Development and Application of InDel Markers in Genetic Diversity Study for Cultivated Peanut

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ABSTRACT To date, nearly 10,000 molecular markers have been identified by various research groups around the world, but less than 14.5% showed polymorphism in peanut and only 5.6% were mapped (Zhao et al. 2012). Low levels of polymorphism limit the application of marker-assisted selection in peanut breeding programs. It has been reported that insertions and deletions (InDels) markers were more polymorphic than SSRs in some crops. The goal of this study is to identify novel InDel markers and evaluate the potential use in peanut. Forty-eight InDel markers were developed from conserved sequences of functional genes and tested in a diverse panel of 118 accessions covering six botanical types of cultivated peanut, of which 104 were from the U.S. mini-core. The results showed that 16 InDel markers were polymorphic, with polymorphic information content (PIC) among InDels ranged from 0.017 to 0.569. In respect to botanical types, PICs varied from 0.176 for *fastigiata* var., 0.181 for *hypogaea* var., 0.306 for *vulgaris* var., 0.556 for *peruviana* var., 0.534 for *aequatoriana* var., to 0.660 for *hirsuta* var., implying that *hirsuta* var., *peruviana* var., and *aequatoriana* var. have higher genetic diversity than the other types and provide a basis for gene functional studies. Single marker analysis was conducted to associate the marker and the trait in facilitating the discovery of functional genes.

INTRODUCTION

Various types of molecular markers, such as random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), inter simple sequence repeat (ISSR) markers, and simple sequence repeats (SSR) have been developed and used for genetic diversity of plant germplasm resources (Jiang et al. 2009, 2012), construction of genetic linkage map (Wang et al. 2012), molecular marker-assisted selection (MAS) and mapping and cloning of genes in peanut. Nearly 10,000 molecular markers have been identified but only 5.6% can be mapped in the linkage maps (Zhao et al. 2012). Low level of polymorphism is due to a narrow genetic basis of cultivated peanut (Xiong et al. 2011). Therefore, it is vital important to develop new type of molecular markers. InDels have been recognized as an abundant source of genetic markers that are widely spread across the genome. (Yu et al. 2005).The objectives of this research were: 1) to develop of gene-specific InDel markers; 2) to evaluate the potential use in genetic diversity study for cultivated peanut; and 3) to identify novel InDel markers that are related to the disease-resistance.

MATERIALS & METHODS

Plant Materials and Phenotyping A total of 118 accessions selected from the U.S. core collection, in which 104 accessions are from mini-core collection and 14 accessions are from *hirsuta* var. and *aequatoriana* var. that are not presented in the mini-core. Twenty seeds of each tested genotype were planted at Dawson, GA (31°45' latitude, -84°30' longitude) in 2010 and 2011 under irrigated conditions. The genotypes were planted in two-row plots 3 m long and 0.91 m between rows at a seeding rate of 3 seed m^{-1} in early May without replication. For TSWV resistance, each PI was visually rated immediately prior to digging in 2010 and 2011 for TSWV foliar symptoms on a percentage basis, similar to the 1 to 10 method described by Tillman et al. (2007). Disease evaluations for leaf spot resistance were conducted in the field under a reduced fungicide-treatment with one application of 1.5 pt/A chlorothalonil in 2010 and no fungicide application in 2011. Plants were rated using the Florida leaf-spot scoring system during flowering, two weeks before harvest, and immediately prior to harvest (Chiteka et al. 1988).

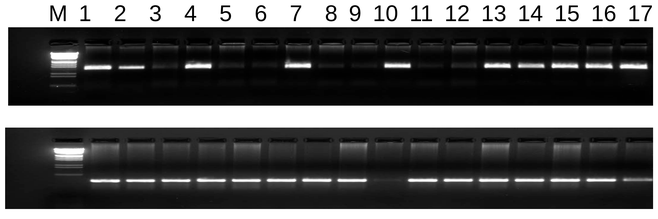

Fig.1 The fragments amplified by InDel-016 (above) and Indel-042 (bottom). The sequences (5' to 3') of Indel-016 primer are TCCTCATCAGGAACTGGGATA(F) and TGCAGCAATAGGACTTCTGG(R). For Indel-042 primer, the sequences (5' to 3') are GGGATTGAGCATGAACGATT(F) and GATAACAAATGGGGGCAAGA(R).

Design of InDels Markers Primers for InDels markers were designed using Primer Express 3.0 (Applied Biosystems, Foster City, CA). Function was identified through BLASTx utilizing peanut ESTs and comparison of the sequences according to conserved sequences of functional genes. Genomic DNA extraction and PCR followed the method of Dang and Chen (2012).

Data Analysis Polymorphism Information Content (PIC) based on allelic frequencies among 118 genotypes was calculated for each InDel markers using the following formula: $PIC=1-\sum xi^2$ where xi is the relative frequency of the ith allele of the SSR loci. Single marker analysis (SMA) (Jansen and Stam 1994) methods were used for trait-marker analysis. It was carried out by PROC GLM of SAS (SAS 9.3 2009) with the following linear model: $Y_{iklm} = u + E_i + M_k + F(M)_{kl} + E \times F(M)_{ikl} + e_{iklm}$, where Y_{iklm} is each observed phenotype, u is the population mean, E_i is the effect of year (i = 1, 2), M_k is the effect of marker genotype (k = 1, 2), $F(M)_{kl}$ is the effect of PIs within marker genotype (l=1, …, 118), $E \times F(M)_{ikl}$ is the interaction between the effect of year and the effect of PIs within marker genotype, and e_{iklm} is residual error. The threshold for declaring a marker significant was chosen to be marker-wise $p < 0.0001$, which is approximately equal to an experiment-wise $p < 0.05$ in this study based on 16 polymorphic markers.

RESULTS

All 48 pairs of InDels markers produced PCR products and 16 were polymorphic (**Fig. 1**). The polymorphic information content (PIC) of those polymorphic markers ranged from 0.0169-0.5960 with an average of 0.1349. The distribution of 16 polymorphic InDel markers among the six botanical types were quite different. *hirsuta* var., *hypogaea* var., *aequatoriana* var., and f*astigiata* var. covered more markers than *vulgaris* var. and *peruviana* var. (**Table 1**). In respect to botanical types, PICs varied from 0.176 for *fastigiata* var., 0.181 for *hypogae*a var., 0.306 for *vulgaris* var., 0.556 for *peruviana* var., 0.534 for *aequatoriana* var., to 0.660 for *hirsuta* var., which implied that *hirsuta* var., *peruviana* var., and *aequatoriana* var. have higher genetic diversity than the other types (**Table 2**). Single marker analysis identified 2 markers (032 and 042) to be significantly correlated to both tomato spotted wilt virus (TSWV) and leaf spot, but InDel-018 and 046 are only for leaf spot (**Table 3**). These markers are designed from conserved sequences of functional genes that were associated with electron transporter/metal ion, dihydroxy-acid dehydratase, arachin *Ahy-3*, and ran GTPase binding, respectively.

Table 1. Distribution of polymorphic markers among six botanical varieties.

Marker	Botanical Variety						Size of Fragment	PIC
	fa	*hy*	*vu*	*pr*	*hi*	*aq*		
InDel-003					+		440	0.0169
InDel-004	+	+			+		310	0.0830
InDel-005	+				+	+	420	0.0666
InDel-007		+					430	0.0169
InDel-011		+					470	0.0169
InDel-016	+	+	+		+	+	320	0.5288
InDel-017		+		+	+	+	320	0.1151
InDel-018	+	+	+	+	+		470	0.5960
InDel-020		+			+		390	0.0336
InDel-029	+	+					300	0.0336
InDel-030	+				+	+	240	0.0502
InDel-032		+				+	400	0.2232
InDel-033					+	+	300	0.0336
InDel-039					+	+	200	0.0666
InDel-042					+	+	250	0.1467
InDel-046	+				+	+	300	0.1310

Table 2. The number of allelic fragments, PIC values of polymorphic markers among six botanical types.

Botanical Type	No. of accessions	Alleles	PIC
fastigiata	34	7	0.1763
hypogaea	55	9	0.1809
vulgaris	12	2	0.3056
peruviana	3	2	0.5556
hirsuta	7	12	0.6597
aequatoriana	7	9	0.5341
Total	118	16	0.1457

Table 3. Signifiance (*P* value) of association between the maker and the trait.

Marker	Leaf spot	TSWV
InDel-018	<0.0001	-
InDel-032	<0.0001	<0.0001
InDel-042	<0.0001	<0.0001
InDel-046	<0.0001	-