MARKER ASSISTED SELECTION FOR FOREGROUND AND BACKGROUND IN BLAST RESISTANT PYRAMID LINES OF RICE [*Oryza sativa* L.]

THESIS

By

MAHESH DURISETI

(A-2007-30-04)

Submitted to

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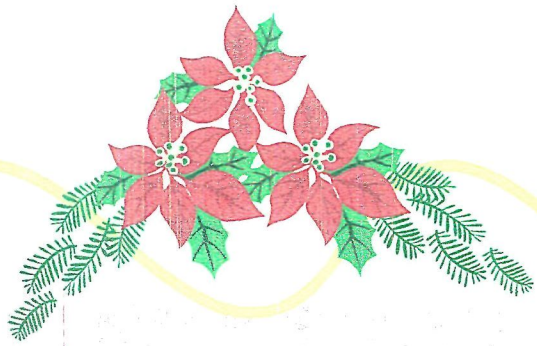

Affectionately

dedicated

To

Late : Dr. P. Plaha

Dr. Kamal Dev Sharma
Associate Professor

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CERTIFICATE-I

This is to certify that the thesis entitled **"Marker assisted selection for foreground and background in blast resistant pyramid lines of rice (*Oryza sativa* L.)"** submitted in partial fulfilment of the requirements for the award of the degree of **Master of Science (Agriculture)** in the discipline of **Agricultural Biotechnology** of CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur is a bonafide research work carried out by **Mr. Mahesh Duriseti (A-2007-30-04)** son of **Shri Veerachari Duriseti** under my supervision and that no part of this thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation have been fully acknowledged.

(Kamal Dev Sharma)
Major Advisor

Place: Palampur
Dated: 28.6.10

CERTIFICATE-II

This is to certify that the thesis entitled, **"Marker assisted selection for foreground and background in blast resistant pyramid lines of rice (*Oryza sativa* L.)"** submitted by **Mr. Mahesh Duriseti (A-2007-30-04)** son of **Shri Veerachari Duriseti** to the CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur, in partial fulfilment of the requirements for the degree of **Master of Science (Agriculture)** in the subject of **Agricultural Biotechnology**, has been approved by the Advisory Committee after an oral examination of the student in collaboration with an External Examiner.

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Needless to say, all omissions and errors are mine.

Mahesh 5th July

(Mahesh Duriseti)
A-2007-30-04

Place: Palampur
Dated: June, 2010

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ABBREVIATIONS USED

%	percent
µg	microgramme
bp	base pair
cM	centi Morgan
CTAB	hexadecyl-trimethyl-ammonium bromide
dATP	deoxyadenosine triphosphate
dCTP	deoxycytosine triphosphate
dGTP	deoxyguanosine triphosphate
DNA	deoxyribonucleic acid
dNTP	deoxynucleotide triphosphate
dTTP	deoxythymidine triphosphate
EDTA	ethylenediamine tetra acetic acid
gm	gramme
h	hour
ISSR	Inter simple sequence repeats
kb	kilobase
l	litre
M	molar
mg	milligramme
min	minute(s)
ml	milliliter
mM	millimolar
NaCl	sodium chloride
ng	nanogramme
°C	degree Celsius
PCA	principal component analysis
PCR	polymerase chain reaction
pH	puissance de hydrogen (ion concentration)
ppm	parts per million
PVP	polyvinypyrrolidone
RAPD	random amplified polymorphic DNA
Sec	second
SSR	simple sequence repeats
STS	sequence tagged site
Taq	*Thermus aquaticus*
TAE	tris/acetate/EDTA buffer
TE	tris EDTA buffer
Tris	tris (hydroxy methyl) amino methane
UPGMA	unweighted pair group method with arithmetic averages
UV	ultraviolet
V	volts

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ABSTRACT

Rice blast caused by fungus "*Magnaporthe grisea*" is one of the most widespread and destructive plant diseases of rice crop throughout the world. High variability in virulence of *M. grisea* combined with favourable environmental conditions prevalent in the North-Western Himalayan region makes the management of rice blast difficult in this area. Hence, breeding for durable resistance by combining many resistance genes in an elite genetic background is expected to provide broad-spectrum resistance. In the present study 44 BC_1F_4 lines generated using high yielding but blast susceptible rice variety 'HPU741' as recurrent parent and 'C101LAC', 'C101A51', and 'Tetep' as donors of blast resistance genes *Pi-1, Pi-2, Pi-ta* and *Pi-k^h* (*Syn. Pi-54*), were characterized for blast resistance genes using gene specific markers. As many as fifteen lines were two and three gene pyramids. The amount of 'HPU741' genome in the individual lines was estimated using marker assisted background selection. Of the three gene pyramid BC_1F_4 lines, eight had more than 80% genome of 'HPU741'. Lines possessing three gene pyramids with high recovery of recurrent parent genome can be evaluated under field conditions for yield and disease resistance to select superior lines for release as new varieties.

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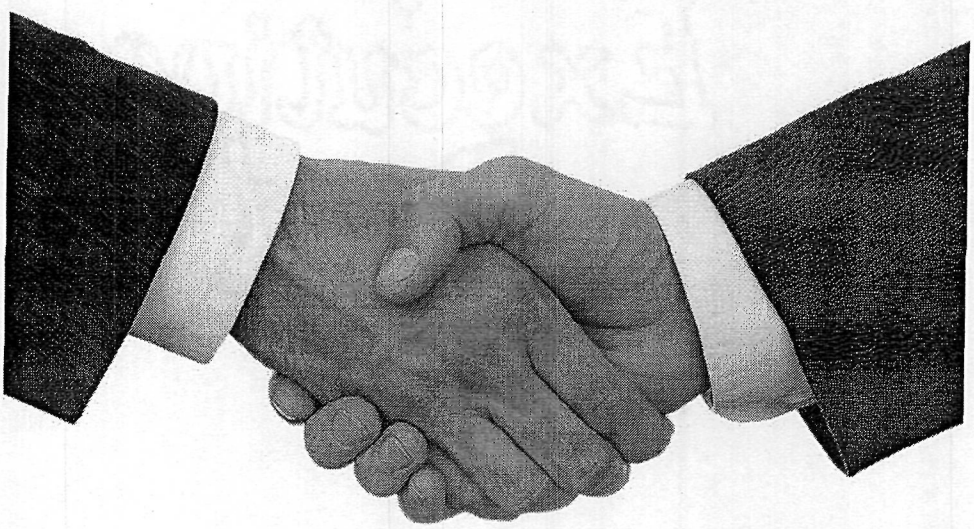
Introduction



1. INTRODUCTION

Rice (*Oryza sativa*) is one of the staple cereal crops of world with total area of 155.711 million hectares and production of 661.811 million tonnes (FAO 2009). In Asia, where 90 per cent of the world's rice is grown and consumed by nearly 2.8 billion people, 35 per cent of calorie requirements are met from this crop. Rice in India is grown on an area of 44 million hectares with a production of 148.365 million tonnes (FAO 2009). In Himachal Pradesh, rice is a major *kharif* crop. The state occupies an area of 77 thousand hectares under rice cultivation with a production of 120 thousand tonnes and an average grain yield of 1.56 q/ha (Anonymous 2008).

In areas such as Asia, Africa and Latin America where demand for rice is a top priority, the population is expected to increase 1.5 fold by 2025. However, capability of land to sustain rice yield is almost saturated. Though, many high yielding varieties of rice have been released in the recent past, yield potential of these varieties is severely affected by various biotic and abiotic stresses. Among the biotic stresses, rice blast caused by *Magnaporthe grisea* (Herbert) Barr. (Barr 1977) is one of the most widespread and destructive disease, infecting rice crop throughout the world (Zeigler *et al.* 1994). The major blast epidemics covering vast area especially in high humid regions occur on a regular basis resulting in 11 to 30% crop losses annually which represents a yield loss of about 157 million tonnes worldwide (Ahn 1994). High degree of variability in virulence of isolates of *M. grisea* prevalent in the Northwestern Himalayan region of India makes the management of rice blast in this area difficult (Rathour *et al.* 2006).

Rapid changes that occur in the virulence characteristics of populations raise a continuous threat to the effectiveness of existing blast resistant varieties. Owing to rapid changes in virulence spectrum of *M. grisea* populations, in many cultivars blast resistance is quite short lived in the field. Hence, there is an urgent need for strategies to develop varieties with durable resistance to the disease. Both major and minor genes can contribute to durable resistance (Zhu *et al.* 1993; Wang *et al.* 1994). Successful exploitation of resistance breeding depends upon careful characterization of resistance spectrum of the genes in question and combining them so that the 'gene pyramid' is effective against the target pathogen populations (Chen *et al.* 1995; Zeigler *et al.* 1994,

1995). Gene pyramiding for rice blast resistance is, however, difficult rather impossible using conventional breeding methods because of epistatic and/or masking effects of individual genes coupled with absence of appropriate blast races for identifying gene pyramids.

Marker assisted selection (MAS) is extremely useful in blast resistance breeding where resistance phenotypes are often encoded by single or a few genes (Young 1996). So far, over 85 such major *Pi* genes conferring resistance to blast disease have been identified (Sharma *et al.* 2010) and over 40 of these have been tagged with molecular markers (Chopra 2006). In general, the sources of resistance are not in agronomically elite backgrounds, therefore, the pyramid lines, though impart resistance, may not be agronomically superior. This warrants pyramiding of resistance genes in the elite genetic backgrounds.

Marker assisted background selection is an approach that facilitates quick recovery of the recipient genome including the desirable genic regions (Hospital *et al.* 1992; Visscher 1996; Liu *et al.* 2003). The Marker assisted background selection can be led with the exclusive objective of monitoring the degree of similarity of the resistant lines to the recurrent parent and predicting the degree of kinship among backcrossed plants. This may be done through the analysis of genetic similarity among the individuals from the progeny and the recurrent parents, as demonstrated by Hagiwara *et al.* (2001) using RAPD markers. In this situation, the number of backcrosses can be reduced by recovering the plants more similar to the recurrent parent, in addition to phenotypic selection for the desired trait, thus, reducing the time frame required for developing new varieties.

Microsatellites are considered ideal markers for genetic studies because they are co-dominant, multiallelic, highly polymorphic even in closely related individuals and have high abundance and fairly uniform distribution in plant genomes (Morgante and Olivieri 1993; Brondani *et al.* 1998; Temnykh *et al.* 2001). The sequence information and map positions of rice SSR markers are publicly available (http://www.gramene.org) and more rice SSR markers are being developed to tag any gene and facilitate MAS (Temnykh *et al.* 2000; McCouch *et al.* 2002; International Rice Genome Sequencing Project 2005). Many research groups have used SSR markers for various purposes, including estimation of the proportion of donor genome in a recurrent parent background (Bernardo *et al.* 2000; Semagn *et al.* 2007; Gopalakrishnan *et al.* 2008).

In addition to SSRs, Inter Simple Sequence Repeat (ISSR) markers (Zietkiewicz *et al.* 1994) based on regions that lie within microsatellite repeats also offer great potential to determine intra and inter-genomic diversity. ISSRs have been widely used to detect polymorphism, analyze phylogenetic relations, evaluate variation within and among the landrace mixtures, identify cultivars and delineate wild and cultivated species (Wolfe and Liston 1998; Camacho and Liston 2001; Nan *et al.* 2003). These markers because of multilocus and highly polymorphic patterns (Tsumura *et al.* 1996; Nagaoka and Ogihara 1997) can be useful for background selection in rice.

In a recently concluded project in our University, pyramid lines with different combinations of blast resistance genes were developed in rice. The F_1s of locally adapted cultivar 'HPU741' with different vertical resistance gene sources were backcrossed with 'HPU741' and then selfed to generate BC_1F_4 populations. The BC_1F_4 lines might have predominantly the elite genetic background of 'HPU741'; however more precise estimates of genomic background of 'HPU741' in these lines can be obtained by background selection based on DNA based markers. There is also a need to ascertain resistance genes present in the selected MAS lines, the homozygous or heterozygous state of these genes, and to identify lines with genomic background of 'HPU741' along with blast resistance genes *Pi-1, Pi-ta* and *Pi-k*^{h} (syn. *Pi-54*, Sharma *et al.* 2010).

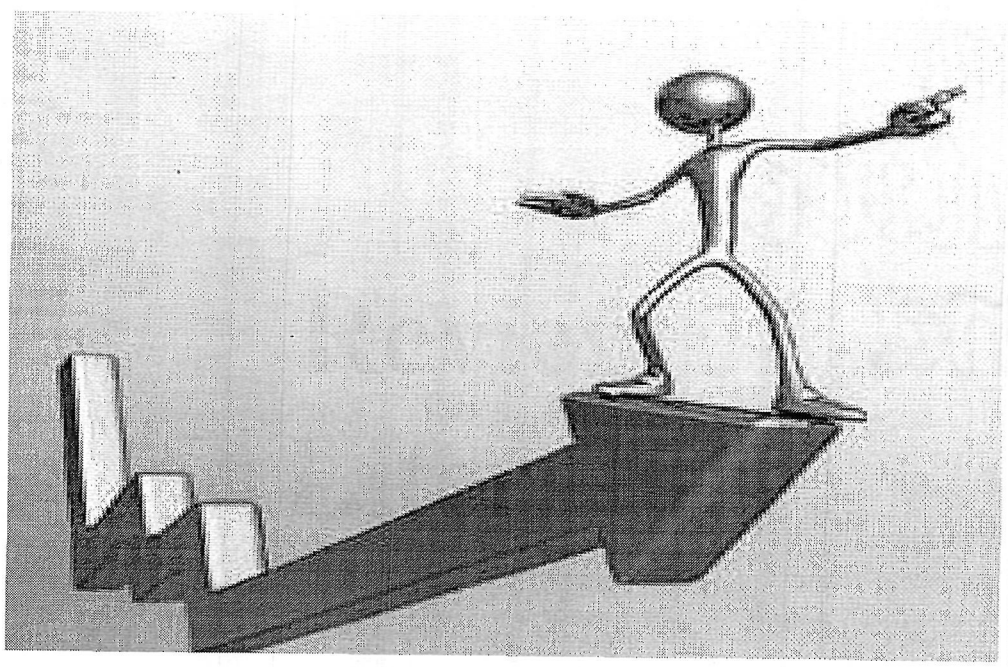
The broad objective of the present study is to exploit molecular marker technology to develop high yielding varieties coupled with durable multigenic disease resistance to rice blast in Himachal Pradesh.

The study was conducted with the following objectives:

i) To identify different combinations of resistance genes, *Pi-1, Pi-k*^{h} and *Pi-ta* in the pyramid lines.

ii) To determine the extent of genomic background of 'HPU741' in the pyramid lines.

Review of Literature



2. REVIEW OF LITERATURE

The literature pertaining to the objectives of present study has been reviewed under following heads:

2.1 Plant material

2.2 Marker assisted foreground selection of blast resistance genes

2.3 Marker assisted background selection for estimation of recurrent parent genome

Rice blast caused by the fungal pathogen *Magnaporthe grisea* is one of the most serious and destructive plant disease in most of the world's rice-growing areas (Zeigler *et al.*1994). Developing rice cultivars with durable resistance to blast is the most economically feasible and environmental friendly management approach in most blast-prone rice ecosystems (Ahn 1994). Because of high degree of variability in pathogenicity of *M. grisea* races, resistance in many cultivars is short-lived in environments that are conducive to this disease (Ou 1979; Pan *et al.* 1998). It is, therefore, necessary to develop cultivars with durable resistance by combining different resistance spectra into individual cultivar (Zeigler *et al.* 1994; Conoway-Bormans *et al.* 2003; Jia *et al.* 2003).

Gene pyramiding i.e. incorporating many disease resistance genes preferably vertical into the elite background of popular or agronomically superior cultivars or genotypes is, however difficult, rather impossible using conventional breeding methods due to epistasis and/or masking effect of individual genes coupled with absence of appropriate blast races for identifying gene pyramids.

The use of molecular markers as a diagnostic tool was first proposed by Tanksley in 1983 and reviewed by Melchinger *et al.* (1990). The term 'foreground selection' to the selection of target allele was suggested by Hospital and Charcosset (1997) whereas the term 'background selection' to identify individuals with a low proportion of undesirable genome from the donor parent was first proposed by Tanksley *et al.* (1989) and Hillel *et al.* (1990).

2.1 Plant material

HPU741 is a high yielding rice variety known for its earliness and uniform maturity is suitable for cultivation in low, mid and high hills of Himachal Pradesh, besides its agronomic superiority is also resistant to leaf blast (Sharma *et al.* 1983)

C101LAC and C101A51 are the two rice NILs that carry broad spectrum blast resistance genes *Pi-1* and *Pi-2* respectively in the background of blast susceptible genotype CO39. Mackill and Bonman (1992) developed these lines by backcrossing LAC23 (donor of *Pi-1*) and A5173 (donor of *Pi-2*) with CO39. They also reported that Tetep the indica rice cultivar carries three broad spectrum resistance genes *Pi-1*, *Pi-ta* and also another resistance gene, *Pi-k*^{h} as reported by Kiyosawa *et al.* (1969). Later these findings were supported by Inukai *et al.* (1994), Kinoshita *et al.* (1994).

2.2 Marker assisted foreground selection of blast resistance genes

During past decade, genetics of blast resistance has been studied extensively and more than 85 resistance genes have been identified (Kumar *et al.* 2010; Sharma et al. 2010). Six resistance genes *Pi-b, Pi-ta, Pi-9, Piz-5, Piz-t and Pi-k*^{h} have been cloned and sequenced (Wang *et al.* 1999; Bryan *et al.* 2000; Ou *et al.* 2006; Zhou *et al.* 2006; Rai *et al.* 2009). Majority of the blast resistance genes and their analogues are located on chromosomes 4, 6, 11 and 12.

Marker assisted selection (MAS) is a low cost and high throughput alternative to conventional phenotypic screening and permits rapid introgression of multiple resistance genes into susceptible varieties (Hittalmani *et al.* 2000; Narayanan *et al.* 2004). The knowledge about the precise chromosomal location of resistance genes and the availability of markers that are tightly linked to the resistance genes are the two essential requirements of a marker assisted breeding programme. The availability of a large number of high density molecular maps of rice, based on microsatellites, sequence tagged sites (STS), expressed sequence tags (EST) and mapping and cloning of several rice blast resistance genes has provided a useful background for MAS of blast resistance genes (Hittalmani *et al.* 1995; Bryan *et al.* 2000; McCouch *et al.* 2002; Fuentes *et al.* 2008; Sharma *et al.* 2010).

Plaha *et al.* (2005) of our department pyramided blast resistance genes *Pi-1, Pi-2, Pi-ta* and *Pi-k*^{h} into the elite background of 'HPU741' following MAS. Pursuing these studies further, BC_1F_4 lines were developed with the objective to select agronomically superior lines possessing combinations of blast resistance genes, *Pi-1, Pi-2, Pi-ta* and *Pi-k*^{h}. The parents of BC_1F_4 lines were HPU741 (recurrent parent), C101LAC (donor for *Pi-1*), C101A51 (donor of *Pi-2*) and Tetep (donor of *Pi-1, Pi-ta* and *Pi-k*^{h}).

2.2.1 MAS for blast resistance genes

Fjellstrom *et al.* (2004) identified microsatellite markers for blast resistance genes *Pi-b, Pi-k* and *Pi-ta*^{2} on rice chromosome 2, 11 and 12, respectively. Two microsatellite markers, RM208 and RM224, were found to co-segregate with the *Pi-b* and *Pi-k* genes, respectively, while OSM89, RM155 and RM7102 were found to closely flank the *Pi-ta*^{2}. These markers are expected to facilitate the introgression and pyramiding of these three blast resistance genes into new rice cultivars and elite lines. Similarly Yi *et al.* (2004) developed three PCR-based dominant markers (JJ80-T_3, JJ81-T_3 and JJ113-T) for rice blast resistance gene *Pi 5(t)*.

Three major genes (*Pi-1, Piz-5* and *Pi-ta*) for blast resistance on rice chromosomes 11, 6 and 12, respectively were fine-mapped using RFLP markers and subsequently these markers were used to pyramid these genes into agronomically superior rice varieties (Hittalmani *et al.* 2000). Jia *et al.* (2002) also developed the dominant markers for blast resistance gene *Pi-ta.* The rice cultivar Katy containing *Pi-ta* gene was resistant to predominant blast races IB-49 and IC-17 and was used as *Pi-ta* donor. Three STS markers, YL153/YL154, YL100/YL102 and YL155/YL87 linked to *Pi-ta* allele were used to detect the introgression of *Pi-ta* in ten advanced breeding lines based on Katy, Drew, and Kaybonnet as parents. Five of the lines contained *Pi-ta* gene.

Sharma *et al.* (2005) mapped and cloned a dominant blast resistance gene (*Pi-k*^{h}) in Tetep. Using 178 sequence tagged microsatellites (STMSs), sequence-tagged sites (STS), expressed sequence tags (ESTs) and simple sequence repeat (SSRs) markers, they genotyped a population of 208 F_2 individuals. The *Pi-k*^{h} was mapped between two SSR markers (TRS26 and TRS33) which were 0.7 and 0.5 cM away, respectively. In a recent report, name of the *Pi-k*^{h} gene was changed to *Pi-54 as* per the standard guidelines of Committee on Gene Symbolization, Nomenclature and Linkage (CGSNL) (Sharma *et al.* 2010).

Fuentes *et al.* (2008) identified new microsatellite markers linked to the blast resistance gene *Pi-1(t)*. They reported that RM1233*I and RM224 markers were mapped at 0.0 cM with the *Pi-1(t)* gene on chromosome 11. Similar findings reported by Prasad *et al.* (2009) used SSR markers RM224 and RM1233A and successfully introgressed blast resistance gene *Pi-1* from NIL C101LAC into the susceptible indica cultivar BPT 5204.

Under marker assisted gene pyramiding programme three rice varieties, Digu, BL-1 and Pi-4, with blast resistance genes *Pi-d(t), Pi-b,* and *Pi-ta2*, respectively were crossed with G46B a high yielding but blast susceptible variety. Fifteen plants selected from F_2 progeny were backcrossed with G46B via MAS to have the background of G46B (Chen *et al.* 2004). Four of the plants were heterozygous for the three resistance genes, ten were heterozygous for two of the three resistance genes and only one was homozygous for two resistance genes. In Indonesia, two blast resistance genes *Pi-1* and *Pi-2* were incorporated into Indonesian cultivars such as Way Rarem, Cabacu and Jambu, by using an RFLP marker RZ536 and a PCR based marker STS-RG64 (Bustamam *et al.* 2005).

Ram *et al.* (2007) performed three way cross involving wild rice *O. rufipogon* accession (Coll-4) with high yielding but blast susceptible rice lines of *O. sativa (indica),* B32-Sel-4 and B29-6. Preliminary screening of F_5 generation lines with blast isolates of Andaman resulted in two genotypes B90-15 and B90-15-4R with superior yield. Later, in a trial on monitoring of virulence of *M. grisea,* these lines (B90-15 and B90-15-4R) were evaluated along with ten near isogenic lines (NILs) possessing blast resistance genes (*Pi-a*, *Pi-1*, *Pi-2*, *Pi-3*, *Pi-4a*, *Pi-4b*, *Pi-5*, *Pi-7*, *Pi-9*, *Pi-12*), four pyramid lines with 2 or 3 gene combinations (*Pi-1, Pi-2* and *Pi-4)*, eight international differentials, five resistant checks and two susceptible checks. The results indicated that line B90-15 had broad spectrum resistance against 16 isolates of *M. grisea* present in India. The study also suggested, broad spectrum resistance in B90-15 was not due to major genes only, but additional QTLs might also have introgressed from *O. rufipogon* resulting in broadening the resistance spectrum. The B90-15 was released under the commercial name Jarava.

Studies were also undertaken to pyramid blast resistance genes as a strategy to improve durability of blast resistance in Latin America. Pathogen characterization of spontaneous mutants of the blast pathogen allowed identification of three new blast

resistance genes *Pi-b, Pi-9* and *Pi-ta*^{2} (Victoria and Martinez 2009). Microsatellite markers tightly linked to these genes were used to detect their introgression and pyramid these along with already known blast resistance genes *Pi-1, Pi-2 and Pi-33* into Latin American rice cultivars.

2.2.3 MAS for bacterial blight resistance genes

Huang *et al.* (1997) used marker assisted selection to pyramid four bacterial blight resistance genes, *Xa-4, Xa-5, Xa-13,* and *Xa-21*. Breeding lines with two, three and four resistance genes showed wider spectrum and a higher level of resistance than the line with a single gene.

2.2.4 MAS for blast and blight resistance genes

Sridhar *et al.* (1996) used a gene pyramiding approach to introduce 'lineage-excluding' genes (*Pi-2, Pi-9*) into elite commercial cultivars of Central Rice Research Institute, Cuttack.

Narayanan *et al.* (2004) identified two major blast resistance genes *Pi-1* and *Piz-5* that conferred resistance to many *M. grisea* lineages. Rice lines C101LAC and C101A51 used in the present study are the near-isogenic lines and these carry blast resistance genes *Pi-1* and *Piz-5* respectively, in the background of a blast susceptible genotype CO39, were used to develop pyramid lines using closely linked RFLP (RZ536) and SAP (RG64) markers. In addition to blast resistance genes, *Xa21* conferring resistance to bacterial blight was also incorporated into the elite background. Three major genes, two for blast resistance (*Pi-1, Piz-5*) and one for bacterial blight resistance (*Xa21*) were stacked into rice using MAS and genetic transformation.

2.3 Marker assisted background selection for estimation of recurrent parent genome

The basis of a marker-assisted backcrossing (MAB) strategy is to transfer a specific allele at the target locus from a donor line to a recipient line while selecting against donor introgressions across the rest of the genome. Selection against donor parent genome is a mean to accelerate the rate of genetic gain in terms of recurrent parent genome achieved with in the backcrossing strategy (Stam and Zeven 1981; Visscher 1996; Ribaut *et al.* 2002; Frisch and Melchinger 2005).

Young and Tanksley (1989) made the first attempt to provide a better estimate of Donor Genome Content by taking marker locations into account; they introduced the concept of `graphical genotype' to portray the parental origin and allelic composition throughout the genome. Subsequently, Tanksley *et al.* (1989) proposed the term "background selection" to identify individuals with a low proportion of undesirable genome from the donor parent. Computer simulations showed that the use of molecular markers for background selection can accelerate the recovery of the recurrent parent genome within 2-3 backcrosses as compared to 5 to 6 required in a conventional backcrossing program. The background selection became a technique that allows identification of individuals with high percentage of recurrent parent genome was shown to be efficient in several theoretical (Hillel *et al.* 1990; Hospital *et al.* 1992; Visscher *et al.* 1996; Servin and Hospital 2002) as well as experimental works (Ragot *et al.* 1995; Benchimol 2005).

Pedigree estimates when compared with marker estimates (RFLP data) of F_2 and BC_1 derived maize inbreds suggested that selection during backcrossing generally favored the recurrent parent over the donor parent (Bernardo *et al.* 1997). In a subsequent study on 13 maize inbreds, Bernardo *et al.* (2000) reported that pedigree and marker data often lead to different estimates of parental contribution and coefficient of co-ancestry. The SSR markers were also found to be superior to RFLP markers for estimating genetic relationship.

Similar to conclusions of Tanksley *et al.* (1989), Hospital *et al.* (1992) showed that, MAS lead to a gain, in time, of about two generations compared to phenotypic selection. It was also concluded that markers were most useful when their map positions are known. Furthermore, in earlier generations increasing the number of markers to more than three for non carrier chromosomes is of not that much use.

2.3.1 Optimal population size for a marker assisted backcross programme

Marker assisted introgression of a QTL combining foreground and background selection was investigated by Hospital and Charcosset (1997). They presented recurrence equations to calculate the minimum population size needed in each back cross programme.

Frisch *et al.* (1999a), in a marker assisted backcrossing programme gave mathematical equations to obtain (i) the optimum distances (d1, d2) between the flanking markers and the target locus and (ii) the minimum number of individuals (n) required to obtain at least one desired individual carrying the donor allele at the target locus and to have a minimum proportion of donor genome on the carrier chromosome. Analytical solutions and tabulated results indicated that minimum marker distance decreased with increasing the number of genotyped individuals. In a similar study Frisch and Melchinger (2005) concluded that in BC_1 populations' marker assisted selection is, within certain limits, more efficient for large populations than for higher marker densities. In continuation of his studies, Frisch *et al.* (1999b) used the published maize map of 80 markers and phenotypic selection of backcrossed progenies and required to a conclusion that increasing population size from BC_1 to BC_3 generation reduced the number of marker data points by as much as 50% without affecting the proportion of recurrent parent genome. The results also indicated that a 4 stage selection approach (involving one foreground selection, recombinant selection and two background selection steps) reduced the required marker data points by as much as 75% when compared to a selection index taking into account all markers across the genome. It was also concluded that two stage selection (involving one foreground selection and one background selection step) is appropriate in early generation backcross programs and is the only option when there is no availability of linkage map.

Like Frisch *et al.* (1999b), Ribaut *et al.* (2002) also performed simulations for different back cross marker assisted selection (BC-MAS) strategies using maize genome as a model. The simulation results indicated that the selection response in the BC_1 could be increased significantly when the selectable population size was less than 50, and that a diminished return was observed when this number was greater than 100. It was also concluded that MAS could be used at non target loci only at one generation and a selection at BC_3 would be more efficient than selection at BC_1 or BC_2 generation, due to the increase over generation of the ratio of the standard deviation to the mean of the donor genome contribution.

2.3.2 Optimization of markers per chromosome for effective background selection

In an effort to predict the contribution of the donor parent to the genome of a backcross individual Visscher *et al.* (1996) assigned different weights to the markers. While doing so selection on markers was not taken into account instead they applied selection index theory proposed by Hazel (1943) to derive weights depending on the recombination frequency between markers. Like Hospital *et al.* (1992) they also concluded that a few well placed markers (two to four markers on a chromosome of 100 cM) would provide adequate coverage of the genome in backcross programs.

Servin and Hospital (2002) suggested an approach of determining optimal positioning of markers, by taking, the selection on markers into account. They define optimal marker positions as the positions that maximize the genome wide proportion of loci that are fixed for homozygous recipient type by the time markers are fixed for recipient type. In doing so he emphasized the importance of selecting polymorphic markers rather than monomorphic markers in recurrent parent studies. The results were in agreement with Hospital *et al.* (1992) obtained by simulations that take selection on markers into account.

The inference based on several studies (Hospital *et al.* 1992; Visscher *et al.* 1996; Servin and Hospital 2002) on the recovery of recipient parent genome is to have optimum number of markers dispersed throughout the genome rather than many markers concentrated on a few regions of the genome. It can be summarized that an average distance of 20 cM between markers and a minimum of 3-4 markers per chromosome are sufficient for accelerated recovery of the recipient parent genome.

2.3.3 Estimation of recurrent parent genome

An unweighted predictor "α" which takes into account the proportion of marker alleles that are identical with the alleles of a parental line was used to predict the contribution of the parental line to the genome of the derived inbred lines (Bernardo *et al.* 2000 and Heckenberger *et al.* 2005). In a similar study Visscher *et al.* (1996) used another weighted predictor "β" which also takes into account the correlation between markers on a chromosome to predict parental genome contribution. Similarly Frisch and Melchinger (2006) in their study on inbreds derived from biparental crosses used a new

marker based predictor "ε" for parental genome contribution which takes into account not only alleles at the marker loci but also their map distance. They concluded "ε" provides substantially greater prediction precision than the commonly used predictor "α", and has a broad range of application in genetics and plant breeding.

MAB as a tool of estimating the content of recurrent genome in backcrossed progenies is already gaining popularity (Gopalakrishnan *et al.* 2008; Semagn *et al.* 2007). Using this approach Semagn *et al.* (2007) released seven BC_2 lines (NERICA1 to NERICA7) for commercial cultivation. The release was preceded by the evaluation 70 BC_2 interspecific inbred lines derived from crosses between a japonica variety (WAB 56-104) as recurrent parent and an *O. glaberrima* variety (CG-14) as the donor parent using microsatellite markers. The average proportion of recurrent parent genome in these lines was 87.4% and donor genome was 6.3% while the remaining 6.3% is of non parental origin.

In another study Kwon *et al.* (2008) developed 13 near-isogenic lines (NILs) of japonica rice via a backcross method (recurrent parent: Chucheong, blast resistant japonica donors: Seolak, Daeseong and Bongkwang). The NILs were scanned using 158 SSR markers. The genetic similarities of the NILs to the recurrent parent Chucheong averaged 0.961, with a range of 0.932 - 0.984. The NILs have the potential as commercial rice varieties because these have broad spectrum resistance to *M. grisea* and have the desirable agronomic traits of Chucheong.

MAB integrated with marker assisted foreground selection was used to improve Indian Basmati rice by Gopalakrishnan *et al.* (2008). They used sequence tagged microsatellite markers (STMS) for background selection along with, foreground selection markers were used to combine bacterial blight resistance gene from a non basmati resistance donor (IRBB55) with grain and cooking quality characteristics of the popular basmati rice variety "Pusa Basmati 1" (PB1) in backcross progenies. An elite selection 'Pusa 1460-01-32-6-67' was released as a new variety in the name of "Improved Pusa Basmati 1" for commercial cultivation in India. "Improved Pusa Basmati 1" records a yield advantage of 11.9% over the "Pusa Basmati 1" in the multilocation agronomic trails in the basmati growing regions of India.

2.3.4 SSR as background selection markers

Simple sequence repeats (SSR), also known as microsatellites or short tandem repeats (STR) are tandemly arranged repeats of mono to hexa nucleotide motifs that are ubiquitous in eukaryotic genomes and frequently exhibit variation in the number of repeats at a locus. Their abundance and inherent potential for variation have made them a valuable source of genetic markers (Temnykh *et al.* 2001; McCouch *et al.* 2002). Microsatellite markers are highly informative and can be rapidly and reliably visualized without the use of radioisotopes (Panaud *et al.* 1996). With the International Rice Microsatellite Initiative (IRMI) the primer sequences, PCR conditions, polymorphism information, and map positions of these markers are publicly available over the Internet with the domain name **"www.gramene.org".**

On the basis of previous investigations (Openshaw *et al.* 1994; Visscher *et al.* 1996; Frisch *et al.* 1999b), an average marker density of about 10-20 cM is sufficient to warrant a good coverage of the genome in marker-assisted selection programs. The SSRs because of higher marker density, almost uniform distribution throughout the genome and their widespread use in construction of genetic maps, make it the marker of choice for conducting MAB selection programs.

2.3.5 Inter Simple Sequence Repeat markers as background selection markers

Inter Simple Sequence Repeat (ISSR) markers were developed by (Zietkiewicz *et al.* 1994). ISSRs are the regions that lie within microsatellite repeats and reveal variations within unique regions of the genome at several loci simultaneously. ISSRs can be highly variable within a species and have the advantage of being longer primers, allowing for more stringent annealing temperatures and revealing a much higher number of polymorphic fragments with good reproducibility. Accordingly, ISSR markers have been widely used to detect polymorphism, analyze phylogenetic relations, evaluate variation within and among the landrace mixtures, identify cultivar and to differentiate between wild and cultivated species (Wolfe and Liston 1998; Camacho and Liston 2001; Nan *et al.* 2003). Several properties of microsatellites, such as a high copy number in eukaryotic genome, make ISSRs extremely useful markers. These are semi arbitrary markers,

amplified by PCR; in the presence of one primer complementary to a target microsatellite amplify the genomic sequences flanked by two inversely oriented repeat elements. Amplification by these does not require genome sequence information and leads to multilocus and highly polymorphic patterns (Tsumura *et al.* 1996; Nagaoka and Ogihara, 1997).

ISSRs have not been used in rice either for MAS or MAB; however their potential use in such studies along with SSRs is immense, primarily because they amplify different regions than SSRs. However, ISSR polymorphism has been used to study genetic diversity and phylogenetic relationships in *Oryza* species (Joshi *et al.* 2000). Forty-two genotypes including 17 wild species, two cultivated species, *O. sativa* and *O. glaberrima*, and three related genera were used in ISSR analysis. Eleven ISSR primers were used to determine the genetic diversity and construct a consensus tree. Keeping in view of its ability to cover large parts of genome, ISSRs can be the marker of choice for MAS or MAB.

Materials and Methods

3. MATERIALS AND METHODS

The present investigation was carried out in the Department of Agricultural Biotechnology, CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur. The materials used and the methodologies adopted to achieve the objectives of the present investigation are given hereunder.

3.1 Plant material

The seed material for the present investigation comprised BC_1F_4 generation lines, (Table 3.1) which were expected to contain individual or combinations of blast resistance genes (*Pi-1, Pi-2, Pi-ta* and *Pi-k^h*). These lines, were derived from crosses of a high yielding but susceptible parent 'HPU741' with resistant donors 'C101LAC' (*Pi-1*), 'C101A51' (*Pi-2*), and 'Tetep' (*Pi-1*, *Pi-ta* and *Pi-k^h*) and were designated as MAS (Marker Assisted Selection) lines. Single plants of 44 elite pyramid lines were used in this research programme. The parentage of the lines is shown in Table (3.1).

3.2 Research methodology

Research programme was carried out in two steps. In the first step called as 'marker assisted foreground selection', all the plants were analyzed using resistance gene specific markers (Table 3.2) for the presence of blast resistance genes (*Pi-1, Pi-ta* and *Pi-k^h*). In the second step called as 'marker assisted background selection', locus specific SSR markers and multilocus ISSR markers were used to quantify background of 'HPU741' in all the lines. The details of the methodologies adopted are described in the proceeding text.

3.3 Marker assisted foreground selection

3.3.1 Isolation of plant genomic DNA

Genomic DNA of the parents (HPU741, C101LAC, C101A51 and Tetep) and 44 BC_1F_4 plants was isolated from young seedlings using CTAB method (Murray and Thompson 1980). Young leaves were excised, rinsed with deionized water and dried on

Table 3.1 Plant material used in the present study

Sr. No.	MAS No.	Parentage	Sr. No.	MAS No.	Parentage
1.	MAS-8	F_1\{RG242[2]× F_1(HPU741×ARBN42)\}	25.	MAS-68	F_1\{RG242[2]× F_1(HPU741×ARBN42)\}
2.	MAS-9	F_1(RG241[1]×GR185[10])	26.	MAS-70Q161	BC_1F_3(HPU741×Tetep)
3.	MAS-10	F_1(RG241[1]×GR185[10])	27.	MAS-70Q163-1	BC_1F_3(HPU741×Tetep)
4.	MAS-14	F_1(RG242-A[3]×RG241[1])	28.	MAS-70Q163-2	BC_1F_3(HPU741×Tetep)
5.	MAS-16	F_1(RG242-A[3]×RG241[1])	29.	MAS-70Q164	BC_1F_3(HPU741×Tetep)
6.	MAS-18	F_1(RG241[1]×RG242-A[3])	30.	MAS-71Q168	BC_1F_3(HPU741×Tetep)
7.	MAS-19	F_1(G335[8]×RG231[6])	31.	MAS-71Q169	BC_1F_3(HPU741×Tetep)
8.	MAS-20	F_1(G335[8]×RG231[6])	32.	MAS-71Q170	BC_1F_3(HPU741×Tetep)
9.	MAS-21	F_1(G273[9]×RG230[5])	33.	MAS-72Q171	BC_1F_3(HPU741×Tetep)
10.	MAS-22-1	F_1(G273[9]×RG230[5])	34.	MAS-72Q172	BC_1F_3(HPU741×Tetep)
11.	MAS-22-2	F_1(G273[9]×RG230[5])	35.	MAS-72Q179	BC_1F_3(HPU741×Tetep)
12.	MAS-29	F_1(GR185[10]×G196[7])	36.	MAS-72Q180	BC_1F_3(HPU741×Tetep)
13.	MAS-31	F_1(RG241[1]×RG242-A[3])	37.	MAS-72Q181	BC_1F_3(HPU741×Tetep)
14.	MAS-33	F_1\{RG231[6]× F_1(HPU741×Tetep)\}	38.	MAS-75Q184	BC_1F_3(HPU741×Tetep)
15.	MAS-34	BC_1F_2(HPU741×Tetep)	39.	MAS-75Q185	BC_1F_3(HPU741×Tetep)
16.	MAS-36	BC_1F_3(HPU741×Tetep)	40.	MAS-75Q186	BC_1F_3(HPU741×Tetep)
17.	MAS-38	BC_1F_3 (HPU741×Tetep)	41.	MAS-76	F_1(RG243[4]×GR185[10])
18.	MAS-40-1	BC_1F_3 (HPU741×Tetep)	42.	MAS-78	F_1(RG243[4]×GR185[10])
19.	MAS-40-2	BC_1F_3 (HPU741×Tetep)	43.	MAS-107	BC_1F_3(HPU741×Tetep)
20.	MAS-41	BC_1F_3 (HPU741×Tetep)	44.	MAS-118	BC_1F_3(HPU741×Tetep)
21.	MAS-43	BC_1F_3 (HPU741×Tetep)	45.	HPU741	Recurrent parent
22.	MAS-44	BC_1F_3 (HPU741×Tetep)	46.	C101LAC	Donor of *Pi-1* gene
23.	MAS-56	F_1(RG242-A[3]×RG241[1])	47.	C101A51	Donor of *Pi-2* gene
24.	MAS-58	F_1(RG242-A[3]×RG241[1])	48.	Tetep	Donor of *Pi-1, Pi-ta & Pi-k[h]* genes

[1, 2, 7, 8, 9] BC_1F_3 (HPU741×ARBN43); [3, 4, 10] BC_1F_3 (HPU741×Tetep); [5, 6] BC_1F_2 (HPU741×Tetep); ARBN42: C101LAC; ARBN43: C101A51

tissue papers. About 0.5-1.0 gm leaf tissue of each plant was frozen in liquid nitrogen and ground to fine powder using autoclaved pestle and mortar. The ground tissue was transferred to 2ml eppendorf tubes containing 700 µl of extraction buffer (2% CTAB, 100 mM Tris, 20 mM EDTA, 1.4 mM NaCl and 1% PVP, pH 8.0) maintained at 60^0C in water bath and mixed vigorously. The mixture was incubated at 60^0C for 1 h with occasional mixing, followed by addition of equal volume of chloroform: isoamyl alcohol (24:1) and gentle mixing. The mixture was centrifuged at 10,000 rpm for 10 min at room temperature. The upper layer (aqueous phase) was transferred to fresh tubes, followed by addition of 600 µl of pre-chilled isopropanol. The contents of the tubes were mixed gently and the mixture incubated at -20^0C for 1 h followed by centrifugation at 10,000 rpm for 10 min. Supernatant was drained and the resulting DNA pellet washed twice with 1 ml of chilled ethanol (70%). The pellet was dried in a stream of sterile air in a laminar air flow cabinet for 3-4 h. The DNA was dissolved in 1 ml TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0) and was treated with 1 µl of RNase A (10 mg/ml) for one hour at 37^0C. The quantity and quality of DNA was estimated by electrophoresis using 1.0 % agarose gel.

3.3.2 Validation of markers linked to resistance genes in parental genotypes and screening of genotypes for blast resistance genes

Previously identified gene specific SSR/STS markers (Table 3.2) were used to identify the three blast resistance genes (*Pi-1*, *Pi-ta* and *Pi-k^h*) in the rice MAS lines used in the present study. These markers were validated in the parental genotypes with respect to their polymorphism in the susceptible parent 'HPU741' and resistance donors, 'C101LAC' (*Pi-1*), 'C101A51' (*Pi-2*), and 'Tetep' (*Pi-1*, *Pi-ta* and *Pi-k^h*).

Two SSR markers RM224 and RM1233A for *Pi-1* gene, one dominant STS marker YL155/YL87 for *Pi-ta* gene and two SSR markers RM206 and TRS26 for *Pi-k^h* gene were validated to detect presence of resistance genes in different genotypes (See Table 3.2 for sequence of the primers that amplify these markers). PCR was performed in 25 µl volume consisting of 2 µl DNA template, 20 ng of each primer, 0.2 µM of each dNTP, 1.5 mM $MgCl_2$, 2.5 µl of 10X PCR buffer, 1 unit *Taq* polymerase (TAQ500, Life Tech) and autoclaved double distilled water. PCR amplification of markers RM224, RM1233A and TRS26 was carried out in a thermocycler (My Cycler, Bio-Rad, USA) using following temperature profile: denaturation at 94^0C for 5 min, followed by 39

cycles at 94^{0}C for 1 min, 55 ^{0}C for 1 min, 72^{0}C for 1.30 min and final extension at 72^{0}C for 5 min, followed by rapid cooling to 4^{0}C. In case of SSR marker TRS26, the annealing temperature was 60^{0}C.

Table 3.2 SSR/STS markers used for MAS of blast resistance genes in BC_1F_4 rice lines

Gene	Marker	Marker type	Primer sequence (5′-3′)	Chromosome No.	Reference
Pi-1	RM224	SSR	F: ATCGATCGATCTTCACGAGG R: TGCTATAAAAGGCATTCGGG	11	Chen *et al.* 1997
	RM1233A	SSR	F: GTGTAAATCATGGGCACGTG R: AGATTGGCTCCTGAAGAAGG		Fuentes *et al.* 2008
Pi-ta	YL155/YL87	STS	F: AGCAGGTTATAAGCTAGGCC R: CTACCAACAAGTTCATCAAA	12	Jia *et al.* 2002
Pi-k^h	RM206	SSR	F: CCCATGCGTTTAACTATTCT R: CGTTCCATCGATCCGTATGG	11	Sharma *et al.* 2005
	TRS26	SSR	F: GGAGAGCCAATCTGATAAGCA R: CAACAAGAGAGGCAAATTCTCA		Sharma *et al.* 2005

For PCR amplification of marker YL155/YL87, the initial denaturation was done at 95^{0}C for 3 min followed by 29 cycles at 95^{0}C for 30 sec, 55^{0}C for 30 sec, 72^{0}C for 30 sec and final extension at 72^{0}C for 7 min, followed by rapid cooling to 4^{0}C.

3.3.3 Analysis of PCR products

PCR products (10 µl) obtained with SSR markers were mixed with 2 µl of 6X gel loading dye (bromophenol blue: 0.2% bromophenol blue, 0.2% Xylene cyanol dye and 30% glycerol in a Tris-EDTA buffer) and electrophoressed in 3% agarose gel in 1X Tris acetate-EDTA (TAE) buffer (40 mM Tris, 40 mM acetic acid glacial, 1 mM EDTA, pH 8.0) at a constant voltage of 120 V for 1 h. Amplification products obtained with *Pi-ta* specific STS marker YL155/YL87 were electrophoressed in 1.5% agarose gel. The gels

were stained with ethidium bromide (0.5µg/ml) for 10 min, destained for 30 min in tap water and visualized using ultraviolet transilluminator (Bio-Rad). The gels were photographed using the Gel-Documentation Unit (Bio-Rad).

Markers linked to three blast resistance genes (*Pi-1, Pi-ta* and *Pi-k^h*) were validated to detect the presence of respective genes in parental genotypes (HPU741, C101LAC, C101A51 and Tetep). All the markers successfully detected the presence of resistance genes in respective donors and were used to screen the 44 BC_1F_4 pyramid lines for the presence of individual or different combination of blast resistance genes.

3.4 Marker assisted background selection.

Forty four lines (Table 3.1) were included in this study to estimate the proportion of recurrent parent genome in the BC_1F_4 lines. SSR and ISSR markers were used in this study.

3.4.1 Primer details

The sequence information of SSR primers was obtained from the publicly available database (http://gramene.org/genome) and from published research papers (Temnykh *et al.* 2000; McCouch *et al.* 2002; International Rice Genome Sequencing Project 2005; Kumar *et al.* 2010). Sequence information of ISSR markers was obtained from Murlidhar (2009). A total of 206 SSR and 30 ISSR markers were used to check parental polymorphism, and those showing polymorphism (Table 3.3 and 3.4) were used to profile BC_1F_4 plants to determine their relatedness to recurrent parent 'HPU741'.

3.4.2 PCR amplification of DNA

The amplification of DNA using SSR primers was carried out in 25 µl volume consisting of 2 µl DNA template, 20 ng of each forward and reverse primer, 0.2 µM of each dNTP, 1.5 mM $MgCl_2$, 2.5 µl of 10X PCR reaction buffer, 1 unit *Taq* polymerase (TAQ500, Life Tech) and autoclaved double distilled water. PCR amplifications were carried out in a thermocycler (My Cycler, Bio-Rad, USA) using the following temperature profile: initial denaturation at 94 ^{0}C for 5 min, followed by 39 cycles at 94^{0}C for 1 min, 55^{0}C for 1 min, 72^{0}C for 1.30 min and final extension at 72^{0}C for 5 min, followed by rapid cooling to 4^{0}C. The standard annealing temperature for the rice SSRs was determined to be 55^{0}C (McCouch *et al.* 2002).

Table 3.3 ISSR primers used for marker assisted background selection

Sr. No.	Primer name	Sequence
1.	ISSR-1	CTCTCTCTCTCTCTCTCTCTG
2.	ISSR-2	CTCTCTCTCTCTCTCTCTCTT
3.	ISSR-3	CTCTCTCTCTCTCTCTCTCTA
4.	ISSR-7	TGTGTGTGTGTGTGTGTGTGG
5.	ISSR-11	AGAGAGAGAGAGAGAGAGAGC
6.	ISSR-12	AGAGAGAGAGAGAGAGAGAGT
7.	ISSR-13	ACACACACACACACACACACG
8.	ISSR-14	ACACACACACACACACACACC
9.	ISSR-15	ACACACACACACACACACACT
10.	ISSR-16	CTCCTCCTCCTCCTCCTCG
11.	ISSR-18	CTCCTCCTCCTCCTCCTCA
12.	ISSR-25	ACCACCACCACCACCACCG
13.	ISSR-26	ACCACCACCACCACCACCT

Amplification of DNA using ISSR primers was carried out in 25 µl volume consisting of 2 µl DNA template, 40 ng of primer, 0.2 µM of each dNTP, 1.5 mM $MgCl_2$, 2.5 µl of 10X PCR reaction buffer, 1 unit *Taq* polymerase (TAQ500, Life Tech) and autoclaved double distilled water. The PCR conditions for ISSRs were standardized as initial cycle of 94°C for 5 min, 39 cycles of 94°C for 1 min, 58°C for 1 min and 72°C for 2 min with final extension at 72°C for 5 min, before cooling to 4^0C.

3.4.3 Analysis of PCR products

Ten µl of each PCR product was mixed with 2 µl of 6X gel loading dye (bromophenol blue: 0.2% bromophenol blue, 0.2% Xylene cyanol dye and 30% glycerol in a Tris-EDTA buffer) and electrophoressed in 3% agarose (Lifetech, India) gel for SSR or 2% agarose for ISSR in 1X Tris acetate-EDTA (TAE) buffer (40 mM Tris, 40 mM

Table 3.4 SSR markers used for marker assisted background selection

Sr. No.	Marker Name	GenBank Accession No.	SSR Motif	No. of Repeats	Forward Primer Sequence	Reverse Primer Sequence	*Chromo-some No.
1.	RM8091	AP001366	TA	22	GGGTATATACTCAATTTCAACAATGGTG	TCCATGTTCAAATATTTGGTAATATGCTAA	1
2.	RM6843	AY023517	TCT	25	GACAAATTCAGCTGTTGACC	ATAAACCACAATGAGCAAGC	2
3.	RM324	AF344149	CAT	21	CTGATTCCACACACTTGTGC	GATTCCACGTCAGGATCTTC	2
4.	RM1358	AY018033	AG	24	GATCGATGCAGCAGCATATG	ACGTGTGGCTGCTTTTGC	2
5.	RM3828	AY020502	GA	21	AAGCATTATTGACCACCAAC	TCTGATGTCCTTATGTCATGG	2
6.	RM7389	AY024064	GATA	7	AGCGACGGATGCATGATC	TTGAGCCGGAGGTAGTCTTG	3
7.	RM5687	AY022362	AAT	17	GATCGCTGGCGATTGATC	GACTTGTGGGGTGGTTTTTG	4
8.	RM6314	AY022989	CTT	11	GATTCGTGTCGGTTGTCAAG	GGTTCAGGGACGAATTTCAG	4
9.	RM1054	AY017729	AC	17	ACTTACATCTGAGGTGCATA	GCATTGCAGATTACAGATAC	5
10.	RM5374	AY022049	TC	13	CATGATGAATGTATTGCTCT	ACATGGTCAACCATTTTAAT	5
11.	RM3827	AY020502	GA	21	TAGTCCTCGAGGACGGATTG	CTGGCCTTTCTTCAATCTGC	6
12.	RM5199	AY021874	TA	40	AATTCATCATATGATGATCG	CTCTGGTCAAACTAGGTGAT	6
13.	RM7193	AY023868	ATAG	7	ATGTGGGAATTTCTAGCCCC	CCCTAGTTTTCCAAATGGCC	6
14.	RM248	AF344068	CT	25	TCCTTGTGAAATCTGGTCCC	GTAGCCTAGCATGGTGCATG	7
15.	RM1253	AY017928	AG	16	CTGAACTTGCCTGAGAACTC	GACGACCTCTCCATGCTCG	7
16.	RM3691	AY020366	GA	15	GCTGATGGTCAAAGATCAGG	ATGTGTCTGCTGGCACAGAG	7
17.	RM8007	AP003939	AT	40	AATAGGATGGATCATGGATA	CATCTCATCAGGAACCTAAC	7
18.	RM6215	AY022890	CGG	9	CAGCAGAGAGATGACGCAAG	AAACCCAAAACCCTCGTCTC	8
19.	RM8266	AP004560	(TTA)(TTG)	7+15	AATAAGAGTCCACACATGCAC	ACAAGTAGTAACGGGTGCTTA	8
20.	RM242	AF344062	CT	26	GGCCAACGTGTGTATGTCTC	TATATGCCAAGACGGATGGG	9
21.	RM553	AQ870017	CT	10	AACTCCACATGATTCCACCC	GAGAAGGTGGTTGCAGAAGC	9
22.	RM2915	AY019590	AT	39	ATCACAAACCCTTGTATAGA	GAGAGATCTTCCTTCATCTG	9
23.	RM5799	AY022474	AGC	9	ATCGAACCATCCAGGATGAC	TTGCACAAGAGGCAACACTC	9
24.	RM7175	AY023850	ATAG	6	ACAGTAAACGTGGTGCCTCC	AGAAGTAGCCTCGAGGACCC	9
25.	RM1375	AY018050	AG	31	CTACACGCGCAAACTCTGTC	ATGAAGGTCTAGGCTGCACC	10
26.	RM4455	AY021130	TA	19	CTCTCAAAGAACTAGGACTC	GAGAAGGTATGATAACCAAT	10
27.	RM4771	AY021446	TA	26	ACGTTGATTTCATTCAGGTC	ACGCTAACTGAGAAACATGG	10
28.	RM4915	AY021590	TA	29	ATGGTAGATTCTTTGTTTTG	CGTATACATTATACGTACGC	10
29.	RM6745	AY023420	TAT	26	GCGCCTTTAGATGCTACTTG	CAGCTCCATCGTAAGCAAAG	10
30.	RM6673	AY023348	TAA	10	CATCGCATCGTATCGTATCG	GCTTCAAACACGCCTTCTTC	10
31.	RM4484	AY021159	TA	20	CACTTTATCAAATCGCAATG	CAGTTCGTCCCAAAATAAAT	11
32.	RM5349	AY022024	TC	13	AGGGCATGCTTACATCCAAC	CATTTGCTTCTATGCCCCAG	11
33.	RRS10	AL731883	CTAT	8	CCTAATTCCGATTGGCTAATTACT	TGTTACCACGTGGCATGTTT	12
34.	RRS23	AL732380	TA	27	TGCTCATCTTGTTTCTTGACG	GCCAACCAAAGCACCTTGTA	12
35.	RRS39	AL954154	GA	25	TGGTCGAAGTGTGTATCGTTT	GCATGTAAGGTGAATGGTCAAG	12
36.	RRS60	AL954152	ATT	23	TGGTTACGATCCTTCCGTGT	GGAGTGCCATATGACAGCTTAG	12
37.	RRS71	AL713907	TATC	11	ATGAAGGGATCGGTTATCTATCT	GCGTTTGTAGGAAGTTTAATGGA	12

* The chromosome number on which marker was situated

acetic acid glacial, 1 mM EDTA, pH 8.0). The gels for SSR as well as ISSR were run at a constant voltage of 120 V for 1 h. The gels were stained with ethidium bromide (0.5 μg/ml) for 10 min and destained for 30 min in tap water. The PCR products were visualized using ultraviolet transilluminator (Bio-Rad) and photographed using the Gel-Documentation Unit (Bio-Rad).

3.5 Estimation of recurrent parent genome

3.5.1 Analysis of SSR and ISSR data

The presence or absence of a band of particular molecular weight in SSR and ISSR profiles was scored manually and the results were entered into an Excel sheet as a binary data matrix with '1' indicating the presence and '0' the absence of that band. The binary data were used to generate a similarity matrix using Jaccard's Coefficient [(J_{ij}, $C_{ij}/(n_i + n_j - C_{ij})$] (Jaccard, 1901) where C_{ij} is the number of positive matches between two genotypes, while n_i and n_j is the total number of bands in the genotypes i and j, respectively in SIMQUAL programme of NTSYS pc version 2.02a (Exeter Software, USA, Rohlf 1998). Cluster analysis based on similarity matrices was performed and the relationship between the 44 BC_1F_4 pyramid lines and their parents was depicted as dendrogram, by the sequential, agglomerative, hierarchical, and nested (SAHN) clustering method using the unweighted pair-group method of arithmetic average (UPGMA). MXCOMP was used to determine the degree of correlation between the original similarity matrix and the cophenetic value matrix derived from the dendrogram using COPH. Principal component analysis (PCA) was used to investigate the overall variation and patterns of relationship among the lines. For PCA, the data were used to derive similarity matrices from which the principal components (PCs) were extracted and projected in three dimensions (EIGEN, PROJ, and MOD3D programs). Cluster analysis, principal component analysis and MXCOMP were performed using NTSYS-pc for windows, version 2.02a (Exeter Software, USA, Rohlf 1998). WINBOOT software (Yap and Nelson 1996) was used to assess robustness of the dendrogram typology and the estimation of faithfulness of cluster analysis.

3.5.2 Estimation of recurrent parent genome

Genetic similarities between pyramid lines and the recurrent parent (here 'HPU741') were an efficient estimate of degree of relatedness between them as these values were generated based on the detection of 'HPU741' specific polymorphic DNA fragments observed in each individual of 44 BC_1F_4 pyramid lines for the assessed SSR & ISSR loci. So the 8similarity matrices generated by NTSYS pc version 2.02a using SSR, ISSR and pooled data of SSR & ISSR were used to estimate the proportion of recurrent parent genome in each individual and, thus identify plants more similar to the recurrent parent.

Results and Discussion

4. RESULTS AND DISCUSSION

In the present study, 44 BC_1F_4 lines supposed to contain individual or combinations of three blast resistance genes (*Pi-1, Pi-ta* and *Pi-k^h*) were selected. Since the name of the gene *Pi-k^h* has recently been changed to *Pi-54* (Sharma *et al.* 2010), we have used *Pi-54* throughout the results and discussion, summary and conclusions. The results obtained on different aspects of the present investigation are presented here under the following heads:

4.1 Foreground selection of BC_1F_4 lines for blast resistance genes

4.2 Estimation of the recurrent parent genome in backcross progenies

4.1 Foreground selection of BC_1F_4 lines for blast resistance genes

4.1.1 Validation of markers in parental genotypes

i) Markers linked to *Pi-1*: Two microsatellite markers, RM224 and RM1233A were validated for detection of *Pi-1* gene in the parental genotypes. *Pi-1* was present in the parental lines C101LAC and Tetep whereas it was abscent in HPU741 and C101A51. Both the markers accurately detected the presence of *Pi-1* gene in C101LAC and Tetep. The allele/loci amplified by RM224 was of 144bp (Fig: 4.1a) whereas the size of this loci amplified by RM1233A was 147 bp (Fig: 4.1 b).

ii) Marker linked to *Pi-ta*: The primer pair YL155 and YL87 was used for PCR amplification of DNA of the four parental genotypes. Amplification of 1,042 bp product was observed only in resistant parent Tetep (Fig: 4.2) but not in susceptible parents HPU741, C101LAC and C101A51, indicating the dominant nature the marker. This marker was used to screen all BC_1F_4 plants for presence of *Pi-ta* gene.

iii) Markers linked to *Pi-54*: The presence of blast resistance gene *Pi-54* in Tetep was validated by using two microsatellite markers, RM206 and TRS26. Amplification of approximately 147 bp (RM206) and 346 bp (TRS26) products were observed only in resistant parent Tetep and was not in susceptible parents' HPU741, C101LAC and C101A51 (Fig 4.3a, b).

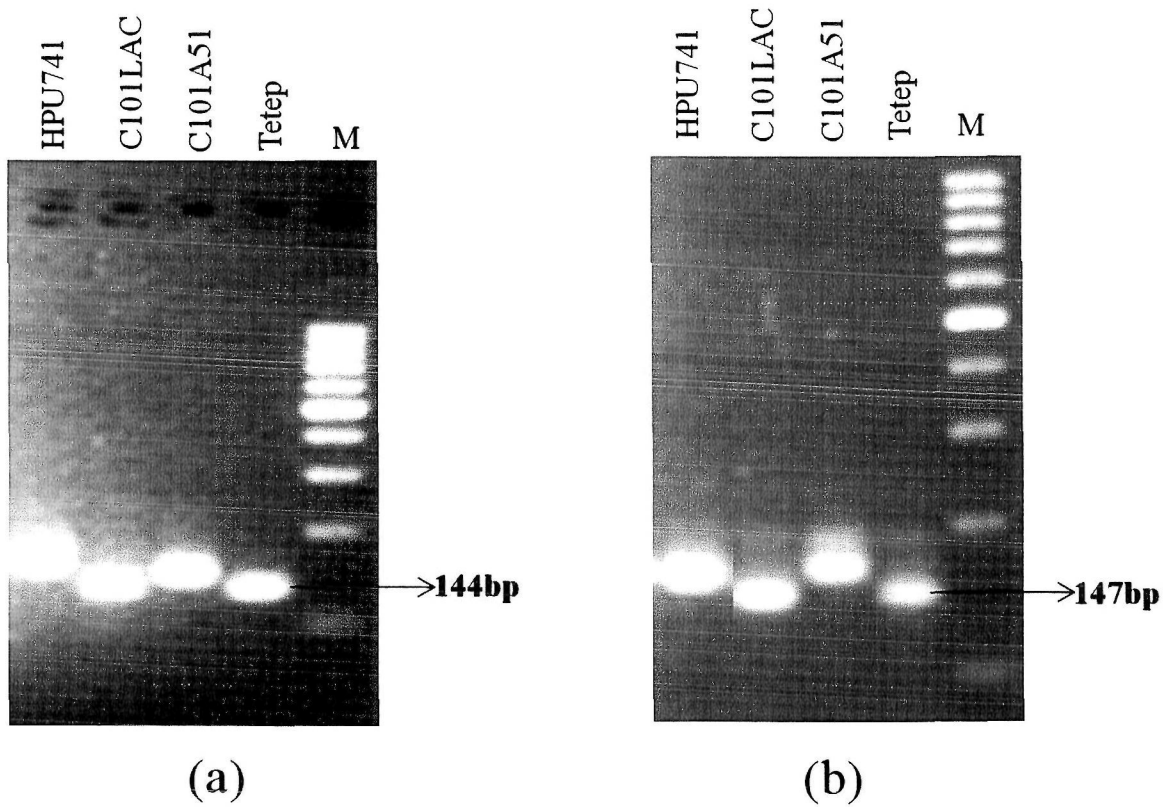


Fig: 4.1 Validation of microsatellite markers (a) RM224 and (b) RM1233A linked to rice blast resistance gene *Pi-1* in parental genotypes, C101LAC and Tetep

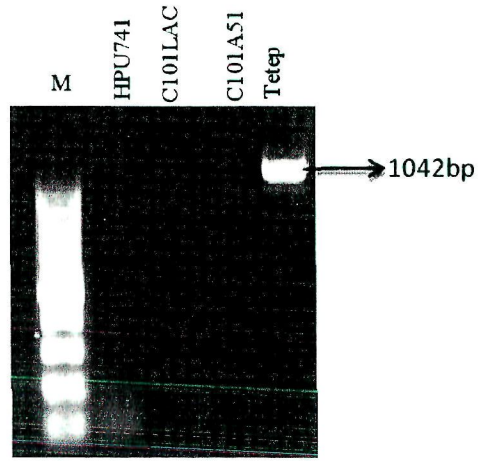


Fig: 4.2 Validation of STS marker YL155/YL87 linked to rice blast resistance gene *Pi-ta* in parental genotype, Tetep

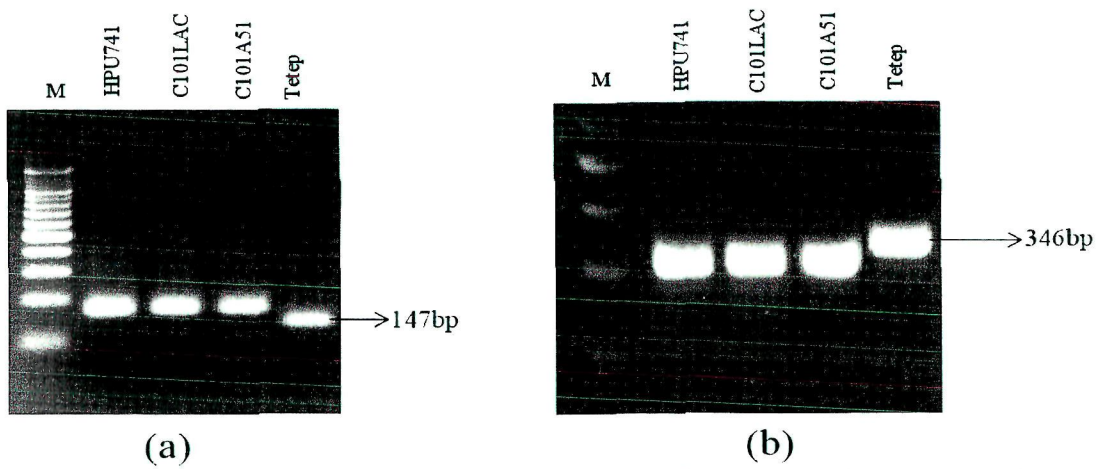


Fig: 4.3 Validation of microsatellite markers (a) RM206 and (b) TRS26 linked to rice blast resistance gene *Pi-54* in parental genotype, Tetep

4.1.2. Identification of pyramid lines harboring different combinations of blast resistance genes

Single plants of 44 elite BC_1F_4 lines were assessed for the presence of three blast resistance genes, *Pi-1*, *Pi-ta* and *Pi-54* using STS and SSR markers validated in the present study.

i) Amplification of *Pi-1* gene: The microsatellite markers RM224 and RM1233A which were reported to be linked to *Pi-1* gene were used for PCR amplification of the DNA of 44 BC_1F_4 plants. In case of RM224, the DNA fragment linked to the *Pi-1* gene was amplified in 32 plants (Fig 4.4a) whereas in case of RM1233A, 31 plants showed amplification product linked to *Pi-1* (Fig 4.4b). Two of the plants amplified only the RM224 marker, one amplified RM1233A marker whereas 30 plants had amplicons with both the markers (RM224 and RM1233A). These 30 plants which possessed both the markers were categorized as possessing *Pi-1*.

ii) Amplification of *Pi-ta* gene: The primer pair for gene specific dominant STS marker YL155/YL87, when used for PCR amplification of the DNA of 44 BC_1F_4 plants, 22 plants amplified the loci linked to *Pi-ta* (Fig 4.5). These 22 plants were, thus, considered to possess the blast resistance gene *Pi-ta*.

iii) Amplification of Pi-*54* gene: The microsatellite markers RM206 and TRS26 validated to be linked to *Pi-54* gene were used for PCR amplification of the DNA of 44 BC_1F_4 plants. The RM206 marker loci, was detected in 22 BC_1F_4 plants (Fig 4.6a) whereas TRS26 marker loci, was amplified in 25 plants (Fig 4.6b). Five of the plants other than 25 (possessing the resistance loci) showed the presence of both the resistance as well as susceptible loci (marker TRS26) indicating the heterozygous state of the marker and the gene. Twenty of the 44 plants amplified both the markers (RM206 and TRS26) linked to resistance gene *Pi-54*, and hence could be categorized as those possessing *Pi-54*.

The markers linked to *Pi-1*, *Pi-ta* and *Pi-54* genes used in the present study were earlier found to be tightly linked to these genes (Fuentes *et al.* 2008, Jia *et al.* 2002 and Sharma *et al.* 2005). Of these markers, RM224 and RM1233A (linked to *Pi-1*) were reported to cosegregate with the Pi-*1* (Fuentes *et al.* 2008). However, in the present

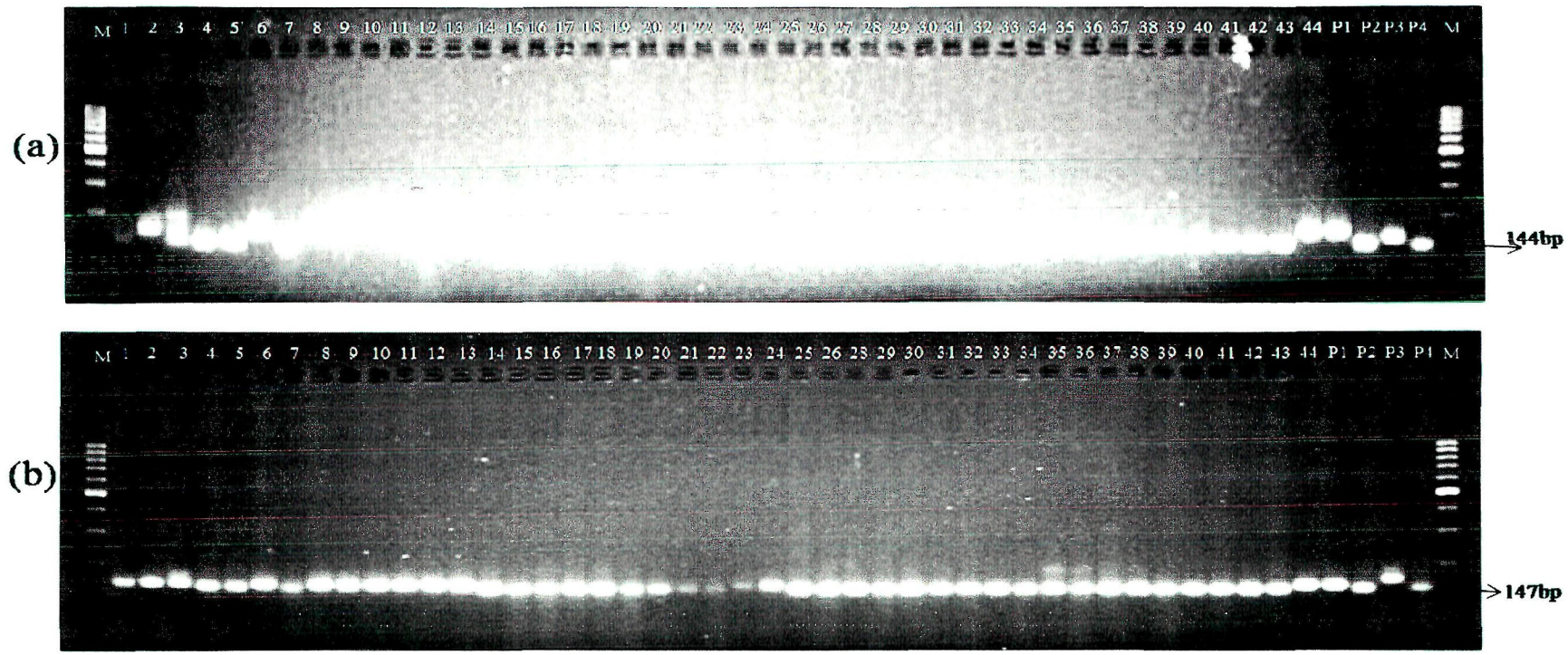


Fig: 4.4 **Genotyping of BC_1F_4 lines with microsatellite markers (a) RM224 and (b) RM1233A for presence of *Pi-1* gene. Lanes 1-44: BC_1F_4 lines (See Table 3.1), P1: HPU741, P2: C101LAC, P3: C101A51, P4: Tetep, M: 100bp Molecular weight marker**

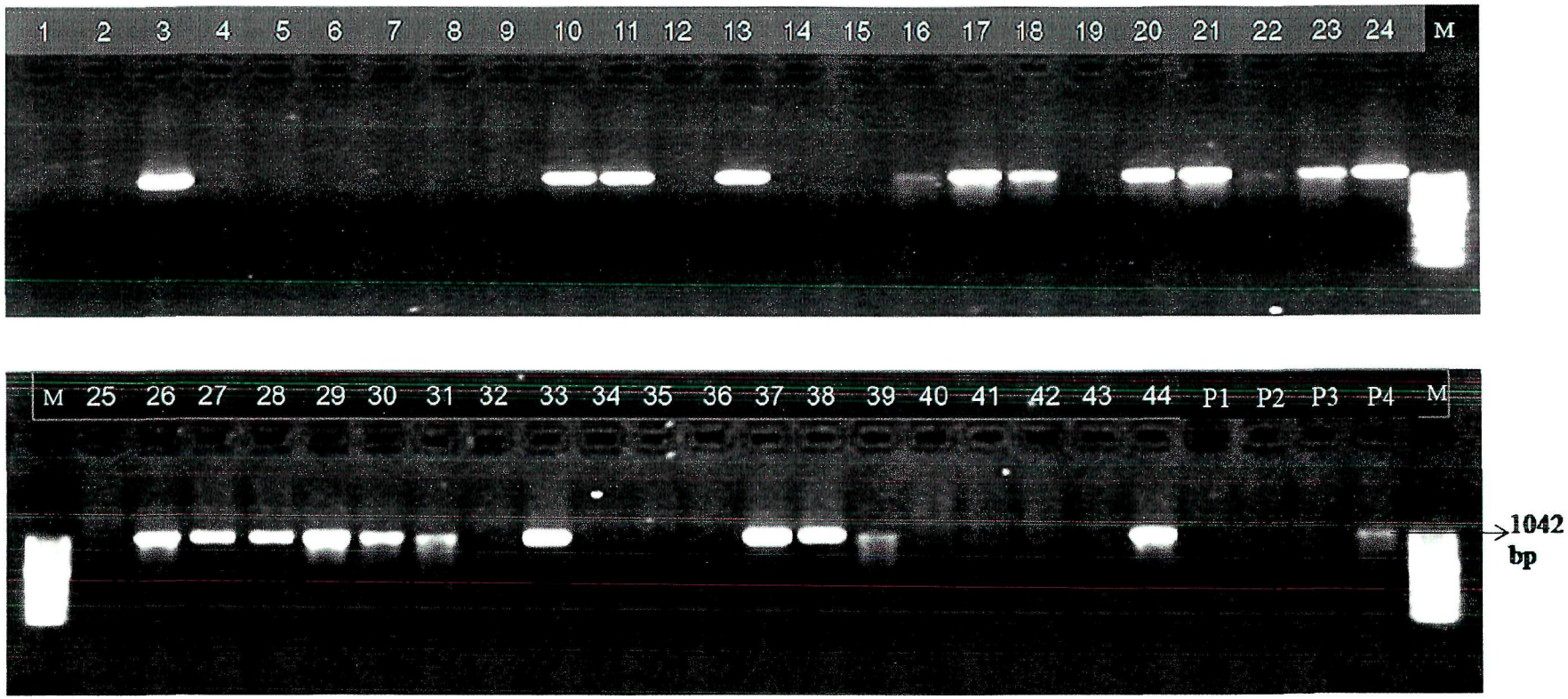


Fig: 4.5 **Genotyping of BC_1F_4 lines with STS marker YL155/YL87 for presence of *Pi-ta* gene. Lanes 1-44: BC_1F_4 lines (See Table 3.1), P1: HPU741, P2: C101LAC, P3: C101A51, P4: Tetep, M: 100bp Molecular weight marker**

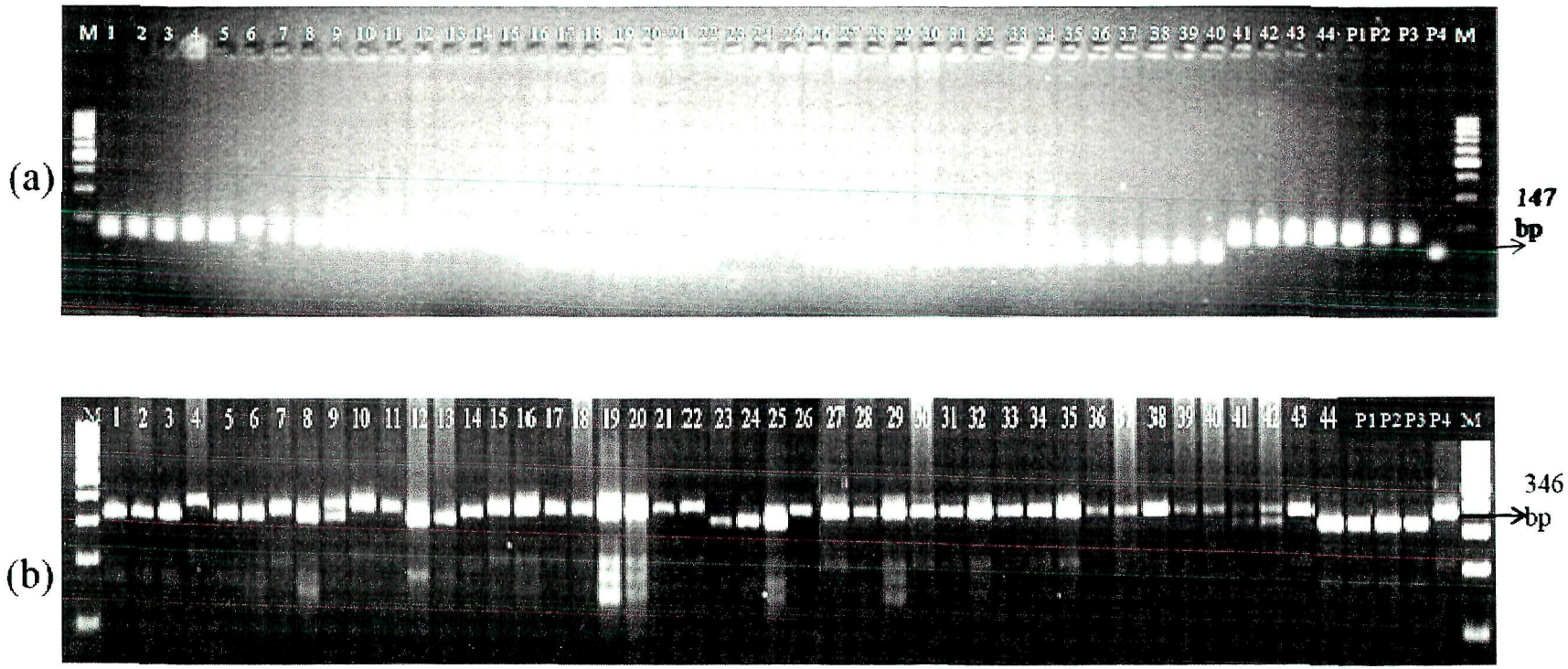


Fig: 4.6 **Genotyping of BC_1F_4 lines with microsatellite markers (a) RM206 and (b) TRS26 for presence of *Pi-54* gene. Lanes 1-44: BC_1F_4 lines (See Table 3.1), P1: HPU741, P2: C101LAC, P3: C101A51, P4: Tetep, M: 100bp Molecular weight marker**

study, three recombination's was recorded between these two markers in the 44 BC_1F_4 plants (Table 4.1) indicating that the RM224 and RM1233A were not amplifying the same locus and hence, were not cosegregating. Using a mapping population of 3060 siblings from cross of IRBLkh-K3xCO39, these two markers were estimated to be about 1 cM away from each other (Xu *et al.* 2008). It is not clear whether these markers are flanking the gene or both of these are on one side of the gene. The markers RM206 and TRS26 which were linked to gene *Pi-54* were also reported to be cosegregating (Sharma *et al.* 2005). These markers were located at 0.7 cM from *Pi-54* on chromosome 11 of rice. These markers in resistant genotype, Tetep, produced 147bp, 346bp amplicons respectively. In the present study, 10 recombinants were recorded in 44 BC_1F_4 lines between these markers. While in five of the lines RM206 was absent, TRS26 was present in all the lines. In two lines RM206 was present and TRS26 amplified both the resistance as well as susceptible alleles. Thus it can be concluded that RM206 and TRS26 were not cosegregating. Like our findings, Sharma *et al.* (2010) have also found that RM206 and TRS26 were about 2.7 cM from each other. However, the genetic distances obtained using different populations may be variable and sometimes not providing good estimates of the physical distances between the markers vis a vis the gene.

The STS marker (YL155/YL87) linked to *Pi-ta* which was earlier found to be cosegregating with the gene (Jia *et al.* 2002), produced 1042bp amplicon. Since it (YL155/YL87) is a dominant marker, it is difficult to establish recombination or heterozygous condition of the alleles using this marker.

Marker assisted selection for these blast resistance genes of 44 blast resistance pyramid lines showed that 7 lines did not carry any resistance gene (Tables 4.2), whereas the combinations of *Pi-1* and *Pi-54* observed in 7 pyramid lines and all the three genes (*Pi-1, Pi-ta* and *Pi-54*) were present in 15 pyramid lines (Table 4.2).

Table 4.1 Foreground selection using DNA based markers to detect presence of blast resistance genes *Pi-1*, *Pi-ta* and *Pi-k^h* in BC_1F_4 progeny plants

Sr. No.	Designation	*Pi-1*		*Pi-ta*	*Pi-k^h* (syn. *Pi-54*)	
		RM224*	RM1233A*	YL155/YL87*	RM206*	TRS26*
	Parents					
1.	HPU741	-	-	-	-	-
2.	C101LAC	+	+	-	-	-
3.	C101A51	-	-	-	-	-
4.	Tetep	+	+	+	+	+
	Lines					
1.	MAS-8	+	-	-	-	-
2.	MAS-9	-	-	-	-	-
3.	MAS-10	+	-	+	-	-
4.	MAS-14	+	+	-	-	+
5.	MAS-16	+	+	-	-	-
6.	MAS-18	-	-	-	-	-
7.	MAS-19	+	+	-	-	-
8.	MAS-20	-	-	-	-	-
9.	MAS-21	-	-	-	-	H
10.	MAS-22-1	-	-	+	-	+
11.	MAS-22-2	-	-	+	-	+
12.	MAS-29	-	-	-	-	-
13.	MAS-31	-	-	+	-	-
14.	MAS-33	-	+	-	-	-
15.	MAS-34	+	+	-	-	+
16.	MAS-36	+	+	+	+	+
17.	MAS-38	+	+	+	+	+
18.	MAS-40-1	+	+	+	+	+
19.	MAS-40-2	+	+	-	+	H
20.	MAS-41	+	+	+	+	H
21.	MAS-43	+	+	+	+	+
22.	MAS-44	+	+	-	+	+
23.	MAS-56	-	-	+	-	-
24.	MAS-58	-	-	+	-	-
25.	MAS-68	+	+	-	-	-
26.	MAS-70Q161	+	+	+	+	+
27.	MAS-70Q163-1	+	+	+	+	+
28.	MAS-70Q163-2	+	+	+	+	+
29.	MAS-70Q164	+	+	+	+	+
30.	MAS-71Q168	+	+	+	+	+
31.	MAS-71Q169	+	+	+	+	+
32.	MAS-71Q170	+	+	-	+	+
33.	MAS-72Q171	+	+	+	+	+
34.	MAS-72Q172	+	+	-	+	+
35.	MAS-72Q179	+	+	-	+	+
36.	MAS-72Q180	+	+	-	+	+
37.	MAS-72Q181	+	+	+	+	+
38.	MAS-75Q184	+	+	+	+	+
39.	MAS-75Q185	+	+	+	+	+
40.	MAS-75Q186	+	+	-	+	+
41.	MAS-76	+	+	-	-	H
42.	MAS-78	+	+	-	-	H
43.	MAS-107	+	+	-	-	+
44.	MAS-118	-	-	+	-	-

* Marker names

Table 4.2 Observed gene combinations in BC_1F_4 plants

Sr. No.	Obs. Gene combinations	Line No.
1	***Pi-1***	MAS-14, MAS-16, MAS19, MAS-34, MAS-68, MAS-76, MAS-78, MAS-107
2	***Pi-ta***	MAS-10, MAS-22-1, MAS-22-2, MAS-31, MAS-56, MAS-58, MAS-118
3	***Pi-54***	-
4	***Pi-1& Pi-ta***	-
5	***Pi-1& Pi-54***	MAS-40-2, MAS-44, MAS-71Q170, MAS-72Q172, MAS-72Q179, MAS-72Q180, MAS-75Q186
6	***Pi-ta & Pi-54***	-
7	***Pi-1, Pi-ta & Pi-54***	MAS-36, MAS-38, MAS-40-1, MAS-41, MAS-43, MAS-70Q161, MAS-70Q163-1, MAS-70Q163-2, MAS-70Q164, MAS-71Q168, MAS-71Q169, MAS-72Q171, MAS-72Q181, MAS-75Q184, MAS-75Q185
8	**No genes**	MAS-8, MAS-9, MAS-18, MAS-20, MAS-21, MAS-29, MAS-33

As stated in the preceding text, *Pi-54* was earlier named as *Pi-k*^{h}. This gene like *Pi-k*^{h} was isolated from Tetep and was mapped by Sharma *et al.* (2005). From the beginning, the identity of *Pi-k*^{h} reported from India (Sharma *et al.* 2005) was a subject of controversy, since the gene was believed to be different from *Pi-k*^{h} already reported by Kiyosawa *et al.* (1969). The markers (RM224, RM144, RM1233A) linked to *Pi-k*^{h} (Fjellstorm *et al.* 2004, Xu *et al.* 2008) were different from those (RM206, TRS26) used to map *Pi-k*^{h} (Sharma *et al.* 2005). Ending this controversy, Sharma *et al.* (2010) reported that the *Pi-k*^{h} gene identified in donor parent Tetep and used in our study was different from that reported by Kiyosawa *et al.* (1969) as this gene was at a distance of more than 10 cM from the *Pi-k* locus. Sharma *et al.* (2010) also revised the name of this gene as *Pi-54* as per the guidelines of Committee on Gene Symbolization, Nomenclature and Linkage. As per our observation, the markers RM206 and TRS26 were also found to different from each other and were at a distance of 2.7 cM (Sharma *et al.* 2010).

4.2 Estimation of the recurrent parent genome in backcross progenies

The recurrent backcrossing programs are planned on the assumption that the proportion of recurrent parent genome recovered is at a rate of $1-(1/2)^{t+1}$ for each of t generations of backcrossing (Babu *et al.* 2004) that means the average recovery of recurrent parent genome in the individuals of BC_1 generation should be around 75%. Molecular markers have been used to estimate the proportion of parental genome contribution in progeny lines. In these applications, the proportion of alleles originating from parent P1 at markers polymorphic between the parental lines P1 and P2 is commonly used as an estimator for the proportion of genome contributed from parent P1 to the offspring (Bernardo *et al.* 2000; Frisch and Melchinger 2006, Gopalakrishnan *et al.* 2008). In the present investigation also, marker assisted backcross selection (MABS) was used to determine the genomic contribution of HPU741 in the 44 BC_1F_4 pyramid lines.

4.2.1 Estimation of recurrent parent genome using SSR markers

Initially a set of 206 SSR primer pairs were used to check parental polymorphism. Of the 206 primer pairs analysed, 41 (19.90%) primers failed to amplify any fragment, whereas the others (165, 80.09%) produced easily interpretable amplification products. Out of these 165, only 47 primers (28.48%) displayed polymorphism with respect to the recurrent parent HPU741. Of the 47 primers exhibiting polymorphism, a set of 37 markers well distributed throughout the genome were chosen to estimate the extent of HPU741 genome in BC_1F_4 pyramid lines. Keeping in view, the coverage of entire genome, a minimum of three markers per chromosome should've been selected however, for chromosomes 1 and 3 only one marker was obtained whereas for chromosomes 4, 5, 8 and 11, two markers per chromosome were obtained (Table 4.3). This is in accordance with the studies of Hospital *et al.* (1992) and Visscher *et al.* (1996), where they suggested using not more than three markers per chromosome at least in earlier generations of back crossing. Furthermore to have thorough genome coverage, ISSR markers in addition to SSRs have also been used for MABS (See section 4.2.2).

Table 4.3 Observed amplicon size in HPU741 as obtained with SSR markers used for MABS

Sr. No.	Marker	*Chromosome No.	**Amplicon Size (bp) in HPU741
1	RM8091	1	210
2	RM6843	2	230
			195
			160
3	RM3828	2	175
4	RM1358	2	190
5	RM324	2	160
6	RM7389	3	145
7	RM6314	4	225
			175
			150
8	RM5687	4	195
9	RM5374	5	130
10	RM1054	5	150
11	RM7193	6	200
12	RM3827	6	180
13	RM5199	6	190
14	RM8007	7	175
15	RM248	7	98
16	RM1253	7	180
17	RM3691	7	150
18	RM6215	8	140
19	RM8266	8	275
20	RM7175	9	98
21	RM242	9	175
22	RM553	9	170
23	RM2915	9	220
24	RM5799	9	190
25	RM1375	10	180
26	RM4455	10	200
27	RM4771	10	210
28	RM4915	10	205
29	RM6745	10	150
30	RM6673	10	190
31	RM4484	11	200
32	RM5349	11	130
33	RRS10	12	180
34	RRS23	12	195
35	RRS39	12	120
36	RRS60	12	195
37	RRS71	12	220

MABS- Marker Assisted Backcross Selection

* The chromosome on which the marker is situated.

** Observed amplicon size.

All the 44 BC_1F_4 plants were genotyped using selected 37 SSR primer pairs (Figs: 4.7 and 4.8). A total of 33 out of 44 BC_1F_4 pyramid lines (75%) contained at least one non-parental band. Non-parental bands may be due to spontaneous mutations at SSR loci (Jain *et al.* 2006). To obtain estimates of genetic similarity between the pyramid lines and to identify the individuals genetically more similar to the recurrent parent (HPU741) the matrix derived from the microsatellite data was constructed with binary data '1' for presence and '0' for absence of bands. The proportion of the recurrent parent genome detected in the BC_1F_4 generation plants ranged from 52.6% to 83.0%, with an average of 67.8% (Table 4.4). Among all the lines MAS-40-1 showed high recovery of recurrent parent genome with 83% similarity to 'HPU741' (Table 4.4).

The dendrogram based on Jaccard's similarity coefficient and SAHN cluster analysis resulted in four main clusters, A, B, C and D with an overall similarity coefficient of 0.24 (Fig: 4.9). Cluster A comprised of a line MAS-8, whereas cluster B had 43 lines along with HPU741. Cluster B could be further sub divided into 9 sub clusters B1, B2, B3, B4, B5, B6, B7, B8 and B9. Sub cluster B1 comprised three lines and B2 had 8 lines whereas B3 comprised a line MAS-14 and the recurrent parent HPU741. MAS-14 was 81.3% similar to recurrent parent but is a single gene (*Pi-1*) line (Fig: 4.9 and Table 4.1, 4.4). Sub cluster B4 with 22 lines formed the largest sub cluster and included most of the two and three blast resistance gene pyramids; one of these lines, MAS-40-1 recovered 83% (Table 4.4) recurrent parent genome and is a three gene pyramid (*Pi-1, Pi-ta* and *Pi-54*). Individuals in sub cluster B4 shared 85% genetic similarity whereas this sub cluster has 73% similarity to individuals in B3. The sub cluster B5 and B6 each had a single line MAS-56 and MAS-16, respectively, whereas B7 was comprised of three lines and the sub clusters B8 and B9 had two lines each. The parental line Tetep was in cluster C whereas the remaining two parental lines, C101LAC and C101A51, formed a separate cluster D.

The cophenetic correlation of the dendrogram with the data matrix derived from SSR markers was 0.96, indicating that there was a very high goodness of fit of cluster analysis to the original data.

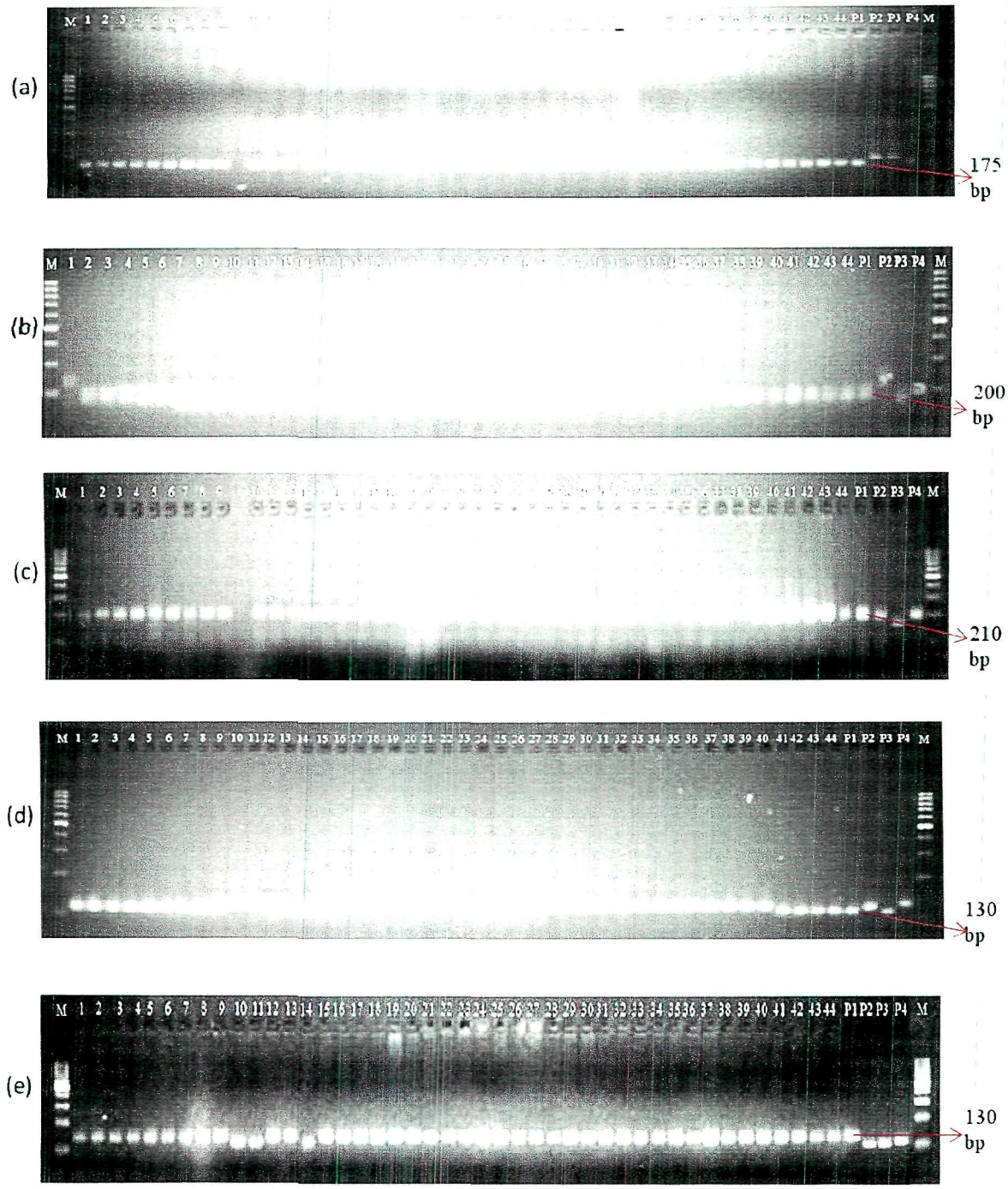


Fig: 4.7 **Genotyping of BC_1F_4 lines with microsatellite primers (a) RM3828, (b) RM4484, (c) RM4771, (d) RM5349 and (e) RM5374 (From top to bottom); Lanes 1-44: BC_1F_4 lines (See Table 3.1), P1: HPU741, P2: C101LAC, P3: C101A51, P4: Tetep; M: 100bp Molecular weight marker**

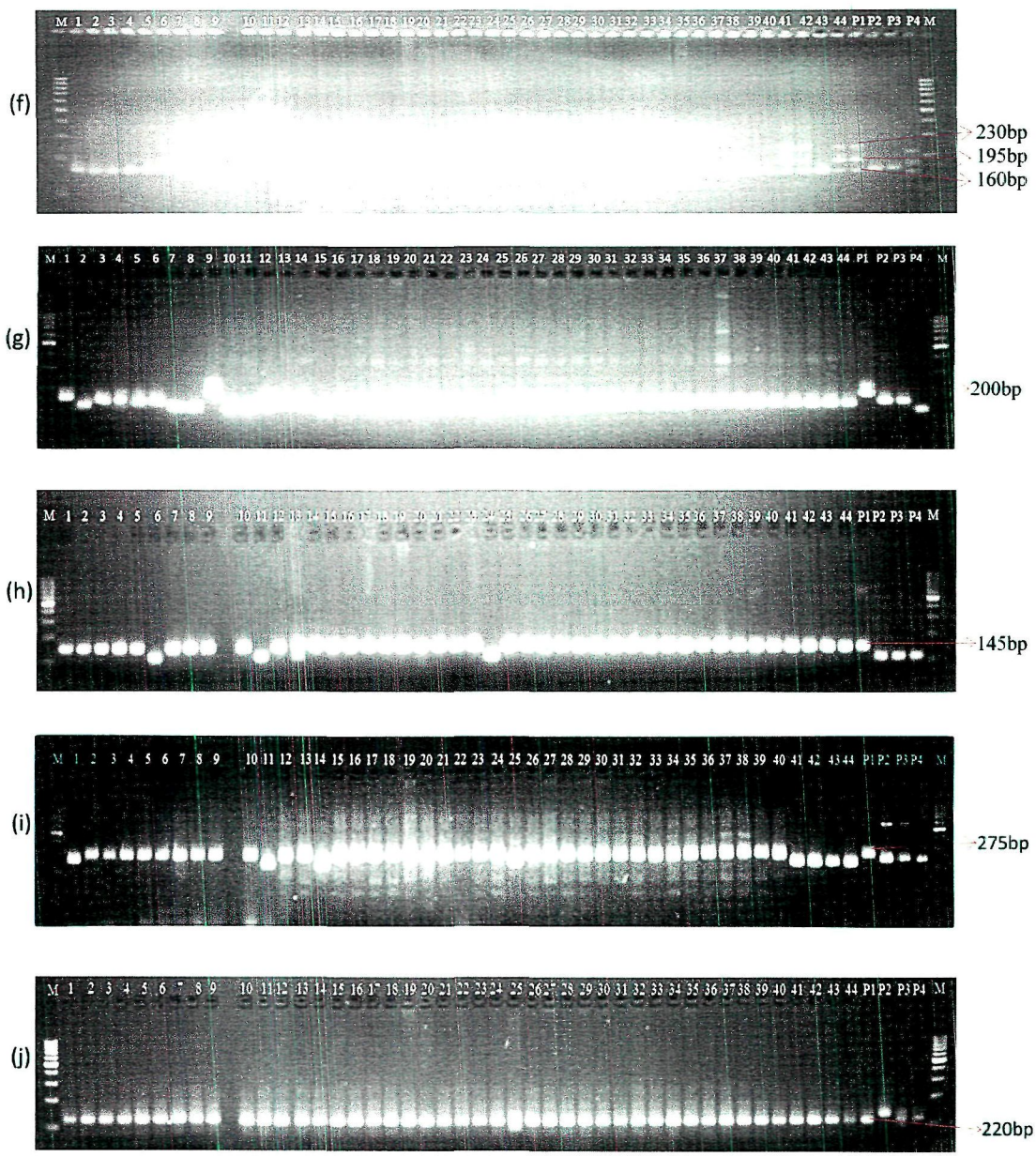


Fig: 4.8 **Genotyping of BC_1F_4 lines with microsatellite primers (f) RM6843, (g) RM7193, (h) RM7389, (i) RM8266 and (j) RRS71 (From top to bottom); Lanes 1-44: BC_1F_4 lines (See Table 3.1), P1: HPU741, P2: C101LAC, P3: C101A51, P4: Tetep; M: 100bp Molecular weight marker**

Table 4.4 Similarity matrix obtained after analysis of SSR data of forty four lines and four parents using UPGMA option of NTSYS programme

0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1	1.000																							
2	0.527	1.000																						
3	0.596	0.660	1.000																					
4	0.574	0.735	0.714	1.000																				
5	0.518	0.518	0.647	0.686	1.000																			
6	0.482	0.537	0.745	0.556	0.556	1.000																		
7	0.582	0.740	0.654	0.600	0.517	0.564	1.000																	
8	0.647	0.615	0.537	0.635	0.518	0.456	0.642	1.000																
9	0.537	0.596	0.708	0.647	0.527	0.640	0.593	0.660	1.000															
10	0.458	0.509	0.604	0.500	0.450	0.518	0.561	0.536	0.635	1.000														
11	0.441	0.491	0.527	0.509	0.387	0.448	0.544	0.545	0.585	0.706	1.000													
12	0.554	0.611	0.593	0.692	0.492	0.509	0.636	0.611	0.593	0.483	0.397	1.000												
13	0.500	0.714	0.729	0.771	0.667	0.660	0.673	0.556	0.627	0.509	0.491	0.642	1.000											
14	0.680	0.647	0.694	0.700	0.545	0.537	0.706	0.585	0.566	0.483	0.545	0.642	0.680	1.000										
15	0.536	0.755	0.735	0.673	0.582	0.574	0.816	0.623	0.635	0.692	0.582	0.618	0.755	0.654	1.000									
16	0.509	0.596	0.673	0.680	0.585	0.608	0.564	0.537	0.577	0.574	0.556	0.564	0.766	0.660	0.700	1.000								
17	0.509	0.596	0.673	0.680	0.585	0.608	0.564	0.537	0.577	0.574	0.556	0.564	0.766	0.660	0.700	1.000	1.000							
18	0.556	0.647	0.694	0.771	0.604	0.566	0.582	0.615	0.660	0.593	0.635	0.582	0.714	0.680	0.654	0.844	0.844	1.000						
19	0.537	0.660	0.673	0.750	0.585	0.547	0.623	0.596	0.640	0.574	0.615	0.623	0.766	0.729	0.700	0.907	0.907	0.930	1.000					
20	0.500	0.585	0.660	0.667	0.574	0.596	0.554	0.527	0.566	0.564	0.545	0.554	0.750	0.647	0.686	0.976	0.976	0.826	0.886	1.000				
21	0.491	0.640	0.653	0.729	0.566	0.500	0.545	0.547	0.588	0.527	0.596	0.545	0.673	0.640	0.615	0.800	0.800	0.907	0.884	0.783	1.000			
22	0.464	0.608	0.653	0.694	0.596	0.528	0.518	0.519	0.558	0.556	0.537	0.518	0.708	0.608	0.647	0.841	0.841	0.864	0.841	0.822	0.905	1.000		
23	0.537	0.566	0.640	0.680	0.647	0.640	0.564	0.537	0.519	0.491	0.448	0.536	0.729	0.596	0.635	0.708	0.708	0.660	0.640	0.694	0.688	0.723	1.000	
24	0.441	0.635	0.585	0.536	0.483	0.585	0.571	0.441	0.474	0.526	0.458	0.571	0.635	0.441	0.642	0.527	0.527	0.466	0.474	0.518	0.482	0.482	0.647	1.000
25	0.615	0.647	0.766	0.771	0.667	0.596	0.642	0.585	0.627	0.536	0.545	0.642	0.826	0.826	0.792	0.804	0.804	0.750	0.804	0.787	0.708	0.745	0.729	0.545
26	0.537	0.596	0.745	0.750	0.647	0.608	0.564	0.596	0.640	0.635	0.615	0.564	0.766	0.660	0.700	0.907	0.907	0.930	0.907	0.886	0.841	0.884	0.673	0.474
27	0.482	0.566	0.673	0.647	0.556	0.577	0.536	0.509	0.547	0.545	0.556	0.536	0.729	0.627	0.667	0.952	0.952	0.804	0.864	0.976	0.800	0.800	0.673	0.527
28	0.491	0.640	0.588	0.694	0.537	0.528	0.545	0.519	0.558	0.500	0.537	0.545	0.673	0.640	0.615	0.884	0.884	0.864	0.884	0.864	0.860	0.860	0.688	0.509
29	0.482	0.596	0.708	0.680	0.585	0.577	0.564	0.537	0.577	0.574	0.585	0.564	0.766	0.660	0.700	0.952	0.952	0.844	0.907	0.930	0.841	0.841	0.673	0.527
30	0.509	0.627	0.708	0.714	0.615	0.577	0.593	0.566	0.608	0.604	0.585	0.593	0.804	0.694	0.735	0.952	0.952	0.886	0.952	0.930	0.841	0.884	0.673	0.500
31	0.519	0.547	0.688	0.694	0.596	0.620	0.518	0.547	0.588	0.585	0.566	0.518	0.708	0.608	0.647	0.929	0.929	0.864	0.841	0.907	0.778	0.860	0.688	0.482
32	0.537	0.627	0.640	0.714	0.556	0.577	0.593	0.566	0.608	0.545	0.585	0.593	0.729	0.694	0.667	0.952	0.952	0.886	0.952	0.930	0.841	0.800	0.673	0.500
33	0.519	0.640	0.653	0.729	0.566	0.528	0.604	0.577	0.620	0.556	0.596	0.604	0.745	0.708	0.680	0.884	0.884	0.907	0.976	0.864	0.860	0.860	0.620	0.456
34	0.509	0.566	0.673	0.680	0.585	0.608	0.536	0.537	0.577	0.574	0.556	0.536	0.729	0.627	0.667	0.952	0.952	0.844	0.864	0.930	0.841	0.884	0.745	0.500
35	0.491	0.577	0.688	0.694	0.596	0.558	0.545	0.547	0.588	0.585	0.566	0.545	0.745	0.640	0.680	0.884	0.884	0.864	0.884	0.864	0.860	0.951	0.688	0.456
36	0.509	0.596	0.708	0.714	0.615	0.577	0.564	0.566	0.608	0.604	0.585	0.564	0.766	0.660	0.700	0.907	0.907	0.886	0.907	0.886	0.884	0.929	0.708	0.474
37	0.509	0.694	0.640	0.750	0.585	0.519	0.593	0.566	0.608	0.545	0.585	0.593	0.729	0.694	0.667	0.864	0.864	0.930	0.952	0.844	0.929	0.884	0.673	0.500
38	0.491	0.608	0.688	0.729	0.627	0.558	0.518	0.547	0.588	0.585	0.566	0.518	0.708	0.608	0.647	0.841	0.841	0.907	0.841	0.822	0.860	0.951	0.688	0.482
39	0.537	0.627	0.640	0.750	0.585	0.577	0.536	0.566	0.608	0.545	0.585	0.536	0.660	0.627	0.604	0.864	0.864	0.930	0.864	0.844	0.884	0.841	0.708	0.500
40	0.519	0.608	0.620	0.694	0.537	0.558	0.574	0.547	0.588	0.527	0.566	0.574	0.708	0.673	0.647	0.929	0.929	0.864	0.929	0.907	0.818	0.818	0.653	0.482
41	0.627	0.694	0.783	0.787	0.615	0.640	0.654	0.627	0.745	0.545	0.647	0.593	0.766	0.766	0.735	0.708	0.708	0.804	0.783	0.694	0.723	0.688	0.640	0.500
42	0.615	0.615	0.804	0.735	0.635	0.694	0.611	0.556	0.660	0.564	0.574	0.554	0.750	0.714	0.755	0.766	0.766	0.714	0.694	0.750	0.640	0.673	0.729	0.574
43	0.545	0.574	0.750	0.623	0.564	0.714	0.571	0.466	0.680	0.582	0.483	0.600	0.700	0.667	0.706	0.714	0.714	0.604	0.647	0.735	0.566	0.596	0.647	0.593
44	0.574	0.700	0.680	0.755	0.564	0.556	0.630	0.574	0.647	0.475	0.564	0.630	0.771	0.771	0.706	0.714	0.714	0.735	0.787	0.735	0.694	0.660	0.615	0.509
P1	0.564	0.623	0.700	0.813	0.611	0.545	0.618	0.623	0.667	0.571	0.582	0.679	0.686	0.686	0.692	0.735	0.735	0.830	0.809	0.720	0.750	0.714	0.635	0.526
P2	0.361	0.258	0.242	0.217	0.313	0.281	0.229	0.239	0.188	0.250	0.235	0.229	0.221	0.297	0.250	0.281	0.281	0.221	0.242	0.277	0.209	0.246	0.242	0.254
P3	0.286	0.246	0.231	0.242	0.344	0.290	0.235	0.209	0.176	0.153	0.206	0.200	0.286	0.286	0.203	0.231	0.231	0.174	0.194	0.227	0.162	0.179	0.270	0.281
P4	0.400	0.424	0.383	0.349	0.371	0.407	0.450	0.424	0.456	0.509	0.441	0.450	0.424	0.377	0.509	0.407	0.407	0.355	0.383	0.400	0.323	0.344	0.297	0.466

Contd../-

25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	P1	P2	P3	P4
1.000																							
0.804	1.000																						
0.766	0.864	1.000																					
0.708	0.800	0.841	1.000																				
0.804	0.907	0.952	0.841	1.000																			
0.844	0.952	0.907	0.841	0.952	1.000																		
0.745	0.929	0.884	0.860	0.884	0.884	1.000																	
0.766	0.864	0.907	0.929	0.907	0.907	0.884	1.000																
0.783	0.884	0.841	0.905	0.884	0.929	0.860	0.929	1.000															
0.766	0.907	0.907	0.841	0.907	0.907	0.929	0.907	0.841	1.000														
0.783	0.929	0.841	0.818	0.884	0.929	0.905	0.841	0.905	0.929	1.000													
0.804	0.952	0.864	0.800	0.907	0.952	0.884	0.864	0.884	0.952	0.976	1.000												
0.766	0.864	0.822	0.929	0.864	0.907	0.800	0.907	0.929	0.822	0.841	0.864	1.000											
0.745	0.929	0.800	0.860	0.841	0.884	0.905	0.800	0.860	0.841	0.905	0.884	0.884	1.000										
0.694	0.864	0.822	0.929	0.822	0.822	0.884	0.907	0.841	0.864	0.800	0.822	0.907	0.884	1.000									
0.745	0.841	0.884	0.951	0.884	0.884	0.905	0.976	0.951	0.884	0.860	0.841	0.884	0.818	0.884	1.000								
0.844	0.783	0.673	0.688	0.708	0.745	0.723	0.745	0.761	0.708	0.723	0.745	0.745	0.723	0.745	0.723	1.000							
0.867	0.766	0.729	0.673	0.729	0.729	0.783	0.729	0.673	0.766	0.708	0.729	0.660	0.708	0.729	0.708	0.886	1.000						
0.809	0.647	0.714	0.627	0.680	0.680	0.660	0.680	0.627	0.680	0.627	0.647	0.615	0.596	0.615	0.660	0.750	0.848	1.000					
0.848	0.714	0.714	0.694	0.714	0.750	0.660	0.750	0.766	0.680	0.694	0.714	0.750	0.660	0.680	0.729	0.867	0.771	0.755	1.000				
0.755	0.809	0.700	0.714	0.735	0.771	0.750	0.771	0.787	0.735	0.750	0.771	0.771	0.750	0.771	0.750	0.771	0.720	0.611	0.706	1.000			
0.277	0.242	0.262	0.266	0.262	0.262	0.266	0.262	0.246	0.262	0.246	0.242	0.242	0.246	0.242	0.266	0.242	0.277	0.292	0.273	0.181	1.000		
0.266	0.194	0.212	0.197	0.212	0.212	0.197	0.212	0.179	0.212	0.179	0.194	0.194	0.179	0.194	0.197	0.231	0.266	0.262	0.281	0.169	0.481	1.000	
0.424	0.383	0.383	0.344	0.383	0.407	0.367	0.383	0.367	0.383	0.367	0.383	0.361	0.344	0.339	0.367	0.407	0.424	0.491	0.417	0.344	0.297	0.266	1.000

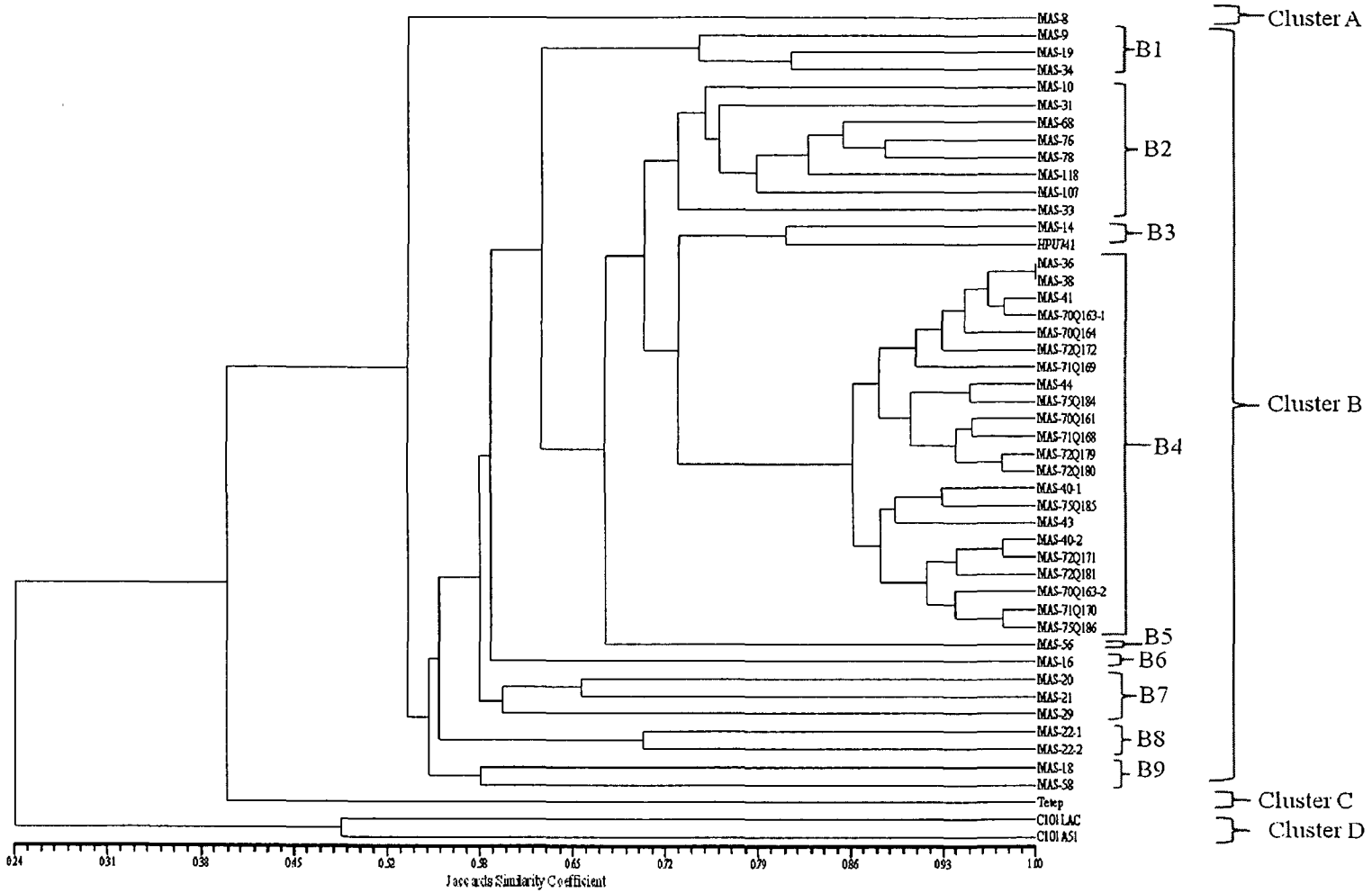


Fig: 4.9 **Dendrogram depicting genetic similarities among BC_1F_4 lines and parents. The dendrogram was generated by analysis of SSR marker data using NTSYS programmee (Rohlf, 1998). The names of the lines and parents are given on the termini of branches**

As per the SSR data, the average proportion of HPU741 was 67.8% (varying from 52.6% to 83.0%) deviated significantly from the expected value 75% (Table 4.4). This may be attributed to less number of markers used to assay genome or possible linkage drag of donor genome along with resistance genes, as a result of selection imposed for blast resistance genes. It may be due to the use of poor resolving agarose gels that led to partial resolution of polymorphic alleles/loci (Collard *et al.* 2008).

4.2.2 Estimation of recurrent parent genome using ISSR markers

A total of 30 ISSR primers were studied. Out of these 30, 13 (43.33%) either did not amplify or fail to generate reproducible and clear bands, the remaining 17 (56.60%) produced easily interpretable amplification products. Out of these 17, 13 (76.47%) displayed polymorphism with respect to the recurrent parent HPU741 (Table 4.5). All the 44 BC_1F_4 plants were genotyped using these 13 ISSR primers (Fig: 4.10). These ISSR primers generated 77 reproducible bands with band size ranging from 260 bp to 2100 bp. Non parental bands were also amplified in certain genotypes by some primers e.g. one band with each ISSR7 and ISSR12 and 2 bands with ISSR13. Null alleles a condition characterised by absence of bands that were observed to be monomorphic among parents was observed at least once in 24 lines out of the 44. Null alleles may be due to mutation in the primer binding site (Tsumara *et al.*1996). The amplification results revealed that 9 bands (maximum) were observed with ISSR13 primer whereas 4 bands were amplified by ISSR7, ISSR15, ISSR18, 5 bands by ISSR3, ISSR14, ISSR26, 6 by ISSR1, ISSR12, ISSR6, 7 by ISSR16 and 8 by ISSR2 and ISSR11. On an average, 6 bands per primer were generated (Table 4.5).

The proportion of the genome of recurrent parent in the BC_1F_4 lines ranged from 65.6 % to 93.8% with an average of 79.7% (Table 4.6). The genome of lines, MAS-118, MAS-20 and MAS-33 were highly similar to HPU741 with similarity coefficients of 0.938, 0.937 and 0.921, respectively; these lines, however, were not blast resistance gene pyramids.

Table 4.5 Results of marker assisted background selection using ISSR markers

Sr. No.	Marker	Bands observed			
		Polymorphic	Monomorpic	Non parental	Total
1	ISSR1	3	3	0	6
2	ISSR2	6	2	0	8
3	ISSR3	2	3	0	5
4	ISSR7	1	2	1	4
5	ISSR11	6	2	0	8
6	ISSR12	1	4	1	6
7	ISSR13	2	5	2	9
8	ISSR14	1	4	0	5
9	ISSR15	2	2	0	4
10	ISSR16	2	5	0	7
11	ISSR18	1	3	0	4
12	ISSR25	2	4	0	6
13	ISSR26	2	3	0	5
		35	**42**	**4**	**77**

Dendrogram generated from the ISSR marker data revealed an overall similarity coefficient of 0.67 (Fig 4.11). All the BC_1F_4 lines with the exception of MAS-41 along with parents HPU741, C101LAC and C101A51 formed four main clusters, A, B, C and D. Cluster A comprised two sub clusters A1, A2 sharing an overall similarity at 0.79. Sub cluster A1 comprised two BC_1F_4 lines MAS-8 and MAS-68 whereas sub cluster A2 comprised parents C101LAC and C101A51. Cluster B consisted four sub clusters, B1, B2, B3 and B4. Sub cluster B1 was consisted of 18 lines and the recurrent parent HPU741, whereas sub cluster B2 was comprised of 21 lines viz., MAS-18, MAS-36, MAS-38, MAS-71Q168, MAS-70Q163, MAS-72Q172, MAS-72Q179, MAS-71Q170, MAS-72Q171, MAS-72Q180, MAS-40-2, MAS-71Q169, MAS-70Q161, MAS-70Q163,

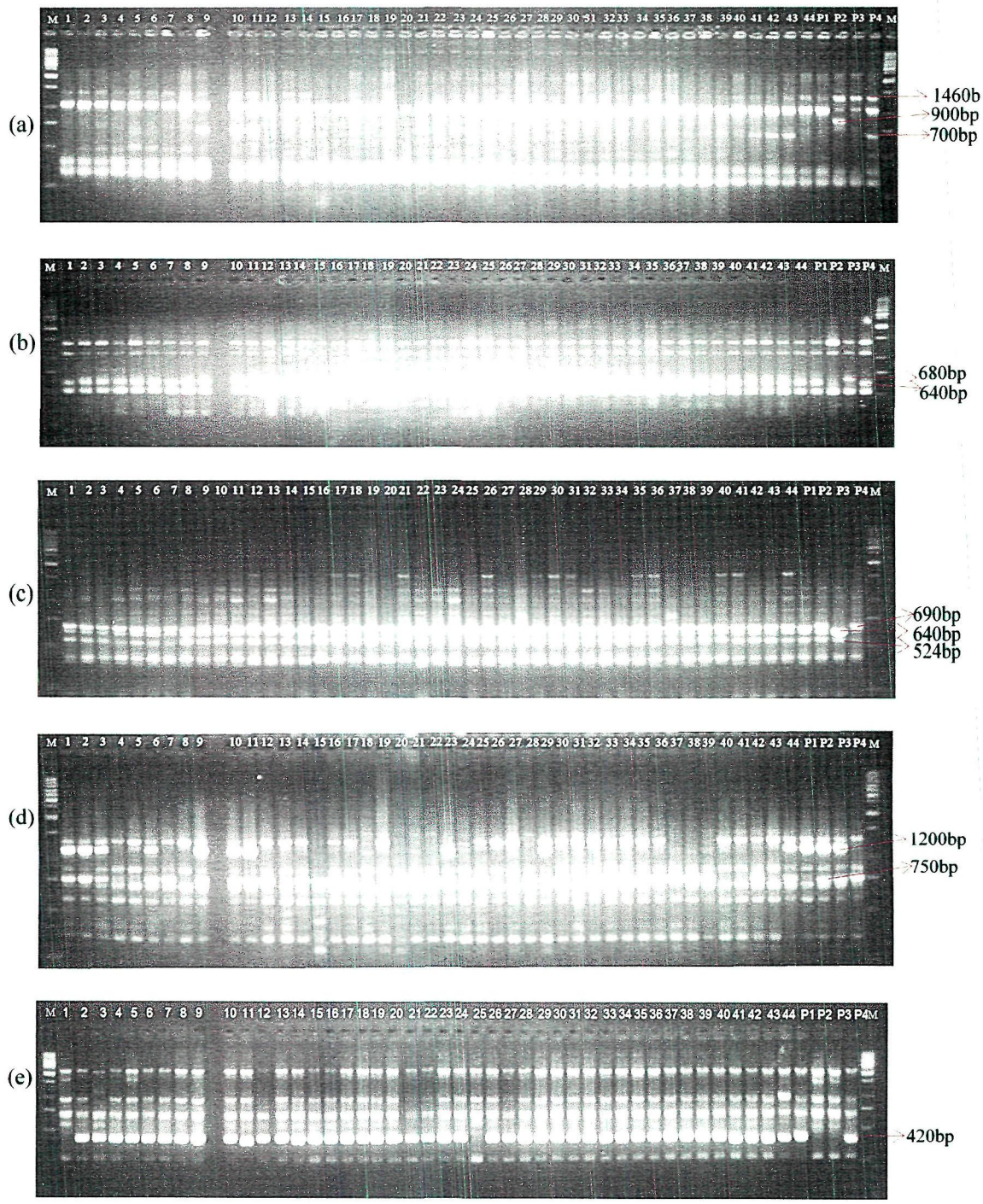


Fig: 4.10 **Genotyping of BC_1F_4 lines with ISSR primers (a) ISSR1, (b) ISSR3 (c) ISSR15, (d) ISSR16, and (e) ISSR18 (From top to bottom); Lanes 1-44: BC_1F_4 lines (See Table 3.1), P1: HPU741, P2: C101LAC, P3: C101A51, P4: Tetep; M: 100bp Molecular weight marker**

Table 4.6 Similarity matrix obtained after analysis of ISSR data of forty four lines and four parents using UPGMA option of NTSYS programme

0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1	1.000																							
2	0.825	1.000																						
3	0.813	0.919	1.000																					
4	0.852	0.841	0.800	1.000																				
5	0.800	0.846	0.891	0.873	1.000																			
6	0.836	0.797	0.813	0.823	0.828	1.000																		
7	0.825	0.844	0.831	0.841	0.846	0.797	1.000																	
8	0.800	0.791	0.833	0.844	0.906	0.857	0.875	1.000																
9	0.857	0.875	0.891	0.844	0.877	0.828	0.875	0.877	1.000															
10	0.831	0.794	0.836	0.791	0.851	0.831	0.821	0.879	0.908	1.000														
11	0.857	0.791	0.833	0.815	0.848	0.857	0.818	0.848	0.906	0.938	1.000													
12	0.803	0.766	0.810	0.790	0.797	0.746	0.823	0.825	0.855	0.857	0.797	1.000												
13	0.810	0.857	0.873	0.855	0.951	0.781	0.828	0.859	0.859	0.806	0.803	0.778	1.000											
14	0.813	0.803	0.846	0.828	0.891	0.871	0.889	0.952	0.891	0.892	0.862	0.869	0.844	1.000										
15	0.703	0.778	0.766	0.746	0.754	0.787	0.750	0.781	0.754	0.731	0.701	0.726	0.708	0.794	1.000									
16	0.727	0.773	0.788	0.769	0.803	0.839	0.773	0.803	0.750	0.779	0.750	0.750	0.758	0.815	0.850	1.000								
17	0.761	0.806	0.848	0.776	0.864	0.844	0.833	0.892	0.836	0.866	0.836	0.813	0.818	0.906	0.769	0.905	1.000							
18	0.778	0.797	0.813	0.823	0.828	0.806	0.797	0.828	0.828	0.831	0.800	0.803	0.810	0.813	0.758	0.839	0.873	1.000						
19	0.750	0.742	0.785	0.823	0.857	0.867	0.769	0.887	0.800	0.831	0.800	0.803	0.810	0.871	0.787	0.900	0.903	0.898	1.000					
20	0.683	0.625	0.641	0.672	0.656	0.741	0.625	0.656	0.631	0.662	0.683	0.623	0.635	0.667	0.719	0.717	0.672	0.683	0.712	1.000				
21	0.723	0.742	0.813	0.766	0.800	0.836	0.769	0.828	0.773	0.803	0.773	0.803	0.754	0.841	0.817	0.900	0.903	0.898	0.931	0.712	1.000			
22	0.738	0.758	0.828	0.781	0.815	0.823	0.785	0.844	0.788	0.818	0.788	0.820	0.769	0.857	0.803	0.885	0.919	0.915	0.915	0.700	0.982	1.000		
23	0.813	0.859	0.875	0.828	0.891	0.841	0.803	0.862	0.862	0.836	0.833	0.781	0.844	0.875	0.823	0.815	0.848	0.841	0.841	0.667	0.841	0.857	1.000	
24	0.769	0.844	0.859	0.841	0.935	0.825	0.815	0.905	0.846	0.821	0.818	0.766	0.887	0.889	0.806	0.800	0.862	0.825	0.855	0.651	0.825	0.841	0.951	1.000
25	0.897	0.794	0.781	0.820	0.769	0.833	0.823	0.797	0.769	0.773	0.769	0.800	0.778	0.839	0.754	0.778	0.785	0.774	0.774	0.678	0.774	0.790	0.781	0.766
26	0.738	0.785	0.828	0.810	0.903	0.823	0.785	0.873	0.815	0.846	0.815	0.790	0.855	0.857	0.746	0.885	0.919	0.883	0.915	0.672	0.852	0.869	0.828	0.871
27	0.750	0.769	0.841	0.823	0.887	0.836	0.797	0.887	0.828	0.859	0.828	0.833	0.839	0.871	0.730	0.839	0.903	0.898	0.931	0.683	0.898	0.915	0.813	0.855
28	0.731	0.803	0.846	0.773	0.862	0.841	0.803	0.862	0.806	0.836	0.806	0.781	0.815	0.875	0.794	0.903	0.968	0.902	0.902	0.694	0.933	0.950	0.875	0.889
29	0.769	0.815	0.859	0.785	0.846	0.825	0.788	0.818	0.818	0.848	0.818	0.794	0.800	0.831	0.778	0.887	0.921	0.917	0.885	0.705	0.885	0.902	0.859	0.815
30	0.746	0.818	0.862	0.788	0.877	0.857	0.818	0.877	0.821	0.851	0.821	0.797	0.831	0.891	0.781	0.919	0.984	0.887	0.918	0.683	0.918	0.934	0.862	0.875
31	0.754	0.773	0.815	0.825	0.889	0.869	0.800	0.919	0.831	0.862	0.831	0.806	0.841	0.903	0.762	0.871	0.935	0.900	0.966	0.689	0.900	0.917	0.844	0.887
32	0.758	0.803	0.846	0.800	0.891	0.871	0.803	0.891	0.833	0.864	0.833	0.810	0.844	0.905	0.766	0.903	0.968	0.871	0.933	0.694	0.902	0.918	0.875	0.889
33	0.769	0.788	0.831	0.813	0.875	0.855	0.788	0.875	0.818	0.848	0.818	0.823	0.828	0.889	0.778	0.918	0.952	0.885	0.949	0.705	0.917	0.933	0.889	0.873
34	0.758	0.803	0.846	0.800	0.862	0.871	0.831	0.891	0.833	0.864	0.833	0.810	0.815	0.905	0.794	0.903	0.968	0.902	0.933	0.694	0.933	0.950	0.846	0.859
35	0.758	0.831	0.875	0.800	0.891	0.841	0.831	0.862	0.833	0.864	0.833	0.810	0.844	0.875	0.766	0.903	0.968	0.902	0.902	0.694	0.902	0.918	0.846	0.859
36	0.781	0.773	0.815	0.797	0.859	0.900	0.773	0.859	0.803	0.833	0.859	0.778	0.813	0.873	0.734	0.871	0.935	0.839	0.900	0.717	0.869	0.885	0.844	0.857
37	0.778	0.825	0.841	0.794	0.828	0.778	0.797	0.800	0.800	0.803	0.773	0.803	0.839	0.813	0.758	0.869	0.903	0.931	0.867	0.712	0.898	0.915	0.841	0.825
38	0.823	0.785	0.828	0.839	0.873	0.823	0.841	0.873	0.873	0.875	0.873	0.850	0.825	0.887	0.774	0.825	0.889	0.883	0.883	0.729	0.883	0.900	0.887	0.871
39	0.716	0.788	0.803	0.785	0.818	0.797	0.761	0.791	0.765	0.821	0.791	0.766	0.773	0.803	0.778	0.887	0.891	0.885	0.885	0.705	0.917	0.902	0.831	0.815
40	0.738	0.813	0.857	0.810	0.903	0.852	0.813	0.873	0.815	0.846	0.815	0.790	0.855	0.857	0.774	0.885	0.919	0.883	0.915	0.700	0.883	0.869	0.828	0.871
41	0.797	0.788	0.831	0.841	0.875	0.797	0.844	0.875	0.846	0.877	0.818	0.852	0.828	0.889	0.778	0.800	0.833	0.855	0.855	0.651	0.825	0.841	0.831	0.844
42	0.839	0.828	0.815	0.855	0.831	0.839	0.828	0.859	0.889	0.862	0.803	0.867	0.813	0.873	0.820	0.813	0.818	0.810	0.839	0.661	0.810	0.825	0.844	0.828
43	0.828	0.818	0.833	0.873	0.906	0.828	0.846	0.906	0.906	0.879	0.848	0.825	0.889	0.891	0.754	0.776	0.836	0.828	0.857	0.631	0.800	0.815	0.862	0.905
44	0.831	0.877	0.922	0.846	0.908	0.889	0.877	0.908	0.908	0.881	0.879	0.800	0.862	0.922	0.813	0.833	0.894	0.859	0.859	0.662	0.859	0.875	0.922	0.906
P1	0.857	0.818	0.862	0.873	0.906	0.857	0.846	0.937	0.906	0.879	0.877	0.825	0.859	0.921	0.781	0.803	0.864	0.857	0.887	0.656	0.828	0.844	0.921	0.905
P2	0.831	0.768	0.783	0.791	0.824	0.750	0.848	0.824	0.851	0.826	0.797	0.800	0.806	0.836	0.657	0.681	0.761	0.750	0.725	0.588	0.700	0.714	0.783	0.768
P3	0.797	0.710	0.725	0.758	0.765	0.797	0.735	0.765	0.765	0.768	0.765	0.712	0.746	0.750	0.647	0.671	0.704	0.716	0.716	0.651	0.691	0.681	0.725	0.710
P4	0.738	0.758	0.746	0.727	0.735	0.687	0.813	0.761	0.815	0.818	0.788	0.790	0.742	0.773	0.618	0.667	0.750	0.712	0.712	0.594	0.687	0.701	0.696	0.706

Contd.../-

25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	P1	P2	P3	P4
1.000																							
0.734	1.000																						
0.746	0.948	1.000																					
0.781	0.918	0.902	1.000																				
0.766	0.902	0.885	0.919	1.000																			
0.797	0.934	0.918	0.984	0.935	1.000																		
0.778	0.949	0.966	0.934	0.887	0.951	1.000																	
0.781	0.950	0.933	0.967	0.919	0.984	0.967	1.000																
0.794	0.933	0.917	0.951	0.934	0.967	0.950	0.983	1.000															
0.810	0.918	0.933	0.967	0.919	0.984	0.967	0.967	0.951	1.000														
0.781	0.950	0.933	0.967	0.951	0.984	0.934	0.967	0.951	0.967	1.000													
0.806	0.917	0.900	0.934	0.887	0.951	0.933	0.967	0.950	0.934	0.934	1.000												
0.803	0.883	0.867	0.933	0.917	0.918	0.869	0.902	0.917	0.902	0.933	0.869	1.000											
0.762	0.869	0.883	0.887	0.871	0.873	0.885	0.887	0.902	0.887	0.887	0.855	0.883	1.000										
0.738	0.871	0.855	0.919	0.903	0.905	0.857	0.889	0.903	0.889	0.919	0.857	0.917	0.871	1.000									
0.762	0.932	0.915	0.918	0.902	0.934	0.917	0.918	0.902	0.918	0.950	0.885	0.883	0.869	0.902	1.000								
0.852	0.841	0.855	0.831	0.844	0.846	0.857	0.831	0.844	0.859	0.859	0.800	0.825	0.841	0.815	0.871	1.000							
0.867	0.797	0.810	0.815	0.800	0.831	0.841	0.844	0.857	0.844	0.815	0.813	0.810	0.825	0.773	0.797	0.887	1.000						
0.825	0.844	0.857	0.833	0.791	0.848	0.889	0.862	0.846	0.862	0.833	0.831	0.800	0.844	0.765	0.844	0.905	0.919	1.000					
0.828	0.846	0.859	0.892	0.877	0.908	0.891	0.892	0.877	0.922	0.892	0.862	0.831	0.875	0.821	0.875	0.877	0.862	0.908	1.000				
0.797	0.844	0.857	0.833	0.846	0.848	0.889	0.862	0.875	0.862	0.833	0.831	0.800	0.903	0.791	0.844	0.875	0.859	0.906	0.938	1.000			
0.773	0.739	0.750	0.732	0.743	0.746	0.754	0.757	0.743	0.757	0.757	0.729	0.725	0.791	0.694	0.739	0.794	0.779	0.824	0.826	0.824	1.000		
0.766	0.706	0.716	0.700	0.710	0.714	0.721	0.725	0.710	0.725	0.725	0.746	0.691	0.706	0.686	0.731	0.735	0.746	0.765	0.768	0.739	0.848	1.000	
0.708	0.727	0.738	0.721	0.706	0.735	0.742	0.746	0.731	0.746	0.746	0.716	0.738	0.781	0.731	0.727	0.758	0.769	0.788	0.739	0.735	0.765	0.657	1.000

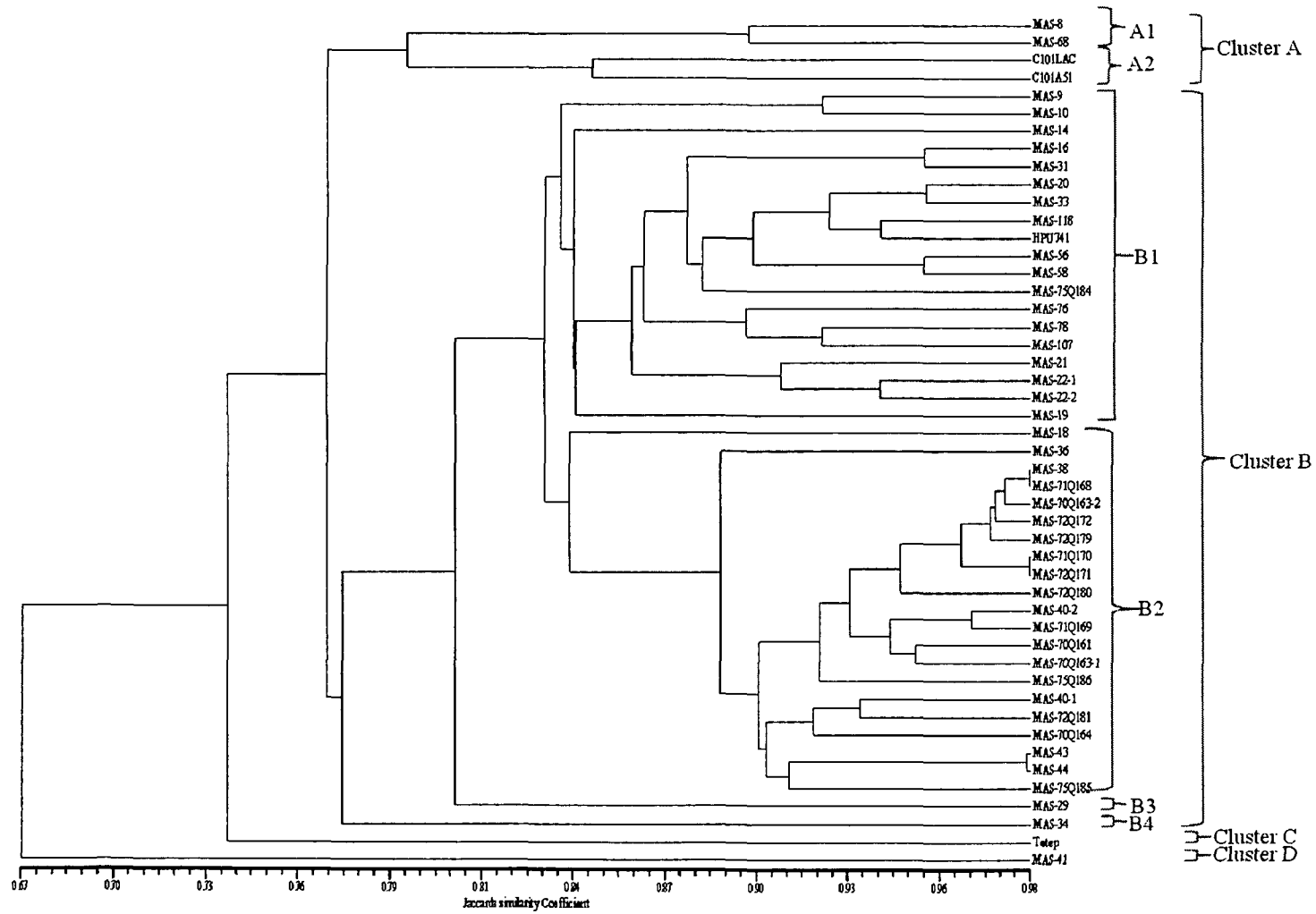


Fig: 4.11 **Dendrogram depicting genetic similarities among BC_1F_4 lines and parents. The dendrogram was generated by analysis of ISSR marker data using NTSYS programme (Rohlf, 1998). The names of the lines and parents are given on the termini of branches**

MAS-75Q186, MAS-40-1, MAS-72Q181, MAS-70Q164, MAS-43, MAS-44, and MAS-75Q185 with an overall similarity coefficient of 0.85. Majority of the two and three gene pyramid lines were in sub cluster B2. Sub clusters B1 and B2 with overall genetic similarity coefficient of 0.83 had genetically highly similar individuals. Sub cluster B3 and B4 had single lines MAS-29 and MAS-34 respectively. Cluster A shared an overall similarity of 0.77 with cluster B. One of the parental lines Tetep formed cluster C whereas MAS-41 formed cluster D.

The cophenetic correlation of the dendrogram with the data matrix derived from ISSR markers was 0.87, indicating there was a good fit of cluster analysis to the original data.

4.2.3 Estimation of recurrent parent genome in pyramid lines using pooled data of SSR and ISSRs

Pooled data of SSR and ISSR data were also used to construct a composite similarity matrix and dendrogram (Table 4.7 and Fig: 4.12). The proportion of the genome of recurrent parent in the BC_1F_4 generation lines using combined data ranged from 68.4% to 85.3% with an average of 76.8% (Table 4.7). The dendrogram revealed three major clusters A, B and C with overall similarity coefficient of 0.49 (Fig: 4.12). The 44 lines and the recurrent parent HPU741 were grouped together in cluster A, which could further be sub divided into 7 sub clusters A1, A2, A3, A4, A5, A6 and A7. Sub clusters A1 and A2 comprised three lines each whereas eleven lines viz., MAS10, MAS-21, MAS-14, MAS-33, MAS-68, MAS-76, MAS-118, MAS-78, MAS-107, MAS-16 and MAS-31 and the recurrent parent HPU741 formed sub cluster A3, with an overall similarity coefficient of 0.78. Twenty one lines viz., MAS-36, MAS-70Q164, MAS-38, MAS-71Q168, MAS-72Q171, MAS-72Q179, MAS-72Q180, MAS-70Q161, MAS-71Q169, MAS-40-2, MAS-71Q168, MAS-72Q171, MAS-75Q186, MAS-70Q163-1, MAS-40-1, MAS-70Q163-3, MAS-72Q181, MAS-75Q185, MAS-43, MAS-44 and MAS-75Q184 formed sub cluster A4. Most of the two and three blast resistance gene pyramids of genes were in sub cluster A4. A single line MAS-41 constituted sub cluster A5 and shared an overall genetic similarity of 0.78 with sub cluster A4 and 0.77 with sub cluster A3. Sub cluster A6 comprises three lines viz., MAS-18, MAS-56 and MAS-58,

Table 4.7 Similarity matrix obtained after analysis of pooled data of SSR and ISSR markers of forty four lines and four parents using UPGMA option of NTSYS programme

0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1	1.000																							
2	0.686	1.000																						
3	0.716	0.804	1.000																					
4	0.722	0.795	0.763	1.000																				
5	0.669	0.694	0.783	0.789	1.000																			
6	0.667	0.678	0.784	0.698	0.703	1.000																		
7	0.712	0.798	0.752	0.729	0.691	0.689	1.000																	
8	0.733	0.714	0.700	0.750	0.725	0.667	0.769	1.000																
9	0.709	0.750	0.813	0.757	0.717	0.746	0.746	0.783	1.000															
10	0.653	0.664	0.733	0.656	0.661	0.686	0.702	0.721	0.786	1.000														
11	0.656	0.653	0.694	0.672	0.625	0.661	0.691	0.711	0.761	0.835	1.000													
12	0.684	0.695	0.709	0.746	0.650	0.633	0.735	0.726	0.733	0.675	0.598	1.000												
13	0.664	0.795	0.811	0.818	0.821	0.728	0.759	0.720	0.757	0.669	0.659	0.716	1.000											
14	0.754	0.735	0.781	0.772	0.731	0.716	0.807	0.783	0.744	0.699	0.717	0.763	0.772	1.000										
15	0.625	0.768	0.752	0.713	0.675	0.687	0.779	0.709	0.701	0.714	0.648	0.675	0.728	0.730	1.000									
16	0.628	0.695	0.739	0.730	0.706	0.735	0.678	0.683	0.675	0.689	0.664	0.664	0.761	0.748	0.782	1.000								
17	0.648	0.714	0.774	0.735	0.739	0.739	0.711	0.731	0.723	0.736	0.711	0.697	0.796	0.798	0.739	0.942	1.000							
18	0.675	0.730	0.761	0.800	0.726	0.696	0.697	0.733	0.754	0.723	0.726	0.698	0.768	0.754	0.711	0.841	0.861	1.000						
19	0.653	0.707	0.737	0.791	0.733	0.717	0.703	0.754	0.730	0.714	0.718	0.719	0.791	0.809	0.748	0.903	0.905	0.912	1.000					
20	0.595	0.607	0.649	0.670	0.619	0.673	0.592	0.597	0.602	0.617	0.619	0.590	0.685	0.658	0.704	0.824	0.792	0.745	0.786	1.000				
21	0.617	0.698	0.743	0.750	0.695	0.678	0.667	0.701	0.692	0.678	0.695	0.681	0.719	0.752	0.723	0.857	0.860	0.902	0.911	0.743	1.000			
22	0.612	0.692	0.752	0.743	0.718	0.687	0.661	0.695	0.686	0.700	0.675	0.675	0.743	0.746	0.732	0.867	0.887	0.893	0.883	0.752	0.949	1.000		
23	0.686	0.726	0.772	0.763	0.783	0.752	0.694	0.714	0.706	0.677	0.653	0.667	0.795	0.750	0.737	0.770	0.789	0.761	0.752	0.679	0.775	0.800	1.000	
24	0.613	0.750	0.735	0.697	0.717	0.716	0.702	0.680	0.672	0.685	0.648	0.675	0.772	0.672	0.730	0.675	0.708	0.653	0.672	0.588	0.664	0.672	0.813	1.000
25	0.764	0.728	0.775	0.798	0.724	0.723	0.739	0.701	0.707	0.664	0.667	0.726	0.798	0.833	0.771	0.789	0.793	0.764	0.787	0.726	0.745	0.771	0.759	0.664
26	0.647	0.701	0.793	0.784	0.788	0.726	0.683	0.748	0.739	0.752	0.726	0.684	0.817	0.770	0.726	0.894	0.914	0.903	0.912	0.762	0.848	0.875	0.761	0.681
27	0.625	0.678	0.768	0.743	0.733	0.717	0.675	0.709	0.701	0.714	0.703	0.690	0.791	0.761	0.702	0.885	0.923	0.857	0.902	0.804	0.856	0.865	0.752	0.701
28	0.623	0.733	0.733	0.739	0.714	0.698	0.686	0.706	0.697	0.683	0.686	0.672	0.754	0.772	0.713	0.895	0.933	0.886	0.894	0.764	0.903	0.913	0.795	0.712
29	0.636	0.718	0.795	0.739	0.729	0.713	0.686	0.692	0.712	0.725	0.714	0.686	0.786	0.757	0.743	0.913	0.933	0.886	0.894	0.798	0.867	0.876	0.779	0.683
30	0.639	0.735	0.796	0.757	0.761	0.730	0.717	0.737	0.729	0.742	0.717	0.703	0.820	0.805	0.761	0.933	0.971	0.887	0.932	0.783	0.886	0.913	0.781	0.700
31	0.647	0.672	0.761	0.768	0.757	0.757	0.669	0.748	0.724	0.737	0.712	0.669	0.784	0.770	0.711	0.894	0.933	0.885	0.912	0.779	0.848	0.893	0.777	0.695
32	0.658	0.726	0.757	0.763	0.737	0.737	0.708	0.744	0.735	0.719	0.723	0.709	0.795	0.813	0.722	0.923	0.962	0.877	0.941	0.790	0.876	0.868	0.788	0.706
33	0.655	0.724	0.754	0.777	0.735	0.704	0.706	0.741	0.733	0.717	0.720	0.722	0.793	0.811	0.735	0.904	0.924	0.894	0.960	0.771	0.893	0.903	0.770	0.675
34	0.645	0.697	0.772	0.748	0.737	0.752	0.694	0.729	0.720	0.733	0.708	0.681	0.779	0.781	0.737	0.923	0.962	0.877	0.904	0.790	0.894	0.922	0.804	0.692
35	0.636	0.718	0.795	0.754	0.759	0.713	0.700	0.720	0.726	0.739	0.714	0.686	0.802	0.772	0.728	0.895	0.933	0.886	0.894	0.764	0.885	0.931	0.779	0.669
36	0.655	0.695	0.770	0.761	0.750	0.750	0.678	0.726	0.718	0.731	0.735	0.678	0.793	0.779	0.719	0.886	0.924	0.858	0.903	0.788	0.875	0.903	0.786	0.675
37	0.653	0.768	0.752	0.775	0.718	0.658	0.703	0.695	0.716	0.686	0.689	0.704	0.791	0.761	0.717	0.867	0.887	0.931	0.902	0.769	0.911	0.902	0.768	0.672
38	0.667	0.707	0.768	0.791	0.763	0.702	0.689	0.724	0.746	0.744	0.733	0.690	0.775	0.761	0.717	0.832	0.869	0.893	0.865	0.769	0.874	0.921	0.800	0.686
39	0.636	0.718	0.733	0.770	0.714	0.698	0.659	0.692	0.697	0.697	0.700	0.658	0.724	0.726	0.698	0.877	0.880	0.904	0.876	0.764	0.903	0.876	0.779	0.669
40	0.639	0.722	0.752	0.759	0.733	0.717	0.703	0.724	0.716	0.700	0.703	0.690	0.791	0.777	0.717	0.903	0.923	0.875	0.921	0.786	0.856	0.848	0.752	0.686
41	0.722	0.748	0.811	0.818	0.759	0.728	0.759	0.765	0.804	0.725	0.744	0.730	0.802	0.836	0.759	0.761	0.781	0.833	0.824	0.670	0.782	0.775	0.748	0.683
42	0.737	0.733	0.811	0.802	0.744	0.775	0.729	0.720	0.788	0.725	0.700	0.716	0.786	0.804	0.791	0.793	0.796	0.768	0.775	0.700	0.735	0.759	0.795	0.712
43	0.697	0.708	0.798	0.759	0.748	0.779	0.719	0.697	0.807	0.744	0.677	0.720	0.805	0.791	0.733	0.750	0.784	0.726	0.763	0.675	0.695	0.718	0.767	0.761
44	0.714	0.800	0.816	0.807	0.750	0.735	0.765	0.756	0.793	0.690	0.736	0.723	0.823	0.857	0.765	0.783	0.817	0.805	0.829	0.693	0.788	0.781	0.784	0.719
P1	0.720	0.731	0.791	0.847	0.771	0.712	0.742	0.793	0.800	0.738	0.742	0.759	0.783	0.816	0.741	0.774	0.809	0.845	0.853	0.684	0.795	0.788	0.791	0.725
P2	0.603	0.519	0.519	0.500	0.576	0.523	0.529	0.533	0.515	0.540	0.518	0.504	0.511	0.573	0.457	0.493	0.533	0.485	0.489	0.436	0.460	0.489	0.519	0.515
P3	0.543	0.485	0.485	0.500	0.566	0.548	0.485	0.489	0.471	0.454	0.485	0.449	0.523	0.527	0.423	0.459	0.478	0.441	0.455	0.434	0.426	0.434	0.508	0.504
P4	0.576	0.600	0.575	0.543	0.562	0.556	0.637	0.603	0.648	0.675	0.624	0.623	0.592	0.583	0.568	0.547	0.591	0.539	0.556	0.500	0.512	0.531	0.504	0.595

Contd../-

25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	P1	P2	P3	P4
1.000																							
0.764	1.000																						
0.755	0.912	1.000																					
0.750	0.868	0.876	1.000																				
0.782	0.904	0.913	0.887	1.000																			
0.817	0.942	0.913	0.924	0.942	1.000																		
0.764	0.941	0.931	0.904	0.886	0.923	1.000																	
0.775	0.913	0.922	0.951	0.914	0.952	0.932	1.000																
0.789	0.913	0.885	0.932	0.913	0.951	0.913	0.961	1.000															
0.791	0.913	0.922	0.914	0.914	0.952	0.951	0.942	0.905	1.000														
0.782	0.941	0.894	0.905	0.923	0.961	0.922	0.914	0.932	0.951	1.000													
0.806	0.931	0.885	0.877	0.895	0.951	0.913	0.923	0.922	0.942	0.951	1.000												
0.787	0.875	0.848	0.931	0.894	0.913	0.840	0.904	0.922	0.868	0.894	0.867	1.000											
0.755	0.893	0.848	0.876	0.858	0.877	0.893	0.850	0.885	0.868	0.894	0.867	0.883	1.000										
0.719	0.868	0.841	0.923	0.869	0.870	0.868	0.896	0.877	0.879	0.869	0.843	0.913	0.876	1.000									
0.755	0.893	0.902	0.931	0.894	0.913	0.912	0.941	0.922	0.904	0.913	0.867	0.883	0.848	0.894	1.000								
0.849	0.817	0.775	0.770	0.786	0.804	0.800	0.795	0.809	0.795	0.802	0.777	0.791	0.791	0.786	0.807	1.000							
0.867	0.784	0.775	0.754	0.770	0.788	0.817	0.795	0.777	0.811	0.770	0.777	0.743	0.775	0.754	0.759	0.887	1.000						
0.818	0.757	0.795	0.744	0.744	0.776	0.788	0.783	0.750	0.783	0.744	0.750	0.718	0.733	0.700	0.763	0.838	0.889	1.000					
0.836	0.789	0.796	0.807	0.807	0.841	0.789	0.832	0.830	0.816	0.807	0.798	0.796	0.781	0.761	0.813	0.873	0.823	0.842	1.000				
0.779	0.829	0.788	0.783	0.798	0.816	0.829	0.823	0.838	0.807	0.798	0.805	0.788	0.836	0.783	0.804	0.830	0.798	0.771	0.835	1.000			
0.527	0.496	0.511	0.511	0.511	0.515	0.519	0.519	0.504	0.519	0.511	0.493	0.489	0.523	0.478	0.511	0.522	0.534	0.564	0.556	0.493	1.000		
0.516	0.452	0.466	0.456	0.467	0.471	0.463	0.474	0.449	0.474	0.456	0.470	0.444	0.444	0.445	0.466	0.489	0.511	0.519	0.534	0.450	0.683	1.000	
0.573	0.563	0.568	0.543	0.555	0.583	0.563	0.575	0.559	0.575	0.567	0.559	0.556	0.568	0.543	0.556	0.592	0.605	0.650	0.589	0.545	0.538	0.470	1.000

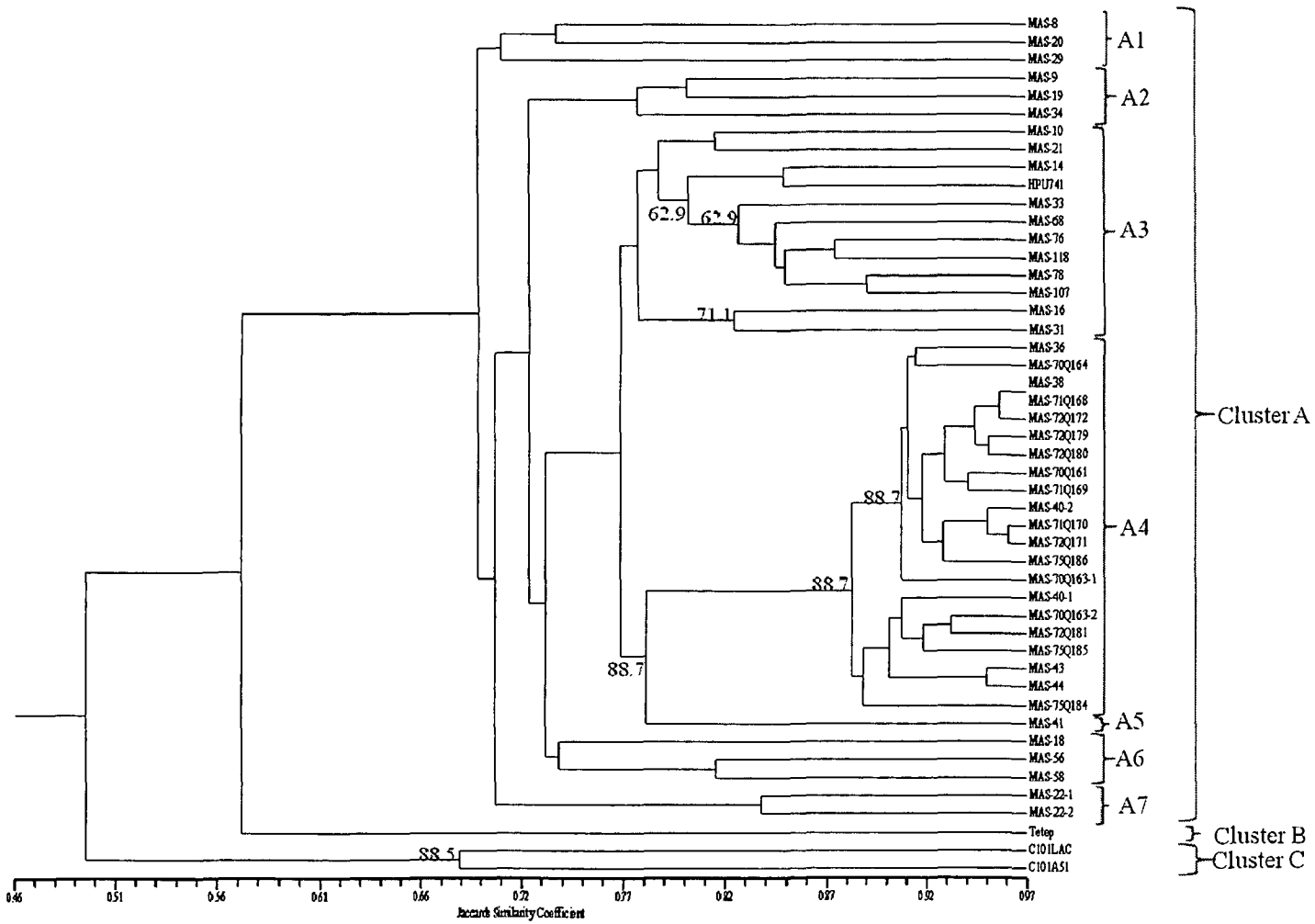


Fig: 4.12 **Dendrogram depicting genetic similarities among BC_1F_4 lines and parents. The dendrogram was generated from pooled data of SSR and ISSR markers analysed using NTSYS programme (Rohlf, 1998). The names of the lines and parents are given on the termini of branches. The numbers of branches represent bootstrap values generated by 1000 replicates using the WINBOOT programme**

whereas sub cluster A7 comprised two lines i.e. MAS-22-1 and MAS-22-2. The donors of blast resistance genes (C101LAC, C101A51 and Tetep) were genetically diverse from the recurrent parent as well as BC_1F_4 lines. While the Tetep formed a separate cluster 'B' with genetic similarity of 0.57 with HPU741 and BC_1F_4 lines, C101LAC and C101A51 shared cluster 'C' with genetic similarity of 0.49 with HPU741 and BC_1F_4 lines.

The cophenetic correlation of the dendrogram with the data matrix derived from pooled data of SSR and ISSR markers was 0.95, indicating that there was a very high goodness of fit of cluster analysis to the original data.

Johns *et al.* (1997) by resampling techniques verified that 50 bands produced the same grouping obtained with 106 bands. Additionally, the accuracy of genetic similarity estimates increases if the number of polymorphic loci is increased (Bonato *et al.* 2006) indicating that pooled data best represent the genome. It can be evident in the average proportion of recurrent parent genome values obtained using pooled data which is 77% close to expected recovery of 75%.

4.2.4 Principal component analysis of pooled data.

The PCA is one of the multivariate approaches of grouping based on similarity coefficients or variance-covariance values that gives more information about major groups, while a cluster analysis provides higher resolution among closely related populations. PCA examines relationships among several quantitative variables by deriving a smaller number of linear combinations (principal components) that retain as much of the information in the original data as possible.

In our study, a total of 62% of the variations in the estimates of genetic similarity were explained by the first three components indicating the suitability of pooled data of SSR and ISSR markers for clustering. The first principal component explained approximately 51% of the variation where the second component explained approximately 7% variation and the third one explained about 4% variation of the data. A total of two distinct clusters were revealed by the first three principal components (Fig: 4.13). PCA successfully delineated all the BC_1F_4 lines into 2 major groups. Group-1 included 22 BC_1F_4 lines and recurrent parent 'HPU741'. Group-2 was comprised of 22

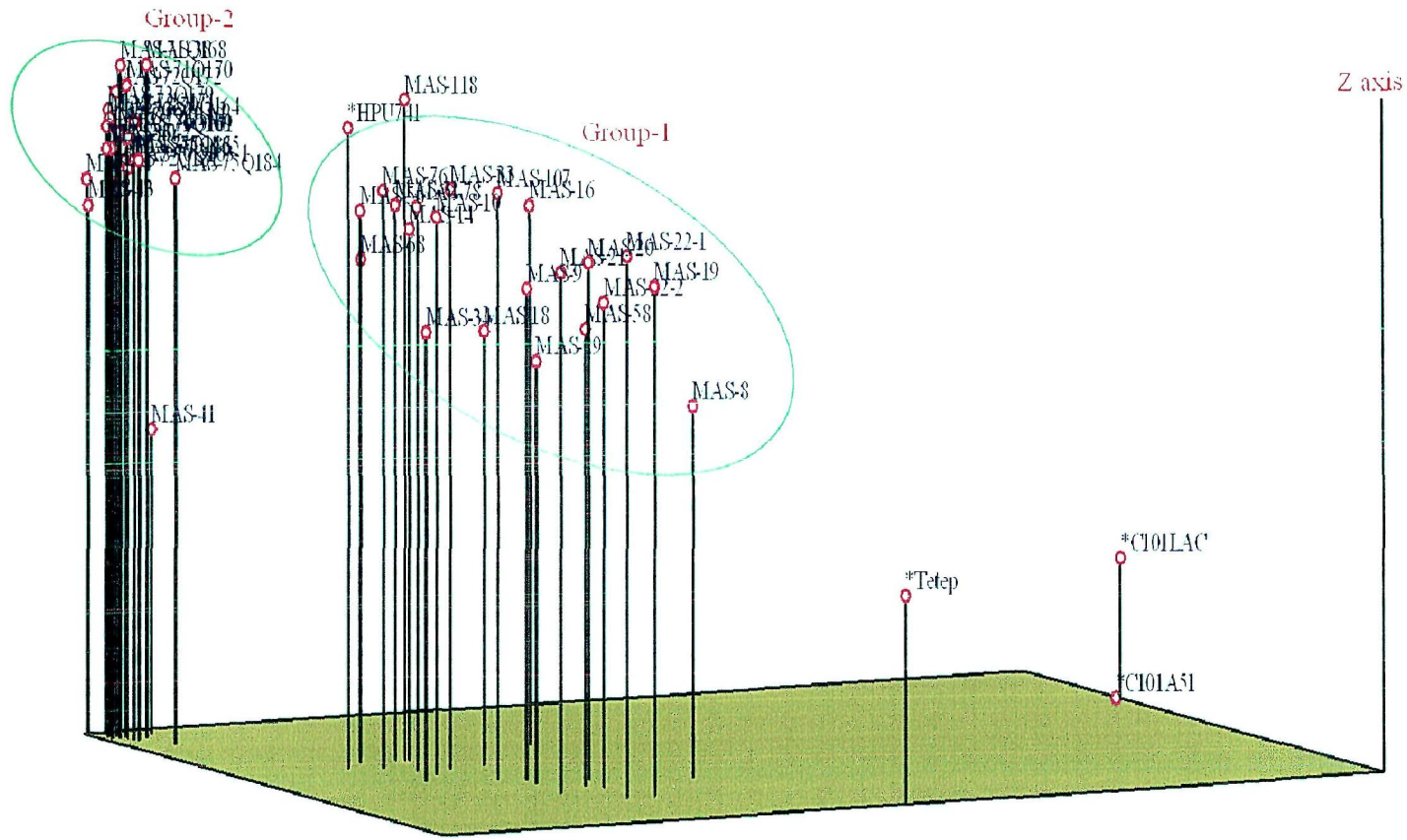


Fig: 4.13 **Three-dimensional plot of genetic similarity among the 44 BC_1F_4 lines and four parents as revealed by the Principle Component Analysis based on pooled data of SSR and ISSR markers**

BC_1F_4 lines. All the lines in Group-2 were two or three resistance gene pyramids. Donors of resistance genes i.e. C101LAC, C101A51 and Tetep did not form any cluster. Similar to dendrogram, PCA placed all two to three gene pyramids in one group and lines possessing single genes or no genes in another group.

PCA analysis using the first three principle components also confirmed the data of the dendrogram as two major groups were also observed in PCA. Group-1 delineated by PCA comprised recurrent parent and 22 pyramid lines with single or no resistance genes and Group-2 comprised 22 lines with two or three resistance gene pyramids. Possibly, the genetic drag along with the resistance gene segments of the chromosomes of donor parents is contributing to increased genetic differences in two and three resistance gene pyramid lines compared to the recurrent parent HPU741.

Overall, the data indicated that transfer of genome of HPU741 to pyramid lines was along expected estimates of pedigree. The average recovery of the recurrent parent genome (HPU741) observed among BC_1F_4 lines using pooled data of SSR and ISSR was 76.85% which didn't deviate significantly from the expected value of 75% for BC_1 generation. However, lines MAS-40-2, MAS-71Q170, MAS-72Q172, MAS-72Q180, MAS-75Q186 and MAS-72Q179, which were pyramids of two genes had 85.3%, 82.3%, 80.7%, 80.5%, 80.4% and 79.8% genetic similarity with HPU741, respectively (Table 4.8) and lines MAS-40-1, MAS-72Q171, MAS-75Q184, MAS-70Q161, MAS-71Q169, MAS-71Q168, MAS-38, MAS-70Q164, MAS-43 and MAS-70Q163-1 which were pyramids of three genes has 84.5%, 83.8%, 83.6%, 82.9%, 82.9%, 81.6%, 80.9%, 79.8%, 79.5% and 78.8% genetic similarity with HPU741, respectively (Table 4.8). These lines because of their high similarity to HPU741 should further be evaluated for agronomic parameters under field conditions with the aim to identify high yielding lines possessing pyramid of blast resistance genes. Such lines may be released as a variety and should provide a durable blast resistance under field conditions.

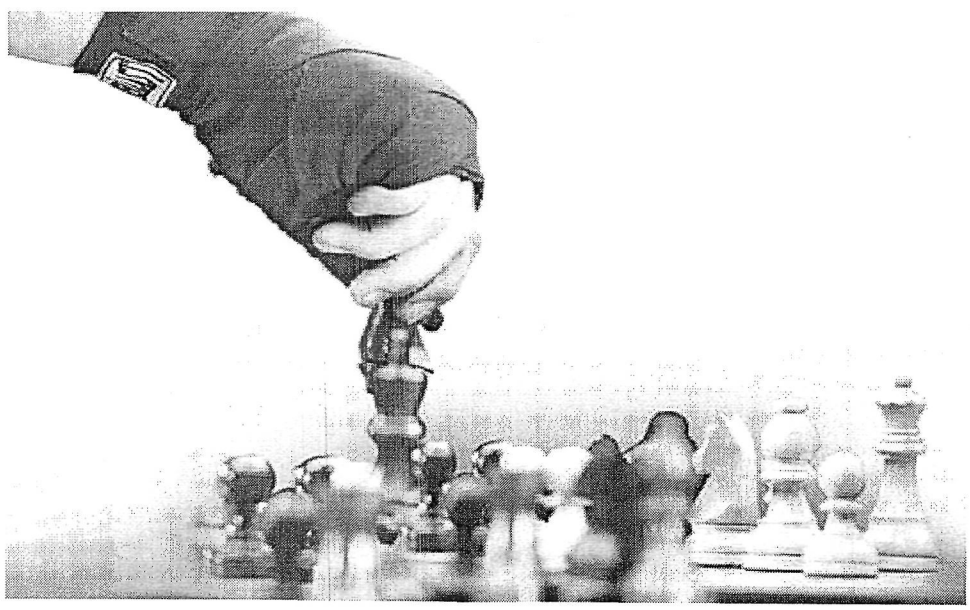
Table 4.8 **Blast resistance gene combinations in BC_1F_4 plants and their genetic similarity to recurrent parent (HPU741) as observed through marker assisted selection**

Sr. No.	MAS No.	Parentage*	Observed gene combinations	Genetic Similarity to HPU741
1.	MAS-8	F_1\{RG242[2]×F_1(HPU741×ARBN42)\}	No genes	0.720
2.	MAS-9	F_1(RG241[1]×GR185[10])	No genes	0.731
3.	MAS-10	F_1(RG241[1]×GR185[10])	*Pi-1*	0.791
4.	MAS-14	F_1(RG242-A[3]×RG241[1])	*Pi-1*	0.847
5.	MAS-16	F_1(RG242-A[3]×RG241[1])	*Pi-1*	0.771
6.	MAS-18	F_1(RG241[1]×RG242-A[3])	No genes	0.712
7.	MAS-19	F_1(G335[8]×RG231[6])	*Pi-1*	0.742
8.	MAS-20	F_1(G335[8]×RG231[6])	No genes	0.793
9.	MAS-21	F_1(G273[9]×RG230[5])	No genes	0.800
10.	MAS-22-1	F_1(G273[9]×RG230[5])	*Pi-ta*	0.738
11.	MAS-22-2	F_1(G273[9]×RG230[5])	*Pi-ta*	0.742
12.	MAS-29	F_1(GR185[10]×G196[7])	No genes	0.759
13.	MAS-31	F_1(RG241[1]×RG242-A[3])	*Pi-ta*	0.783
14.	MAS-33	F_1\{RG231[6]×F_1(HPU741×Tetep)\}	No genes	0.816
15.	MAS-34	BC_1F_2(HPU741×Tetep)	*Pi-1*	0.741
16.	MAS-36	BC_1F_3(HPU741×Tetep)	*Pi-1, Pi-ta & Pi-54*	0.774
17.	MAS-38	BC_1F_3 (HPU741×Tetep)	*Pi-1, Pi-ta & Pi-54*	0.809
18.	MAS-40-1	BC_1F_3 (HPU741×Tetep)	*Pi-1, Pi-ta & Pi-54*	0.845
19.	MAS-40-2	BC_1F_3 (HPU741×Tetep)	*Pi-1&Pi-54*	0.853
20.	MAS-41	BC_1F_3 (HPU741×Tetep)	*Pi-1, Pi-ta & Pi-54*	0.684
21.	MAS-43	BC_1F_3 (HPU741×Tetep)	*Pi-1, Pi-ta & Pi-54*	0.795
22.	MAS-44	BC_1F_3 (HPU741×Tetep)	*Pi-1&Pi-54*	0.788
23.	MAS-56	F_1(RG242-A[3]×RG241[1])	*Pi-ta*	0.791
24.	MAS-58	F_1(RG242-A[3]×RG241[1])	*Pi-ta*	0.725
25.	MAS-68	F_1\{RG242[2]× F_1(HPU741×ARBN42)\}	*Pi-1*	0.779
26.	MAS-70Q161	BC_1F_3(HPU741×Tetep)	*Pi-1, Pi-ta & Pi-54*	0.829
27.	MAS-70Q163-1	BC_1F_3(HPU741×Tetep)	*Pi-1, Pi-ta & Pi-54*	0.788
28.	MAS-70Q163-2	BC_1F_3(HPU741×Tetep)	*Pi-1, Pi-ta & Pi-54*	0.783
29.	MAS-70Q164	BC_1F_3(HPU741×Tetep)	*Pi-1, Pi-ta & Pi-54*	0.798
30.	MAS-71Q168	BC_1F_3(HPU741×Tetep)	*Pi-1, Pi-ta & Pi-54*	0.816
31.	MAS-71Q169	BC_1F_3(HPU741×Tetep)	*Pi-1, Pi-ta & Pi-54*	0.829
32.	MAS-71Q170	BC_1F_3(HPU741×Tetep)	*Pi-1&Pi-54*	0.823
33.	MAS-72Q171	BC_1F_3(HPU741×Tetep)	*Pi-1, Pi-ta & Pi-54*	0.838
34.	MAS-72Q172	BC_1F_3(HPU741×Tetep)	*Pi-1&Pi-54*	0.807
35.	MAS-72Q179	BC_1F_3(HPU741×Tetep)	*Pi-1&Pi-54*	0.798
36.	MAS-72Q180	BC_1F_3(HPU741×Tetep)	*Pi-1&Pi-54*	0.805
37.	MAS-72Q181	BC_1F_3(HPU741×Tetep)	*Pi-1, Pi-ta & Pi-54*	0.788
38.	MAS-75Q184	BC_1F_3(HPU741×Tetep)	*Pi-1, Pi-ta & Pi-54*	0.836
39.	MAS-75Q185	BC_1F_3(HPU741×Tetep)	*Pi-1, Pi-ta & Pi-54*	0.783
40.	MAS-75Q186	BC_1F_3(HPU741×Tetep)	*Pi-1&Pi-54*	0.804
41	MAS-76	F_1(RG243[4]×GR185[10])	*Pi-1*	0.830
42.	MAS-78	F_1(RG243[4]×GR185[10])	*Pi-1*	0.798
43.	MAS-107	BC_1F_3(HPU741×Tetep)	*Pi-1*	0.771
44.	MAS-118	BC_1F_3(HPU741×Tetep)	*Pi-ta*	0.835

* See Table 3.1 for details

The results obtained in the present investigation are in accordance with previous studies in rice where molecular markers were used as a complementary strategy to phenotypic analysis in order to improve and accelerate the backcross breeding programs (Semagn *et al.* 2007). It can be concluded that marker assisted selection is very effective in the early generation of crosses to accelerate the backcross breeding programme by selecting plants that recover most of the recurrent parent genome along with introgressed traits.

Summary & Conclusions



5. SUMMARY AND CONCLUSIONS

Blast is one of the major rice diseases that cause severe economic damage in all rice growing parts of the world. High genetic variability in pathogen combined with environmental conditions conducive to this disease, makes it difficult to control this disease in the North- Western Himalayan region. Gene pyramiding is one of the successful strategies used to obtain durable blast resistance in rice. MAS, provides a quick and efficient alternative to conventional phenotypic selection to obtain pyramids of resistance genes in backcross progenies and to select lines with required quantity of recurrent genome.

For the present study 44 BC_1F_4 lines resulting from crosses between recurrent parent 'HPU741' and donors of rice blast resistance genes i.e. 'C101LAC' (*Pi-1*), 'C101A51' (*Pi-2*) and 'Tetep' (*Pi-1, Pi-ta* and *Pi-k^h*) were evaluated using MAS to detect the presence of blast resistance genes '*Pi-1*', '*Pi-ta*' and '*Pi-k^h*'(Syn. *Pi-54*) and to estimate the proportion of recurrent parent (HPU741) genome in the individual lines.

Foreground selection i.e. detection of presence of *Pi-1, Pi-ta* and *Pi-54* gene combinations was done using SSR and STS markers already reported to be linked to these resistance genes. SSR markers RM224 and RM1233A were used to detect *Pi-1*, SSR markers RM206 and TRS26 to detect *Pi-54* and STS marker YL155/YL87 to detect *Pi-ta*. Of the 44 BC_1F_4 pyramid plants, 15 (MAS-10, MAS-16, MAS-19, MAS-34, MAS-68, MAS-76, MAS-78, MAS-107, MAS-22-1, MAS-22-2, MAS-31, MAS-56, MAS-58 and MAS-118) possessed single resistance gene, either *Pi-1* or *Pi-ta* or *Pi-54*; seven plants had two gene combinations and 15 (MAS-36, MAS-38, MAS-40-1, MAS-41, MAS-43, MAS-70Q161, MAS-70Q163-1, MAS-70Q163-2, MAS-70Q164, MAS-71Q168, MAS-71Q169, MAS-72Q171, MAS-72Q181, MAS-75Q184, MAS-75Q185) had all the three resistance genes i.e. *Pi-1, Pi-ta* and *Pi-54*. Among plants having two gene combinations all were *Pi-1* and *Pi-54* combination (MAS-40-2, MAS-44, MAS-71Q170, MAS-72Q172, MAS-72Q179, MAS-72Q180 and MAS-75Q186) and none were *Pi-1* and *Pi-ta* or *Pi-1* and *Pi-54* gene combinations. Seven plants (MAS-8, MAS-9, MAS-18, MAS-20, MAS-21, MAS-29, and MAS-33) did not possess any resistance genes.

Recurrent parent genome proportion in the 44 BC_1F_4 pyramid lines was estimated through background selection using 37 SSR and 13 ISSR markers. The results obtained with combined SSR and ISSR data showed that proportion of the recurrent parent genome in the BC_1F_4 lines ranged from 68.4% to 85.3% with an average of 76.8%, which does not deviate significantly from the expected recovery of 75%. While all the BC_1F_4 plants had high similarity to recurrent parent HPU741 than the donors of resistance genes, majority of plants possessing pyramids of two and three resistance genes had less similarity (formed a separate sub cluster) compared to plants with high proportion of the recurrent genome (fell in another sub cluster) and possessed either single resistance gene or no resistance gene. PCA analysis also successfully divided all lines into two groups with Group-1 comprising of the recurrent parent and 22 pyramid lines with no or single resistance gene and group-2 comprised of remaining 22 lines that are two and three resistance gene pyramids. In case of two gene pyramids, seven lines (MAS-72Q172, MAS72-Q179, MAS-72Q180, MAS-40-2, MAS-71Q170, MAS-75Q186 and MAS44) recovered more than 80% recurrent parent genome. Similarly, eight of the three gene pyramids (MAS-29, MAS-36, MAS-70Q164, MAS-38, MAS-71Q168, MAS-70Q161, MAS-71Q169, MAS-72Q171, MAS-75Q185, MAS-43 and MAS-41) had more than 80% of genome of HPU741. From these results, it is also evident that marker assisted selection cannot alter the expected recovery of the recurrent genome, but it has advantage in choosing plants with maximum proportion of recurrent parent genome and having desired gene pyramids, thus accelerating the backcrossing of selected individuals and considerably reducing time required to get plants with high proportion of desired genome.

However, all the two and three gene pyramid lines had more than 77% genome of the recurrent parent indicating that these can be subjected to agronomic evaluation under field conditions to explore the possibility of identification of a cultivar with durable blast resistance under the North-Western Himalayan region. Data in similarity matrix indicated that some of the three resistance gene pyramids i.e. MAS-38 (80.9%), MAS-71Q168 (81.6%), MAS-70Q161 (82.9%), MAS-71Q169 (82.9%) and MAS72Q171 (83.9%) had high proportion of the genome of HPU741 and are ideal candidates for agronomic evaluation.

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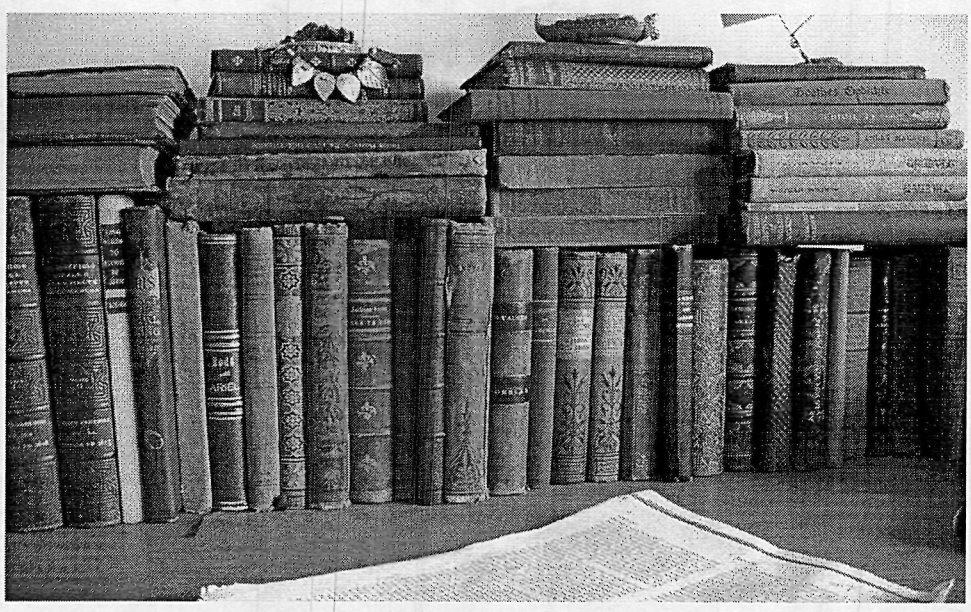

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