# MARKER-ASSISTED SELECTION ON SOYBEAN RUST RESISTANCE GENOTYPES
## Part I: *DNA Survey and Genetic Divergence Analysis*

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Soybean rust is disease caused by *Phakospora pachyrhizi*. It is one of the major diseases of soybeans in Asia and significant yield losses occur from most soybean producing countries throughout Asia. Nearly all tropical and subtropical countries have reported the occurrence of soybean rust where it is endemic. *Phakospora pachyrhizi* was in a list of "The 100 most dangerous exotic pests and diseases" (number 22) and the estimated loss in yield was 50% (McGregor 1973). In Vietnam, the disease is spreading from Tay Nguyen to Mekong Delta.

A large number of legume species are host plants for *Phakospora pachyrhizi* with 17 different genera, 31 species (Sinclair and Hartman 1996), and 18 genera, 35 species (CABI 2001).

The Asian Vegetable Research and Development Center (AVRDC) started a cooperative project in 1978 to study the epidemiology of soybean rust. The soybean rust newsletter was first published in 1977. Soybean SSR loci (microsatellite markers), linkage group, PCR primers were used from *http://bldg6.arsusda.gov/~pooley/soy/cregan/soymap1.html*. Several cultivars carrying resistance genes as donors have been recognized as followed:

- Rpp1: PI 200492, Tainung 3, Tainung 4, L85-2378
- Rpp2: PI 230970, L86-1752, PI 197182, PI 230971, PI 417125
- Rpp3: PI 462312
- Rpp4: PI 459025, L87-0482,

Rpp5, 6…: Abura, PI 200487, Shiranui, PI 416819, PI 417503, PI 416810, PI 417115, PI 417421,PI 423966, GC84058-21-4

Linkage maps based on restriction fragment length polymorphism (RFLP) and soybean genetic map of RAPD markers were published by Ferreira et al. (2000). A 356-marker linkage map of *Glycine max* (L.) Merr. (2n=20) was established by anchoring 106 RAPD markers to an existing RFLP map built with a large recombinant inbred line population (330 RILs). This map comprises 24 major and 11 minor linkage groups for this genome, which is estimated to be approximately 3275 cM (Ferreira et al. 2000). Single nucleotide polymorphisms (SNPs) in soybean provide an abundant source of DNA polymorphism. Approximately 28.7 kb of coding sequence, 37.9 kb of non coding perigenic DNA, and 9.7 kb of random non-coding genomic DNA were sequenced in each of 25 diverse soybean genotypes (Zhu et al. 2003)

Genetic divergence analyse was conducted among nine soybean accessions from CLRRI gene bank and 81 accessions from IAS. Susceptible genotypes were MTD176 and OMDN110 (score 9). Tolerant genotypes were OMDN1, OMDN117 and OMDN111 (score 1), especially OMDN 29 (score 0).

Of 81 soybean accessions form IAS were identified into three genetic clusters with genetic distance value of 2.92. Cluster A included 37 soybean rust tolerance genotypes (score 0 and 1). Cluster B composed of 30 soybean genotypes with score of and 5 for rust disease. Cluster C remained susceptible genotypes to rust with score 7 and 9.

Figure 1: Inoculum of *Phakospora pachyrhizi* in the appropriate medium

**DNA isolation**

A crude DNA preparation suitable for PCR analysis was prepared using a simplified miniscale procedure (Lang 2002). The DNA was air dried and re-suspended in 50 $\mu$l of TE buffer (10mM Tris-HCl, pH 8.0, 1 mM EDTA, pH 8.0). One ml of aliquot was used for PCR analysis and the remaining solution was stored at –20$^{\circ}$C for any further use.

**BC populations** were created for genetic mapping, in 2006-2007 at Hung Loc with three crosses as

HL203 x DT2000
HL203 x Stuart 99084B-28
HL203 x PI 56128A

Where HL203: high yielding variety, all male parents: soybean rust resistant varieties

$BC_2F_1$s were completely produced and $BC_3$s will be obtained in early 2008 for genetic mapping

**Data analysis**: Data (RAPD and SSR bands) were scored as discrete variables, using 1 to indicate presence and 0 to indicate absence of a band. A pairwise similarity matrix was generated with SPSS 10.0 software (SPSS Inc., Chicago, IL, USA) using Jaccard's coefficient values as follows: $J_{ij} = a/(a+b)$, where a is the number of bands common to both accessions and b is the number of missing bands in one accession, but present in the other (Lang 2002). The GD between two samples was calculated. The degree of concordance between SSR and RAPD markers was determined by visual appraisal of graphic depictions generated from the analysis of GD values and correlation analysis using Microsoft Excel 2000. Cluster analysis was performed based on RAPD marker data using the PHYLIP version 3.5c software package (Scientific American, Inc., Seattle, Washington, USA) with an unweighted pair-group method using an arithmetic averaging (UPGMA) algorithm.

Table 1: List of RADPs and SSRs used in genetic divergence analysis

| Locus | Primer | Sequence (5'----------3') | Size (bp) |
|---|---|---|---|
| 1 | AA11 | CAATCGCCGT | 600 – 2300 |
| 2 | AC14 | GTCGGTTGTC | 900 – 2500 |
| 3 | OPD03 | GTCGCCGTCA | 800 – 230 |
| 4 | OPD05 | TGAGCGGACA | 500 – 3000 |
| 5 | OPD07 | TTGGCACGGG | 600 – 2000 |
| 6 | OPD11 | AGCGCCATTG | 500 – 3200 |
| 7 | OPD18 | GAGAGCCAAC | 600 – 3200 |
| 8 | RAPD03 | GTAGACCCGT | 300 – 2800 |
| 9 | RAPD05 | AACGCGCAAC | |
| 10 | RAPD02 | GTTTCGCTCC | |
| 11 | RAPD04 | GTAAACCCGT | |
| 12 | RAPD06 | CCCGTCAGCA | |
| 13 | PC 11 | AAAGCTGCGG | |
| 14 | LPF<br>LPR | TATAGCAATGTGTGCGCTGG<br>GTTCCCTTCCAGCAGCTAAC | 700-1,500 |
| 15 | S35F<br>S35R | GCTCCTACAAATGCCATCA<br>GATAGTGGGATTGTGCGTCA | 240-245 |
| 16 | SSVF<br>SSVR | GTAATCT(TA)ACCACTGTGTGTG<br>TGGTCTCCTTTGGA(AG)GCCCCC- | 280-300 |
| 17 | Satt05F<br>Satt05R | TATCCTAGAGAAGAACTAAAAAA<br>GTCGATTAGGCTTGAAATA | 190 – 200 |
| 18 | Satt20F<br>Satt20R | GAGAAAGAAATGTGTTAGTGTAA<br>CTTTTCCTTCTTATTGTTTGA | 100 – 150 |
| 19 | Satt83F<br>Satt83R | ACCATTGGAATGTTCTACA<br>TTGAAGTTATAAAAAAGTTTACATC | 280 – 300 |

**Phenotyping**

Field screening at CưMgar, Daclac, with 160 entries in 2007 wet season; there were 16 genotypes expressing their resistance to rust disease. Three of them exhibited high resistance were noticed as AGS367, Sutart 99084B-28, DT 2000; ten of resistance as PI 230970, MSBR 17, Vir 72, BR 23, DT 93, Cao Bang, GC 960345-5-1-13, IAC 100, HL 2, OMDN 29; and three of moderate resistance as PI 561287A, PI 567430, 96033-2.

Field screening at O Mon, Can Tho, with 30 entries in 2007 dry season; there were five genotypes expressing their resistance to rust disease: AFT15, OMDN 31, OMDN 29, OMDN 33, and OMDN 176. Three genotypes were noticed to be moderately resistant as OMDN 115, OMDN 34 and OMDN 109

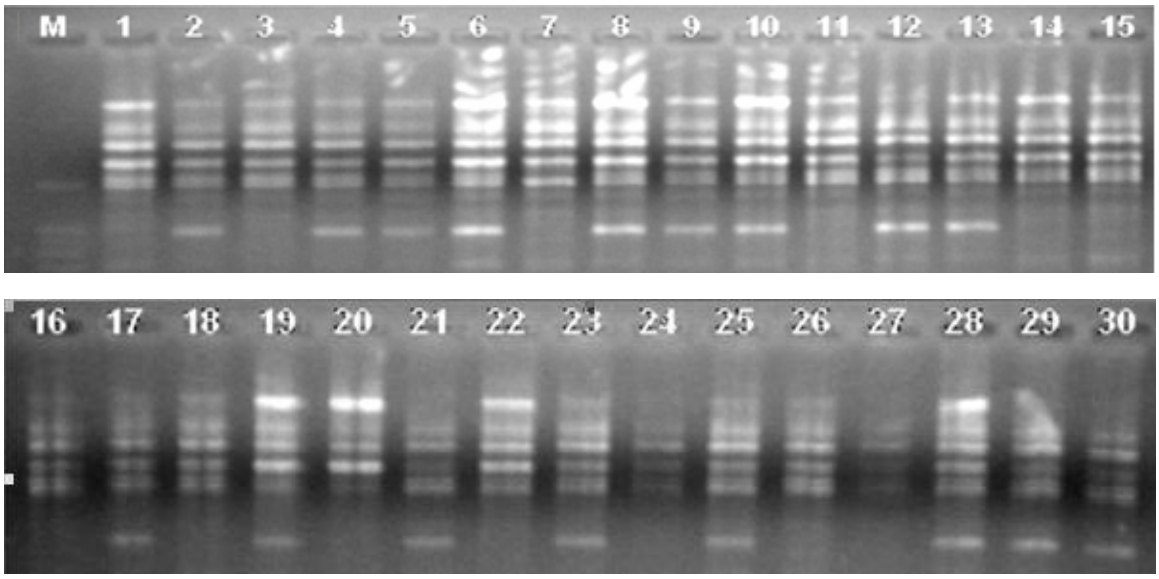


Figure 2: Polymorphism at locus AC14 in agarose gel of 1.5%

| | | | | |
|---|---|---|---|---|
| 1/OMDN64 | 7/Nam Vang | 13/OMDN59 | 19/OMDN117 | 25/OMDN112 |
| 2/ATF15 | 8/OMDN109 | 14/OMDN115 | 20/OMDN14 | 26/OMDN33 |
| 3/OMDN32 | 9/DT84 | 15/OMDN34 | 21/OMDN29 | 27/OMDN113 |
| 4/OMDN83 | 10/OMDN118 | 16/OMDN62 | 22/OMDN110 | 28/MTD176 |
| 5/OMDN31 | 11/OMDN116 | 17/OMDN111 | 23/OMDN114 | 29/TL57 |
| 6/OMDN87 | 12/OMDN86 | 18/OMDN36 | 24/OMDN85 | 30/OMDN01 |

**Genotyping**

Polymorphism at 19 loci was obtained with genetic distance value of 2.92. Three main genetic cluster and other sub-clusters were classified as

Cluster A

*SubCluster* $A_1$ included 27 accessions: PI 0830881, LS201, AGS374, GC84058, OMDN1, BR23, HL203, 9800410, SSE137559, , BR24, MTD483-4, PI200429, Leirchart, MTD164-1, OMDN111, DT85, Aliant, ATF15, MTD652-5, Quang Phu, MTD652-2, AGS360, ATS16, AGS371, DT94, PI085089, PI548484, with reaction score to rust of 1 (resistant genotypes)

*SubCluster* $A_2$ included 10 accessions: GLS2111, CPAC365-76, OMDN29, DT2000, AGS376, DH4, GC990013-12-15-10, IAC100, 96033B, AGS365, with reaction score to rust of zero (highly resistant genotypes)

Cluster B

*SubCluster* $B_1$ included 14 accessions: DT200, L07515, OMDN109, ATF8, 13176, PRANA, DT93, 9907A-4, MSBR22, 9005A-7, GC90013-21-23, MTD664, MANTA, MTD517-8, with reaction score to rust of 5 (moderately resistant)

*SubCluster* $B_2$ included 16 accessions: 903551CR, 9603331-1-1-1, TL57, AGS367, HL2, GC990013-1-1-39, GC90013-12-15-6, 9804512, G85-5126, OMDN64, MSBR17, 96033B, OMDN87, PI417088, HL92, OMDN130, Đậu trắng DT, with reaction score to rust of 3 (resistant)

Cluster C

*SubCluster* $C_1$ included 14 accessions: 980464, PI518759, MTD176, Đậu đen DT, MSPR20, OMDN110, MTD514-6, with reaction score to rust of 9 (very susceptible)
*SubCluster* $C_2$ included 7 accessions: 95389, 95389-1, AGS129, 5113, Nam Vang, HQ1, PI103, with reaction score to rust of 7 (susceptible).

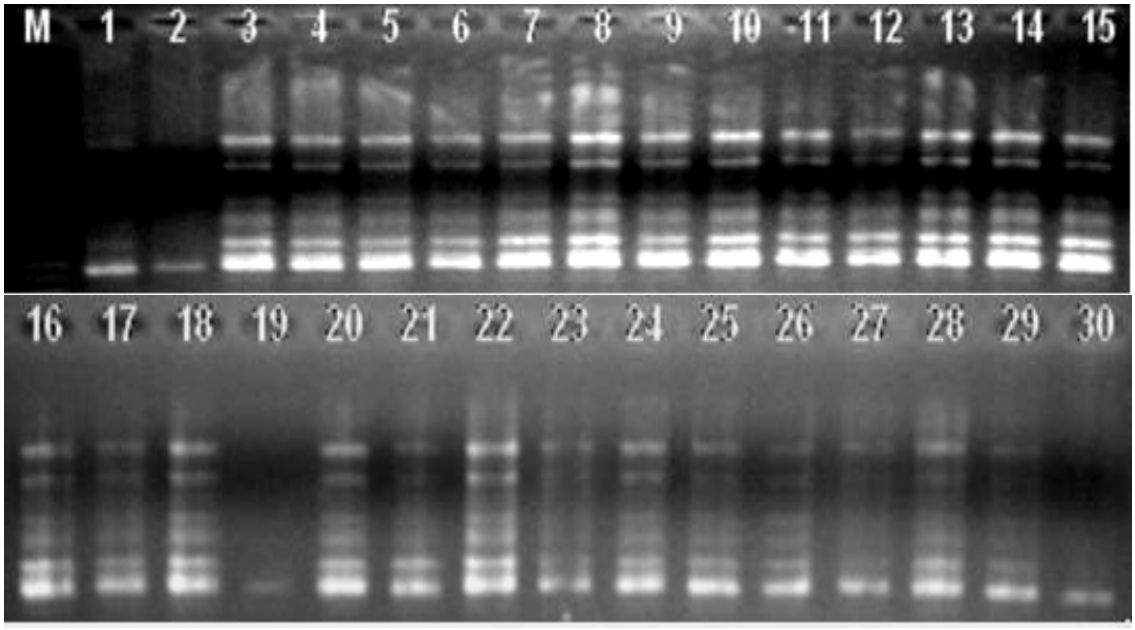


Figure 3: Polymorphism at locus RADP05 in agarose gel of 1.5% (column number referring to fig.2)

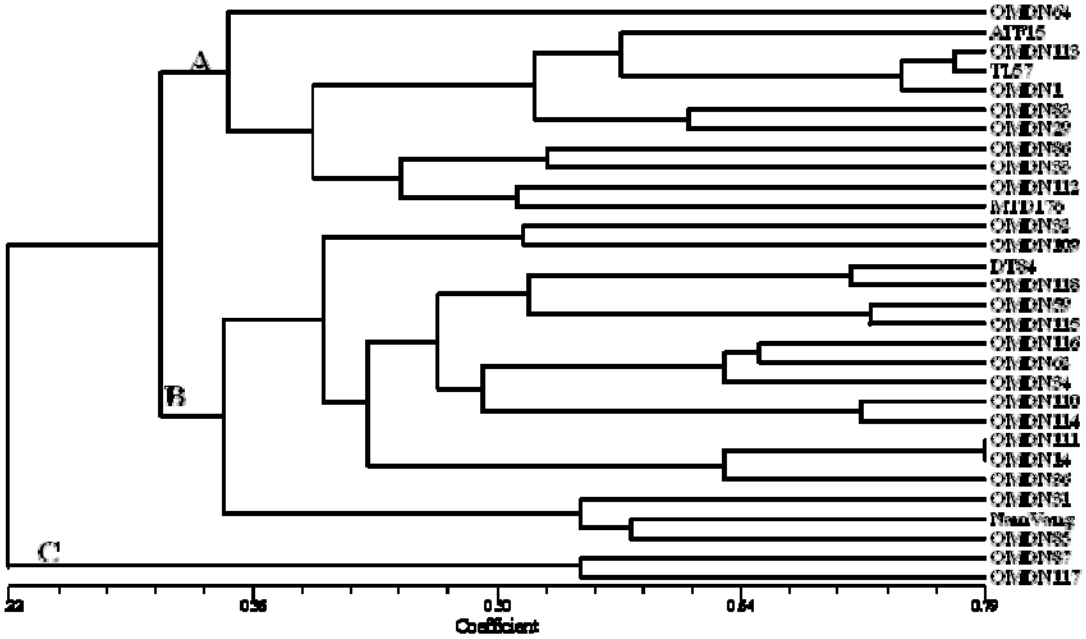


Figure 4: Dendrogram indicates three genetic clusters among 30 soybean accessions due to polymorphism by RADP and SSR markers at 19 loci

Table 2: Genetic divergence analysis by NTSYSpc of 30 soybean genotypes at 19 polymorphic RADP and SSR markers

| Parameter | RAPD | SSR |
|---|---|---|
| Polymorphic percentage (P) | 69.23 | 100.00 |
| Diversity index (H) | 0.124 | 0.312 |
| Correlation coefficient between loci and alleles | 0.77 | 0.59 |

The clustering analysis shows the existence of three main groups of varieties at the level of similarity of 0.80. Molecular extension of PCR products varied from 100 bp (min.) to 3,200 bp (max.).

Random amplified polymorphism DNA (RAPD) and microsatellite marker were used as a DNA fingerprinting technique in soybean germplasm evaluation. Accurate classification of soybean germplasm into the three major clusters and many subclusters can provide essential information for selecting parents in the development of intercluster crossing program.

Attentions will be continuously paid to genetic map, fine map, and marker-assisted selection after the DNA survey to identify good progenies in our soybean hybridization program resistant to rust disease in 2008.

## References


CABI. 2001. *Phakospora pachyrhizi*. From Crop Protection Compendium, a CD-ROM available from CAB International, Wallinford, Oxon OX10 8DE. E-mail: cabi@cabi.org

Ferreira AR, KR Foutz, P Keim. 2000. Soybean genetic map of RADP markers assigned to an existing scaffold RFLP map. The American Genetic Association 91:392-396

Lang NT. 2002. Basic protocols in biotechnology. Nông Nghiệp Publisher, Ho chi Minh, Vietnam

McGregor R. 1973. The Emigrant Pests. A report to the administrator of APHIS by Dr. Russell McGregor

Sinclair JB, GL Hartman (Editors). 1996. Proceedings of the soybean rust workshop (1995). National Soybean Research Laboratory, Publication No.1

Zhu YL, QJ Song, DL Hyten, CP van Tassell, LK Matukumalli, DR Grimm, SM Hyatt, EW Fickus, ND Young, PB Creagan. 2003. Single-Nucleotide Polymorphism in Soybean. Genetics 163: 1123-1134