Original Research Article

Molecular Analysis of Selected Aromatic Rice Germplasm Lines for Known Bacterial Blight Resistance Genes

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Abstract

Molecular analysis of 30 aromatic lines revealed presence of Xa₄, xa₅ and xa₁₃ in twelve, two and five germplasm lines, respectively. Moreover, only xa₅+xa₁₃ gene combination was found to be present in two lines. Germplasm lines, ANP-44 (RAU 3073) and ANP-204 (Improved Pusa Basmati) showed presence of xa₅ and xa₁₃. Nine lines namely ANP-70 (ASGPC 14), ANP-143 (UPRI 93-101), ANP-144 (Khazia Dhan), ANP-176 (Shyam Jeera), ANP-317 (R-1498-747-358-2-1), ANP-323 (NDR 8497-2), ANP-445 (Jao Mali), ANP-526 (IR 74717-3-3-1-3) and ANP-536 (IET 13548) did not contain any of the genes tested but as they impart resistance against different pathotypes so, they might contain some other known gene(s). The information generated will be helpful in accelerating elite breeding program including pyramiding of different disease resistance genes in basmati varieties.

Keywords
Aromatic rice, Bacterial blight, Molecular marker, Resistance genes, Xanthomonas oryzae pv oryzae

Introduction

Rice is the staple food of almost half of the world's population. More than 90% of rice is grown in Asian countries, such as China, Japan, India, Pakistan, Vietnam and Thailand (Sumithra et al., 2012). Among the biotic stress affecting rice, bacterial blight (BB) caused by Xanthomonas oryzae pv. oryzae (Xoo) is a major devastating disease that limit rice yields significantly across the world (Ou, 1985; Mew et al., 1993) and limits rice production up to 81% in countries like India (Kumar et al., 2012). Aromatic rice constitutes a small but special group of rice which is considered best in quality. Among the aromatic rice, basmati has more demand in international market due to its unique features. Basmati rice is highly favored and fetches higher prices in world markets due to its special taste, aroma and flavor. Diseases, insects and weeds cause as much as 25 percent yield losses annually in cereal crops (Khush 2005). Basmati, besides being a natural low
yielder, has a number of other constraints like abiotic and biotic factors which limit crop productivity. In India, the losses in grain yield of aromatic rice due to disease and insect pests are as high as 35 per cent (Siddiq 1993). Out of these diseases, bacterial blight (BB) caused by the pathogen Xanthomonas oryzae pv. oryzae (Xoo) is one of the most destructive diseases of rice throughout the world and causes as much as 80% yield reduction (Arunakumari et al., 2016). This disease can cause yield losses up to 60-70 per cent in the Punjab state during the epiphytotic years (Raina et al., 1981). Breeding for BB resistance is the only effective, economical and eco-friendly strategy for the management of this disease. To date, at least 38 BB resistance genes conferring host resistance against various strains of Xoo have been identified (Bhasin et al., 2012 2012). In basmati germplasm, however, there are few reports for resistance to BB disease (Singh et al., 2004) which has necessitated the transfer of resistance genes from non- basmati sources (Bhatia et al., 2011), which often leads to deterioration of quality characteristics and consumer acceptability. It is therefore, essential to search useful and diverse gene(s) within aromatic germplasm available in the country. Tremendous progress has been made in mapping many agriculturally important genes with DNA markers in many crop plants (Mohan et al., 1997). These molecular markers linked to known BB resistance genes can be used for surveying the presence of different genes and this information can be subsequently exploited in the improvement. In Punjab, no single gene is effective against all the prevalent ten pathotypes of BB pathogen. Bacterial blight resistant genes like Xa4, xa5, xa13, Xa38 in combination are more effective and durable. A set of 30 aromatic rice lines was screened against two pathotypes of bacterial blight to identify potential donors and characterized for presence of known BB genes using molecular markers.

Materials and Methods

Plant material

The plant material comprised of a set of aromatic rice lines. These lines were evaluated against two predominant pathotypes PbXo-7 and PbXo-8 of bacterial blight pathogen at maximum tillering stage under artificial inoculation conditions Cultivars Basmati 386 and IRBB60 were used as susceptible and resistant check, respectively. Data was recorded as per SES on 0-9 scale (IRRI, 1996).

Extraction of DNA

DNA from selected material was isolated following a CTAB based DNA extraction method as follows (Murray and Thomson, 1980). Quantification of nucleic acids was performed by using NanoDrop™ 1000 spectrophotometer (ThermoScientific, Wilmington, USA) and the quality of DNA was checked by agarose gel electrophoresis. Finally the concentration of DNA was adjusted to make the working concentration of 25 ng/μl.

PCR amplification

In vitro amplification using Polymerase Chain Reaction (PCR) was performed in an Eppendorf master cycler for confirmation of Xa4, xa5 and xa13 genes. Standard lines with known presence of these genes were used as checks. PCR analysis was carried out using Xa4 (Wang et al., 2001), xa5 (IRRI personal communication) and xa13 (Sundaram et al., 2008) gene-linked primers.

Visualization of PCR amplified product

The amplified product for Xa4 was visualized by electrophoresis on 4 percent agarose gel while rest two primers were resolved on 1.5
percent agarose gel. The scoring of test material was done accordingly. Test entries carrying the resistant band of appropriate size were classified as positive for the particular gene while the lines showing the susceptible band were classified as negative for that particular gene.

Results and Discussion

Against PbXo-7, out of 30 lines tested, 18 lines showed resistant to moderately resistant reaction (1-5) while the rest of lines were susceptible (Table 1). On the other hand against PbXo-8, 12 lines exhibited resistant to moderately susceptible reaction. About 9 lines showed resistance against both the pathotypes. High level of resistance was shown by ANP-44, ANP-154, ANP-155, ANP-204, ANP-445 against PbXo-7 and ANP-144, ANP-154 and ANP-155 against pathotype PbXo-8. Ten lines namely ANP-44, ANP-143, ANP-144, ANP-154, ANP-155, ANP-203, ANP-204, ANP-205, ANP-219 and ANP-317 showed high to moderate level of resistance against both the pathotypes studied. The resistance in these lines may be either due to known genes or combination of known genes or as yet undescribed gene(s). ANP-154 and ANP-155 are resistant derivative lines of Basmati 386 and Basmati 370. According to Bhatia et al., (2011), they transferred Xa13, Xa21 and sd1 genes in Basmati 386 and Basmati 370. ANP-154 (RYT 3267) was later released as Punjab Basmati 3 variety (Singh et al., 2014a). In basmati germplasm however there are few reports for resistance to BB (Singh et al., 2004) and only few resistant donors in basmati are known. Similar results for evaluation of aromatic rice germplasm have been shown by Singh et al., (2014b). Punjab Basmati 3 is reported to carry xA13+Xa21 (Singh et al., 2014a). Rajpurohit et al., (2011) reported that pyramiding of more than one major resistance gene has proven to deliver durable resistance against BB disease. The information gained will be useful in basmati breeding programme for bacterial blight resistance.

The total 30 selected germplasm lines and two controls namely Basmati 386 (negative control), IRBB 60 (positive control). The details of these are given in Table 1. IRBB 60 is known to carry combination of four genes i.e. Xa4+xa5+xa13+xa21 (Laon et al., 2006). BB resistance gene Xa4 was first characterized in the rice variety TKM 6 on long arm of rice chromosome 11 (Wang et al., 2001). Amplification of sequence tagged site (STS) marker MP4 which is linked to Xa4 gene revealed the presence of a 150bp fragment specific for Xa4 mediated BB resistance in the positive control IRBB 60 and 120bp fragment corresponding to the negative control Basmati 386. This could be easily resolved on 4% agarose gel using 50bp DNA ladder. Based on the banding pattern of germplasm lines, BB resistance gene Xa4, was found to be present in twelve lines namely ANP -37 (Sonachoor), ANP -62 (IGSR-2-1-6), ANP -124 (CB 06550), ANP -203 (Rajendra Basmati), ANP -207 (Pusa 834), ANP -218 (IET 22289), ANP -219 (1601-105-1-46-1-1), ANP -222 (IET22778), ANP 227 (Sumati), ANP -239 (NWGR 3045), ANP -254 (Mugad Sugandh) and ANP -498 (Mugad Sugandha) as shown in Table 1 while the remaining 18 germplasm lines were found to be without Xa4 lines. Bacterial blight resistance gene Xa4 is one of the most widely exploited resistance gene in many rice breeding program (Sun et al., 2003). According to Khush et al., (1989), the exploitation of gene Xa4 resulted in development of many BB resistant rice cultivars that played significant role in protecting rice from Xoo. But presently, this gene alone is ineffective but shows resistance when present in combination. It has been reported by many workers that the pyramided lines with Xa4 and other bacterial blight resistance genes showed a wider spectrum and a higher level of resistance than the lines with
single resistance gene (Huang et al., 1997; Zheng et al., 1998, Arif et al., 2008). This implies that these 12 germplasm lines are source of Xa4 and can be transferred to different basmati breeding lines during crossing and breeding procedure.

Screening of recessive xa5 resistance gene by the amplification of microsatellite markers specific for resistant as well susceptible allele at 190bp revealed presence of xa5 only in two germplasm lines (Table 1) namely ANP-44 (RAU 3073) and ANP-204 (Improved Pusa Basmati). The bacterial blight resistance gene xa5 has been mapped on chromosome 5 with restriction fragment length polymorphism (RFLP) markers RG556 and RZ390 and microsatellite markers RM122 and RM390 (Blair and McCouch, 1997). Ramalingam et al., (2001) performed similar type of molecular survey for the presence of bacterial blight resistance genes xa5, xa13 and Xa21 in Chinese rice germplasm. Naveed et al., (2010) also detected xa5 gene in Pakistani germplasm including Basmati varieties.

Similarly, the amplified product with primer xal3promoter (xa13 gene) from resistant line IRBB 60 was of 500bp, while that from susceptible lines was about 300bp which could be easily resolved on 2.5% agarose gel by using standard 50bp DNA ladder to confirm proper size of amplified product. Based on the banding pattern of germplasm lines, it was observed that in case of xa13 BB resistance gene, only ANP-155 (RYT 3275), ANP-204 (Improved Pusa Basmati), ANP-309, ANP-44 (RAU 3073) and ANP-154 (RYT 3267) germplasm lines showed presence of the gene (Fig. 1). The recessive resistance gene, xa13 was first characterized in rice variety BJ 1 and fine mapped to a genomic region ~4cM on long arm of chromosome 8 (Sanchez et al., 1999). The gene linked markers MP4 linked to Xa4 (Wang et al., 2001), ‘xa5 R’ and xa5 S’ linked to xa5 (IRRI, personal communication) and xa13 promoter linked to xa13 (Sundaram et al., 2008) were used to select lines carrying Xa4, xa5 and xa13 genes, respectively. The details of amplification pattern of selected lines and checks for Xa4, xa5 and xa13 genes are given in Table 1.

Out of 30 germplasm lines tested, only two lines showed presence of combination of xa5+xa13 namely ANP–44 (RAU 3073) and ANP-204 (Improved Pusa Basmati). Goel et al., (1998) reported that none of the known genes were fully effective against all the pathotypes of BB pathogen from northern India.

Similar work has been reported by Mangat et al., (2012) and Khanna et al., (2014) in which they identified Xa4, xa13 and Xa21 genes through molecular analysis in elite non basmati rice breeding lines and showed effectiveness of multiple BB resistance genes in rice varieties. Sombunjitt et al., (2017) identified Xa-resistance genes such as Xa4, xa5, Xa7 and xa13 using PCR-based gene-linked and gene-specific markers in Thai local rice germplasm.

The narrow genetic base of cultivated rice will cause vulnerability to BB because of an increased frequency of newly evolved pathotypes of greater virulence. As a result, increasing attention is needed to focus on the accumulation of major disease resistance genes in crop plants. Pyramided lines carrying more resistance genes showed broad spectrum and higher resistance than the lines with a single resistance gene (Suh et al., 2009, 2013). Fourteen lines did not contain any of the known genes tested i.e. Xa4, xa5 and xa13. Out of these, nine lines namely ANP-70 (ASGPC 14), ANP-143 (UPRI 93-101), ANP-144 (Khazia Dhan), ANP-176 (Shyam Jeera), ANP-317 (R-1498-747-358-2-1), ANP -323 (NDR 8497-2), ANP-445 (Jao Mali), ANP-526 (IR 74717-3-3-1-3) and ANP-536 (IET 13548) impart resistance against different pathotypes.
Table 1 Reaction of aromatic germplasm lines against two BB pathotypes and molecular analysis showing presence (+) and absence (-) of Xa4, xa5 and xa13 gene

| S. No. | ANP Number | Pusa 386 | PbXo-7 | PbXo-8 | Xa4 | Xa5 | Xa13 |
|--------|------------|----------|--------|--------|-----|-----|------|
| 1.     | ANP-124   |           | 6±1.41 |        | -   | -   | -    |
| 2.     | ANP-143   |           | 6±1.41 |        | -   | -   | -    |
| 3.     | ANP-344   |           | 6±1.41 | 2±1.41 | -   | +   | +    |
| 4.     | ANP-344   |           | 6±1.41 | 2±1.41 | -   | +   | +    |
| 5.     | ANP-344   |           | 6±1.41 | 2±1.41 | -   | +   | +    |
| 6.     | ANP-344   |           | 6±1.41 | 2±1.41 | -   | +   | +    |
| 7.     | ANP-344   |           | 6±1.41 | 2±1.41 | -   | +   | +    |
| 8.     | ANP-344   |           | 6±1.41 | 2±1.41 | -   | +   | +    |
| 9.     | ANP-344   |           | 6±1.41 | 2±1.41 | -   | +   | +    |
| 10.    | ANP-344   |           | 6±1.41 | 2±1.41 | -   | +   | +    |
| 11.    | ANP-344   |           | 6±1.41 | 2±1.41 | -   | +   | +    |
| 12.    | ANP-344   |           | 6±1.41 | 2±1.41 | -   | +   | +    |
| 13.    | ANP-344   |           | 6±1.41 | 2±1.41 | -   | +   | +    |
| 14.    | ANP-344   |           | 6±1.41 | 2±1.41 | -   | +   | +    |
| 15.    | ANP-344   |           | 6±1.41 | 2±1.41 | -   | +   | +    |
| 16.    | ANP-344   |           | 6±1.41 | 2±1.41 | -   | +   | +    |
| 17.    | ANP-344   |           | 6±1.41 | 2±1.41 | -   | +   | +    |
| 18.    | ANP-344   |           | 6±1.41 | 2±1.41 | -   | +   | +    |
| 19.    | ANP-344   |           | 6±1.41 | 2±1.41 | -   | +   | +    |
| 20.    | ANP-344   |           | 6±1.41 | 2±1.41 | -   | +   | +    |
| 21.    | ANP-344   |           | 6±1.41 | 2±1.41 | -   | +   | +    |
| 22.    | ANP-344   |           | 6±1.41 | 2±1.41 | -   | +   | +    |
| 23.    | ANP-344   |           | 6±1.41 | 2±1.41 | -   | +   | +    |
| 24.    | ANP-344   |           | 6±1.41 | 2±1.41 | -   | +   | +    |
| 25.    | ANP-344   |           | 6±1.41 | 2±1.41 | -   | +   | +    |
| 26.    | ANP-344   |           | 6±1.41 | 2±1.41 | -   | +   | +    |
| 27.    | ANP-344   |           | 6±1.41 | 2±1.41 | -   | +   | +    |
| 28.    | ANP-344   |           | 6±1.41 | 2±1.41 | -   | +   | +    |
| 29.    | ANP-344   |           | 6±1.41 | 2±1.41 | -   | +   | +    |
| 30.    | ANP-344   |           | 6±1.41 | 2±1.41 | -   | +   | +    |

Susceptible Check

Resistant Check
Fig. 1 Banding patterns showing presence and absence of xa13 gene in selected aromatic germplasm lines amplified 500bp and 3000bp fragments respectively. Lane M = 50bp DNA ladder, Lane R = IRBB60, Lane S = Bas386, Lane 1-22 = selected germplasm lines and Lane C = control

So, it is suggested that they either contain some other known gene(s) or undescribed genes. Several BB resistance genes have been identified and characterized in non-aromatic rice and incorporated and pyramided through MAS to develop resistant cultivars (Perumalsammy et al., 2010, Rajpurohit et al., 2011, Singh et al., 2014a). The information emanating from this work will help to utilize the aromatic lines for basmati breeding program directed towards pyramiding different disease resistant genes without compromising aroma and quality.

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