Single Marker Analysis for Oil Yield and Component Traits in Groundnut (\textit{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 (\textit{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 \textit{et al}., 1991; Kochert \textit{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 \textit{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 \textit{Arachis} species. Many hundreds of SSR makers have been developed during recent years (Jayashree \textit{et al}., 2005; Luo \textit{et al}., 2005; Ma \textit{et al}., 2007), with less than 30 per cent being polymorphic among \textit{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 \textit{et al}., 2007, Srivastava \textit{et al}., 2007) and increasingly adopted in many plants (Butler \textit{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. 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_2 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 polymeric 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 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; \( \mu \) =
population mean and \( f(\text{marker}) \) = 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
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 1. Particulars of parents studied

<table>
<thead>
<tr>
<th>Pedigree</th>
<th>ICGV 03128</th>
<th>ICGV 00440</th>
</tr>
</thead>
<tbody>
<tr>
<td>Habit</td>
<td>(ICGV 99160x ICGV 99240)</td>
<td>(ICGV 88386x ASHFORD) X ICGV 95172</td>
</tr>
<tr>
<td>Oil content (%)</td>
<td>52-55</td>
<td>42-45</td>
</tr>
<tr>
<td>Special features</td>
<td>Drought tolerant</td>
<td>Foliar diseases resistant line</td>
</tr>
</tbody>
</table>

(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
in the PVE 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 SEQ4E08 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 2. Variability for different characters among 71 F2:3 progenies

<table>
<thead>
<tr>
<th>Characters</th>
<th>Mean</th>
<th>Minimum</th>
<th>Maximum</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of branches per plant</td>
<td>5.5</td>
<td>3.3</td>
<td>7.0</td>
<td>11.6</td>
</tr>
<tr>
<td>Number of pods per plant</td>
<td>21.9</td>
<td>7.4</td>
<td>37.2</td>
<td>27.8</td>
</tr>
<tr>
<td>100-pod weight (g)</td>
<td>83.6</td>
<td>52.0</td>
<td>116.4</td>
<td>15.5</td>
</tr>
<tr>
<td>100-kernel weight (g)</td>
<td>26.5</td>
<td>16.8</td>
<td>35.4</td>
<td>15.0</td>
</tr>
<tr>
<td>Shelling weight (g)</td>
<td>3.1</td>
<td>1.8</td>
<td>5.5</td>
<td>24.9</td>
</tr>
<tr>
<td>Shelling percentage</td>
<td>63.6</td>
<td>51.0</td>
<td>73.3</td>
<td>18.5</td>
</tr>
<tr>
<td>Pod yield per plant (g)</td>
<td>17.9</td>
<td>3.9</td>
<td>29.6</td>
<td>32.5</td>
</tr>
<tr>
<td>Kernel yield per plant (g)</td>
<td>11.7</td>
<td>2.3</td>
<td>21.7</td>
<td>34.1</td>
</tr>
<tr>
<td>Oil content (%)</td>
<td>46.1</td>
<td>32.7</td>
<td>54.8</td>
<td>19.5</td>
</tr>
<tr>
<td>Oil yield per plant (g)</td>
<td>5.4</td>
<td>0.5</td>
<td>11.2</td>
<td>39.7</td>
</tr>
</tbody>
</table>

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 F2:3 population of cross ICGV 00440 x ICGV 03128

<table>
<thead>
<tr>
<th>Character</th>
<th>Primer</th>
<th>PVE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of branches/plant</td>
<td>SEQ18B08</td>
<td>5.8</td>
</tr>
<tr>
<td></td>
<td>SEQ19G05</td>
<td>7.0</td>
</tr>
<tr>
<td></td>
<td>SEQ11H01</td>
<td>9.6</td>
</tr>
<tr>
<td></td>
<td>PMd60</td>
<td>6.4</td>
</tr>
<tr>
<td></td>
<td>PM36</td>
<td>4.2</td>
</tr>
<tr>
<td>Number of pods/plant</td>
<td>Ah51</td>
<td>7.7</td>
</tr>
<tr>
<td></td>
<td>SEQ4F04</td>
<td>6.1</td>
</tr>
<tr>
<td>100-pod weight (g)</td>
<td>Ah2562</td>
<td>7.1</td>
</tr>
<tr>
<td></td>
<td>GM1369</td>
<td>4.3</td>
</tr>
<tr>
<td></td>
<td>SEQ4EO8</td>
<td>8.5</td>
</tr>
<tr>
<td></td>
<td>PMc348</td>
<td>6.8</td>
</tr>
<tr>
<td></td>
<td>PGP03A08</td>
<td>7.2</td>
</tr>
<tr>
<td>100-kernel weight (g)</td>
<td>GM1369</td>
<td>7.8</td>
</tr>
<tr>
<td></td>
<td>PM238</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td>SEQ4EO8</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td>SEQ15C10</td>
<td>7.6</td>
</tr>
<tr>
<td></td>
<td>GM2528</td>
<td>4.7</td>
</tr>
<tr>
<td></td>
<td>PGP03A08</td>
<td>12.8</td>
</tr>
<tr>
<td>Shell weight (g)</td>
<td>Ah2562</td>
<td>8.4</td>
</tr>
<tr>
<td></td>
<td>SEQ4EO8</td>
<td>6.4</td>
</tr>
<tr>
<td></td>
<td>TC2C11</td>
<td>8.6</td>
</tr>
<tr>
<td></td>
<td>PMc348</td>
<td>8.5</td>
</tr>
<tr>
<td>Shelling percentage</td>
<td>Ah51</td>
<td>8.1</td>
</tr>
<tr>
<td></td>
<td>SEQ4EO8</td>
<td>9.4</td>
</tr>
<tr>
<td></td>
<td>SEQ4F04</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td>TC5A06</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>TC2C11</td>
<td>5.3</td>
</tr>
<tr>
<td></td>
<td>TC2B09</td>
<td>6.0</td>
</tr>
<tr>
<td></td>
<td>PMc348</td>
<td>5.2</td>
</tr>
<tr>
<td>Pod yield per plant (g)</td>
<td>Ah51</td>
<td>4.8</td>
</tr>
<tr>
<td></td>
<td>SEQ4EO8</td>
<td>7.1</td>
</tr>
<tr>
<td></td>
<td>PMc348</td>
<td>4.2</td>
</tr>
<tr>
<td></td>
<td>PGP03A08</td>
<td>4.9</td>
</tr>
</tbody>
</table>

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 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%) and 100- 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 F2, 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.

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