Development and Characterization of Blast Resistant Genetic Stocks in Rice for Pi 9 Gene through Backcross Breeding

Usha Kiran¹*, Manmohan Sharma¹*, Punya¹, R. K. Salgotra¹, Bikram Singh² and Amrinder Singh¹

¹School of Biotechnology, ²Division of Plant Breeding and Genetics, SKUAST-J, Chatha, Jammu (J&K), India

*Corresponding author

A B S T R A C T

A major issue in rice production is the control of Magnaporthe oryzae, the causal agent of rice blast. K 343 being a well adapted variety in the hill zone of Jammu and Kashmir region is susceptible to blast. Present study was undertaken to introgress the broad spectrum resistance gene Pi9 through marker assisted backcross breeding in order to avoid the losses due to this fungus. Foreground selection Pi9 gene in BC²F₁ (K 343³/RML 22) population with marker AP5930 identified 30 target gene positive plants. Background selection for analysis of recurrent parent genome in target gene positive plant using genome wide polymorphic SSR markers led to identification of three plants three plants (P3, P11, P28) in BC²F₁ which had recurrent parent genome recovery more than 85 percent. These plants exhibited broader similarity with recurrent parent with respect to agro-morphological traits and resistant to highly resistant reaction to PLP-1 strain of rice blast fungus M. oryzae. It indicates the effectiveness of resistance provided Pi9 gene. These plants would serve as genetic stocks for development of blast resistant lines/varieties or donor for development of blast resistant varieties.

Keywords: Rice, Blast disease, Pi9 gene, Foreground selection, Background selection

Introduction

Rice (Oryza sativa L.) is a self-pollinated diploid plant (2n=24) with a genome size of 430 Mb and is the most important staple cereal food crop in world. Asia accounts for 90 percent of global rice consumption, and total rice demand continues to rise. To ensure food security, the efficient way is the continuous improvement of rice yield (Liang et al., 2014) and the current level of production needs to be increased to 130 million tonnes by the year 2025. Inspite of being an important staple crop, rice is affected by many biotic and abiotic stresses. Of all the biotic stresses, blast caused by the heterothallic ascomycete fungus Magnaporthe oryzae (Hebert), is a major restriction on rice production in both tropical and temperate rice growing regions of the world (Raghu et al., 2018). This is a polycystic disease spread by asexual spores (conidia) that infect above ground tissues of rice plants (Ou et al., 1985; Talbot and Wilson, 2009; Pennisi, 2010).
Several rice blast epidemics have occurred in different parts of the world, resulting in heavy yield losses up to 90 percent (He et al., 2012; Singh et al., 2015; Li et al., 2019). The incidence of the disease has been reported in 85 countries, particularly in the irrigated and rainfed lowlands of temperate and subtropical Asia, Latin America and Africa (Sharma et al., 2012). The disease is a serious production constraint for rice in north western Himalayan region of India comprising the Union Territory Jammu and Kashmir, Uttrakhand and Himachal Pradesh (Sharma et al., 2002). Most of the popular rice varieties under cultivation in the hills of Jammu and Kashmir show variable reaction to blast varying from moderately resistant to highly susceptible response (Ali et al., 2009). Blast frequently affects coarse grain Kashmiri Japonica/Indica rice cultivars. Disease severity varies with weather, location, crop growth stage and the innate level of partial resistance of cultivars (Anwar et al., 2009). The disease can be managed through agronomic practices, use of fungicides, planting resistant cultivars and biotechnological interventions (Ribot et al., 2008). The excessive and indiscriminate use of fungicides prompts the evolution of resistance in the fungus, which in turn leads to disease resurgence, hence, breeding for host resistance is the most reliable, simple, economical and eco-friendly approach for the management of this disease (Khush and Jena, 2009). Thus, the present study was planned to introgress Pi9 blast resistance gene into well adapted rice variety K 343 using marker assisted selection.

**Genotyping of research material generated**

Genomic DNA was isolated following Doyle and Doyle (1990) method, with slight modifications. For foreground selection of Pi9 gene with SSR marker AP5930 (0.05cM) was done in BC2F1 population based on earlier studies (Sharma et al., 2005; Fjellstrom et al., 2006; Hangloo, 2018). A total of 51 SSR markers which had shown parental polymorphism between the parents K 343 and RML 22 were used for background selection of the BC2F1 population (K 343*RML 22). It was done to assess the recovery of recurrent parent genome and to select only those plants having maximum recovery of recurrent parent genome.

Amplification of DNA was carried out in PCR tubes with total volume of master mix 10μl containing 5.3μl of nuclease free water, 2.2 μl 5X PCR buffer with 15mM (MgCl2), 0.3 μl of 2.5 mM/μl dNTP, 0.5 μl of each forward and reverse primers, 5 U of Taq polymerase. An initial denaturation step (94°C) of 5 min was programmed in the thermo Cycler, followed by a loop of 35 cycles each consisting of denaturation (94°C for 30 sec), annealing (55°C – 58°C for 30 sec depending on the marker used) and extension (72°C for 30 sec). The final extension was performed at 72°C for 7 min.

**Evaluation of recurrent parent genome recovery in BC2F1 using GGT 2.0 software**

The SSR bands for all the plants in BC2F1 populations were counted and scored manually as “A” for their resemblance with the one parent, “B” for its resemblance with the other parent, “H” if both the bands were present i.e. resembled with both the parents.
and “-” if no band was present. The sizes of the bands were estimated by comparing them with 100bp standard marker along with the both the parents. The graphical representation of molecular marker data was done using computer programme GGT 2.0 (an acronym for Graphical GenoTypes) developed by Van Berloo (1999) at Wageningen University, The Netherlands. GGT 2.0 software is able to graphically represent chromosome wise and overall recovery of recurrent parent genome and also gives numerical representation of recurrent parent genome recovery (%) of each plant genotyped.

Phenotyping for agro-morphological traits in BC₂F₁ gene positive plants

The BC₂F₁ population along with parents K 343 and RML 22 were evaluated at Experimental Research Farm and Greenhouse at School of Biotechnology, SKUAST-Jammu during Kharif seasons of 2017. The 25 days old selected plants were transplanted with spacing of 15 × 20 cm in augmented design-II design in the field. Observations on single plants were recorded as per the DUS guidelines of IIRR, Hyderabad (Rani et al., 2006).

To test the significance of variations among different genotypes evaluated in the study, data with respect to blocks and treatments (including checks and test genotypes) were subjected to analysis of variance as per augmented design-II (Federer, 1956) to obtain adjusted trait values for checks as well as test genotypes.

Pathotyping of BC₂F₁ populations for blast symptoms

The pathotypic screening of the BC₂F₁ plants population was done using the PLP-1isolate of *Magnaporthe oryzae*, which is the predominant biotype in the North Western Himalayan region. All BC₂F₁ plants along with parents were inoculated with PLP-1 using spray as standardized by Bonman et al., (1986) under greenhouse at School of Biotechnology. The seedlings were inoculated with conidial suspension (1×10⁵ spores/ml) of *Magnaporthe oryzae* at the three to four leaf stages as described by (Sharma et al., 2005b). The inoculated plants were then placed in dark at high relative humidity (> 90%) for 24 h, and subsequently transferred to a polyhouse, under a regime of 16 h light/8 h dark at 80 per cent relative humidity. Day and night temperatures were maintained at 35± 2°C and 21± 2°C, respectively.

Disease reactions of inoculated plants were recorded on a scale of 0–5 (Bonman et al., 1986), 6–7 days after inoculation. The plants exhibiting reactions that scored 0-2 were considered resistant while those showing reactions that scored 3-5 were categorized as susceptible.

Results and Discussion

K 343 being a predominant rice variety in the hill zone of Jammu region shows moderate to susceptible response to blast fungus over the years depending upon the prevailing weather conditions. With the sequencing of whole rice genome closely linked/gene derived markers and potential donors for the genes are available. Thus, these genes can be easily deployed for generating resistance response in susceptible temperate rice variety (K 343). The marker assisted backcross breeding approach coupled with phenotypic selection helped in improving the elite genotype with blast resistance genes.

Foreground and background selections in backcross progenies

Advances in rice genomics and sequencing of whole rice genome have enabled the use of
DNA marker system mainly to track the trait of interest like disease resistance in segregating generations (Jena and Mackill, 2008). In the present investigation 42 BC$_2$F$_1$ plants were grown and screened for the presence of $Pi9$ gene by using closely linked marker AP5930. Out of the 42 BC$_2$F$_1$ plants (K 343*3 /RML 22), 30 plants were found positive for $Pi9$ gene using foreground selection (Plate1).

The purpose of background selection was to know the recovery of recurrent parent’s genome content in the backcross derived lines. Background selection was performed in 30 foreground positive BC$_2$F$_1$ plants using 51 polymorphic SSR markers (Plate 2).

The BC$_2$F$_1$ plants confirmed positive for the gene $Pi54$ and $Pi9$ were subjected to background selection to identify the plants with maximum percentage of recurrent parent genome. Foreground selection is often followed by recombinant selection process to select for recurrent parent alleles at markers flanking target regions with the aim of reducing linkage drag. Similar studies have carried out earlier by Singh et al., (2012a); Patroti et al., (2019) and Sagar et al., (2020).

**Background selection in BC$_2$F$_1$ stocks**

Background selection is the process of using markers to minimize the length of the donor segment around a target locus to accelerate the recovery of recurrent parent genome during backcrossing. Background selection in target gene ($Pi9$) positive plants in each of the genetic stock K 343*3/RML 22 led to estimation of percent recurrent parent genome recovery using about 51 genome wide polymorphic SSR markers. Genotypic data when analyzed using GGT 2.0 software (Van Berloo, 1999) identified 3 plants as P3 (86.4%), P11 (85.8%) and P28 (93.25%) with chromosomes 1, 2 and 10 showing 85-90 percent of recovery in most of the plants in the stock population (Table 1), (Figure 1). Thus marker assisted background selection is a potential tool to identify the plants among the large population having more than average recurrent parent genome recovery and thus accelerates the pace of selection and development of varieties in comparison to conventional breeding approaches of selection. Integration of foreground, background and/or phenotypic selection to achieve high recovery of recurrent parent genome and phenome has been practiced in various studies Neeraja et al., (2005); Sundaram et al., (2008); Gopala Krishnan et al., (2008); Singh et al., (2012a); Divya et al., (2014) and Miah et al., (2014); Patroti et al., (2019) and Sagar et al., (2020).

Analysis of variance for morphological/agronomical traits in both BC$_2$F$_1$ populations exhibited non-significant variations for most of the agro-morphological traits except for plant height, number of effective tillers and grain length which gave indication about uniformity of traits in genetic stocks (Table 2). Many test entries with the target genes showed grain yield slightly higher than the recurrent parent, K 343 (Table 3). Most of the test entries were similar in various morpho-physiological traits like the recipient parent, K 343. Maximum grain yield was recorded in P12 (27g) followed by P25 (26.9g), P11 (26.5g) and P13 (26.3g) whereas a minimum grain yield of 24g was recorded in P30. The average grain yield per plant was recorded as 25.60g with the range varying from 24g- 27g (Table 3). In case of plant height, the maximum value was recorded in P2 (133.1cm), followed by P21 (132.1cm) and P23 (131.7) where as P29 recorded a minimum plant height i.e. 121.21cm. The range for plant height in the BC$_2$F$_1$ population was between 121.21- 133.10cm with an average of 128.77cm. The number of effective tillers per plant ranged between 8-9.
with an average of 8.83. The maximum numbers of effective tillers per plant were recorded in P1 (9) where as the minimum numbers of effective tillers per plant were recorded in P4 (8). In case of panicle length, the maximum value was recorded in P3 (26.20cm) followed by P12 (25cm) and P13 (24.4cm) whereas the minimum value was recorded in P16 (18.90cm). The panicle length had a range varying from 18.90 - 26.20cm with an average value of 22.12 cm. Highest value of 1000-grain weight was observed in P9 (28.50g), followed by P15 (28.10g) and P17 (28g) whereas the lowest 1000- grain weight was recorded in P25 (23.60g).

Table 1 Recurrent parent genome recovery in BC$_2$F$_1$ population (K 343$^*$3/RML 22)

| Genotypes | A%  | B%  |
|-----------|-----|-----|
| P1        | 84.85 | 15.15 |
| P2        | 55.80 | 44.10 |
| P3        | 86.40 | 13.50 |
| P4        | 80.80 | 19.20 |
| P5        | 70.50 | 29.50 |
| P6        | 59.05 | 40.95 |
| P7        | 70.05 | 29.95 |
| P8        | 79.50 | 20.50 |
| P9        | 70.50 | 29.50 |
| P10       | 69.20 | 30.70 |
| P11       | 85.80 | 14.20 |
| P12       | 63.90 | 36.10 |
| P13       | 74.35 | 25.65 |
| P14       | 68.55 | 31.45 |
| P15       | 61.60 | 38.40 |
| P16       | 65.30 | 34.70 |
| P17       | 29.80 | 70.20 |
| P18       | 33.50 | 66.50 |
| P19       | 60.95 | 39.05 |
| P20       | 73.30 | 26.70 |
| P21       | 56.75 | 43.25 |
| P22       | 64.40 | 35.60 |
| P23       | 58.75 | 41.25 |
| P24       | 63.60 | 36.40 |
| P25       | 71.70 | 28.20 |
| P26       | 65.50 | 34.50 |
| P27       | 65.40 | 34.60 |
| P28       | 93.25 | 6.75  |
| P29       | 74.65 | 25.35 |
| P30       | 64.35 | 35.65 |
Table 2 Analysis of Variance of genotypes BC$_2$F$_1$ (K 343*/RML 22) for yield and yield contributing traits

| Source of variation | DF | Plant height (cm) | Days to 50% flowering | Days to maturity | Duration of grain filling | Panicle length (cm) | Effective tillers | Grain length (mm) | Grain breadth (mm) | Grain yield/plant (g) | 1000 grain weight (g) |
|---------------------|----|------------------|-----------------------|-----------------|--------------------------|---------------------|-----------------|-----------------|-------------------|----------------------|----------------------|
| Blocks              | 2  | 0.071            | 2.666                 | .000            | 2.66                     | 4.80                | 0.000*          | 0.025           | 0.0034            | 1.349                | 2.101                |
| Treatment           | 31 | 7.906*           | 6.282                 | 7.322           | 0.561                    | 3.47                | 0.198*          | 0.073*          | 0.0353            | 1.654                | 1.538                |
| Tests               | 29 | 7.870*           | 7.322                 | 2.107           | 0.868                    | 0.355               | 2.86            | 0.141*          | 0.066*            | 0.0189               | 0.538                | 0.975                |
| Checks              | 1  | 11.76*           | 6.282                 | 60.16*          | 4.16                     | 8.16*              | 1.50*           | 0.20*           | 0.17*             | 12.24*               | 6.82                 |
| Test v/s checks     | 1  | 5.08*            | 105.80                | 73.47*          | 2.93                     | 16.56*             | 0.55*           | 0.16*           | 0.374*            | 23.42*               | 12.55                |
| Error               | 2  | 0.01             | 0.66                  | 0.00            | 0.66                     | 0.41               | 0.00            | 0.003           | 0.005             | 0.37                 | 0.22                 |

Table 3 Mean performance of genotypes BC$_2$F$_1$ (K 343*/RML 22) for yield and yield contributing traits

| Genotypes | Plant height(cm) | Days to 50% Flowering | Days to maturity | Duration of grain filling | Panicle length (cm) | No. of effective tillers/plant | Grain length (mm) | Grain breadth (mm) | Grain yield/plant (g) | 1000 grain weight (g) |
|-----------|------------------|-----------------------|-----------------|--------------------------|---------------------|-------------------------------|------------------|-------------------|----------------------|----------------------|
| K 343     | 129.2            | 93                    | 128             | 35                       | 19.5                | 9                             | 5.44             | 2.47              | 26.1                 | 25.8                 |
| RML 22    | 126.5            | 86                    | 120             | 34                       | 18                  | 8                             | 5.11             | 2.01              | 22.51                | 24                   |
| P1        | 129.20           | 93                    | 128             | 35                       | 20.90               | 9                             | 5.70             | 2.48              | 25.30                | 27.40                |
| P2        | 133.10           | 93                    | 128             | 35                       | 21.20               | 9                             | 5.31             | 2.54              | 25.70                | 25.10                |
| P3        | 133.10           | 93                    | 128             | 35                       | 26.20               | 9                             | 5.21             | 2.52              | 26.00                | 25.50                |
| P4        | 125.30           | 93                    | 128             | 35                       | 23.30               | 8                             | 5.92             | 2.63              | 26.20                | 27.40                |
| P5        | 133.10           | 93                    | 128             | 35                       | 22.90               | 9                             | 5.44             | 2.67              | 26.00                | 25.20                |
|   | P6     | P7     | P8     | P9     | P10    | P11    | P12    | P13    | P14    | P15    | P16    | P17    | P18    | P19    | P20    | P21    | P22    | P23    | P24    | P25    | P26    | P27    | P28    | P29    | P30    | Mean   | CV     | SE(m)  |
|---|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
|   | 129.30 | 130.30 | 129.10 | 127.10 | 130.30 | 128.90 | 126.30 | 127.30 | 127.30 | 131.30 | 129.10 | 130.10 | 130.90 | 129.80 | 129.30 | 132.10 | 127.30 | 131.70 | 129.20 | 129.00 | 121.51 | 125.52 | 125.20 | 121.21 | 130.30 | 128.77 | 92.66  | 0.17   |
|   | 93     | 93     | 93     | 93     | 93     | 94     | 94     | 94     | 94     | 94     | 94     | 89     | 89     | 89     | 89     | 93     | 93     | 93     | 93     | 93     | 93     | 93     | 93     | 93     | 93     | 128.60 | 35.93  | 0.17   |
|   | 128    | 128    | 128    | 128    | 128    | 131    | 131    | 131    | 131    | 131    | 131    | 128    | 128    | 128    | 128    | 128    | 128    | 128    | 128    | 128    | 128    | 128    | 128    | 128    | 35.93  | 8.83   | 0.17   |
|   | 35     | 35     | 35     | 35     | 35     | 37     | 37     | 37     | 37     | 37     | 37     | 39     | 39     | 39     | 39     | 35     | 35     | 35     | 35     | 35     | 35     | 35     | 35     | 35     | 35     | 0.17   | 0.17   | 0.17   |
|   | 21.70  | 21.20  | 21.00  | 21.60  | 23.50  | 22.20  | 25.00  | 24.40  | 19.50  | 22.90  | 18.90  | 21.30  | 21.60  | 22.90  | 22.30  | 18.10  | 20.80  | 21.10  | 22.30  | 21.70  | 20.80  | 21.70  | 21.00  | 21.60  | 23.50  | 22.12  | 8.83   | 0.17   |
|   | 8      | 9      | 9      | 9      | 9      | 9      | 8      | 9      | 9      | 9      | 9      | 9      | 8      | 8      | 9      | 9      | 9      | 9      | 8      | 9      | 8      | 8      | 8      | 9      | 9      | 9      | 9      | 9      | 8      | 5.64   |
|   | 5.55   | 5.22   | 5.19   | 5.61   | 5.39   | 5.59   | 5.99   | 5.99   | 5.25   | 5.57   | 5.01   | 5.95   | 5.34   | 5.25   | 5.87   | 5.43   | 5.21   | 5.22   | 5.55   | 5.21   | 5.56   | 5.42   | 5.22   | 5.19   | 5.61   | 5.45   | 5.64   |
|   | 2.45   | 2.47   | 2.52   | 2.61   | 2.53   | 2.37   | 2.70   | 2.65   | 2.18   | 2.65   | 2.36   | 2.43   | 2.58   | 2.45   | 2.65   | 2.68   | 2.57   | 2.53   | 2.60   | 2.30   | 2.60   | 2.45   | 2.47   | 2.21   | 2.21   | 2.50   | 2.51   |
|   | 26.10  | 25.00  | 26.20  | 26.60  | 26.50  | 27.00  | 26.40  | 26.50  | 25.70  | 25.50  | 26.10  | 25.20  | 25.60  | 25.60  | 25.20  | 25.20  | 25.50  | 24.30  | 24.12  | 24.01  | 25.51  | 24.00  | 24.21  | 24.01  | 25.60  | 25.91  |
|   | 26.60  | 27.50  | 28.50  | 28.50  | 28.50  | 26.50  | 28.00  | 26.00  | 25.70  | 28.10  | 25.30  | 25.00  | 25.00  | 25.50  | 25.20  | 25.20  | 25.50  | 25.20  | 24.30  | 23.60  | 25.61  | 25.40  | 24.21  | 25.51  | 25.60  | 25.91  |
| Mean|        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| CV  | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      |
| SE(m)| 0      | 0.17   | 0      | 0.17   | 0.17   | 0      | 0      | 0      | 0      | 0.17   | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0.17   | 0      |
Table 4 Pathotyping of BC$_2$F$_1$ (K 343*3/RML 22) plants for blast symptoms

| S. No. | Genotype | Score | Disease reaction |
|--------|----------|-------|------------------|
| 1      | K 343    | 3     | Susceptible      |
| 2      | RML 22   | 0     | Resistant        |
| 3      | P1       | 0     | Highly Resistant |
| 4      | P2       | 1     | Resistant        |
| 5      | P3       | 0     | Highly Resistant |
| 6      | P4       | 0     | Highly Resistant |
| 7      | P5       | 2     | Moderately Resistant |
| 8      | P6       | 2     | Moderately Resistant |
| 9      | P7       | 2     | Moderately Resistant |
| 10     | P8       | 0     | Highly Resistant |
| 11     | P9       | 2     | Moderately Resistant |
| 12     | P10      | 1     | Resistant        |
| 13     | P11      | 2     | Moderately Resistant |
| 14     | P12      | 2     | Moderately Resistant |
| 15     | P13      | 2     | Moderately Resistant |
| 16     | P14      | 2     | Moderately Resistant |
| 17     | P15      | 2     | Moderately Resistant |
| 18     | P16      | 2     | Moderately Resistant |
| 19     | P17      | 0     | Highly Resistant |
| 20     | P18      | 1     | Resistant        |
| 21     | P19      | 2     | Moderately Resistant |
| 22     | P20      | 2     | Moderately Resistant |
| 23     | P21      | 2     | Moderately Resistant |
| 24     | P22      | 2     | Moderately Resistant |
| 25     | P23      | 2     | Moderately Resistant |
| 26     | P24      | 2     | Moderately Resistant |
| 27     | P25      | 2     | Moderately Resistant |
| 28     | P26      | 2     | Moderately Resistant |
| 29     | P27      | 2     | Moderately Resistant |
| 30     | P28      | 0     | Highly Resistant |
Table 5 Agronomical and pathological status of genetic stocks K 343*3/RML 22 with maximum RPG recovery

| K 343*3/RML 22 | Gene positive plants Pi9 | RPG (%) | Disease score | Plant height (cm) | Days to 50 percent flowering | Days to maturity | Duration of grain filling | Panicle length (cm) | Effective tillers | Grain length (mm) | Grain breadth (mm) | Yield per plant (g) | 1000 grain weight (g) |
|----------------|--------------------------|---------|---------------|------------------|----------------------|------------------|-------------------------|------------------|-----------------|-----------------|-----------------|------------------|-------------------|
|                | RML 22                   | K 343   | P 3           | P 11             | P 28                 |                  |                         |                  |                 |                 |                 |                  |                   |
| RPG (%)        | 86.4                     | 85.8    | 93.2          |                  |                      |                  |                         |                  |                 |                 |                 |                  |                   |
| Disease score  | 0                        | 3       | 0             | 0                | 0                    |                  |                         |                  |                 |                 |                 |                  |                   |
| Plant height (cm) | 126.5                   | 129.2   | 133.1         | 128.9            | 125.2                |                  |                         |                  |                 |                 |                 |                  |                   |
| Days to 50 percent flowering | 86            | 93      | 93            | 94               | 93                   |                  |                         |                  |                 |                 |                 |                  |                   |
| Days to maturity | 120                      | 128     | 128           | 131              | 128                  |                  |                         |                  |                 |                 |                 |                  |                   |
| Duration of grain filling | 34                      | 35      | 35            | 37               | 35                   |                  |                         |                  |                 |                 |                 |                  |                   |
| Panicle length (cm) | 18                      | 19.5    | 26.2          | 22.2             | 21                   |                  |                         |                  |                 |                 |                 |                  |                   |
| Effective tillers | 8                       | 9       | 9             | 9                | 9                    |                  |                         |                  |                 |                 |                 |                  |                   |
| Grain length (mm) | 5.1                      | 5.4     | 5.2           | 5.3              | 5.2                  |                  |                         |                  |                 |                 |                 |                  |                   |
| Grain breadth (mm) | 2.0                      | 2.4     | 2.5           | 2.5              | 2.4                  |                  |                         |                  |                 |                 |                 |                  |                   |
| Yield per plant (g) | 22.5                   | 26.1    | 26            | 26.5             | 24.2                 |                  |                         |                  |                 |                 |                 |                  |                   |
| 1000 grain weight (g) | 24                      | 25.8    | 25.5          | 26.4             | 24.1                 |                  |                         |                  |                 |                 |                 |                  |                   |
The mean value of 1000-grain weight recorded was 25.91g and ranged between and 23.60-28.50g respectively. In case of days to 50 percent flowering the BC$_2$F$_1$ which took maximum days to flowering were P11 (94 days) where as the minimum number of days to 50 percent flowering were recorded in P17 (89) and ranged between 89-94 with an average value of 92.66 days. Duration of grain filling in the BC$_2$F$_1$ ranged from 35-39 with an average value of 35.93 days. P17 took maximum duration of grain filling (39days) followed by P11 (37days) whereas the P1 took minimum number of duration of grain filling (35 days).

**Pathotyping of BC$_2$F$_1$ population (K 343*/3 /RML 22)**

All the 30 gene positive plants carrying $Pi9$ gene in the background of K 343 in BC$_2$F$_1$ generation along with the donor and recipient parents were inoculated with PLP-1 strain of *M. oryzae*. These plants showed 0-2 score depicting resistant reaction while the recipient parent K 343 showed susceptible reaction with the score 3 (Table 4).

The genetic stocks of K 343*/3/RML 22 with maximum recovery of recurrent parent genome were compared agronomically and pathologically with the recurrent parent (Table 5). The maximum recovered recurrent parent genome in plant numbers P3, P11 and P28 had broader agronomical similarity to the recurrent parent and pathologically related to the donor parent. The results confirmed the accuracy of marker assisted selection (MAS) for the gene $Pi9$ using the corresponding marker AP5930. These results revalidate the findings of Sharma *et al.*, (2005a) and Rathour *et al.*, (2008). These plants would serve as genetic stocks for development of blast resistant lines/varieties or donor for development of blast resistant varieties.
References

Ali, A., Teli, M. A., Bhat, G. N., Parray, G. A., and Wani. 2009. Status of rice blast (Pyricularia grisea), cultivar reaction and races of its causal fungus in temperate agro-ecosystem of Kashmir, India. SAARC Journal of Agriculture. 7(2): 25-37.

Anwar, A., Teli, M. A., Bhat, G. N., Parray, G.A., and Wani. 2009. Status of rice blast (Pyricularia grisea) cultivar reaction and races of its causal fungus in temperate agro-ecosystem of Kashmir, India. SAARC Journal of Agriculture. 7(2): 25-37.

Bonman, J. M. 1986. Physiologic Specialization of Pyricularia oryzae in the Philippines. Plant Dis. 70(8): 767.

Divya, B., Robin, S., Rabindran, R., Senthil, S., Raveendran, M., and Joel, A. J. 2014. Marker assisted backcross breeding approach to improve blast resistance in Indian rice (Oryza sativa) variety ADT43. Euphytica 200: 61–77.

Doyle, J.J., and Doyle, J.L. 1990. Isolation of plant DNA from fresh tissue. Focus. 12:13-15.

Federer, W. T., and Raghavarao, D. 1975. On Augmented Designs. Biometrics. 31(1): 29.

Fjellstrom, R., McClung, A. M., and Shank, A. R. 2006. SSR Markers Closely Linked to the Pi-z Locus are Useful for Selection of Blast Resistance in a Broad Array of Rice Germplasm. Molecular Breeding. 17(2): 149-157.

Frisch, M., Bohn, M., and Melchinger, A. A. 1999. Minimum Sample Size and Optimal Positioning of Flanking Markers in Marker- Assisted Backcrossing for Transfer of a Target Gene. Crop Science. 39(4): 967-975.

Gopala, Krishnan, S., Sharma, R.K., Rajkumar, K.A., Joseph, M., Singh, V.P., Singh, A.K., Bhat, K.V., Singh, N.K., Mohapatra, T. 2008. Integrating marker assisted background analysis with foreground selection for identification of superior bacterial blight resistant recombinants in Basmati rice. Plant Breeding. 127:131-139.

Hangloo, S. 2018. Generation of Genetic Stocks for Blast Resistance in Susceptible Temperate Variety of Rice (Oryza sativa) L. Using Marker Assisted Selection. Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Jammu and Kashmir, India.

He, X., Liu, X., Wang, L., Lin, F., Cheng, Y., and Pan, Q. 2012. Identification of the novel recessive gene pi55(t) conferring resistance to Magnaporthe oryzae. Science China Life Sciences. 55:141-149.

Jena, K. K., and Mackill, D. J. 2008. Molecular Markers and Their Use in Marker-Assisted Selection in Rice. Crop Science, 48(4): 1266-1276.

Khush, G.S., and Jena, K.K. 2009. Current status and future prospects for research on blast resistance in rice (Oryza sativa L.) In: Wang, G.L., valent, B. Advances in genetics, genomics, and control of rice blast disease. New York, pp1-10.

Li, W., Zhu, Z., Chern, M., Yin, J., Yang, C., Ran, L., Chen, X. 2017. A Natural Allele of a Transcription Factor in Rice Confers Broad-Spectrum Blast Resistance. Cell, 170(1): 114-126 e115.

Li, Y., Li, J., Zhang, M., Tian, L., Yang, S., Li, P., and Zhang, Y. 2019. Analysis of blast resistance genes in japonica rice core collection and progeny in Ningxia. J Plant Genet Res. 20(2): 321–334.

Liang, W.H., Shang, F., Lin Q.T., Lou C., and Zhang, J. 2014. Tillering and panicle branching genes in rice. Gene. 537: 1-5.

Miah, G., Rafii, M.Y., Ismail, M.R., Putheh, A.B., Rahim, H.A., and Latif, M.A. 2014. Improvement of MR219-rice variety for blast resistance through marker-assisted backcross breeding. Molecular Breedin., 27:129–135.

Neeraja, C.N., Hariprasad, A.S., Malathi, S. and Siddiq, E.A. 2005. Characterization of tall landraces of rice (Oryza sativa L.) using gene-derived simple sequence repeats. Current Science. 88:149-152.

Ou, S. H. 1985. Rice Diseases. 2nd Edn, Commonwealth Agricultural Bureaux., Commonwealth Mycological Institute, Kew, England.

Patroti, P., Vishalakshi, B., Umakanth, B., Suresh,
J., Senguttuvel, P., and Madhav, M. S. 2019. Marker-assisted pyramiding of major blast resistance genes in Swarna-Sub1, an elite rice variety (Oryza sativa L.). Euphytica. 215(11).

Penniset, E. 2010. Armed and dangerous. Science. 327(5967), 804-805.

Raghu, S., Yadav, M. K., Prabhukarthikeyan, S. R., Baite, M. S., Lenka, S., and Jena, M. 2018. Occurrence, pathogenicity, characterization of Fusarium fujikuroi causing rice bakanae disease from Odisha and in vitro management. Oryza. 55, 214–23.

Ramkumar, G., Srinivasarao, K., Madhan Mohan, K., Sudarsha, I., Sivaranjani, A.K.P., Gopalakrishna, K., Neeraja, C.N., Balachandran, S.M., Sundaram, R.M., Prasad, M.S., Shobha Rani, N., Rama Prasad, A.M., Viraktamath, B.C., Madhav, M.S. 2011. Development and validation of functional marker targeting an InDel in the major rice blast disease resistance gene Pi54 (Pikh). Molecular Breeding. 27:129–135.

Rani, N. S., Rao, L. V.S., and Viraktamath, B. C. 2006. National Guidelines for the conduct of tests for Distinctness, Uniformity and Stability: Rice (Oryza sativa L.) – Zero Draft, Directorate of Rice Research, Hyderabad Andhra Pradesh, India, PP: 39.

Rathour, R., Chopra, M., and Sharma, T. R. 2008. Development and validation of microsatellite markers linked to the rice blast resistance gene Pi-z of Fukunishiki and Zenith. Euphytica. 163(2): 275-282.

Ribot, C., Hirsch, J., Balzergue, S., Tharreau, D., Notteghem, J. L., Lebrun, M. H., and Morel, J. B. 2008. Susceptibility of rice to the blast fungus, Magnaporthe grisea. J Plant Physiol. 165(1): 114-124.

Sagar, V., Dhawan, G., Gopala Krishnan, S., Vinod, K. K., Ellur, R. K., Mondal, K. K., Singh, A. K. 2020. Marker assisted introgression of genes governing resistance to bacterial blight and blast diseases into an elite Basmati rice variety, ‘Pusa Basmati 1509’. Euphytica. 216(1).

Sharma, T.R., Chauhan, R.S., Singh, B.M., Paul, R., Sagar, V., and Rathore, R. 2002. RAPD and pathotype analysis of Magnaporthe grisea population from North-western Himalayan region of India. Journal of Phytopathology. 150: 649–656.

Sharma, T. R., Madhav, M. S., Singh, B. K., Shanker, P., Jana, T. K., Dalal, V., and Singh, N. K. 2005a. High-resolution mapping, cloning and molecular characterization of the Pi-k (h) gene of rice, which confers resistance to Magnaporthe grisea. Mol Genet Genomics. 274(6): 569-578.

Sharma, T. R., Shanker, P., Singh, B. K., Jana, T. K., Madhav, M. S., Gaikwad, G., Singh, N. K., Plaha, P., and Rathour, R. 2005b. Molecular mapping of rice blast resistance gene Pi-kh in rice variety Tetet. Journal of Plant Biochemistry and Biotechnology. 14: 127–133.

Sharma, T. R., Rai, A. K., Gupta, S. K., Vijayan, J., Devanna, B. N., and Ray, S. 2012. Rice Blast Management Through Host-Plant Resistance: Retrospect and Prospects. Agricultural Research. 1(1): 37-52.

Singh, A., Singh, V.K., Singh, S.P., Pandian, R.T.P., Ellur R.K., Singh, D., Bhowmick, P.K., Gopala Krishnan, S., Nagarajan, M., Vinod, K.K., Singh, U.D., Prabhu, K.V., Sharma, T.R., Mohapatra, T and Singh, A.K. 2012. Molecular breeding for the development of multiple disease resistance in Basmati rice. Annals of Botany Plants. 1-13.

Singh, S. K., Bhatia, P. K., Sharma, A., and Sahu, V. 2015. Super hybrid rice in china and India: current status and future prospects. International Journal of Agriculture and Biology. 17: 221-232.

Sundaram, R.M., Vishnupriya, R.M., Biradar, S.K., Laha, G.S., Ashok Reddy, G., Shobha Rani, N., Sarma, N.P., Sonti, R.V. 2008. Marker assisted introgression of bacterial blight resistance in Samba Mahsuri, an elite indica rice variety. Euphytica. 160:411-422.

Talbot, N. J., and Wilson, R. A. 2009. Under pressure: investigating the biology of plant infection by Magnaporthe oryzae. Nature Reviews Microbiology. 7(3): 185-195.

Van Berloo, R. 1999. Computer note. GGT: software for the display of graphical
genotypes. Journal of Heredity. 90(2): 328-329.

Visscher, P. M., Haley, C. S., and Thompson, R. 1996. Marker-assisted introgression in backcross breeding programs. Genetics. 144(4): 1923-1932.

Wang, B.-h., Ebbole, D. J., and Wang, Z.-h. 2017. The arms race between Magnaporthe oryzae and rice: Diversity and interaction of Avr and R genes. Journal of Integrative Agriculture. 16(12): 2746-2760.

Zhao, H., Wang, X., Jia, Y., Minkenberg, B., Wheatley, M., Fan, J., Yang, Y. 2018. The rice blast resistance gene Prr encodes an atypical protein required for broad-spectrum disease resistance. Nat Commun. 9(1): 2039.

Singh, A., Singh, V.K., Singh, S.P., Pandian, R.T.P., Ellur R.K., Singh, D., Bhownick, P.K., Gopala Krishnan, S., Nagarajan, M., Vinod, K.K., Singh, U.D., Prabhu, K.V., Sharma, T.R., Mohapatra, T and Singh, A.K. 2012. Molecular breeding for the development of multiple disease resistance in Basmati rice. Annals of Botany Plants. 1-13.

Patroti, P., Vishalakshi, B., Umakanth, B., Suresh, J., Senguttuvel, P., and Madhav, M. S. 2019. Marker-assisted pyramiding of major blast resistance genes in Swarna-Sub1, an elite rice variety (Oryza sativa L.). Euphytica. 215(11).

Sagar, V., Dhanwan, G., Gopala Krishnan, S., Vinod, K. K., Ellur, R. K., Mondal, K. K., Singh, A. K. 2020. Marker assisted introgression of genes governing resistance to bacterial blight and blast diseases into an elite Basmati rice variety, ‘Pusa Basmati 1509’. Euphytica. 216(1).

Neeraja, C.N., Hariprasad, A.S., Malathi, S. and Siddiq, E.A. 2005. Characterization of tall landraces of rice (Oryza sativa L.) using gene-derived simple sequence repeats. Current Science. 88:149-152.

Sundaram, R.M., Vishnupriya, R.M., Biradar, S.K., Laha, G.S., Ashok Reddy, G., Shobha Rani, N., Sarma, N.P., Sonti, R.V. 2008. Marker assisted introgression of bacterial blight resistance in Samba Mahsuri, an elite indica rice variety. Euphytica. 160:411-422.

Gopala, Krishnan,S., Sharma, R.K., Rajkumar, K.A., Joseph, M., Singh, V.P., Singh, A.K., Bhat, K.V., Singh, N.K., Mohapatra, T. 2008.Integrating marker assisted background analysis with foreground selection for identification of superior bacterial blight resistant recombinants in Basmati rice. Plant Breeding. 127:131-139.

Divya, B., Robin, S., Rabindran, R., Senthil, S., Raveendran, M., and Joel, A. J. 2014. Marker assisted backcross breeding approach to improve blast resistance in Indian rice (Oryza sativa) variety ADT43. Euphytica. 200: 61–77.

Miah, G., Rafii, M.Y., Ismail, M.R., Puteh, A.B., Rahim, H.A., and Latif, M.A. 2014. Improvement of MR219-rice variety for blast resistance through marker-assisted backcross breeding. Molecular Breeding. 27:129–135.

How to cite this article:

Usha Kiran, Manmohan Sharma, Punya, R. K. Salgotra, Bikram Singh and Amrinder Singh. 2020. Development and Characterization of Blast Resistant Genetic Stocks in Rice for Pi 9 Gene through Backcross Breeding. Int.J.Curr.Microbiol.App.Sci. 9(10): 310-322. doi: https://doi.org/10.20546/ijcmas.2020.910.039