



# Selection of monogenic race protection from shoot infection in rice

\*Le Pham, Ngo Vu and Duong Vu

Cuulong Delta Rice Research Institute, Codo - Cantho -Vietnam..

## Abstract

Blast, caused by *Pyricularia grisea* Cav., is one of the major fungal diseases infected rice (*Oryza sativa* L.) in Vietnam. This disease occurs in Vietnam which causes the yield loss of up to 20% particularly in a year with long wet season. Local varieties have been considered as genetic sources of disease resistance among crops. The breeding program was aimed at improvement of blast resistant varieties. Six crosses of plants, OM 24/IR 64, IR 24/OM 2514, C 53/IR 64, C53/OM 2514, OM 1308/TeTep and IR 36/C 53 were obtained. Four crosses were mapped using molecular marker. The resistance genes are inherited dominant and located on chromosomes 6, 8 and 11. A simple sequence repeat (SSR) marker (RM 483) was used to detect 100 local varieties to find resistance with some race at Mekong delta. Phenotypic selection was used to compare with genotype in order to check how accurate the polymorphisms in varieties show that marker assisted selection (MAS) reached an accuracy of 100% in SSR marker with RM 483. These methods can be applied in practice to select varieties that have blast resistance genes for breeding rice, because of their high precision levels. Polymorphisms also show that MAS reached an accuracy of 100% in sequence tagged site (STS) marker with RG64 and 99.49% in SSR marker with RM21. Several blast resistant rice varieties [P(OM 1), OMP 2, OM P4, OMP 5, and OMP 6] have been reported by many researchers. These are considered as valuable material for pyramiding resistance genes to create durable resistant varieties.

**Keywords:** *Oryza sativa*, blast resistance, polymerase chain reaction, simple sequence repeat, sequence tagged site (STS) marker.

## INTRODUCTION

Rice blast disease, caused by *Pyricularia grisea* Cav., is one of the most devastating diseases of rice (Oush, 1985). The genetic constitution for blast resistance of rice varieties bred at the VietNam is poorly characterized. The genetic characterization of resistance in varieties was undertaken following a differential system based on the gene for gene relationship between rice resistance genes and avirulence genes in the blast pathogen. A number of genes conferring resistance to blast have been identified

and utilized in rice breeding programs. However, large-scale and long-term cultivation of varieties carrying a single gene for resistance resulted in a significant shift in pathogen race frequency with consequent breakdown of resistance in these cultivars. The most economical protection from the diseases is planting resistant varieties. The breeding of resistant varieties is an effective approach to eliminate the use of pesticides and minimize crop losses due to this disease. However, resistant varieties often break down within 3 to 5 years after they are released for cultivation. To breed rice varieties with more durable blast resistance, multiple resistance genes must be incorporated into individual varieties. The availability of molecular maps in rice has opened new avenues to tag genes governing agronomic traits with molecular markers. This has led to major advances in marker assisted selection and pyramiding of useful genes.

**Abbreviations:** SSR; Simple sequence repeat, MAS; marker assisted selection, STS; sequence tagged site, PCR; polymerase chain reaction, RFLP; restriction fragment length polymorphism, EDTA; ethylenediamine tetra -acetic acid, SDS; sodium dodecyl sulfate, TE; tris HCl-EDTA buffer, TAE; tris acetate- EDTA buffer, LOD; limit of detection, CLRR; Cuulong delta rice research institute, cM; centi-Morgan.

**Table 1.** Progenies studied for genetic analysis of resistance to Blast.

| Cross         | Resistance parent | Resistance to   | Susceptible | No. of progeny plants |
|---------------|-------------------|-----------------|-------------|-----------------------|
| IR 24 / IR 64 | IR 64             | OM P1           | IR 24       | 109 F2                |
| IR 24/OM 2514 | OM 2514           | OMP 1           | IR 24       | 46F2                  |
| C 53/ IR 64   | IR 64             | OM P2           | -           | 61F2                  |
| C 53/ OM 2514 | OM 2514           | OMP 2           | -           | 101F2                 |
| IR 36/ C 53   | C 53              | OMP 1 and OMP 2 | IR 36       | 48F2                  |
| Om1308/Te Tep | Te Tep            | OM P1, OMP 2,   | OM 1308     | 92 F2 ,56 Bc1F2       |

Some markers used for blast resistance – *Pil*, *Pi 2(t)*, *Pita*, *Pit*, *Pi7(t)*, *Pi9(t)*, *Pi1(t)* and *Pib*, have available molecular maps (Khush and Brar, 2004). In the present article, we report the development of molecular markers for selection of resistant mono race, a goal of many rice breeding programs.

## MATERIAL AND METHODS Collection

### of diseased rice samples

Blast leaves with typical lesions were collected from farmers field at irrigated regions in 6 provinces of Mekong Delta (MD) area (Can Tho = race OMP 01), AnGiang=OMP 02, Long An = OMP03, Dong Thap = OMP4, Hau Giang= OMP 5 and Vinh Long =OMP 6) from season Dong Xuan 2005. All monocultures were maintained in medium for long term storage at Genebank at CLRRRI.

## Genetic material and disease evaluation

Six crosses involving 3 different resistant varieties (Table 1) out of 100 local and 32 improved varieties using this experiment. Te Tep and IR 24 for checked the 138F2 individuals of each cross were grown in a plastic tray under standard green house conditions for phenotypic disease scoring. The parental resistant and susceptible cultivars were also included as controls. 21 day old seedlings were inoculated with blast isolates *OM P1*, *OMP 2*, *OMP 3*, *OMP 4*, *OMP 5*, and *OM P6* (from VietNam). The rice seedlings were sprayed with 50 ml of blast inoculum suspension per tray ( $5 \times 10^4$  conidia/ml) and incubated for 24 h in a controlled temperature of 25°C. The seedlings were then transferred to a temperature and humidity controlled chamber. Plants were observed for blast disease symptoms for 1 week after inoculation and scored for resistance and susceptibility when the typical blast lesions developed on the susceptible parental cultivar IR 24. This phenotypic evaluated was repeated 3 times.

## Rapid isolation of rice DNA

Suitable rice DNA for PCR analysis was isolated using the method of Lang (2002). The young leaf was ground using a polished glass rod in a well of a Spot Test plate (Thomas Scientific) after adding 400 µl of extraction buffer (50 mM Tris-HCl pH 8.0, 25 mM EDTA, 300 mM NaCl and 1% SDS). Grinding was done until the buffer turned green which is an indication of cell breakage and release of chloroplasts and cell contents. Another 400 µl of the extraction buffer was added and mixed into the well using a pipette. Then 400µl of the lysate was transferred to the original tube of the leaf sample. The aqueous supernatant was transferred to a new 1.5 ml tube and DNA precipitated using absolute ethanol. DNA was air-

dried and resuspended in 50 µl of TE buffer (10 mM Tris - HCl pH 8.0, 1 mM EDTA pH 8.0). An aliquot of 1 µl was used for the PCR analysis and thereafter, DNA quality and quantity were spectrophotometrically determined.

## PCR amplification

The genomic DNA from both of the 148F2 plants and the parents were subjected to PCR amplification using the synthesized primers. The PCR buffer consisted of: 10 mM Tris pH 8.4, 50 mM KCl, 1.8 mM MgCl<sub>2</sub>, 0.01 mg/ml gelatin and *Taq* [5 units of *Taq* polymerase in a volume of 25 µl]. Template DNA were initially denatured at 94°C for 5 min, followed by 30 cycles of PCR amplification under the following parameters: 1 min denaturation at 94°C, 1 min primer annealing at 55°C and 2 min primer extension at 72°C. Final 5 min incubation at 72°C was allowed for completion of primer extension on a 480-thermalcycler. The amplified products were electrophoretically resolved on 1% agarose gel using 1X TAE buffer.

## Linkage analysis

Linkage analysis for the segregating polymorphic markers and the blast resistance gene was conducted with MAPMAKER V.2.0 (Lander et. al., 1987) for each of the individual populations. All map distances (centi -Morgans, cM) are reported in Kosambi units (Kosambi, 1994) and critical LOD score thresholds of 3.0 and 0.05 were used for determining linkage groups and for the calculation of map distance in JOINMAPV.14. The segregation ratios of individual markers were calculated with the software program QGENE V.2.0 (Nelson 1994), and skewing was indicated when the ratio deviated significantly from the expected.

## RESULTS AND DISCUSSION

### Resistance tests and genetic mapping

#### Analysis of the F2 population

DNA genome from the F2 population, two of the cross between IR24/IR 64 (109 F2 plants), IR 24/ Om 2514 (42 plants of F2) were assessed through PCR amplification using 13 primers (RM 225, RM 314, RM 111, RM 253, RM 50, RM 276, RM 136, RM 83, RM 541, RM 162, RM 276, RM 343 and RM 30) on chromosome 6. Four (4) fragments were resolved on agarose gels and the banding patterns were scored with reference to those of the parents. The banding pattern of the F2 individuals could be classified into homozygote for the IR 24 type marker size 210 fragment, homozygote for Ir64 type mar-

**Table 2.** Segregation ratios for Blast resistance.

| <b>Cross</b>   | <b>Segregation R : S</b> | <b>Chi- square test (3:1)</b> | <b>Infection rate (%)</b> |
|----------------|--------------------------|-------------------------------|---------------------------|
| IR 24 / IR 64  | 79:30                    | 0.25                          | 95                        |
| IR 24/ OM 2514 | 35:11                    | 0.00                          | 98                        |
| C53/ IR64      | 46:15                    | 0.003                         | 92                        |
| C53/ OM 2514   | 84:17                    | 2.56                          | 92                        |
| IR 36/ C53     | 36:12                    | 0.00                          | 95                        |
| OM 1308/ TeTep | 83:16                    | 2.56                          | 92                        |

ker 200 bp fragment with primers RM 162.

In the first step, the resistant reaction of 148F2 plants against blast was monitored. In all crosses the segregation displayed a 3:1 ratio (resistance: susceptible) as expected from previous experiments which indicated the presence of single dominance genes for all the resistant parents. Infection rates varied from 92-98%; the progeny of resistant F2 plants was tested in plants in F3 generation. Segregation ratios for resistance to blast were summarized in Table 2.

According to the resistance data from the F2 generation phenotypic pools, mapped molecular markers were used for simple sequence repeat (SSR) in chromosome until a region was identified in each cross where the markers showed a clear differentiation between resistance and susceptible (Figure 1).

#### **Gene to race OMP 1**

The co-dominant SSR 16 markers have been mapped in both two populations at a distance of 3.9 cM with cross IR 24/IR 64 (102 F2) and 3 cM cross IR 24/OM 2514 (46F 2), respectively (Figure 1).

#### **Gene to race OM P2**

The resistance gene has been localized on chromosome 11 with marker RM21 in population F2 from C 53/OM 2514 at distance of 7.7 cM (Figure 2).

#### **Gene to race OM P 3**

The population F2 from the cross IR 36/ C53 resistant race OM P3 polymorphism with 11 markers and was located on chromosome 8 with marker RM 483 at a distance of 0.7 cM (Figure 2) .

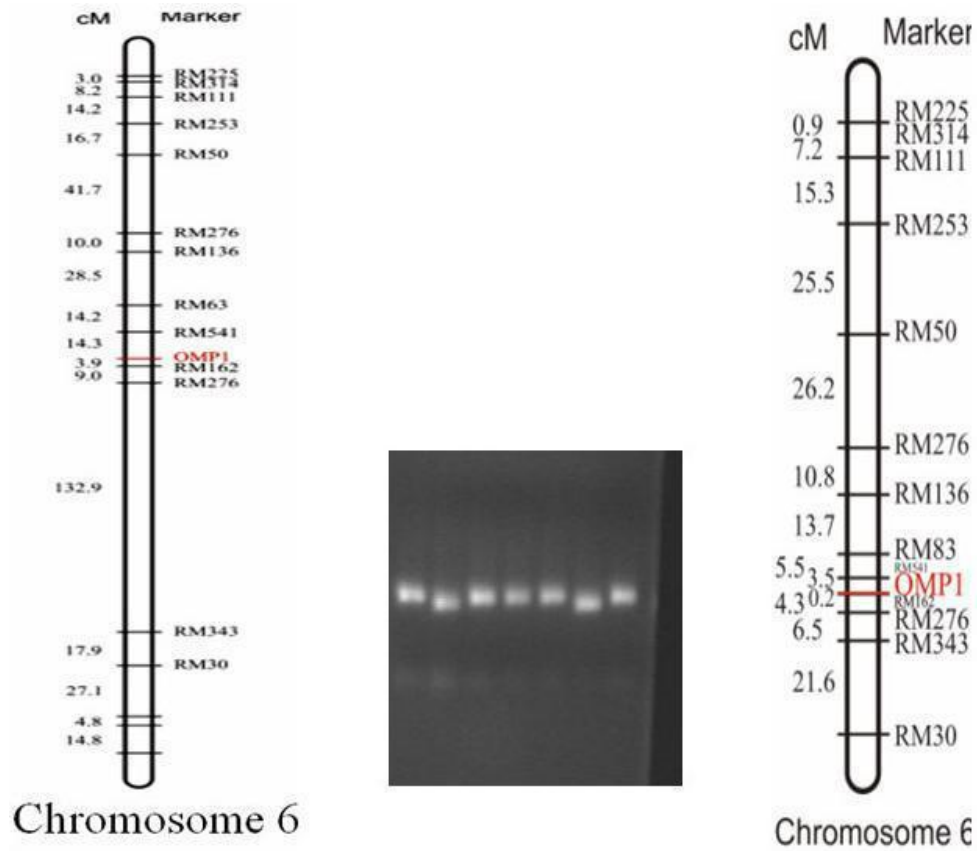
#### **Molecular marker for marker assisted breeding strategies**

DNA markers that are useful for marker-assisted selection inbreeding programs for the introgression of resistance genes from donor lines into adapted germplasm

have been identified. SSR markers are linked to blast races OM P1, OMP 2, and OMP 3 in VietNam in the coupling phase and therefore are suitable for selecting homozygous resistance as well as heterozygous susceptible plants, thereby avoiding the necessity of two backcrossing steps which would be indispensable to identify carriers of dominance genes in classical breeding programs. Similarly, SSR marker RM 21 is useful for the identification of lines carrying the blast, and this is in correspondence with (C53 / OM 2514) in the homozygous or heterozygous state. On the other hand, RM 162 is linked to OMP 1 in repulsion and hence allows selection of homozygous resistant lines which are characterized by the lack of the SSR fragment. In this case, however, samples that failed to amplify can not be differentiated from resistant plants. Therefore, stringent controls have to be included into the experimental design. Some of the RG 64 markers on chromosome 6 were linked with population on F2 from OM 1308/ Te Tep (Lang et. al., 2001) . Population from OM 1038/ Te Tep were evaluated and compared to identify a resistant plant with race OM P4, based on the genotype linked with RG 64 marker that indicated a matching of up to 96:49. There was accuracy in identifying individuals carrying the resistance gene by selecting from 92 lines F2: 37/92 reaction (R); 43/92 reaction (S) score and 12 /92 reaction (MR) score heterozygote. In this population, a series of micro-satellite markers in close linkage to resistance genes were effective against blast. Molecular markers with SSR primers (RM 162, RM 541) were detected in F2 (92 plants) and BC1 F2 population (56 plants) from Om 1308/TeTep .There may be differences regarding the mechanisms of resistance between individual resistance genes against the blast complex. This can be deduced from the observation that some resistant lines, such as Te Tep, were resistant in the field under natural infection conditions but became infected in the greenhouse upon mechanical inoculation of the leaves.

In order to further characterize the molecular mechanisms of resistance to pathogens, high-density molecular linkage maps can be a starting point for cloning of the corresponding genes via a map-based cloning approach.

Closely linked DNA markers have been identified for the Blast resistance genes. A single marker, RM 162, was used to identify blast genes in a segregating popula-



From IR 24/ IR 64

IR 24/ Om 2514

Figure 1. Molecular linkage maps of chromosomal regions harbouring the resistance gene *OMP-1*.

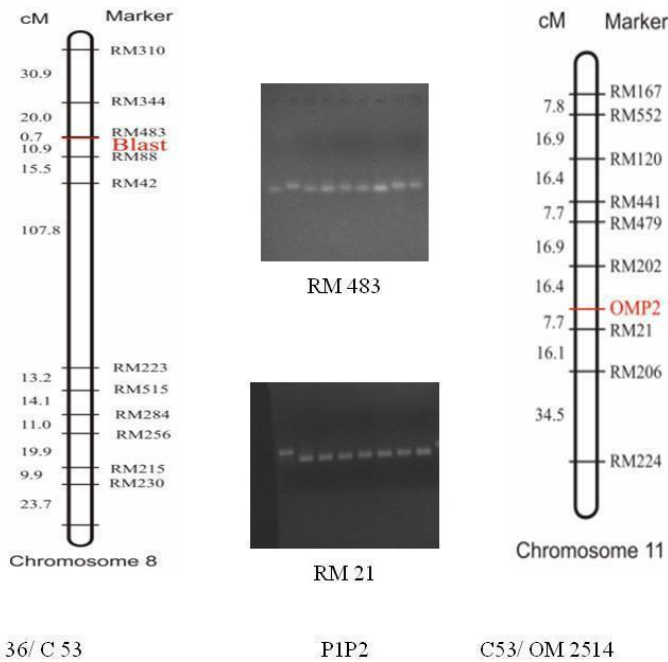


Figure 2. Molecular linkage maps of chromosomal regions harbouring the resistance gene *OMP-2* and *OMP-1 + OMP*

**Table 3.** Prediction of genotypes based on RM 162 at the blast (*OMP 1*) and *OMP 4* locus in F3 population of OM 1308/ Te Tep.

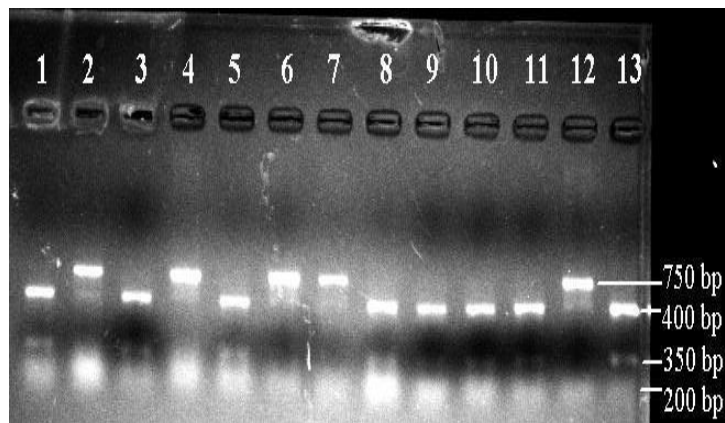
| Phenotype    | No. Plants | Genotype |    | Accuracy (%) |
|--------------|------------|----------|----|--------------|
|              |            | R        | S  |              |
| <i>OMP 4</i> |            |          |    |              |
| S            | 52         | 1        | 51 | 98.07        |
| R            | 19         | 16       | 3  | 84.21        |
| <i>OMP1</i>  |            |          |    |              |
| S            | 49         | 2        | 47 | 95.91        |
| R            | 22         | 17       | 5  | 77.27        |

tion of 71 F3 individuals (OM 1308/ Te Tep) (Table 3).

### Germplasm analysis

The genetic diversity of a population depends upon number and frequency of alleles (allelic composition). Microsatellite markers have extensively been used in molecular characterization, genetic diversity studies and management of genetic resources in seed gene banks. Blast resistance genotypes were measured for four microsatellites (RM 21, RM 162, and RM 483) and one STS marker (RG 64F-R). One of the blast resistance genes mapped in rice *Pi-2(t)* were mapped on chromosome 6 (Mackill and Bonman, 1992), and located 2.8 cM distance from the RFLP marker, RG 64 (Yu et. al., 1991) to check the closeness between the genotype and phenotype and confirm these markers. Several DNA markers have been identified which are useful for marker assisted selection in breeding program for the introgression of resistance genes from donor lines into adapted germplasm. 100 local varieties, with 57 accessions, have resistance and 43 susceptible with 6 races from CLRRRI (*OMP 1*, *OMP 2*, *OMP 3*, *OMP 4*, *OMP 5*, and *OMP 6*).

Table 4 shows the reaction of phenotype and genotype for the *OMP 4*, *OMP 5*, *OMP 6*, *OMP 1*, *OMP 2* and *OMP 3* blast resistance loci. The genotype was compared with the phenotype: + 57/100 reactions (R) score 57% and + 43/100 reaction (S) scores 43%. The results of all 105 lines detected by sequence tagged site marker (RG 64F-R) with the same phenotypic selection were used to compare with the genotype to check for 60% accuracy with RG 64 and race *OMP 4*. At the same time, the phenotypic selection and comparison with the genotype was also used to ascertain how accurate the methods were. Polymorphisms in varieties showed that MAS reached an accuracy of 99.49% in SSR marker with RM21. These methods are, therefore, accurate enough to be applied in practice to select varieties that have blast resistance genes for breeding rice. Several blast resistant



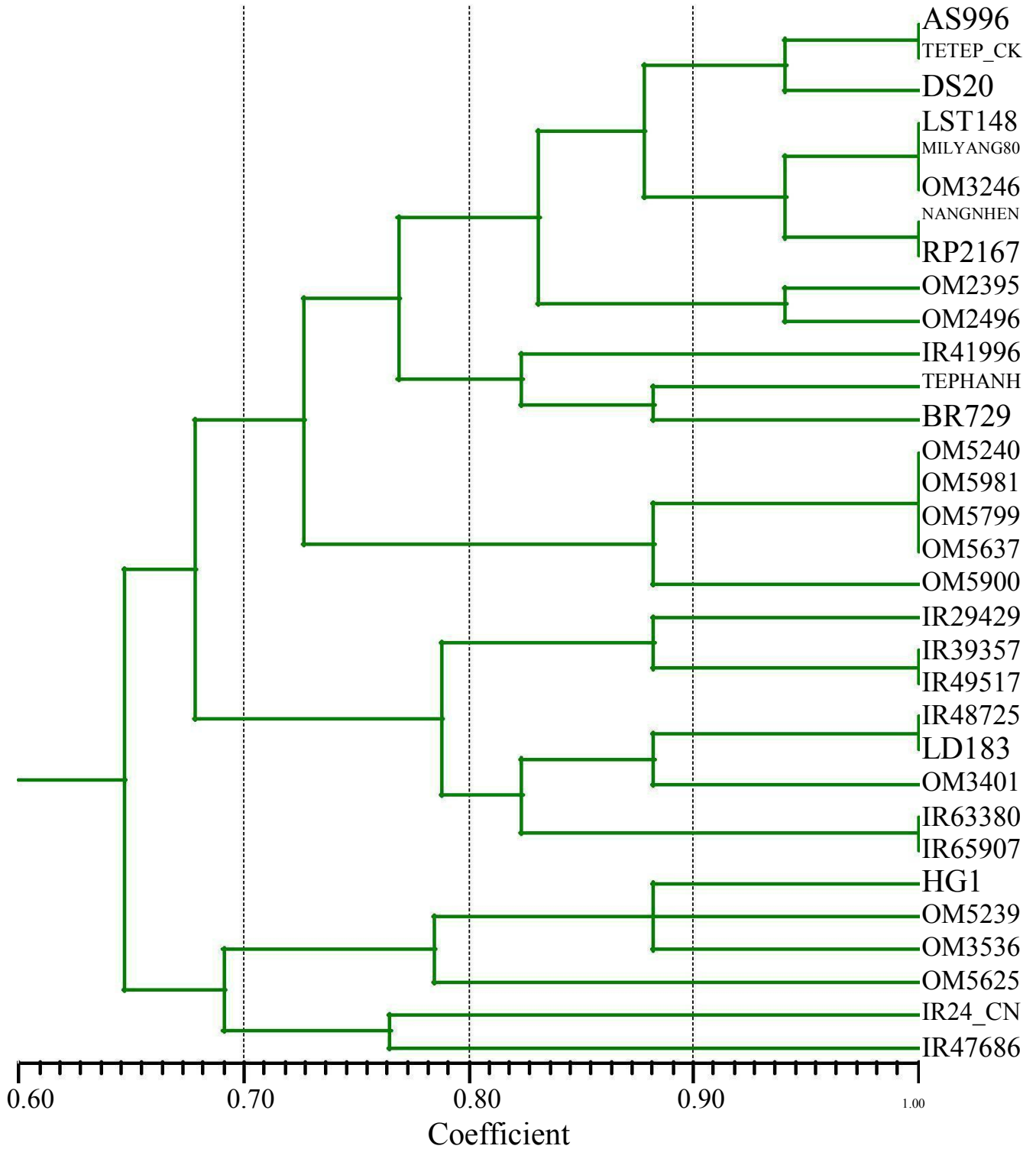
**Figure 3a.** PCR production of the local varieties based on the banding pattern of the RG64 locus digested with *HeaIII*, gel agarose 1.5%, TAE 1X. M: marker: kb ladder: lines : 1: Te Tep, 2: IR64, 3: Soc Nau, 4: Tiêu ôi, 5: Tai Nguyen, 6: Nang Quot, 7: Nep Mu U, 8: Lem B i, 9: Soi Da, 10: Nang Tra, 11: Nmag Tra Ran Doc, 12: Nang Huong, 13: Nang Thom Cho Dao

rice varieties [*P(OM 1)*, *OMP 2*, *OMP 4*, *OMP 5*, and *OMP 6*] have been reported by many researchers (Nông et. al., 2004). These are considered as valuable materials for pyramiding resistance genes to create durably resistant varieties (Table 4).

The diversity of alleles in blast resistant genotypes was also measured for 32 improved varieties using three micro-satellites and 1 STS marker (RM 21, RM 162, RM 483, and RG 64F- R). A Similarity matrix based on pair wise comparisons of pooled data was made using NTSYSp. The dendrogram showed 4 major clusters with 32 varieties (Figures 3a and 3b and Table 5). AS 996 and Te Tep group (D) together are distinct from all other accessions at 60% similarity. Three allelic conditions of the plants susceptible to the disease were detected: Homozygotes for resistance allele, homozygotes for susceptible allele and heterozygotes.

### DISCUSSION

In theory, this minor inaccuracy in single-marker-based selection can be corrected if flanking markers are used for marker-aided selection. We tested this with the restorer gene blast in the field, which has been previously mapped with RM162. We found a flanking marker, RM162, and used both the flanking markers, RM 162 and RG 64, to perform marker-aided selection. Selection accuracy was 100% in identifying homozygous blast plants from a segregating F2 population. These results demonstrate the usefulness of marker assisted selection to precisely identify the genotype of a linked target gene in a segregating population, especially when the selected plants are to be used for further crosses. PCR methodology provides a more effective means of performing marker-aided selection than screening by PCR based



**Figure 3b.** Tree dendrogram shows genetic interaction of thirty –two varieties in term of genotype evaluation.

PCR based marker have opened up a new avenue to map genes of economic importance and the mapping of blast gene provided the opportunity for marker-assisted selection in hybrid rice. The tools of molecular biology

now provide the opportunity to develop large numbers of phenotypically neutral genetic markers in any organism from which DNA can be extracted. The significant progress in the introgression of exotic germplasm into well-

**Table 4.** Reaction of phenotype and genotype for the OM *P4*, OM *P5*, OM *P6*, OMP 1 OM *P2* and OMP 3 blast resistance locus.

| No | Accession | Name            | RaceOM <i>P4</i> | RaceOM <i>P5</i> | Race OM <i>P6</i> | Race OMP 1 | Race OMP2 | Race OMP 3 |
|----|-----------|-----------------|------------------|------------------|-------------------|------------|-----------|------------|
| 1  | 1672      | TaiNguyen       | R                | S                | S                 | S          | R         | S          |
| 2  | 1694      | NangThomChoDao  | R                | S                | S                 | S          | R         | S          |
| 3  | 1559      | NepDauKim       | S                | R                | R                 | S          | R         | S          |
| 4  | 1564      | Nep             | S                | R                | R                 | S          | R         | S          |
| 5  | 1574      | ThanNong Lun    | S                | R                | R                 | S          | R         | S          |
| 6  | 1575      | NangNgocTienNu  | -S               | R                | R                 | S          | R         | S          |
| 7  | 1576      | LunKienGiang1   | S                | R                | R                 | S          | R         | S          |
| 8  | 1578      | Nep Phu         | S                | R                | R                 | S          | R         | S          |
| 9  | 1580      | BaCo            | S                | R                | R                 | S          | R         | S          |
| 10 | 1588      | NepChuotChe     | S                | R                | R                 | S          | R         | S          |
| 11 | 1591      | Lua Mua504      | S                | R                | R                 | S          | R         | S          |
| 12 | 1603      | MotBuiDo        | S                | R                | R                 | R          | R         | R          |
| 13 | 1606      | ReHanh          | S                | R                | R                 | S          | R         | S          |
| 14 | 1609      | MotBuiMua       | S                | R                | R                 | S          | R         | S          |
| 15 | 1610      | VangNghe        | S                | R                | R                 | S          | R         | S          |
| 16 | 1612      | MotBuiTrang     | S                | R                | R                 | S          | R         | S          |
| 17 | 1613      | ThangCon        | S                | R                | R                 | S          | R         | S          |
| 18 | 1614      | NepAoVang       | S                | R                | R                 | S          | R         | S          |
| 19 | 1618      | Mashuri         | S                | R                | R                 | S          | R         | S          |
| 20 | 1632      | NepDaiLoan      | S                | R                | R                 | S          | R         | S          |
| 21 | 1636      | MotBui          | S                | R                | R                 | S          | R         | S          |
| 22 | 1638      | NangThomPhuocLy | S                | R                | R                 | S          | R         | S          |
| 23 | 1641      | NangHuong2      | -S               | R                | R                 | S          | R         | S          |
| 24 | 1643      | NangThomGiua    | -S               | R                | R                 | S          | R         | S          |
| 25 | 1652      | NangThomChoDao  | S                | R                | R                 | S          | R         | S          |
| 26 | 1658      | NangThomGiau    | -S               | R                | R                 | S          | R         | S          |
| 27 | 1664      | NangTraRan      | -S               | R                | R                 | S          | R         | S          |
| 28 | 1673      | NangThom        | -S               | R                | R                 | S          | R         | S          |
| 29 | 1676      | SoiDa           | -S               | R                | R                 | -S         | R         | S          |
| 30 | 1693      | TauHuong        | -S               | R                | R                 | -M         | R         | S          |
| 31 | 1696      | TrangTep        | S                | R                | R                 | R          | R         | S          |
| 32 | 1697      | NeMuU           | S                | R                | R                 | S          | R         | S          |
| 33 | 1700      | NepRuoi Xanh    | S                | R                | R                 | S          | R         | S          |
| 34 | 1702      | NangLoanDoc     | S                | R                | R                 | S          | R         | S          |
| 35 | 1703      | TrangHoaBinh    | S                | R                | R                 | S          | R         | S          |

**Table 4.** Contd.

|    |      |                |   |   |   |   |    |    |
|----|------|----------------|---|---|---|---|----|----|
| 36 | 1706 | NepRuoi        | S | R | R | S | R  | S  |
| 37 | 1708 | NangDen        | S | R | R | S | S  | S  |
| 38 | 1711 | ChumDoc        | S | R | R | S | S  | S  |
| 39 | 1713 | RuoiXanhDoc    | S | R | R | S | S  | S  |
| 40 | 1714 | MuaDoc         | S | R | R | S | S  | S  |
| 41 | 1715 | SoiDaDoc       | S | R | R | S | -M | -M |
| 42 | 1716 | Nmang Thom Doc | S | R | R | S | -M | -M |
| 43 | 1722 | NangLoanDoc    | S | R | R | S | S  | S  |
| 44 | 1724 | LemBuiDoc      | S | R | R | S | S  | S  |
| 45 | 1725 | TrangTepDoc    | S | R | R | S | S  | S  |
| 46 | 1727 | NepMuUDoc      | M | R | R | S | M  | M  |
| 47 | 1729 | NepMoDoc       | S | R | R | S | S  | S  |
| 48 | 1730 | NangHuongDoc   | S | R | R | S | S  | S  |
| 49 | 1666 | NangHuong      | R | R | R | S | S  | S  |
| 50 | 1687 | LemBui         | R | R | R | S | S  | S  |
| 51 | 1689 | SoiDa          | R | R | R | R | R  | R  |
| 52 | 1704 | NangTra        | R | R | R | S | S  | S  |
| 53 | 1710 | NangTraRanDoc  | R | R | R | S | S  | S  |
| 54 | 1800 | TeTep          | R | R | R | R | R  | R  |
| 55 | 1536 | Trang Tep      | S | S | S | S | S  | S  |
| 56 | 1544 | Nang Huong     | S | S | S | S | S  | S  |
| 57 | 1545 | B40            | S | S | S | S | S  | S  |
| 58 | 1551 | Nep Tuong      | S | S | S | S | S  | S  |
| 59 | 1557 | Lun Thong      | S | S | S | S | S  | S  |
| 60 | 1560 | MotBui o       | S | S | S | S | S  | S  |
| 61 | 1567 | TrangTron      | S | S | S | S | S  | S  |
| 62 | 1579 | LunKienGiang2  | S | S | S | S | S  | S  |
| 63 | 1585 | TroiCho        | S | S | S | S | S  | S  |
| 64 | 1586 | NepBaTap       | S | S | S | S | S  | S  |
| 65 | 1587 | KT15           | S | S | S | S | S  | S  |
| 66 | 1591 | LuaMua504      | R | R | R | R | R  | R  |
| 67 | 1592 | NgocNu         | S | S | S | S | S  | S  |
| 68 | 1593 | TrangPhieu     | S | S | S | S | S  | S  |
| 69 | 1597 | MongChimRoi    | S | S | S | S | S  | S  |
| 70 | 1600 | NepMauLuon     | S | S | S | S | S  | S  |



**Table 4.** Contd.

|     |      |                 |   |   |   |   |   |   |   |
|-----|------|-----------------|---|---|---|---|---|---|---|
| 71  | 1601 | LunVangSom      | S | S | S | S | S | S | S |
| 72  | 1602 | MongChimO       | S | S | S | S | S | S | S |
| 73  | 1606 | ReHanh          | R | R | R | R | R | R | R |
| 74  | 1609 | MotBuiMua       | R | R | R | R | R | R | R |
| 75  | 1612 | MotButTrang     | R | R | R | R | R | R | R |
| 76  | 1614 | NepAoVang       | R | R | R | R | R | R | R |
| 77  | 1620 | NangNhenThom    | S | S | S | S | S | S | S |
| 78  | 1621 | BaThiet         | S | S | S | S | S | S | S |
| 79  | 1636 | MotBui          | R | R | R | R | R | R | R |
| 80  | 1640 | DiTruyen2       | S | S | S | S | S | S | S |
| 81  | 1643 | NangThomGiua    | R | R | R | R | R | R | R |
| 82  | 1645 | NangThomSom     | S | S | S | S | S | S | S |
| 83  | 1646 | NhoThom         | S | S | S | S | S | S | S |
| 84  | 1648 | NangHuong       | S | S | S | S | S | S | S |
| 85  | 1650 | MotBuiLun       | S | S | S | S | S | S | S |
| 86  | 1652 | NangThomCho ao  | R | R | R | S | S | S | S |
| 87  | 1656 | TaiNguyen       | S | S | S | S | S | S | S |
| 88  | 1658 | NangThomGiua    | R | R | R | S | R | R | R |
| 89  | 1660 | Tieu oi         | S | S | S | S | S | S | S |
| 90  | 1667 | NangThom        | S | S | S | S | S | S | S |
| 91  | 1671 | NangQuot        | S | R | S | S | S | S | S |
| 92  | 1675 | NangTraRan      | S | S | S | S | S | S | S |
| 93  | 1677 | NepMo           | S | S | S | S | S | S | S |
| 94  | 1683 | NongNghipChum   | S | S | S | S | S | S | S |
| 95  | 1684 | NepRuoiXanh     | S | S | S | S | S | S | S |
| 96  | 1686 | NepNgheAn       | S | S | S | S | S | S | S |
| 97  | 1689 | Soi a           | R | R | R | R | R | R | R |
| 98  | 1690 | NangThom        | S | S | S | S | S | S | S |
| 99  | 1698 | TauHuongVoTrang | S | S | S | S | S | S | S |
| 100 | 1715 | Soi a oc        | R | R | R | S | R | R | R |
| 101 | 1729 | NepMo oc        | R | R | R | S | R | R | R |
| 102 | 1731 | TrangHoaBinh oc | S | S | S | S | S | S | S |
| 102 | 1731 | TrangHoaBinh oc | S | S | S | S | S | S | S |
| 103 | 1736 | NanhChon        | S | S | S | S | S | S | S |
| 104 | 1737 | NepVo en        | S | S | S | S | S | S | S |
| 105 |      | IR 24           | S | S | S | S | S | S | S |

|   | Race<br>OM P4 | Race<br>OM P5 | Race<br>OM P 6 | Race<br>OMP 1 | Race<br>OMP2 | Race<br>OMP 3 |
|---|---------------|---------------|----------------|---------------|--------------|---------------|
| S | 20            | 65            | 64             | 12            | 49           | 14            |
| R | 85            | 40            | 41             | 93            | 56           | 91            |

S: Susceptible. R: Resistance, M: Medium.

**Table 5.** Blast resistance genes estimated and identified in CLRR1 variety group.

| Variety group | Cultivars   | Reaction pattern       |
|---------------|---|------------------------|
| A             | IR 24 IR 47686<br>HG 1  | OM P2                  |
| B             | OM 5239<br>OM 3536<br>OM 5625<br>IR 29426<br>IR 39357<br>IR 49517<br>IR 48725 | OM P1                  |
| C             | LD 183<br>OM 3401<br>IR 63380<br>IR 65907                                     | OM P3                  |
| D             | AS 996<br>Te Tep  | OMP 1, OMP 2,<br>OMP 3 |

adapted cultivars is also anticipated. The use PCR technology to rapidly amplify genomic DNA fragment will a significant.

## REFERENCES

- Khush GS, Brar DS (2004). Rice genetics from Mendel to functional genomics. Rice Geneyoc IV. International Rice Research Institute (IRRI) pp.3-25
- Kosambi DD (1944). The estimation of map distance from Recombination values. Ann. Eugen. 12: 172 - 175
- Lander ES, Green P, Abrahamson J, Barow A, Daly MJ, Lincoln Newberg SEL (1987). MAPMAKER: an interactive computer Program for constructing primary genetic maps of experimental and natural populations. Genomics: 174-181
- Mackill DJ, Bonman JM (1992). Inheritance of blastresistance in near isogeniclines of rice. Phytopathology 82: 746-749
- Nelson RJ, Baraoidan MR, Vera Cruz CM, Yap IV, Leach JE, Mew TW, Leung H (1994). Relationship between phylogeny and pathotype for the bacterial blight pathogen of rice. Appl. Environ. Microbiol. 60: 3275-3283
- Nguyen thi L (2002). Protocol for basic of biotechnology. Agricultural Publishing House, Ho chi Minh City, Vietnam
- Nông TC, Nguyễn MC, Nguyễn TC (2004). Using molecular marker to detect blast resistance in local rice (*Oryza sativa* L). Vietnam Info. Sci. Technol. Adv. 12: 1669-1672.
- Oush (1985). Blast in rice disease, 2nd edn. Common wealth agricultural Bureax, Wallingford pp 109-201
- Yu ZH, Mackill DJ, Bonman JM (1987). Inheritance of resistance to blast in some traditional and improved rice cultivars. Phytopathology 77: 323-326.