. 430-439.

HE, Guohao and PRAKASH, Channapatna. Evaluation of genetic relationship among botanical varieties of cultivated peanut (*Arachis hypogaea* L.) using AFLP markers. *Genetic Resources and Crop Evolution*, August 2001, vol. 48, no. 4, p. 347-352.

HE, Guohao; MENG, Ronghua; NEWMAN, Melanie; GAO, Guoqing; PITTMAN, Roy. N. and PRAKASH, Channapatna S. Microsatellites as DNA markers in cultivated peanut (*Arachis hypogaea* L.). *BMC Plant Biology*, 2003, vol. 3, no. 3.

HOLBROOK, C.C. and STALKER, H.T. Peanut breeding and genetic resources. *Plant Breeding Reviews*, 2003, vol. 22, p. 297-356.

LAGERCRANTZ, Ulf; ELLEGREN, Hans and ANDERSSON, Leif. The abundance of various polymorphic microsatellite motifs differs between plants and vertebrates. *Nucleic Acids Research*, March 1993, vol. 21, no. 5, p. 1111-1115.

LEAL-BERTIOLI, Soraya C.M.; JOSE, Ana Carolina V.F.; ALVES-FREITAS, Dione M.T.; MORETZSOHN, Márcio C.; GUIMARÃES, Patricia M.; NIELEN, Stephan; VIDIGAL, Bruna S.; PEREIRA, Rinaldo W.; PIKE, Jodie; FÁVERO, Alessandra P.; PARNISKE, Martin; VARSHNEY, Rajeev K. and BERTIOLI, David J. Identification of candidate genome regions controlling disease resistance in *Arachis*. *BMC Plant Biology*, 2009, vol. 9, no. 112.

LI, Men-Jun; LI, Ai-Quin; XIA, Han; ZHAO, Chuan-Zhi; LI, Chang-Sheng; WAN, Shu-Bo; BI, Yu-Ping and WANG, Xing-Jun. Cloning and sequence analysis of putative type II fatty acid synthase genes from *Arachis hypogaea* L. *Journal of Biosciences*, June 2009, vol. 34, no. 2, p. 227-238.

LI, Meng-Jun; XIA, Han; ZHAO, Chuan-Zhi; LI, Ai-Qi; LI, Chang-Sheng; BI, Yu-Ping; WAN, Shu-Bo and WANG, Xing-Jun. Isolation and characterization of putative acetyl-CoA carboxylases in *Arachis hypogaea* L.



*Plant Molecular Biology Reporter*, March 2010, vol. 28, no. 1, p. 56-58.

LIANG, Xuanqiang; CHEN, Xiaoping; HONG, Yanbin; LIU, Haiyan; ZHOU, Guiyuan; LI, Shaoxiong and GUO, Baozhu. Utility of EST-derived SSR in cultivated peanut (*Arachis hypogaea* L.) and *Arachis* wild species. *BMC Plant Biology*, 2009, vol. 9, no. 35.

MORETZSOHN, M.C.; LEOI, L.; PROITE, K.; GUIMARÃES, P.M.; LEAL-BERTIOLI, S.C.M.; GIMENES, M.A.; MARTINS, W.S.; VALLS, J.F.M.; GRATTAPAGLIA, D. and BERTIOLI, D.J. A microsatellite-based, gene-rich linkage map for the AA genome of *Arachis* (Fabaceae). *TAG Theoretical and Applied Genetics*, October 2005, vol. 111, no. 6, p. 1060-1071.

MURRAY, M. and THOMPSON, W. Rapid isolation of high-molecular weight plant DNA. *Nucleic Acids Research*, 1980, vol. 8, no.19, p. 4321-4325.

PROITE, Karina; LEAL-BERTIOLI, Soraya C.M.; BERTIOLI, David J.; MORETZSOHN, Márcio C.; DA SILVA, Felipe R.; MARTINS, Natalia F. and GUIMARÃES, Patricia M. ESTs from a wild *Arachis* species for gene discovery and marker development. *BMC Plant Biology*, 2007, vol. 7, no. 7.

SIMPSON, C.E.; STARR, J.L.; NELSON, S.C.; WOODARD, K.E. and SMITH, O.D. Registration of TxAG-6 and TxAG-7 peanut germplasm. *Crop Science*, November 1993, vol. 33, no. 6, p. 1418.

SIMPSON, C.E. and STARR, J.L. Registration of COAN peanut. *Crop Science*, May 2001, vol. 41, no. 3, p. 918.

STALKER, H.T. and BEUTE, M.K. Registration of four leaf spot resistant peanut germplasm lines. *Crop Science*, September 1993, vol. 33, p. 1117.

SUBRAMANIAN, V.; GURTU, S.; NAGESWARA RAO, R.C. and NIGAM, S.N. Identification of DNA polymorphism in cultivated groundnut using random amplified polymorphic DNA (RAPD) assay. *Genome*, August 2000, vol. 43, no. 4, p. 656-660.

TALLURY, S.P.; HILU, K.W.; MILLA, S.R.; FRIEND, S.A.; ALSAGHIR, M.; STALKER, H.T. and QUANDT, D. Genomic affinities in *Arachis* section *Arachis* (Fabaceae): molecular and cytogenetic evidence. *Theoretical and Applied Genetics*, November 2005, vol. 111, no. 7, p. 1229-1237.

WANG, Z.; WEBER, J.L.; ZHONG, G. and TANKSLEY, S.D. Survey of plant short tandem DNA repeats. *Theoretical and Applied Genetics*, April 1994, vol. 88, no. 1, p. 1-6.

WANG, X.J.; SU, L.; QUAN, X.Q.; SHAN, L.; ZHANG, H.T. and BI Y.P. Peanut (*Arachis hypogaea* L.) EST sequencing, gene cloning an Agrobacteria-mediated transformation. In: International groundnut conference on groundnut Aflatoxin and genomics. (5<sup>th</sup> -9<sup>th</sup> November, 2006, Guangzhou, China). Abstracts, 2006. p. 59.