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RESEARCH ARTICLE

Differential gene effectiveness and blast disease progression in MAS derived NILs in the background of aromatic rice landrace *Mushk Budji*

Haroon R. Wani, Saba Mir[1], Raheel S. Khan, Noor-ul-Ain, Gazala H. Khan[1], Najeeb-ul-Rehman Sofi[1] and Asif B. Shikari[*]

Abstract

Mushk Budji is a premium quality scented rice landrace of Kashmir, which is highly susceptible to rice blast cause by *Magnaporthe oryzae* with more than 70% grain yield loss. Near-isogenic lines (NILs), namely, SKUA-27-4-40-9, SKUA-27-20-10-2 and SKUA-27-13-5-1, for the blast resistance genes, *Pi54, Pi1*and *Pita* were successfully developed in the background of *Mushk Budji*. Marker-assisted foreground selection was carried out using gene-based and closely linked markers viz., Pi54 MAS (*Pi54*), RM224 (*Pi1*) and YL155/87 (*Pita*). The background analysis was done with 90 genome-wide distributed SSR markers linked to previously tagged SNPs, which helped in estimating the recurrent parent genome (RPG) recovery in the NILs. An area under the disease progress curve (AUDPC) was drawn to test the effectiveness of the individual-resistance genes in the developed NILs. A differential reaction pattern exhibited by the individual genes helped validate the respective genes' effectiveness under the Kashmir conditions. The *Pi54* and *Pita* were found to be effective in conferring resistance towards *M. oryzae* infection in the NILs of *Mushk Budji.*

Keywords: Rice (*Oryza sativa* L), rice blast, *Mushk Budji*, NILs, *Pi1, Pita, Pi54*

Introduction

Short-grained fragrant rice is popular throughout the country and epitomizes a rich cultural heritage of inhabited ecological niches. Apart from the cultivation of high-yielding varieties, fragrant rice landraces in Jammu and Kashmir occupy specific locations and pockets. The most notable among them is *Mushk Budji*, which is known for its aroma and carries substantial demand in local markets. However, *Mushk Budji* is highly susceptible to rice blast caused by *Magnaporthe oryzae* and suffers more than 70% grain yield loss on a seasonal basis. Frequent crop failures due to blast disease have become a major challenge to the farmers and remain a cause for decline in its area. At the global level rice blast has become the most serious disease of rice, causing yearly output losses ranging from 10 to 30% (Zeigler et al., 1994). Previously, we developed a genetically improved three-gene pyramided line carrying *Pi54+Pi1+Pita* in the background of *Mushk Budji* through marker-assisted backcross breeding approach (Khan et al. 2018). Subsequently, the pyramided line ($BC_2F_{4:6}$) was again backcrossed to the recurrent parent, *Mushk Budji* to yield BC_3F_1 NILs carrying three major genes viz., *Pi54, Pi1* and *Pita* to study the response of resistant genes individually and their effectiveness against *Magnaporthe oryzae* isolates under open field conditions from seedling to adult plant stage. We were interested in generating information on disease progress throughout the different growth stages and noting the behavior of genes against neck blast. The isolation of advanced-generation NILs was aimed at developing stable gene donors in the japonica rice background.

Division of Genetics & Plant Breeding, Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir, Wadura, Jammu & Kashmir, India.

[1]Mountain Research Centre for Field Crops, Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir, Wadura, Jammu & Kashmir, India.

***Corresponding Author:** Asif B. Shikari, Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir, Wadura, Jammu & Kashmir, India, E-Mail: asifshikari@skuastkashmir.ac.in

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Materials and methods

The experiment was conducted at Mountain Research Centre for Field Crops, SKUAST-Kashmir, Khudwani (34.5°N, 77.0°E; 1560 msl). The investigations were carried out using $BC_3F_{2:3}$ plants generated from a cross between improved *Mushk Budji* pyramided line, namely, SKUA-485-27 ($BC_2F_{4:6}$) carrying genes *Pi54+Pi1+Pita* and recurrent parent (RP) *Mushk Budji*. The pyramided line was previously developed from crossing a medium slender donor DHMAS 70Q 164-1b carrying blast resistance genes, *Pi54+Pi1+Pita* and aromatic landrace *Mushk Budji* (Khan et al.,2018).

Marker analysis

The plant DNA was extracted using the CTAB (Cetyl-Tri Methyl Ammonium Bromide) method published by Murray and Thompson (1980). PCR assay was carried out in a 10 µL reaction volume using 25 ng of DNA,10x PCR buffer (10 mMTris, pH 8.4, 50 mMKCl, 1.8 mM $MgCl_2$), 2 mMdNTPs (MBI, Fermentas, USA), 5 pmol each of forward and reverse primer and 5U of Taq DNA polymerase (MBI, Fermentas, USA). The derived lines in BC_3F_2were subjected to foreground selection with the help of InDel marker Pi54 MAS (*Pi54*), linked SSR marker RM224 (*Pi1*) and gene-based SNP marker YL155/87 (*Pita*). The background selection was carried out using 90 genome-wide STMS markers. The SSR markers were selected based on the previously known SNP locations on 'OsSNPnks' 50K Axiom® 2.0 SNP array (Singh et al. 2015) identified for the $BC_2F_{4:6}$-line SKUA-485-27 (Khan et al., 2018). Accordingly, the derived NILs in $BC_3F_{2:3}$ was evaluated for the extent of Recurrent Parent Genome (RPG%) recovery with the help of SSR/ STS markers tagged near already validated SNPs (Khan et al. 2018). RPG recovery was represented using Graphical Geno Typing (GGT 2.0) software (Van Berloo 2008).

Disease phenotyping

The selected homozygous plants in BC_3F_2were advanced to $BC_3F_{2:3}$. The designated leaves tagged on the plants within the lines were evaluated for disease progress throughout the crop cycle. The blast symptoms were scored on five different dates from 2-3 leaf stage to post-flowering stage of development. Differential response and effectiveness of genes towards *M. oryzae* was noted under open field conditions and was explained through AUDPC (Area Under Disease Progress Curve) for each NIL as per the method followed by Campbell and Madden (1990). AUDPC was estimated as:= $\sum_{i=1}^{n}\left(\frac{y_i+y_{i+1}}{2}\right)(t_{i+1}-t_i)$, where, n = total number of observations, y_i = Disease intensity at the i^{th} observation, and t = time at the i^{th} observation. Since the unit for y in the sample data was expressed as a percent and the unit for t denoted the development stage, AUDPC was, therefore, reflected as 'per cent-development stage'. The NILs were evaluated for neck blasts following the SES-IRRI (2013).

Evaluation for agronomic and quality traits

The individual plants in $BC_3F_{2:3}$ generation were evaluated for grain yield and the contributing traits like plant height (PH), number of effective tillers per plant (ET), panicle length (PL), number of spikelets per panicle (SP), percent Spikelet fertility (SF) and grain yield per plant (GY). Approximately five grams of paddy from selected plants was de-hulled using a palm huller. The brown rice was milled with rice polisher (Kett Rice Polisher, AI43590, Ota-ku, Tokyo). The NILs were evaluated for amylose content (AC) and gel consistency (GC). A standard curve was plotted on different amylose concentrations on X-axis against OD_{620} on Y-axis. The regression equation of y = 0.1877x + 0.1984 was obtained and used to estimate amylose content (%).

Results

Marker-assisted foreground selection

Foreground selection was exercised on $BC_3F_{2:3}$ plants in order to identify homozygous individuals. A total of 12 plants were screened for the genes *Pi54, Pi1* and *Pita*, of which three plants carried genes *Pi54+Pi1* (SKUA-485-27-13-5-1, SKUA-485-27-13-5-11, SKUA-485-27-13-5-13), two carried *Pi54+ Pita* (SKUA-485-27-4-38-2, SKUA-485-27-13-5-2), one carried *Pi1+ Pita* (SKUA-485-27-13-5-7), two were homozygous for *Pi54* (SKUA-485-27-4-40-9, SKUA-485-27-13-5-4), two for *Pi1* (SKUA-485-27-13-1-1, SKUA-485-27-13-5-9) and two for *Pita* (SKUA-485-27-20-10-2, SKUA-485-27-13-5-1) (Fig. 1, Table 1).

Disease reaction response using AUDPC of NILs

The NILs were screened for blast disease under open field conditions with the purpose of evaluating the effectiveness of three blast resistance genes, namely, *Pi54, Pi1* and *Pita,* towards prevalent *M. oryzae* isolates. The reaction of individual genes across 12 NILs was verified through the computation of AUDPC (Area Under Disease Progress Curve). The derived NILs SKUA-485-27-4-40-9 (*Pi54*), SKUA-485-27-20-10-2 (*Pita*) and SKUA-485-27-13-5-1 (*Pita*) showed severity of less than 3, 5 and 4%, respectively. However, NILs SKUA-485-27-13-1-1, SKUA-485-27-13-5-9 carrying gene *Pi1* showed severity of more than 35 and 25%, respectively. SKUA-485-27-13-5-13 carrying *Pi54+Pi1* showed the lowest AUDPC (109.13) and SKUA-485-27-13-1-1 carrying gene *Pi1* showed the highest AUDPC (996.55). The RP *Mushk Budji* showed high susceptibility score and recorded the AUPDC of 2870.85. The donor DHMAS 70Q 164 (control) carrying genes *Pi54, Pi1* and *Pita* showed AUDPC of 36 (Fig. 2, Supplementary Table S1).

Marker-assisted background analysis of NILs

A polymorphism survey was carried out between recurrent parent *Mushk Budji* and donor *DHMAS70Q 164-1b*, utilising SSRs and gene-based markers equally dispersed across the genome. The markers previously reported to be polymorphic (Khan et al., 2018) were revalidated on

Table 1. Foreground analysis of NILs for blast resistance genes Pi54, Pi1 and Pita

Plant ID	Phase
SKUA-485-27-4-40-9	*Pi54*
SKUA-485-27-20-10-2	*Pita*
SKUA-485-27-13-5-1	*Pita*
SKUA-485-27-13-1-1	*Pi1*
SKUA-485-27-4-38-2	*Pi54+Pita*
SKUA-485-27-13-5-2	*Pi54+Pita*
SKUA-485-27-13-5-4	*Pi54*
SKUA-485-27-13-5-7	*Pi1+Pita*
SKUA-485-27-13-5-9	*Pi1*
SKUA-485-27-13-5-10	*Pi54+Pi1*
SKUA-485-27-13-5-11	*Pi54+Pi1*
SKUA-485-27-13-5-13	*Pi54+Pi1*
DHMAS 70Q 164	*Pi54+Pi1+Pita*
MushkBudji	-

M: 100 bp DNA Ladder (ThermoFischer Scientific, New Delhi, India); M: Mushk Budji, D: DHMAS 70Q 164-1b, 1: SKUA-485-27-4-40-9, 2: SKUA-485-27-20-10-2, 3: SKUA-485-27-13-5-1, 4: SKUA-485-27-13-1-1, 5: SKUA-485-27-4-38-2, 6: SKUA-485-27-13-5-2, 7: SKUA-485-27-13-5-4, 8: SKUA-485-27-13-5-7, 9: SKUA-485-27-13-5-9, 10: SKUA-485-27-13-5-10, 11: SKUA-485-27-13-5-11, 12: SKUA-485-27-13-5-13.

Fig. 1. Marker-assisted foreground selection in BC_3F_2

parents using available primer sequences (McCouchet al., 2002). Marker-assisted background analysis was carried out for five NILs carrying genes *Pi54*, *Pi1* and *Pita* using 90 genome-wide markers. The markers used were distributed throughout the genome with eight markers screened each for chromosomes 1 and 6. The background selection was carried out using six markers each on chromosomes 2, 5, 7 and 10, besides five markers per chromosome for linkage groups 3, 4, 8 and 9. The carrier chromosomes 11 and 12 had fifteen and twelve polymorphic markers, respectively. The RPG recovery ranged from 86.95% for SKUA-485-13-1-1 (*Pi1*)

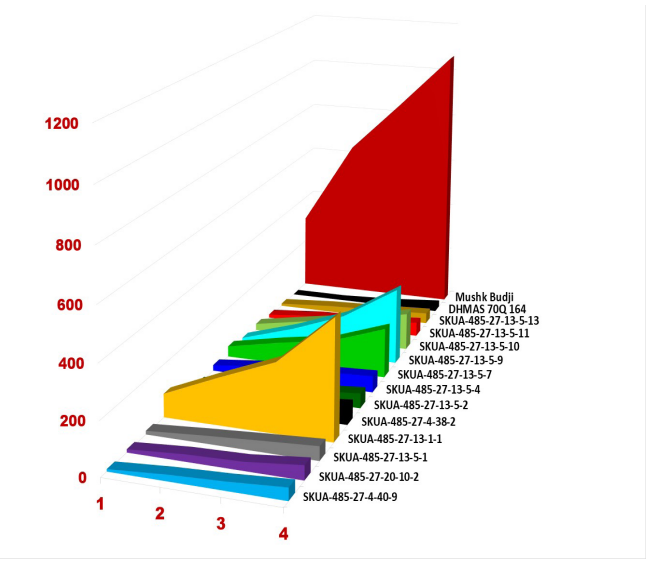


Fig. 2. Area Under Disease Progress Curve of MAS derived NILs

to the maximum value of 94.98% for SKUA-485-27-20-10-2 (*Pita*) and SKUA-485-27-13-5-1 (*Pita*). SKUA-485-27-4-40-9 was heterozygous at PNK10, PKN7, RM314, RM204, RM202, and RM7003. SKUA-485-20-10-2 showed heterozygosity at HV-02-68, PKN10, RM202, RM204, RM314, RM598, RM201 and RM7048. SKUA-485-27-13-5-1 showed heterozygosity at PKN7, RM598, HV-02-68, PKN10, RM314, RM204, RM7048 and RM7003. SKUA-485-27-4-40-9, SKUA-485-20-10-2, SKUA-485-27-13-5-1, SKUA-485-27-4-38-2 and SKUA-485-27-13-1-1 showed RPG recovery of 91.89, 94.98, 94.98, 90.65 and 86.95%, respectively. Linkage drag around target gene *Pi54* was reduced within 2.8 Mb on chromosome 11 in line SKUA-485-27-4-40-9. Gene *Pi1* was incorporated in SKUA-485-27-13-1-1 within a chromosomal segment of 4.4 Mb on the telomeric end of chromosome 11. Similarly, *Pita* was delimited within 5.8 Mb of chromosome 12 in SKUA-485-27-13-5-1 4 (Figs 3-5).

SSR/STS marker-based validation of recovery at SNP loci linked to agronomic and rice quality traits

Single nucleotide polymorphisms (SNPs) are plant genomes' most abundant DNA sequence variation. Previously, SNP genotyping was carried out on $BC_2F_{2:3}$ pyramided lines using OsSNPnks' 50 K Axiom® 2.0 SNP array (Khan et al., 2018). The pyramided lines were subsequently used as parents for the development of NILs in $BC_3F_{2:3}$. The chip assay was carried across 50,051 SNPs from 18,980 different genes spanning 12 rice chromosomes, which included 194 agronomically important cloned rice genes. The derived NILs were revalidated for recovery at the screened SNP loci with the help of linked SSR/ STS markers. A total of 25 genes related to yield and quality were investigated using linked SSR markers. The data from the SNP genotyping performed earlier (Khan et al. 2018) was further analyzed here to investigate the

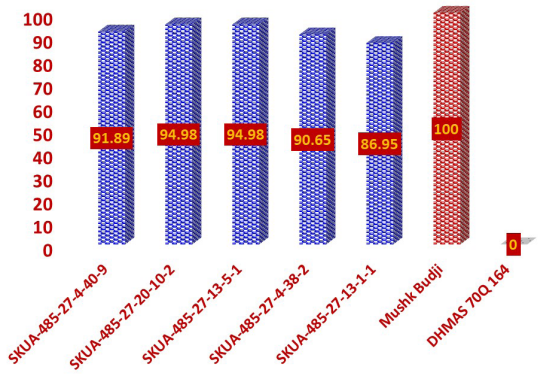
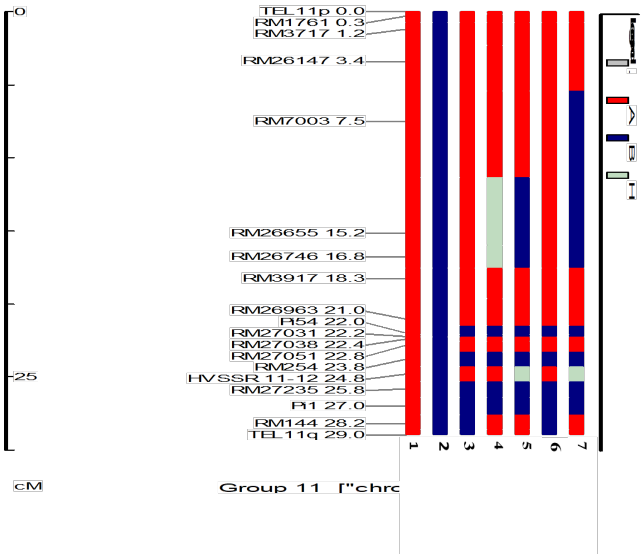


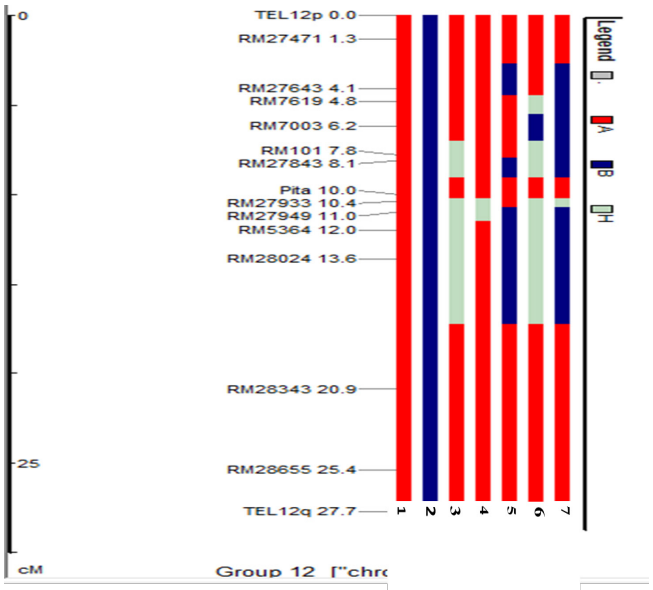
Fig. 3. RP Genome (%) recovery of NILs against MushkBudji parent



Bars from left to right: 1) *Mushk Budji;* 2). *DHMAS 170Q 164-1b* 1; 3). SKUA-485-27-4-40-9 2; 4). SKUA-485-27-20-10-2; 5). SKUA-485-27-13-5-1; 6). SKUA-485-27-4-38-2; 7). SKUA-485-27-13-1-1

Fig. 4. Graphical genotype depicting recipient parent genome recovery of NILs for carrier Chromosome 11

recovery at selected loci related to important traits. For instance, the locus for plant height governing gene, namely, *Dwarf 18* present on chromosome 1 showed RPG recovery in pyramided lines with allele CC and *Mushk Budji* against GG for the donor. The locus was revalidated in the present NILs using RM1033 which recorded complete recovery for RP. On chromosome 2, gene *GID2,* responsible for dwarfness showed RP allele in the derived $BC_3F_{2:3}$ NILs, SKUA-485-27-4-40-9 and SKUA-485-27-20-10-2. Gene *d1* present on chromosome 5, responsible for dwarfness showed donor allele in SKUA-485-27-4-40-9. On chromosome 4 gene *GPP-4* for yield and productivity showed recovery across all the NILs. Gene *GIF1,* present responsible for yield and productivity, showed RPG recovery in pyramided lines and was again validated through SSR marker RM16938 in $BC_3F_{2:3}$ NILs. Gene *UXS2* present on chromosome 1, responsible for cold tolerance and seed germination, showed RPG recovery



Bars from left to right: 1) *Mushk Budji;* 2). *DHMAS 170Q 164-1b* 1; 3). SKUA-485-27-4-40-9 2; 4). SKUA-485-27-20-10-2; 5). SKUA-485-27-13-5-1; 6). SKUA-485-27-4-38-2; 7). SKUA-485-27-13-1-1

Fig. 5. Graphical genotype depicting recipient parent genome recovery of NILs for carrier Chromosome 12

in both parents and NILs. Quality-related genes *SSIVa, SSIIb, GBSS1, AGPL1, SDBE, PUL, SSIIIb, SSS1, ISA2, AGPL1, SDBE, PUL, SSIIa, SSIVb* were screened for RPG recovery using linked SSR/ STS markers. Of these the SSR marker RM276 linked to the genes *SSIIa* showed donor allele for all the four NILs. The NILs (SKUA-485-27-4-40-9, SKUA-485-27-20-10-2) showed recovery at *SSIIIb* locus (PKN7) while NILs (SKUA-485-27-13-5-1, SKUA-485-27-4-38-2) carried donor fragment. The SSR marker RM153 linked to PPKL2 for grain length showed complete recovery of RP allele in the derived $BC_3F_{2:3}$ NILs (Supplementary Table 2).

Agronomic traits of NILs

$BC_3F_{2:3}$ plants were grown in the field under irrigated conditions along with two parents *DHMAS70Q 164-1b* and *Mushk Budji*, at Mountain Research Centre for Field Crops, Khudwani, SKUAST-Kashmir. The observations were recorded on agronomic traits, which included plant height (PH), effective tillers per plant (ET), Spikes per panicle (SP), panicle length (PL); spikelet fertility (SF), grain yield per plant (GY). The plants SKUA-485-27-4-40-9, SKUA-485-27-20-10-2, SKUA-485-27-13-5-1, SKUA-485-27-4-38-2 and SKUA-485-27-13-1-1 recorded the PH in the range of 114 to 127cm and were statistically in line with the recurrent parent *Mushk Budji* (123cm). ET was highest (24) in SKUA-485-27-13-1-1, while the lowest value (7) was recorded for SKUA-485-27-20-10-2. PL ranged from 17.5 to 24.5cm with 24.5cm for SKUA-485-27-13-1-1. SP was recorded to be highest (157) in SKUA-485-27-20-10-2 and lowest (65) in SKUA-485-27-4-40-9.

Table 2. Agronomic performance of $BC_3F_{2:3}$ NILs of Mushk Budji/DHMAS 70Q 164-1b

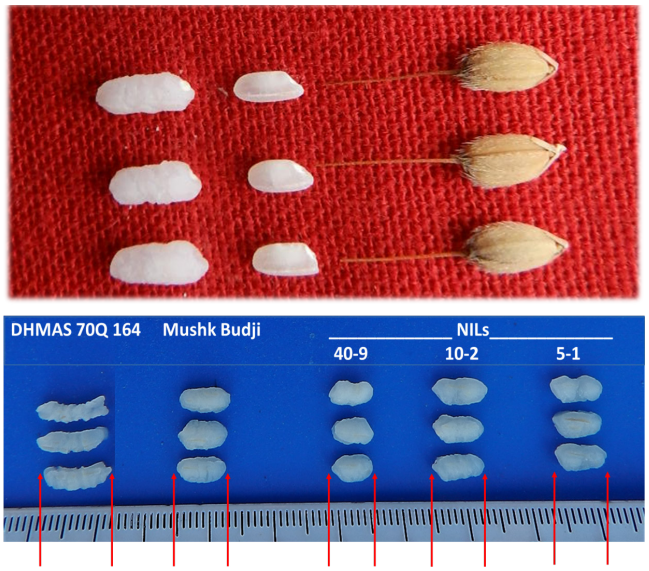
Plant#ID	PH (cm)	ET	PL (cm)	SP	SF (%)	GY(g)
SKUA-485-27-4-40-9	114	9	17.5	65	80.0	7.4
SKUA-485-27-20-10-2	116	7	27.0	157	91.1	6.9
SKUA-485-27-13-5-1	127	13	22.5	139	84.2	11.3
SKUA-485-27-4-38-2	123	16	19.0	80	82.3	9.5
SKUA-485-27-13-1-1	117	24	24.5	142	82.4	21.3
Mushk Budji	123	10	19.0	156	86.0	13.8
DHMAS 70Q 164-2b	128	12	23.0	114	66.0	8.6

SF ranged from 80 to 91.1% for SKUA-485-27-4-40-9 and SKUA-485-27-20-10-2, respectively (Table 2). Family mean values of PH, ET, PL, SP, SF and GY were found to be 114 cm, 13, 21.79 cm, 121.86, 81.71% and 21.26 g/plant, respectively.

Physico-chemical properties, cooking quality and aroma of NILs

At the time of harvest, the plants in $BC_3F_{2:3}$ wereselected on the basis of grain and plant type. Three plants with grain type similar to *Mushk Budji* were selected and subjected to cooking quality analysis. The milled rice length (KLBC) ranged from 4.70 mm to 5.47 mm in SKUA-485-27-13-5-1 and SKUA-485-27-20-10-2, respectively. SKUA-485-27-20-10-2 recorded highest (1.91) value for LBR. KLAC ranged from 6.1 to 8.6 in SKUA-485-27 and SKUA485-4, respectively. KLAC ranged from 6.40 to 9.30 in SKUA-485-27-13-5-1 and SKUA-485-27-4-40-9, respectively. KER was found to be highest (1.92) in SKUA-485-27-4-40-9 and lowest (1.36) in SKUA-485-27-13-5-1. The selected plants recorded the mean values for KLBC, LBR, KLAC, KER as 5.29, 1.99, 8.25 mm and 1.56, respectively (Supplementary Table 3). SKUA-485-27-4-40-9, SKUA-485-20-10-2 and SKUA-485-27-13-5-1 showed an aroma score of 2 similar to *Mushk Budji* (Fig, 6).

Amylose content of the lines SKUA-485-27-4-40-9, SKUA-485-20-10-2 and SKUA-485-27-13-5-1 was recorded at 18.45, 20.39 and 21.31%, respectively, against 16.64% for RP *Mushk Budji* and 23.01% for *DHMAS 170Q 164-1b*. GC (Gel consistency) of a set of five NILs for the genes *Pi54, Pi1* and *Pita* ranged from 85 mm for SKUA-485-27-20-10-2 to 95 mm for SKUA-485-27-4-40-9 was similar to *Mushk Budji*. All the selected NILs, therefore, recorded soft GC like *Mushk Budji,* whereas the donor *DHMAS 170Q 164-1b* recorded medium hard GC (60mm) (Supplementary Table 3). The NILs showed aroma score of 2 like RP under panel test after cooking.



NILs from left to right: SKUA-485-27-4-40-9, SKUA-485-20-10-2, SKUA-485-27-13-5-1

Fig. 6. Kernel length and cooking quality of selected NILs

Discussion

NILs can be utilized as genetic resources for studying differential disease phenotypes, response of the constituent genes and pathogen virulence. Mackill and Bonman (1992) developed the first set of differential varieties (NILs) in the background of indica variety Co39. It addressed four blast resistance genes: *Pita, Piz5, Pi1* and *Pi3*. Ling et al. (2001) developed set of NILs with five resistance genes *Pib, Pik, Pikm, Pikp, Pita2*in the background of susceptible japonica variety LTH. One of the strategies to combat rice blast disease is the transfer of genetic resistance into susceptible cultivars through the use of conventional or molecular breeding approaches (Singh et al. 2011). . Foreground selection for gene *Pi54* was carried out using gene based InDel marker Pi54 MAS developed by Ramkumar et al. (2011). The marker Pi54 MAS amplified 216 bp fragment specific to *Pi54* resistance and 359 bp allele for susceptible plants. A total of 12 plants were screened for the genes *Pi54, Pi1* and *Pita*, of which three plants carried genes *Pi54+Pi1*, two carried *Pi54+ Pita*, one carried *Pi1+ Pita*, two were heterozygous for *Pi54*, two for *Pi1* and two for *Pita*. RM224 a linked SSR marker, was used to select the *Pi1* (Feuntes et al. 2008). The gene *Pita* is located at the centromeric region of chromosome 12 and was targeted using gene-based marker YL 155/87 (Jia et al. 2002). The use of gene-based markers allows transfer of gene of interest with high precision and accuracy. AUPC

was used for comparison of the effectiveness of blast resistance genes. Disease intensity was recorded on a 0-9 scale, in which 0-3 was categorized as resistant response and 5-9 as susceptible under field conditions. SKUA-485-27-4-40-9, SKUA-485-27-20-10-2 and SKUA-485-27-13-5-1 were selected for background analysis on the basis of disease severity. Comparatively, these lines expressed resistance towards blast compared to other NILs. SKUA-485-27-13-1-1 and SKUA-485-27-13-5-9 carrying *Pi1* gene were susceptible to blast. SKUA-485-27-4-40-9 carried *Pi54* and showed high degree of resistance to blast. SKUA-485-27-20-10-2 and SKUA-485-27-13-5-1 carried *Pita*. The response of 11 different blast resistance genes was studied (Shikari et al., 2014) to reveal varied phenotypic response across a large germplasm set. In the present study, five selected NILs with the lowest disease score (AUPC) were subjected to background analysis using 23 SSR / gene-based markers that carried heterozygous alleles in BC_2 derived NILs in previous generation. The RPG recovery ranged from 86.95% for SKUA-485-13-1-1 (*Pi1*) to the maximum value of 94.98% for SKUA-485-27-20-10-2 (*Pita*) and SKUA-485-27-13-5-1 (*Pita*). Linkage drag around target gene *Pi54* was reduced within 2.8 mb on chromosome 11 in line SKUA-485-27-4-40-9. Gene *Pi1* was incorporated within a chromosomal segment of 4.4 mb in SKUA-485-27-13-1-1 on telomeric end of chromosome 11. Similarly, *Pita* was delimited within 5.8 mb of chromosome 12 in SKUA-485-27-13-5-14. Previously the background analysis of pyramided lines developed was carried out using 'OsSNPnks' 50 K Axiom® 2.0 SNP array (Singh et al. 2015) in BC_2F_3 generation. After advancement to $BC_3F_{2:3}$, the derived NILs were again verified at 25 SNP loci (associated with 25 genes) with the help of linked SSR /STS markers. The traits that were examined in the present study included plant height, grain yield, rice quality and tolerance to cold stress. The locus for plant height governing gene, *Dwarf 18* present on chromosome 1 produces protein GA 3 beta hydroxylase-2 and showed RPG recovery in pyramided lines with allele CC for lines and *Mushk Budji* against GG for the donor. The locus was revalidated in the present NILs using RM1033 which recorded complete recovery for RP. On chromosome 2 gene *GID2* produces protein Gibberellin-insensitive dwarf2 responsible for dwarfness and was not recovered in (pyramided) parental lines in BC_2F_3 generation but showed RP allele in the derived $BC_3F_{2:3}$ NILs, SKUA-485-27-4-40-9 and SKUA-485-27-20-10-2. Gene *d1* present on chromosome 5 produces Guanine nucleotide binding protein alpha 1 subunit (GP-alpha-1) responsible for dwarfness and showed donor allele in SKUA-485-27-4-40-9 that explains the short stature of the derived NIL and its corresponding pyramided line. On chromosome 4 gene *GPP-4* produces protein methyltransferase responsible for yield and productivity and showed recovery in $BC_2F_{2:3}$ derivedlines. The observation recorded for GY in NILs correlated with the allelic phase. Gene *GIF1*present on chromosome 4 codes for Glucose-6-phosphate 1-dehydrogenase responsible for yield and productivity and showed recovery in pyramided lines. The locus was again validated through SSR marker RM16938 in $BC_3F_{2:3}$ NILs. Gene *UXS2* present on chromosome 1 is responsible for cold tolerance and seed germination. The SNP and SSR marker showed RPG recovery in both parents and NILs for the corresponding generations. The locus explains the cold stress adaptability of NILs compared to original donor DHMAS 70Q 164-1b. Among the genes responsible for starch and amylose properties, the loci screened through SNP chip included *SSIVa, SSIIb, GBSS1, AGPL1, SDBE, PUL, SSIIIb, SSS1, ISA2, AGPL1, SDBE, PUL, SSIIa, SSIVb*. Of these the SSR marker RM276 linked to the genes *SSIIa* showed donor allele for all the four NILs. The NILs SKUA-485-27-4-40-9 and SKUA-485-27-20-10-2 showed recovery at *SSIIIb* locus (PKN7) while NILs SKUA-485-27-13-5-1 and SKUA-485-27-4-38-2 carried donor fragment. SSII and SSIII are starch synthase genes that condition rice's various starch properties. The partial recovery at these two loci explains the intermediate amylose in the derived NILs compared to RP. However, at the same time other genes mentioned above which are related to quality showed complete recovery in all the NILs. The recovery in NILs at such loci, particularly, *SSIVa*, *SSIIb* and GBSS1 explained good recovery for GC, GT and other starch properties. The two loci PPKL2 and *GL3* have been reported to explain grain size in rice (Zhang et al., 2012). For *PPKL2*, the parents *Mushk Budji* and DHMAS 70Q 164-1b carried AA and GG, respectively. All the pyramided lines in $BC_2F_{2:3}$ showed recovery at the said locus. The SSR marker RM153 linked to PPKL2 at a distance of 2.39 Mb showed complete recovery of RP allele in the derived $BC_3F_{2:3}$ NILs. The locus correlates with the early recovery of grain type in pyramided lines and NILs, similar to *Mushk Budji*. The genotype exhibits a wide difference of at least 1.63 mm concerning milled rice length (KLBC) and 2.53mm concerning cooked kernel length (KLAC) compared to *Mushk Budji*. The stringent phenotypic selection for these traits and background selection for grain type, KLAC, KLBC, KER and aroma in BC3F3 NILs were employed. Grain size is governed by GS3 locus on chromosome 3 which explains 80–90% of phenotypic variability (Fan et al., 2005). Fast recovery of grain type in our population was possibly due to elimination of mutant fine-grained allele specific to donor. KLAC for recurrent parent *Mushk Budji* was about 7.70mm whereas for selected lines it ranged from 6.40mm to 9.30mm. The elongation ratio of the selected NILs was at par with recurrent parent in the range of 1.36mm to 1.92mm, the lower values in some of the derived lines could be explained as an interaction between various QTL loci spread across genome. The NILs were evaluated for amylose content (AC) and gel consistency (GC). The AC is responsible for texture and appearance of cooked rice and GC explains the nature of starch packaging in rice kernel. Amylose content for the selected lines was observed to be 18.19, 20.39

and 21.31% for SKUA-485-27-4-40-9, SKUA-485-20-10-2 and SKUA-485-27-13-5-1, respectively, against 16.64% for RP *Mushk Budji* and 23.01% for DHMAS 170Q 164-1b. *Mushk Budji* carries Wx_b allele on chromosome 6 against Wx_a allele for donor. Wx_b encodes for low amylose trait although, nature of starch is also conditioned by other loci such as SSI, SSII, Alk, etc (Fujita et al. 2006; Tanaka et al. 1995). The variation in recovery at these loci may be responsible for difference in NILs for AC. *Mushk Budji* possesses soft GC as compared to donors DHMAS 170Q 164-1b which had medium GC of 60mm. Gel consistency (GC) of NILs ranged from 85mm to 95mm which falls in soft GC range. Most importantly, the derived NILs had similar aroma like that of RP, the trait which would be a major criterion at the time of introduction in farmer's field.

The present study aimed to develop a set of Near-isogenic lines, SKUA- 27-4-40-9, SKUA-27-20-10-2 and SKUA-27-13-5-1, for genes *Pi54, Pi1*and*Pita* in the background of *Mushk Budji*. NILs in $BC_3F_{2:3}$ possessed higher RPG recovery (>91%) than previously developed gene pyramided lines. The background analysis using the SSR markers linked to previously tagged SNP's helped to estimate RPG recovery of NILs. Further, it could be demonstrated that SSR markers can be reliably used to select the important loci linked to traits of agronomic importance and those related to rice quality. A differential reaction pattern exhibited by individual genes helped us validate the respective genes' effectiveness under Kashmir conditions. *Pi54* and *Pita* were effective in conferring resistance towards M. oryza infection, while Pi1was was a comparatively weak gene. Therefore, the information shall help determine the choice of genes to be brought into combination through gene pyramiding to achieve durable resistance to rice blast. The developed NILs can be used as donors for resistance in future breeding programmes particularly, which aim at the improvement of cold tolerant high-altitude cultivars for resistance to blast.

Supplementary material

Supplementary Tables S1, S2 and S3 are provided, www.isgpb.org

Author's contributions

Conceptualization of research and designing of the experiments (ABS); Contribution of experimental materials (ABS, GK, NR); Execution of field/lab experiments and data collection (HR, SM, RK, NA); Analysis of data and interpretation (ABS, GK); Preparation of the manuscript (ABS, HR, SM).

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SupplementaryTable S1. AUDPC score for leaf blast across a set of NILs

Gene	Plant#ID	1-2	2-3	3-4	4-5	AUDPC
Pi54	SKUA-485-27-4-40-9	8.75	23.75	33.15	47.18	112.83
Pita	SKUA-485-27-20-10-2	12.00	27.00	33.08	54.68	126.75
Pita	SKUA-485-27-13-5-1	13.75	28.75	41.70	53.93	138.13
Pi1	SKUA-485-27-13-1-1	90.00	181.25	267.13	458.18	996.55
Pi54 + *Pita*	SKUA-485-27-4-38-2	20.00	45.00	50.88	71.63	187.50
Pi54 + *Pita*	SKUA-485-27-13-5-2	10.50	27.00	39.83	57.23	134.55
Pi54	SKUA-485-27-13-5-4	22.50	45.00	54.15	63.53	185.18
Pi1 + Pita	SKUA-485-27-13-5-7	41.79	84.64	119.96	194.70	441.09
Pi1	SKUA-485-27-13-5-9	21.25	86.25	165.95	298.20	571.65
Pi54 + Pi1	SKUA-485-27-13-5-10	26.25	63.75	99.38	146.85	336.23
Pi54 + Pi1	SKUA-485-27-13-5-11	13.50	31.50	37.35	55.88	138.23
Pi54 + Pi1	SKUA-485-27-13-5-13	9.38	24.38	32.70	42.68	109.13
Pi54 + Pi1 + Pita	*DHMAS 70Q 164*	0.00	0.00	9.38	26.63	36.00
-	*MushkBudji*	303.00	645.75	853.88	1068.23	2870.85

Supplementary Table S2. SSR/ STS marker based validation of SNP loci associated withagronomic and rice quality traits in backcross derived NILs

Trait class	Trait	Gene Name	Chr.	Marker	LOC_ID	Annotations	SSR-SNP distance (Mb)	Marker-assietd background selection (SSR based)						Marker-assietd background selection (SNP based)					
								MB	*D*	*1*	*2*	*3*	*4*	*MB*	*D*	*1*	*2*	*3*	*4*
Plant height	Plant height, Cold tolerance, Flooding	*DWARF 18*	1	RM10333	LOC_ Os01g08220	GA 3 beta-hydroxylase2, GA metabolism	1.52	A	B	A	A	A	A	CC	GG	CC	CC	CC	CC
	Plant height (Dwarfess)	*GID2*	2	RM13377	LOC_ Os02g36974	G-box factor 14-3-3e protein, Gibberellin-insensitive dwarf2,	2.15	A	B	A	A	A	A	CC	TT	TT	TT	CC	CC
	Dwarfess, Blast resistance	*d1*	5	RM598	LOC_ Os05g26890	Guanine nucleotide-binding protein alpha-1 subunit (GP-alpha-1).	2.07	A	B	B	A	A	A	GG	GG	GG	GG	GG	GG
	Yield and productivity	*GPP-4*	4	HV-04-21	LOC_ Os04g31000	Methyltransferase domain containing protein	1.94	A	B	A	A	A	A	AG	AA	GG	GG	GG	AG
	Yield and productivity	*GIF1*	4	RM16938	LOC_ Os04g33740	Glucose-6-phosphate 1-dehydrogenase	0.03	A	B	A	A	A	A	AA	GG	AA	AA	AA	AA
	Development of flag leaves, ears and non-tip roots	*ORC4*	1	RM11456	LOC_ Os01g49010	Origin recognisation complex 4	1.46	A	B	A	A	A	A	AA	GG	AA	AA	AA	AA
Grain yield	Yield and productivity	*GPP-4*	4	HV-04-21	LOC_ Os04g31000	Methyltransferase domain containing protein	1.94	A	B	A	A	A	A	AG	AA	GG	GG	GG	AG
	Yield and productivity	*GIF1*	4	RM16938	LOC_ Os04g33740	Glucose-6-phosphate 1-dehydrogenase	0.03	A	B	A	A	A	A	AA	GG	AA	AA	AA	AA
	Grain length	*PPKL2*	5	RM153	LOC_ Os05g05240	Serine/threonine-specific protein phosphatase and bis(5-nucleosyl)-tetra phosphatase	2.39	A	B	A	A	A	A	AA	GG	AA	AA	AA	AA
	Grain length	*GL3*	3	RM15350	LOC_ Os03g44500	Serine/threonine protein phosphatase	3.84	A	B	A	A	A	A	GG	GG	GG	GG	GG	GG
Rice and cooking quality	Cooking quality glycaemic index	*SSIVa*	1	RM6666	LOC_ Os01g52250	Soluble starch Synthase IVA	1.34	A	B	A	A	A	A	GG	AA	GG	GG	GG	GG
	Cooking quality	*SSIIb*	2	HV-02-68	LOC_ Os02g51070	Starch synthase, putative	2.43	A	B	A	A	A	A	GG	AA	AG	GG	GG	GG
	Amylose content, grain quality	*GBSS1*	3	RM14275	LOC_ Os06g0133000	Granule bound starch synthase 1	1.29	A	B	A	A	A	A	CC	AA	CC	AC	AC	CC
	Grain quality, AC and viscosity	*AGPL1*	3	RM156	LOC_ Os03g52460	Glucose-1-phosphate adenylyl transferase	1.35	A	B	A	A	A	A	GG	GG	GG	GG	GG	GG
	Cooking quality glycaemic index	*SDBE, PUL*	4	RM16301	LOC_ Os04g0164900	R-enzyme, starch debranching enzyme	3.3	A	B	A	A	A	A	AA	CC	AA	AA	AA	AA

	Grain quality	*SSIIIb*	4	PKN7	LOC_ Os04g53310	Tetratricopeptide repeat domain containing protein	0.06	A	B	A	A	B	B	GG	GG	GG	GG	GG	GG
	Grain quality	*SSS1*	6	RM204	LOC_ Os06g06560	Soluble starch synthase 1	0.02	A	B	A	A	A	A	CC	CC	CC	CC	CC	CC
	Grain quality	*ISA2*	5	RM18758	LOC_ Os05g32710	Alpha amylase, catalytic domain containing protein	2.19	A	B	A	A	A	A	AA	GG	AA	AA	AA	AA
	Grain quality, AC and viscosity	*AGPL1*	3	RM156	LOC_ Os03g52460	Glucose-1-phosphate adenylyl transferase	1.35	A	B	A	A	A	A	GG	GG	GG	GG	GG	GG
	Cooking quality glycaemic index	*SDBE, PUL*	4	RM16301	LOC_ Os04g0164900	R-enzyme, starch debranching enzyme	3.3	A	B	A	A	A	A	AA	CC	AA	AA	AA	AA
	Grain quality starch properties	*SSIIa*	6	RM276	LOC_ Os06g12450	Soluble starch synthase 2	0.55	A	B	B	B	B	B	CC	CC	CC	CC	CC	CC
	Cooking quality	*SSIVb*	5	RM3616	LOC_ Os05g45720	Starch synthase	0.27	A	B	A	A	A	A	TT	GG	TG	TG	GG	TT
	Cold tolerance and seed germination	UXS-2	*1*	RM580	LOC_ Os01g21320	UDP-glucuronic acid decarboxylase	1.39	A	B	A	A	A	A	AA	CC	AA	AA	AA	AA
	Pollen viability and pathogen resistance	SWEET12	*3*	RM218	LOC_ Os03g22590	Nodulin MtN3 family protein.	4.09	A	B	B	B	B	B	TT	TT	TT	TT	TT	TT
Others	Nutritional quality, Flavonoid content	ANS1	*1*	RM11018	LOC_ Os01g27490	Leucoanthocyanidin dioxygenase	2.62	A	B	A	A	A	A	AA	CC	AA	AA	AA	AA

Supplementary Table S3: Kernel traits, cooking quality, Amylose content and gel consistency of $BC_3F_{2:3}$ NILs of *MushkBudji / DHMAS 70Q 164-1b*

Plant#ID	*KLAC (mm)*	*KBAC (mm)*	*KLBC (mm)*	*KBBC (mm)*	*LBR*	*KER*	*AC (%)*	*Class*	*Gel consistency (mm)*	*Class*
SKUA-485-27-4-40-9	9.30 ± 0.28	3.70 ± 0.14	4.83 ± 0.06	2.83 ± 0.08	1.71 ± 0.57	1.92 ±0.64	18.45	Low	95	Medium
SKUA-485-27-20-10-2	8.87 ± 0.11	4.30 ± 0.03	5.47 ± 0.02	2.87 ± 0.09	1.91 ±0.64	1.62 ±0.54	20.39	Intermediate	85	Medium
SKUA-485-27-13-5-1	6.40 ± 0.14	3.15 ± 0.06	4.70 ± 0.08	2.87 ± 0.04	1.64 ±0.55	1.36 ±0.45	21.31	Intermediate	90	Medium
MushkBudji	7.07 ± 0.03	3.73 ± 0.22	4.90 ± 0.05	2.80 ± 0.07	1.75 ±0.58	1.44 ±0.48	16.24	Low	120	Soft
DHMAS 70Q 164-2b	9.60 ± 0.14	2.83 ± 0.08	6.53 ± 0.17	2.20 ± 0.07	2.97 ±0.99	1.47 ±0.49	23.01	Intermediate	60	Medium

KLAC= Kernal Length After Cooking, KBAC= Kernal Breadth After Cooking, KLBC= Kernal Length Before Cooking, KBBC= Kernal Breadth Before Cooking, LBR= Length Breadth Ratio, KER= Kernal Elongation Ratio, AC= Amylose Content and GC= Gel Consistency