# Transferability of Soybean SSR Markers in Peanut (Arachis hypogaea L.) 

Guohao $\mathrm{He}^{1 *}$, F.E. Woullard ${ }^{1}$, I. Marong ${ }^{1}$, and B.Z. Guo ${ }^{2}$


#### Abstract

Simple sequence repeats (SSRs) are useful DNA markers in plant genetic research. However, they are not fully exploited in peanut because of the high cost and labor intensity involved in their development. Many studies have showed that DNA markers could be transferable among related species due to the conserved regions in their genomes. The objective of this study was to investigate the transferability of soybean SSR markers to peanut because of the availability of a large number of soybean SSRs. Four hundred thirty-two soybean SSR primer pairs were used to amplify peanut genomic DNAs extracted from four cultivated peanut lines. The result showed that $25 \%$ of soybean SSR primer pairs tested in this study could amplify peanut genomic DNA. Among these transferable SSR markers, 28\% have detected polymorphism among these peanut lines. These transferable markers will benefit peanut genome research by not only providing additional DNA markers in peanut, but also allowing comparative mapping to be possible between peanut and soybean.


Key Words: Transferable marker, soybean SSR, peanut.

Simple sequence repeat (SSR) markers are a valuable tool in genetic mapping, genotyping, and marker-assisted selection in breeding due to their characterization of co-dominant loci, high allelic variation, even distribution, and be easily used by PCR. In peanut, SSR markers have been developed by several groups (Hopkins et al., 1999; He et al., 2003; Ferguson et al., 2004; Luo et al., 2005a), however, they are still not fully exploited and developed compared to other crops. Peanut genome research has made less progress than other legumes because of insufficient genomic tools available (Luo et al., 2005a, 2005b). Thus, one of the pressing needs in peanut genomic research is to take advantage of progress made in the wellcharacterized legumes. Searching for transferable SSR marker developed from model legumes, such

[^0]as soybean, Medicago truncatula, and Lotus japonicus is a cost-effective way to increase DNA markers for peanut genomic studies and genetic linkage map development.

Many studies have shown that RFLP and SSR markers were transferable among cereal crops (Saghai Maroof et al., 1996; Devos et al., 1998; Zhang et al., 1998; Hernandez et al., 2001; Babula et al., 2003; Kuleung et al., 2004; La Rota and Sorrells 2004; Spielmeyer et al., 2004), and legume crops (Boutin et al., 1995; Humphry et al., 2002; Isobe et al., 2003). The transferable markers are developed from the conserved genomic regions among related species. Transferability of DNA markers between the genomes of different species not only provides researchers with large pools of available markers, but also allows us to better understand the evolution and speciation of crops through comparative mapping. However, the transferability of DNA markers from other crops to peanut is unknown. Therefore, the objective of this study is to examine the transferability of soybean SSR markers to cultivated peanut.

## Materials and Methods

Plant Material. Two peanut cultivars (GK7 and COAN) and two breeding lines (C11-2-39 and 448A) were used in this study to test the transferability of soybean SSR markers in peanut. These four peanut lines were selected because they are used as parental lines in current peanut breeding programs and mapping population development. The cultivar, COAN, was developed by introgression from $A$. cardenasii, A. duranensis and $A$. batizocoi to cultivated peanut (Simpson and Starr, 2001).

DNA Extraction. DNA was extracted from young leaf tissue using the CTAB method of Murray and Thompson (1980) with some modifications. Leaves ( $3-5 \mathrm{~g}$ ) were ground in liquid nitrogen and suspended in a buffer containing $2 \%$ CTAB, 30 mM Tris-HCl ( pH 8.0 ), 10 mM EDTA, and 0.1 M NaCl . One-third volume of 5 M potassium acetate was added, and the supernatant was extracted twice with phenol and once with chloroform. Nucleic acid was precipitated with two volumes of ethanol and suspended in 0.5 ml TE ( 10 mM Tris- $\mathrm{HCl}, \mathrm{pH} 8.0$, and 1 mM EDTA) containing $5 \mu \mathrm{~g} / \mathrm{ml}$ RNaseA (Sigma). This mixture was then extracted twice with phenol:chloroform
Table 1. List of transferable soybean SSR markers in peanut.

| SSR locus | Repeat motif | Upper primer sequence ( $5^{\prime}->3^{\prime}$ ) | Lower primer sequence ( $5->3^{\prime}$ ) | Allele size in Williams (soybean) |
| :---: | :---: | :---: | :---: | :---: |
| Sat_119 | (AT) 25 | TAGGCTTTCAATTTGCAGAACT | GTTAGGTGTCCCAAGCAACTTA | 152 |
| Sat_143* | (AT) 13 | GAAGATTGGGTAGATACTTCAACAC | GGATGGATGGTCCATTGATTCTTT | 177 |
| Sat_146 | (AT) 20 | GGGATCAAGTTACTTCAAAATCAT | GGCGATGGAAATAGGGCAAATAAT | 257 |
| Sat_147* | (AT) 12 | GTGCGACGTCATGCCTTACTCAAT | GCGCTCCGTACACTTAAAAAAGAA | 265 |
| Sat_149 | (AT) 21 | GCGGAGCAACCACTTGTGTCTTCCTGT | GCGCGTAGTTGAATTAATTAAATTACT | 208 |
| Sat_150* | (AT) 24 | GCGCACATGCTCACCAAGCAAAGTAT | GCGGTAGAGCGGATTAAACTTGTC | 212 |
| Sat_151* | (AT) 13 | GCTGCATCAGATCACCCATCCTTC | CATGCCATGTTGTATGTATGT | 230 |
| Sat_155 | (AT) 19 | GGGACAGCACCGTCAAGGAGGAGA | TGGGAAAGAAATTGTAGC | 171 |
| Sat_156 | (AT) 17 | GCGGTGTGGATCCAAAAACTCAAACTT | GCGTGCTAGTTCGATCAGCTTAGTTTC | 214 |
| Sat_159 | (AT) 22 | GCGCCTAGAAGTAATTAACTCTCT | GCATTCCCGTGCCCCACATATGCT | 173 |
| Sat_160 | (AT) 25 | GCGCATGATAACCTATAATGAGAT | CCAGCAAGCAATGCTCGGTCTACT | 204 |
| Sat_162 | (AT) 36 | GCGTGGTTTTTCGCTGGATATA | GCGCATTTCGTAACATATTTTTCAC | 175 |
| Sat_169 | (AT) 17 | ATTCGTTAAATACTCCACATCAATA | TTATGCTTTGTTGTTTTTCAGTT | 162 |
| Sat_172* | (AT) 18 | GCGTTCTAATTTCCTGACACTGTT | GCGGGACGTAAACGGATAATAAGGT | 208 |
| Sat_180 | (AT) 23 | GATCTAGGGCAAACAAGGTT | CTCGCTCTTCGCAACATA | 235 |
| Sat_184 | (AT) 23 | GCGGAATTTGAGTCCTCTAAAGTG | GCGTGGCACCTCAAGATGGAAGT | 264 |
| Sat_187 | (AT) 29 | GCGTAACGGTGATACAAACAGATT | GCGGCGACTCTGATAACAACA | 239 |
| Sat_189 | (AT) 10 | GCGCACCTAGTTGACTCTTG | ACTACCCCACATACTTCCTTTTAT | 117 |
| Sat_192* | (AT) 13 | GCGGAATGGCAATAGTTGATGAGTA | GCGGGATGGGATATGAGAGTAAG | 173 |
| Sat_197 | (AT) 33 | GCGATTTTGGTTTTGTTTTATTAG | GCGGTTAACAGCCAAGTTCTTTC | 194 |
| Sat_205 | (AT) 26 | GCGCCTTTTCGTCTGTTCTGTTC | GCGAGCTTTTAAAAATTTAGAAATCAAT | 219 |
| Sat_217 | (AT) 21 | GCGAAAAATTGTCAATGATATGATCAGTAAG | GCGGTCCTAGATGAAAAATGCTTTGTAA | 293 |
| Sat_218 | (AT) 26 | GCGCACGTTAAATGAACTGGTATGATA | GCGGGCCAAAGAGGAAGATTGTAAT | 290 |
| Sat_219* | (AT) 26 | GCGTCATGCCACGTGATATTTTAT | GCGTGTGTCCCAAATGTGATTCA | 262 |
| Sat_222 | (AT) 21 | GCGGTCATGTGTCCCATTTAATTTAATCAA | GCGATGTGCCTCAAAAACTAACATCAATAA | 168 |
| Sat_228 | (AT) 24 | GCGTGACTACGGGAAGTTGGAAC | GCGTTGGCGGTAAGAGCACTATA | 252 |
| Sat_229* | (AT) 21 | GCGTGTGCTACTTCACATCTTGAGAGAAAGA | GCGAGGGTTTAGAAAAAGATTCACCAAATAT | 257 |
| Sat_230 | (AT) 26 | GCGGTGGGACATTGGTTTAAGTTATTTT | GCGGGATATCTTCAGCGATGGATTTTA | 283 |
| Sat_234* | (AT) 22 | GCGATGCGTTTAATAAGTTTTGAAAAATGCC | GCGGAAACCATCCTTATATGTCAATTGCTCA | 332 |
| Sat_235* | (AT) 26 | GCGTTGGGATGGGTGTAAAACATT | GCGGAAGGCAAGTCAAGTTGATGAG | 281 |
| Sat_237* | (AT) 25 | GCGTTCCTGAATTTTCTTCTTTGTTGTA | GCGTTTTGGTTTTACTTGCTATTTATCCT | 223 |
| Sat_240 | (AT) 15 | GCGGGCAGAAGTCTAATGAATGTGAAATGA | GCGGTTGTGACCGAAATAGATGTTATTTAAT | 232 |
| Sat_241 | (AT) 21 | GCGTATTTTCTAATTCCACTATAACTTCAAT | GCGGGTATAAGTATCCATCAAATGTCAG | 301 |
| Sat_242* | (AT) 18 | GCGGATCCACCACTTGTTCTAAGAATCTC | GCGTAGGGTCGGGTTTTAGTATGTCATT | 265 |
| Sat_244 | (AT) 27 | GCGTCAACCGGTGAAAAAACCTA | GCGTGGCTGGCAGTAGTCTATATCA | 224 |
| Sat_247 | (AT) 21 | GCGGGGCAGGATATGATATTGTT | GCGTATTCGCCAAGCACTACTTTTT | 260 |
| Sat_250* | (AT) 19 | GCGGTTTTTGCTTTAGGACATTTTGATA | GCGTTGGGTACAACATATAATATTTTGGA | 296 |
| Sat_252 | (AT) 25 | GCGTTTTTCTGTCATGTCTTTGAATTTT | GCGGCAGGTCTCATACAAGTCATCATCT | 192 |
| Sat_253 | (AT) 22 | GCGATTGGTTGGGTGTTTAATTTTAAGAT | GCGTGTTGATGGTATAAAGATCGCTACTCT | 275 |
| Sat_260 | (AT) 25 | GCGCCGTTAGTTGTCGAGGTGTCAACC | GCGTCGGTGATTAAAAATAAGTATCAAAG | 300 |
| Sat_279 | (AT) 28 | GCGTTGCGTTGTTACGTGAAAGCACAGAAAC | GCGACTGGTTAATCTAGTCAGACTTAACAGA | 276 |

Table 1. continued.

| SSR locus | Repeat motif | Upper primer sequence ( $5^{\prime}->3^{\prime}$ ) | Lower primer sequence ( $5->3^{\prime}$ ) | Allele size in Williams (soybean) |
| :---: | :---: | :---: | :---: | :---: |
| Sat_283 | (AT)26 | GCGTGGTGCACGATCATATAGAG | GCGTCTCCTTCGCTATCTCAAAC | 195 |
| Sat_285 | (AT) 34 | GCGATCCCACAATATTTCTATTTCTTT | GCGGGCAAAATGCAGATGTATAAAC | 289 |
| Sat_286 | (AT) 32 | GCGTTGCTTGCTAAGTAGTGTTTTTAATCCT | GCGTCTCCCATCATGCAACTTCAATA | 161 |
| Sat_290 | (AT) 34 | GCGATGCCAAACTAGCTGAAGAGAAAT | GCGTAGCCTGCTTGGATGGTAGATTC | 265 |
| Sat_293 | (AT)26 | GCGTTAGGCAAATGAGATGTCAA | GCGCAGGGCAGTCATCGGAGGTAT | 278 |
| Sat_296* | (AT) 32 | GCGAGACCCATTTAATTCTCAATATCAGACA | GCGCCCGTGAATGAGTCAAACAAGTA | 233 |
| Sat_298 | (AT) 28 | GCGCGTCGAAGCAAAAATTAAA | GCGGCGAAACCCACAAAGCATA | 282 |
| Sat_299* | (AT) 23 | GCGACAAGGCACTCACATCTCTTCTC | GCGCTACCCATAACAAAAAGTTCAAATC | 292 |
| Sat_351 | (AT) 21 | GCGCCACCCAAGGGCATCTTTCG | GCGGGCCGCAACTATGAAAAGAC | 277 |
| Sat_355 | (AT) 27 | GCGATAACACTAAATGACCAGCAGGATT | GCGGAGCCAAGTATCAAACCAAAACAAC | 213 |
| Sat_357* | (AT) 17 | GCGAGGGTTTAAGGTGTAGGTTGT | GCGCACCGCTTTTGTTTCTTTTTG | 260 |
| Sat_361 | (AT) 19 | GCGTTAGATTTCCTTAGAATACATTGCTTCC | GCGTTGACACTCATGATGTTATCTTACACC | 275 |
| Sat_362 | (AT)23 | GCGCAAACAAGTTATACCTTTATATTGGTGA | GCGAAGGGAACCTAACGTATGTCTTTTA | 196 |
| Satt503 | (ATT) 18 | GGGTGGCCATGGAATAAT | TTTCGGGTAGATGAGTGTAGG | 256 |
| Satt504 | (ATT)21(ATTT) 9 | GCGCATGTGCAACTTGAAAAACA | TCGTTGGTTGACCCAATGTCATC | 210 |
| Satt507 | (ATT) 22 | GCGCTCAGCCTTGTTAAATCACTT | GCGCTACTCTCGTGTCGTTAGTTA | 217 |
| Satt509 | (ATT) 30 | GCGCTACCGTGTGGTGGTGTGCTACCT | GCGCAAGTGGCCAGCTCATCTATT | 238 |
| Satt520 | (ATT) 12 | GCGGTGTGCAAGAGTGACA | GCGCATTTGGACTTTCTA | 271 |
| Satt522* | (ATT) 16 | GCGAAACTGCCTAGGTTAAAA | TTAGGCGAAATCAACAAT | 262 |
| Satt523 | (ATT) 15 | GCGATTTCTTCCTTGAAGAATTTTCTG | GCGCTTTTTCGGCTGTTATTTTTAACT | 168 |
| Satt524 | (ATT)14 | GCGAATTATCCAAAGATACACTTAGTC | GCGGGTCTTACGAACGTGTCACATTAT | 168 |
| Satt530 | (ATT) 12 | CATGCATATTGACTTCATTATT | CCAAGCGGGTGAAGAGGTTTTT | 220 |
| Satt531* | (ATT)14 | GCATGCAACTGAGGGAGCAGAT | GCCACAAATTATGCAGAATATA | 240 |
| Satt535 | (ATT) 10 | GCGCCCAACAACTTATAGTTATATA | GCGCTAGATTTTAGGCAGAGATTAA | 253 |
| Satt550* | (ATT) 16 | CGTCAATTAAGCAAAAATGTGA | GCGCGGATGAGCGTGCGTTTTTA | 210 |
| Satt552 | (ATT)14 | CGAACCGGCAAAACCAAGAT | GATCCGCATTGGTTTCTTACTT | 154 |
| Satt556 | (ATT) 14 | GCGATAAAACCCGATAAATAA | GCGTTGTGCACCTTGTTTTCT | 167 |
| Satt570 | (ATT) 11 | CTCATGTGGTCCTACCCAGACTCA | CGCTATCCCTTTGTATTTTCTTTTGC | 105 |
| Satt573 | (ATT) 10 | GCGGATTTCGATTTGAATATACTTAC | CCTGTGGCTGTTATACTATGCATATA | 167 |
| Satt576* | (ATT) 19 | GCGGGACACACACAAACACCTACA | GCGGGTTTGCGTTCTTATATTATC | 297 |
| Satt593 | (ATT)15(TTG)10(TTC)4 | GCGGGGTTGTTGATCTATAATGTAA | GCGGGTTTGGATTTTATAATGTGAT | 190 |
| Satt596 | (ATT) 17 | TCCCTTCGTCCACCAAAT | CCGTCGATTCCGTACAA | 252 |
| Satt597* | (ATT) 13 | GCTGCAGCGTGTCTGTAGTAT | CGAGGCACAACCATCACCAC | 155 |
| Satt622 | (ATT) 27 | GCGGTGTAGGTAATAATTTTAATTCTCAT | GCGGTGTAGGTTTCACACTTCATTCAC | 236 |
| Satt623 | (ATT) 15 | GCGGTGCAATGATTTTAATGATATGAT | GCGCGTGTAAAAGGTTATAACGTGTAA | 238 |
| Satt624 | (ATT) 23 | GCGATGGCTTGTGGGAACACTAAT | GCGGACGTGGGACCAACACACTAA | 151 |
| Satt631* | (ATT)21 | GGTAGATCCAGGAGCTTGAGTCAG | GCGCATCTCACTGCACTTGATTTT | 175 |
| Satt633 | (ATT) 12 | GGGACACTATCGGCCTAGAAAGTT | GGGTGATAAAGTTCCCCCTCTAAG | 131 |
| Satt641 | (ATT) 10 | GCGGAACATCACGGTTATA | GCGGGAGGCTCTGTCTCTTAGA | 318 |
| Satt650* | (ATT) 10 | CAGTTGGCTGGTCAAATC | TCTGGGTTACTTTTATTGTCA | 247 |

Table 1. continued.

| SSR locus | Repeat motif | Upper primer sequence ( $5^{\prime}->3^{\prime}$ ) | Lower primer sequence ( $5->3^{\prime}$ ) | Allele size in Williams (soybean) |
| :---: | :---: | :---: | :---: | :---: |
| Satt652 | (ATT)18 | GCGAACATTCCAAAATTAATGATAAAAA | GCGGGGTAATATGCACTCTCCAGTAT | 217 |
| Satt658 | (ATT) 11 | GCGTTGAGTGGTAAAATTTATAATTAAA | ACTTGGCCCGCGAAGTGCTCAATTG | 227 |
| Satt569 | (ATT) 14 | GCGCAAATTGCTTCACGCATCCAAAT | GCGGCCTACTATAGTGAAGGGTATA | 177 |
| Satt678 | (ATT) 10 | CTAAGCGTGACAAACAGACCATTA | CGGCCATATCTACCAATCAGA | 158 |
| Satt679* | (ATT) 15 | GCGAACAAAGGAAGAATAGAG | CAATTACCCCCAACAACTAAGT | 262 |
| Satt681 | (ATT) 20 | GCGGTGCACTTGTCAATCTGTT | GCGGTGAGGCATATGTCAGTC | 241 |
| Satt688 | (ATT) 11 | ATGCCTCCAAAGAGAAAT | CTGCCCATTGACCCATCT | 175 |
| Satt692* | (ATT) 12 | GCGAAGATTGGTCTTTTATGTCAAATG | GCGGAGGAATACAAGTCTCTATTCAA | 231 |
| Satt699* | (ATT) 9 | GCGGATATTTTTGTCCTCAATAAT | GCGTACCGTATGTGGAGTTT | 181 |
| Satt700 | (ATT) 12 | GCGGGGGTTAAGAGGAGGAAAAATA | GCGCACTTTGCAAATGAGAGAT | 147 |
| Satt702 | (ATT)26 | GCGGGGTTCTGTGGCTTCAAC | GCGCATTGGAATAACGTCAAA |  |
| Satt709* | (ATT)20 | GCGTGACGAATTCTGTTCTAACTC | GCGCATACGCCACTCCACTCA | 281 |
| Satt712 | (ATT)21 | GCGAATATAGCCAAATTTAGGTTGAATGACA | GCGACCACCCATCACCTCCACCTCAAACAAC |  |
| Satt716* | (ATT) 10 | GCGTTTGCAGTTTGGATGATGTTGAT | GCGAACCCTTGAGTTGGACATGTTGA |  |
| Satt718 | (ATC)15(ATT) 15 | GCGTGCAACACCTCAAGTTTCAAATAC | GCGTAGCTCTTTCCAAAGTTTTCATC |  |
| Satt721 | (ATT) 13 | GCGTGGTTGGAAGGAAGAGAATGAC | GCGAAAGGCTGGCTGACACTGACT | 206 |
| AW132402 | (AT) 17 | GCGCCTCCCTCCTCTCCTTTCTT | GCGTTTCCCACATATTCTATCATTTGTT | 153 |
| AW781285 | (CGA) 8 | GCGTCTTTTGCACGATGAA | GCGAATGGTGGGAGAAA | 138 |
| BE021153 | (GT) 10 | GCGAAACTGCTTGTATTTTA | GCGCTCCAATTGAAAGTG | 153 |
| BE475343* | (GT)11 | GCGTCTCCCTGTCTCTC | GCGAGCTTAAAACAATCATC | 181 |
| AW186493 | (CTT) 13 | GCGGTGATCCGTGAGATG | GCGGAAAGTAGCACCAAGAG | 219 |
| BE806387 | (CTT) 14 | GCGACCCCTTTTGTCTTCTT | GCGGAGGCCAGAGATGAA | 205 |
| AF162283 | (CT) 11 | GCGAGTTCTGGATGTAGG | GCGTGGCGGCTTTGGTAG | 221 |
| BE823543* | (CT) 10 | GCGAAATGCCGAAAGAG | GCGGGGATAAGAAAAACAAT | 156 |
| AZ536570 | (AT) 12 | GCGGCATGACAAGGAAATCT | GCGAATTAAAGGCAAAAGGAAAA | 175 |
| AW620774 | (CTT) 9 | GCGATTTCCCCTCTTACTC | GCGAAAAACCAAGTTC | 152 |
| AZ302047* | (AT) 15 | GCGTGGAGCGAAAATCAACTCTT | GCGATGACCCCGTAATGGTGA | 234 |
| AW756935 | (ATT) 18 | GCGGCTGGTGATTGTGTAAT | GCGTAATATAGTTTTGTATTGAAAT | 232 |

[^1](1:1) and precipitated with two volume of ethanol, dissolved in 0.2 ml of TE, and diluted to $50 \mathrm{ng} / \mu \mathrm{l}$ for PCR amplification.

PCR Amplification. Soybean SSR primer pairs were provided by Drs. Cregan and Song at the USDA-ARS, Beltsville, MD. A total of 432 soybean SSR primer pairs including $200(A T)_{n}$ motifs (named as Sat \#), 197 (ATT) ${ }_{n}$ motifs (named as Satt \#), and 35 EST-SSR (named as GenBank accession \#) primer pairs were used. PCR amplification was carried out in $10 \mu \mathrm{l}$ solution containing 0.25 units of AmpliTaq polymerase (Applied Biosystems, CA), $1.5 \mu \mathrm{M}$ of each primer, $5 \mu \mathrm{l}$ of FailSafe PCR 2X PreMix-B (Epicentre, WI), and 50 ng peanut genomic DNA. Amplification was carried out under the following conditions: 3 min at 94 C for initial denaturation; two cycles of 30 sec at $94 \mathrm{C}, 30 \mathrm{sec}$ at 65 C , and 1 min at 72 C ; two cycles of 30 sec at $94 \mathrm{C}, 30 \mathrm{sec}$ at 56 C , and 1 min at $72 \mathrm{C} ; 30$ cycles of 15 sec at $94 \mathrm{C}, 30 \mathrm{sec}$ at 55 C , and 1 min at 72 C ; and 10 min at 72 C for final extension (Mellersh and Sampson, 1993). PCR products were analyzed on a $6 \%$ denaturing polyacrylamide gel and visualized by silver staining (He and Prakash, 1997).

## Results and Discussion

Out of 432 soybean SSR primer pairs tested, 109 ( $25 \%$ ) were amplifiable in peanut (Table 1). Among 109 soybean SSR markers, $28 \%$ detected polymorphism among 4 cultivated peanut lines (Fig. 1). The polymorphism detection rate in this study is higher than the previous report (He et al. 2003). This difference may be due to the fact that cultivar
'COAN' contains wild species segments of chromosomes, from which additional genetic variation could be detected. For $54(A T)_{n}$ markers, $30 \%$ detected genetic variation, while $28 \%$ of 43 (ATT) ${ }_{n}$ markers detected a polymorphism. The transferability of EST-SSR in peanut was higher ( $34 \%$ ) than genomic SSR markers, but only $25 \%$ of ESTSSR markers could detect polymorphism (Table 2). The result of more transferable markers from the coding regions (EST-SSR) was consistent with the previous study (Liewlaksaneeyanawin et al., 2003) because coding regions are more conserved between related species.

These amplifiable markers implied that $25 \%$ of primer-binding sites were conserved between soybean and peanut. However, most banding patterns amplified by these transferable markers were similar to multiple-band patterns produced by random amplified polymorphic DNAs (RAPDs) rather than typical SSR banding patterns (Fig. 1). Choumane et al. (2004) reported that $54.4 \%$ of chickpea SSR primer-binding sites were conserved among the three genera, chickpea, dry pea, and lentil. They also found that SSR motifs were present in chickpea, but absent either in dry pea or lentil, after sequencing the amplicons produced by the same chickpea SSR primer. In this study, we speculate that amplicons from soybean SSR primers that produced complex patterns may not contain SSR motifs. This speculation needs to be confirmed by sequencing these amplicons. Nevertheless, these transferable SSR markers derived from soybean could be used to detect genetic variation in peanut.

Among legume crops, there is a high level of conservation between cowpea and mungbean,


Fig. 1. Genetic variation among four peanut lines detected by soybean SSR markers Sat 151, Satt 692, and BE475343. Lane 1-4 are cultivars C11-2-39, GK7, 448A, and COAN, respectively.

Table 2. Transferability of soybean SSR markers in peanut.

| Soybean marker | Total marker | Amplifiable marker | Polymorphic marker | Transferability $\%^{\text {a }}$ | Poly/ampl $\%^{\mathrm{b}}$ |
| :--- | :---: | :---: | :---: | :---: | :---: |
| (AT)n | 200 | 54 | 16 | 27 | 30 |
| (ATT)n | 197 | 43 | 12 | 22 | 28 |
| EST-SSR | 35 | 12 | 3 | 34 | 25 |
| Total | 432 | 109 | 31 | 25 | 28 |

${ }^{\text {a }}$ Transferability $=$ Amplifiable markers/total markers.
${ }^{\mathrm{b}}$ Poly/ampl $=$ Polymorphic markers/amplifiable markers.
mungbean and common bean, and mungbean and lablab genomes (Menancio-Hautea et al., 1993; Boutin et al., 1995; Humphry et al. 2002). In contrast, the homology between soybean and common bean was retained only in dispersed blocks throughout their genomes (Boutin et al., 1995). Although the comparative mapping has been progressed in legume crops, the comparison of peanut genome with other legumes could not be carried out due to the lack of a set of common DNA markers. The level of transferability observed in this study would provide genome tools for comparative mapping between peanut and soybean because there are a large number of soybean SSR markers available. The comparative mapping between peanut and soybean will allow us to gain deeper insight into the degree of chromosome colinearity between them, and to elucidate the biological relationship among legume crops.

In our previous study, we have searched for SSR markers in 1350 peanut ESTs. Three hundred fiftythree ESTs were found to contain SSRs. Primers were designed for 44 EST-SSRs and 9 of them detected polymorphism, for a polymorphism rate of $20 \%$ in coding regions (Lou et al. 2005a). This study shows that $25 \%$ of transferable soybean ESTSSR markers detected a polymorphism in peanut genome. The results from both studies may suggest that there might be an abundance of mutations in coding regions in peanut. Using EST-SSR markers in comparative mapping between peanut and soybean will allow us to predict the location of genes of interest from soybean which has a well developed genetic map compared to peanut for which such information is scarce.

Peanut and most legume crops belong to the same subfamily Papilionoideae of the Leguminosae family. However, peanut is isolated in a different clade (dalbergioid) from most legume crops in the legume phylogenetic tree (Doyle and Luckow, 2003). There is a relatively under-developed infrastructure of genomic tools in peanut. Therefore, in peanut, there is a great need to integrate the knowledge gained from the study of model legume
genomes with the important biological and agronomic questions of peanut. Identification of transferable DNA markers from model legumes in peanut would be useful for peanut improvement through comparative genome research because the information of these transferable markers from other legumes is known. The common transferable DNA markers among legumes would also provide us tools to compare legume genomes, gain insight into relationship of legumes, and elucidate legume evolution.

## Acknowledgements

The authors thank the support from the George Washington Carver Agricultural Experiment Station in Tuskegee University. The authors also express gratitude to Drs. Cregan and Song for providing soybean SSR markers. This work has been partially funded by a grant from USDA/ CSREES/CBG (No. 00-38814-9541) and funds provided by National Peanut Foundation and Georgia Peanut Commission. Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply approval to the exclusion of other products that may be suitable.

## Literature Cited

Babula, D., M. Kaczmarek, A. Barakat, M. Delseny, C.F. Quiros, and J. Sadowski. 2003. Chromosomal mapping of Brassica oleracea based on ESTs from Arabidopsis thaliana: complexity of the comparative map. Mol Gen Genomics 268:656-665.
Boutin, S.R., N.D. Young, T.C. Olson, Z.H. Yu, R.C. Shoemaker, and C.E. Vallejos. 1995. Genome conservation among three legume genera detected with DNA markers. Genome 38:928-937.
Choumane, W., P. Winter, M. Baum, and G. Kahl. 2004. Conservation of microsatellite flanking sequences in different taxa of Leguminosae. Euphytica 138:239-245.
Devos, K.M., Z.M. Wang, J. Beales, T. Sasaki, and M.D. Gale. 1998. Comparative genetic maps of foxtail millet (Setaia italica) and (Oryza sativa). Theor Appl Genet 96:63-68.
Doyle, J.J., and M.A. Luckow. 2003. The rest of the iceberg. Legume diversity and evolution in a phylogenetic context. Plant Physiology 131:900-910.
Ferguson, M.E., M.D. Burow, S.R. Schulze, P.J. Bramel, A.H. Paterson, S. Kresovich, and S. Mitchell. 2004. Microsatellite iden-
tification and characterization in peanut (A. hypogaea L.). Theor Appl Genet 108:1064-1070.
He, G.H., and C.S. Prakash. 1997. Identification of polymorphic DNA markers in cultivated peanut (Arachis hypogaea L.). Euphytica 97:143-149.
He, G.H., R.H. Meng, M. Newman, G.Q. Gao, R.N. Pittman, and C.S. Prakash. 2003. Microsatellites as DNA markers in cultivated peanut (Arachis hypogaea L.). BMC Plant Biology 3:3.
Hernandez, P., G. Dorado, P. Prieto, M.J. Gimenez, M.C. Ramirez, D.A. Laurie, J.W. Snape, and A. Martin. 2001. A core genetic map of Hordeum chilense and comparisons with maps of barley (Hordeum vulgare) and wheat (Triticum aestivum). Theor Appl Genet 102:1259-1264.
Hopkins, M., A. Casa, T. Wang, S. Mitchell, R. Dean, G. Kochert, and S. Kresovich. 1999. Discovery and characterization of polymorphic simple sequence repeats (SSRs) in peanut. Crop Sci. 39:1243-1247.
Humphry, M.E., V. Konduri, C.J. Lambrides, T. Magner, C.L. McIntyre, E.A.B. Aitken, and C.J. Liu. 2002. Development of a mungbean (Vigna radiata) RFLP linkage map and its comparison with lablab (Lablab purpireus) reveals a high level of colinearity between the two genomes. Theor Appl Genet 105:160-166.
Isobe, S., I. Klimenko, S. Ivashuta, M. Gau, and N.N. Kozlov. 2003. First RFLP linkage map of red clover (Trifolium pratense L.) based on cDNA probes and its transferability to other red clover germplasm. Theor Appl Genet 108:105-112.
Kuleung, C., P.S. Baenziger, and I. Dweikat. 2004. Transferability of SSR markers among wheat, rye, and triticale. Theor Appl Genet 108:1147-1150
La Rota, Mauricio, and M.E. Sorrells. 2004. Comparative DNA sequence analysis of mapped wheat ESTs reveals the complexity of genome relationships between rice and wheat. Funct Integr Genomics 4:34-46.

Liewlaksaneeyanawin, Cherdsak, C.E. Ritland, Y.A. El-Kassaby, and K. Ritland. 2003. Single-copy, species-transferable microsatellite markers developed from loblolly pine ESTs. Theor Appl Genet 109:361-369.
Luo, M., P. Dang, B.Z. Guo, G. He, C.C. Holbrook, M.G. Bausher, and R.D. Lee. 2005a. Generation of expressed sequence tags (ESTs) for gene discovery and marker development in cultivated peanut. Crop Sci. 45:346-353.
Luo, M., P. Dang, C.C. Holbrook, M.G. Bausher, R.D. Lee, R.E. Lynch, and B.Z. Guo. 2005b. Identification of transcripts involved in resistance responses to leaf spot disease Caused by Cercosporidium personatum in peanut (Arachis hypogaea). Phytopath. 95:381-387.
Mellersh, C., and J. Sampson. 1993. Simplifying detection of microsatellite length polymorphisms. BioTechniques 15:582-584.
Menancio-Hautea, D., C.A. Fatokun, L. Kumar, D. Danush, and N.D. Young. 1993. Comparative genome analysis of mungbean (Vigna radiata L. Wilczek) and cowpea (Vigna unguiculata L. Walpers) using RFLP mapping data. Theor Appl Genet 86:797-810.
Murray, M.G., and W.F. Thompson. 1980. Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res 8:4321-4326.
Saghai Maroof, M.A., G.P. Yang, R.M. Biyashev, P.J. Maughan, and Q. Zhang. 1996. Analysis of the barley and rice genomes by comparative RFLP linkage mapping. Theor Appl Genet 92:541-551.
Simpson, C.E., and J.L. Starr. 2001. Registration of 'COAN'. Crop Sci. 41:118.
Spielmeyer, W., M. Ellis, M. Robertson, S. Ali, J.R. Lenton, and P.M. Chandle. 2004. Isolation of gibberellin metabolic pathway genes from barley and comparative mapping in barley, wheat and rice. Theor Appl Genet 109(4):847-855.
Zhang, H., J. Jia, M.D. Gale, and K.M. Devos. 1998. Relationships between the chromosomes of Aegilops umbellulata and wheat. Theor Appl Genet 96:69-75.


[^0]:    ${ }^{1}$ Dept. of Agricultural Sciences, Tuskegee University, Tuskegee, AL 36088.
    ${ }^{2}$ USDA-ARS, Crop Protection and Management Research Unit, Tifton, GA 31793.
    *Corresponding author (email: hguohao@tuskegee.edu).

[^1]:    *The primers detected polymorphism among four peanut lines

