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Peanut Genetics

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NPB Final Report

Title: **Identification of Markers for Maturity and O/L Ratio, Leafspot Resistance, and Resistance to Heat and Drought for Varietal Development**

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Objectives.

The proposed research aims to develop markers for cultivated × cultivated crosses to complement the peanut breeding programs in Texas. In the past, we have identified RFLP (restriction fragment length polymorphism) markers in wild species for nematode resistance and are currently evaluating markers for resistance to leafspot resistance using lines developed from wild species. In this continuing project, we will develop markers for cultivated × cultivated crosses. The applications expected to benefit the soonest are early maturity, disease resistance, and high O/L content.

Results:

The experimental plan was to use microsatellite (SSR, simple-sequence repeat) markers to find differences that can be used for DNA fingerprints that identify plants that have specific traits. We are identifying markers that can be used to make a microsatellite map of peanut, using the same BC1 population developed by Dr. Simpson and used for making the previous peanut RFLP map. We are also identifyng markers for use in a cross made for developing early-maturing runner lines. Finally, we used markers to identify hybrids in attempted crosses for heat stress and leafspot resistance.

(1) Find markers that distinguish between early and late-maturing parents in our breeding populations. A total of 75 peanut SSR markers were selected to screen the two parents. Out of 75 SSR primers pairs only 56 primers produced good amplification. Among these, 30 primers could distinguish the two parents in a 6% non-denaturating polyacrylamide gel. We have found 53 % polymorphism in this cross. We expect to test polymorphism with 225 additional SSR primers. It is expected that, using polyacrylamide gels, 120 polymorphic markers can be obtained, which should be enough to make a map from the TxAG-6 x Florunner cross. We expect that additional markers can be detected using the Beckman CEQ8000 DNA analyzer in the lab.

For initial experiments at mapping, genomic DNA was isolated from 18 BC_1 progenies and we started progeny screening of using polymorphic SSR markers. Initially nine polymorphic markers were evaluated individually by the chi square test for goodness of fit against a 1:1 segregation ratio at 0.01 and 0.05 probability levels (See Table 1). All the markers fit expected segregation of 1:1 based on chi-square test at 0.05 and 0.01 per cent probability. Currently we are progressing progeny screening with remaining polymorphic markers.

Table 1 . Segregation of polymorphic SSR markers in the BC_1 population

Marker	No. of Florunner alleles	No. of Heterozygous allele	χ^2 value
PM 3	7	6	0.08 ns
PM 32	5	6	0.09 ns
PM 36	8	10	0.22 ns
PM 42	6	7	0.08 ns
PM 45	9	8	0.06 ns
PM 145	9	6	0.60 ns
PM 201	8	7	0.06 ns
PM 204	7	8	0.06 ns
PGS14D01	5	8	0.46 ns

χ^2 Tabulated value
** Significant at 0.01 probability level = 6.63
* Significant at 0.05 probability level = 3.84

(2) Find markers distinguishing parents resistant and susceptible to heat and water stress, and advance populations made for drought and heat tolerance. We have developed three populations for studying the genetics of heat stress, and have tested polymorphism in two of these crosses, plus an additional population for mapping maturity and quality-related traits. We had tried to detect polymorphism using SFR agarose gels, but the lack of resolution made it difficult to detect polymorphisms. Therefore, we have used polyacrylamide gels, and found >50% polymorphism between wild and the cultivated species, and up to 23% polymorphism among parents in cultivated peanut (Table 2). We expect the actual value to be higher using a DNA analyzer, because some of the polymorphisms between parents are only 2bp and such small differences cannot be detected without use of large sequencing gels.

Table 2. Polymorphism Observed in Parents of Population

Population	Primers Tested	Primers Amplified	Polymorphic Primers	Percent Polymorphism
TxAG-6 x Florunner	75	56	30	53%
BSS56 x TamrunOL02	48	36	5	13.8%
Cultivated #2	48	35	8	22.8%
Cultivated #3	48	36	3	8.3%

(3) Identify markers that can be used to distinguish hybrids from selfs. A total of 24 oligonucleotide primer pairs flanking microsatellite repeat sequences were used in the present study. The PCR products were separated on 6 % non denaturing polyacrylamide gel using horizontal electrophoresis unit for 2- 3 hrs with chilled buffer and visualized by ethidium bromide staining on a UV trans illuminator. Polymorphic SSR markers (see Table 3) were used to fix the true hybrids. For the F_1 heat stress cross, we transplanted plants possessing the heterozygous allele for that marker. For the F_2 population made for introgression of leafspot resistance, we have confirmed the segregation of marker alleles. The PM210 and PM 3 markers are differentiating most of the hybrids studied and is a potential marker for fixing true hybrids in various crosses involving cultivated peanut.

Table 3. Development of mapping population and SSR analysis to test true hybrids

Cross	Number of F_1 Individuals (Seeds)	Number of F_1/F_2 Individuals used for SSR analysis (pods)	% of selfing	SSR primer used for testing
ICGS76× TAMRUNOL2 (F_1)	25	16	38	PM 210
ICGV87157×TAMRUNOL2 (F_1)	10	9	30	PM 210
ICGS76 × SPANCO (F_1)	14	10	50	PM 3
41-10-0103 × TAMRUNOL2 (F_2)	6	30	33	PGS12 A07
TAMRUNOL2 × 43-09-03-02(F_2)	2	12	0	PGS12A07
63-04-02-02×TAMRUNOL2(F_2)	9	54	22	PM 42
55-437 × 43-09-03-02(F_2)	6	36	0	PM 3
55-437 × 45-04-02-01 (F_2)	2	12	0	PM 210

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