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Single Marker Analysis for Oil Yield and Component Traits in Groundnut (*Arachis hypogaea* L.)

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In groundnut, marker-trait association was studied for ten yield and yield component traits using 60 SSR markers. Single marker analysis was performed to ascertain the relationship between the marker and traits. The genotype and phenotype of 71 $F_{2:3}$ population were subjected to single marker analysis (SMA) using single factor regression analysis. A total of 20 markers gave significant association with at least one of the 10 traits studied. Most of the markers were found to be related to more than one trait. The results indicated that markers PGP03A08 and SEQ11HO1 weight recorded about 12.8 and 9.6 per cent phenotypic variance explained for the trait 100- kernel weight and number of branches per plant. In addition, these markers also showed association with other traits. Marker PGP03A08 was also associated with 100-pod weight, pod yield per plant, kernel yield per plant and oil yield per plant. Similarly other markers *viz.,* SEQ4EO8, Ah2562, GM1369, Ah51, PMc348 and GM1369 had recorded association with more traits. Hence, these markers could be considered as a potential tool in the marker assisted breeding in groundnut.

Key words: Groundnut, SSR, Regression, Oil yield.

Groundnut (*Arachis hypogaea* L.) occupies an area of 5.26 million ha in India with a production of 6.96 million tonnes, which accounts for a productivity of 1323 kg/ha during 2011-12. In India, 72 per cent of the groundnut area and 78 per cent of the production are concentrated in the four states of Gujarat, Tamil Nadu, Andhra Pradesh and Rajasthan. In Tamil Nadu, the area under groundnut is about 3.86 lakh ha with a production of 10.61 lakh tonnes and productivity of 2751 kg/ha during 2011-12 (Anon, 2012). Groundnut is an important crop for both its versatility and value. The high protein and energy contents make groundnut valuable as a subsistence crop in some countries, and the demand for its oil allows groundnut to be sold as a cash crop.

Molecular markers and genetic linkage maps are pre-requisites for molecular breeding in any crop; such tools would speed up the process of introgression of beneficial traits into preferred varieties. A large number of studies in various crop species have used molecular markers as a tool to identify major genes / QTLs to introduce a new character into elite germplasm. Knowing the location of these genes and specific alleles offer the possibility to apply in marker assisted selection (MAS). Groundnut molecular breeding has been hindered by a shortage of polymorphic genetic markers due to a very narrow genetic base (Halward *et al*., 1991; Kochert *et al.,* 1991).

In spite of substantial effort over the last few years by a number of research groups, the number of SSRs that are polymorphic for *A. hypogaea* is still limiting for routine application, creating the demand for the discovery of more markers polymorphic within cultivated germplasm. New efforts for the development of SSR genomic markers are important in order to increase the availability of this class of markers for genetic studies of the *Arachis* species. Many hundreds of SSR makers have been developed during recent years (Jayashree *et al.,* 2005; Luo *et al.,* 2005; Ma *et al.,* 2007), with less than 30 per cent being polymorphic among *A. hypogaea* lines. However, many markers identified in preliminary genetic mapping studies are not suitable for direct use in marker-assisted selection. In order to overcome these limitations, molecular marker-trait association have been analyzed through regression technique (Pradeep *et al.,* 2007, Srivastava *et al.,* 2007) and increasingly adopted in many plants (Butler *et al.,* 2007). In the present study, marker analysis was carried out for oil yield and component traits in groundnut.

Materials and Methods

Two parents *viz.,* ICGV00440 (low oil genotype) and ICGV03128 (high oil genotype) (Table 1) were crossed and forwarded to F_2 generation. The F_2 population was raised at the Oilseeds farm, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore during July 2012
- Nov 2012. Normal agronomic practices were followed under irrigated condition. DNA samples of individual plants of F_2 were collected. F_3 progenies were raised as progeny rows in augmented block

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design during Jan 2013 - May 2013. Phenotyping was done for individual plants of F_3 progenies of cross ICGV00440 x ICGV03128. The data were recorded for 10 traits *viz.,* number of branches/plant, number of pods/plant, 100-pod weight (g), 100-kernel weight (g), shell weight (g), shelling per cent, pod yield/plant (g), kernel yield/plant (g), oil per cent (estimated using soxhlet method) and oil yield/plant
(g) (derived from kernel yield per plant and oil content). The progeny mean was used for single marker analysis.

SSR Markers

DNA of two parents were collected and parental polymorphic study was carried out with 778 markers. A total of 60 SSR markers were identified polymorphic and used for F_2 genotyping (Figure 1).

DNA extraction

Leaves were collected from 71 genotypes of F_2 population in two leaf stage and genotyping was done in single plant basis. DNA extraction was performed according to the cetyl-trimethyl ammonium bromide (CTAB) method (Doyle and Doyle, 1990). The extracted DNA content was measured using DNA standards in agarose gel (0.8 % w/v). The 10 µl PCR cocktail contained 20 ng of 2 µl DNA, 1 µl of 10XTaq buffer, 0.2 µl of 25 mM $Mgcl_2$, 1.0 µl of 0.2 mM of dNTP, 0.5 µl of 0.5 uM of each forward and reverse primer, 4.5 µl of sterile water and 0.3 µl of 0.03 IU Taq DNA polymerase. DNA amplification was performed in a Veriti® 96-Well Fast Thermal Cycler (Applied Biosystems Inc., Foster city, CA). DNA samples were denatured initially at 94°C for 3 min, then subjected to the following 20 cycles: 94°C for 30 s, 63°C for 30 s with a decrement of 0.5°C per cycle, and 70°C for 1 min. This was followed by another 20 cycles of 94°C for 15 s, 55°C for 30 s, and 70°C for 1 min. A 10 min extension was performed at 72°C as the last step. Amplified products were analyzed using 6% polyacrylamide gel electrophoresis at 150 volts DC for 4 hrs and silver stained in accordance with the protocol described by Benbouza *et al.* (2006).

Data scoring and data analysis

Clear and unambiguous bands were scored for their presence or absence with the score 1 indicating their presence and 0 indicating their absence. The data matrix of binary codes thus obtained was subjected to further analysis. Phenotypic mean values of all the 71 $F_{2:3}$ population were subjected to associate with corresponding marker score for its significance by using simple regression in SPSS software (version.16).

Simple linear regression method (Haley and Knott, 1992) was used to identify significant marker trait association. The linear equation formed was

$$Y = \mu + f(\text{marker}) + \text{error}$$

where, Y = phenotypic trait value; µ = population mean and f (marker) = function of the molecular marker.

The potential relationship between the marker and trait was established considering the significance of the regression coefficient at 5 and 1 per cent probability. Adjusted R_2 values were used to express phenotypic variance as explained (PVE).

Results and Discussion

Phenotype

The phenotypic variation observed among 71 $F_{2:3}$ progenies is summarized in the Table 2. The traits number of pods per plant, shell weight, pod yield, kernel yield and oil yield per plant had high coefficient of variation (>20 %). Whereas, number of branches per plant, 100-pod weight, 100-kernel weight, shelling percentage and oil content recorded medium level coefficient of variation (10-20%).

Simple linear regression was calculated for each of the phenotypic traits with all the marker classes (Table 4). The potential relationship between the marker and trait was established considering the significance of the regression coefficient. The marker which is having the strongest relationship can be judged from its PVE. The PVE will give the overall percentage of variability of that particular trait explained by the marker.

The number of associated markers varied from seven SSR primers (shelling percentage) to two SSR primers (number of pods per plant). The PVE value varies from 4.2 to 12.8 per cent. Among various traits, the trait 100- kernel weight and number of branches per plant recorded 12.8 per cent (PGP03A08) and 9.6 per cent (SEQ11HO1), respectively.

In this study, most of the markers were found to be related to more than one trait. Primer PGP03A08 is associated with oil yield per plant (8.0%), 100-pod weight (7.2%), kernel yield per plant (5.6%) and pod yield per plant (4.9%). The primer SEQ4EO8 recorded 9.0, 8.5, 7.1, 6.4 and 5.9 per cent for shelling percentage, 100-pod weight, pod yield per plant,

Table 1. Particulars of parents studied

	ICGV 03128	ICGV 00440
Pedigree	(ICGV 99160x ICGV 99240)	(ICGV 88386x ASHFORD) X ICGV 95172
Habit	Virginia bunch	Virginia bunch
Oil content (%)	52-55	42-45
Special features	Drought tolerant	Foliar diseases resistant line

Table 2. Variability for different characters among 71 $F_{2:3}$ progenies

Characters	Mean	Minimum	Maximum	CV (%)
Number of branches per plant	5.5	3.9	7.0	11.6
Number of pods per plant	21.9	7.4	37.2	27.8
100-pod weight (g)	83.6	52.0	116.4	15.5
100-kernel weight (g)	26.5	16.8	35.4	15.0
Shell weight (g)	3.1	1.8	5.5	24.9
Shelling percentage	63.6	51.0	73.3	18.5
Pod yield per plant (g)	17.9	3.9	29.6	32.5
Kernel yield per plant (g)	11.7	2.3	21.7	34.1
Oil content (%)	46.1	33.7	54.8	19.5
Oil yield per plant (g)	5.4	0.5	11.2	39.7

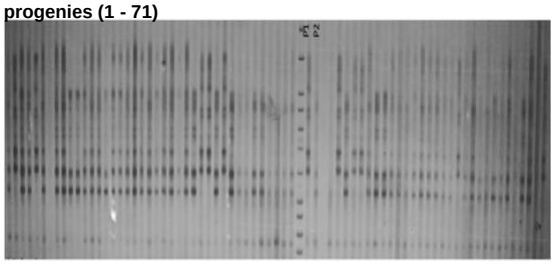
shell weight and oil yield per plant, respectively. Primer Ah2562 is associated with shell weight (8.4%) and 100-pod weight (7.1%). Primer GM1369

Table 3. Single marker analysis for various characters of $F_{2:3}$ population of cross ICGV 00440 x ICGV 03128

Character	Primer	PVE (%)
Number of branches/plant	SEQ18B08	5.8
	SEQ19G05	7.0
	SEQ11HO1	9.6
	PMc660	6.4
	PM36	4.2
Number of pods/plant	Ah51	7.7
	SEQ4F04	6.1
100- pod weight (g)	Ah2562	7.1
	GM1369	4.3
	SEQ4EO8	8.5
	PMc348	6.8
	PGP03A08	7.2
100- kernel weight (g)	GM1369	7.8
	PM238	4.5
	SEQ4EO8	4.5
	SEQ15C10	7.6
	GM2528	4.7
	PGP03A08	12.8
Shell weight (g)	Ah2562	8.4
	SEQ4EO8	6.4
	TC2C11	8.6
	PMc348	8.5
Shelling percentage	Ah51	8.1
	SEQ4EO8	9.4
	SEQ4F04	4.5
	TC5A06	5.0
	TC2C11	5.3
	TC2B09	6.0
	PMc348	5.2
Pod yield per plant (g)	Ah51	4.8
	SEQ4EO8	7.1
	PMc348	4.2
	PGP03A08	4.9
Kernel yield per plant (g)	Ah51	7.1
	SEQ4F04	4.9
	PGP03A08	5.6
Oil yield per plant (g)	Ah51	5.1
	GM1369	7.0
	GM1971	4.6
	SEQ4EO8	5.9
	PGP03A08	8.0

recorded 7.8, 7.0 and 4.3 per cent for 100- kernel weight, oil yield per plant and 100- pod weight respectively. Primer Ah51 is associated with shelling

Fig 1. Segregation pattern for marker AC2C08 in F2 progenies (1 - 71)



P1- ICGV 00440; P2- ICGV 04128

percentage (8.1%), number of pods per plant (7.7%), kernel yield per plant (7.1%), oil yield per plant (5.1%) and pod yield per plant (4.8%). Primer PMc348 recorded 6.8, 5.2 and 4.2 per cent for 100- pod weight, shelling percentage and pod yield per plant. Primer GM1369 is associated with for 100- kernel weight (7.8%), oil yield per plant (7.0%) and100- pod weight

(4. 3%). This indicates that the same gene(s) may be controlling the expression of these characters. Moreover, phenotypically these characters have more association with each other. Hence, these markers may be useful for yield improvement programme.

Molecular markers linked with QTL/major genes for traits of interest are being routinely developed in several crops using materials derived from planned crosses such as F_2, RIL, DH populations, etc. However, non-availability of mapping populations and substantial time needed to develop such populations are sometimes major limitations in the identification of molecular markers for specific traits. Another limitation is the absence of tight linkage observed in these studies. Also, it is difficult to eliminate false positives with available methods. Therefore, markers identified during the present study need to be subjected to validation and/or functional analysis of respective traits. Sun *et al.* (2003) highlighted that this approach could have advantages over the use of mapping populations as the markers are more likely to be applicable to a large number of breeding programmes. However, it is expected that at least some of the markers identified during the present study would be validated and used for MAS of groundnut breeding programme.

References

Anonymous. 2012. dacnet.nic.in

Benbouza, H., Jacquemin, J.M., Baudoin J.P. and Mergeai, G. 2006. Optimization of a reliable, fast, cheap and sensitive silver staining method to detect SSR markers in polyacrylamide gels. *Biotechn., Agron. Soc. Environ.,* **10(2):** 77-81.

Butler, M.I.J., Udall, J.A. and Osborn, T.C. 2007. Analysis of a multi-parent population derived from two diverse alfalfa germplasm: testcross evaluations and phenotype-DNA associations. *Theor. Appl. Genet***. 115:** 859-867.

Doyle J.J. and Doyle J.L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.,* **19:** 11-15.

Haley C.S. and Knott S.A. 1992. A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. *Heredity* **69:** 315–324.

Halward T.M., Stalker H.T., La Rue E., Kochert, G. 1991. Genetic variation detectable with molecular markers among unadapted germplasm resources of cultivated peanut and related wild species. *Genome* **34:** 1013– 1020.

Jayashree, B., Ferguson, M., Ilut, D., Doyle, J. and Crouch J.H. 2005. Analysis of genomic sequences from peanut (*Arachis hypogaea*). *Electron. J. Biotechnol.* **8:** 226–237.

Kochert, G., Halward, T., Branch W.D. and Simpson C.E. 1991. RFLP variability in peanut (*Arachis hypogaea* L.) cultivars and ,wild species. *Theor. Appl. Genet.* **81:** 565– 570.

Luo M., Dang, P., Guo B.Z., He, G., Holbrook, C.C., Bausher, M.G. and Lee, R.D. 2005. Generation of expressed sequence tags (ESTs) for gene discovery and marker development in cultivated peanut. *Crop Sci.* **45:** 346– 353.

Ma, W., Li, Y., Guo, B., Culbreath, A.K, Lewis, M.S, Tallury, S., Holbrook, C.C., Isleib, T., Stalker, H.T. and Knapp, S.J. 2007. Simple sequence repeat polymorphisms in peanut. Plant & Animal Genomes XV Conference, San Diego, California.http://www.intl-pag.org/ 15/ abstracts/PAG15_P03b_113.html (abstr.).

Pradeep, A.R., Jingade, A.H. and Urs, R.S. 2007. Molecular markers for biomass traits: association, interaction and genetic divergence in silkworm *Bombyx mori*. *Biomarker Insights* **2:** 197-217.

Srivastava, P.P, Kar, P.K, Awasthi, A.K. and Urs, S.R. 2007. Identification and association of ISSR markers for thermal stress in polyvoltine silkworm *Bombyx mori*. *Rus. J. Genet.* **43:** 858-864.

Sun, G., Bond, M, Nass, H, Martin, R. and Dong, Z. 2003. RAPD polymorphism in spring wheat cultivars and lines with different level of Fusarium resistance. *Theor Appl Genet* **106:**1059–1067.

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