

Transgenic basmati rice transformed with the *Xa21* gene shows resistance against bacterial leaf blight

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Abstract: Rice (*Oryza sativa* L.) cultivar Super Basmati was transformed with a cloned gene, *Xa21*, through particle bombardment method. Twenty-two plants were recovered from the selected calli. Initial screening through PCR confirmed the presence of the transgene in 21 plants. Southern analysis further confirmed the integration of the *Xa21* gene into the genome of these transgenic plants. Analysis of the T₁ generation revealed that both the *hpt* and *Xa21* genes were successfully transmitted from the primary transformants to the progeny. Bioassays with two local isolates of bacterial leaf blight pathogen, *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), showed that 14 transgenic lines were resistant to both *Xoo* isolates. No significant differences were observed in yield and grain characteristics between transgenic lines and the control; however, the transgenic lines took 8–12 days longer to flower and showed reduced plant height compared to the control plants. In conclusion, we have developed bacterial blight-resistant transgenic lines that can be further exploited by breeders for rice improvement.

Key words: Bacterial leaf blight, particle bombardment, rice, Super Basmati, transgenic, *Xoo*

1. Introduction

Bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is one of the most devastating diseases of rice worldwide (Dossa et al., 2016) and yield losses due to this disease can reach up to 50% in some areas (Verdier et al., 2012). In Pakistan, the incidence of this disease is increasing in famous rice-growing areas (Khan et al., 2009) and no natural resistance against this disease could be found in basmati cultivars. All basmati varieties cultivated in Pakistan are susceptible to this disease, especially Super Basmati, which is a valuable source of foreign exchange earnings (Khan et al., 2014). In a search to identify bacterial blight resistance genes in basmati landraces and cultivars, Ullah et al. (2012) identified the *Xa4*, *xa5*, *Xa7*, and *xa13* genes in five basmati cultivars and 52 basmati landraces using PCR. They observed that *Xa7* was the most prevalent gene among the cultivars and landraces. Khan et al. (2015) also reported the presence of the *Xa7* gene in Super Basmati. Some of the basmati cultivars (Basmati-385 and Basmati-2000) also contain the *Xa4* gene; however, *xa5* and *xa13* were confined to landraces only (Ullah et al., 2012). Recently, Xiao et al. (2016) reported that *Xa4* shows an incomplete dominance

nature while *Xa21* generally confers resistance at the late tillering stage and the resistance levels offered by these genes are affected by the genetic background. Hence, the development and use of resistant varieties and pyramiding of two or more resistance genes into elite rice cultivars is the only strategy available to combat BLB (Vikal et al., 2014). Incorporation of other resistance (*xa*) genes in basmati rice either through breeding or genetic transformation is therefore highly desirable. Conventional breeding is the most obvious choice to evolve a BLB-resistant rice variety; however, it involves lengthy crossing and back-crossing procedures and sometimes may also result in the incorporation of undesirable genetic components, which are difficult to remove from the parental background. Genetic engineering, on the other hand, provides us with an opportunity to transfer either one or more cloned genes to the regenerable tissues from which resistant plants can be developed, hence providing us with relatively controlled engineering for improvement of desired traits.

Xa21 was the first resistance gene to be cloned (Song et al., 1995; Wang et al., 1996) and transferred into rice, conferring a high level of resistance to the BLB pathogen (Tu et al., 1998; Zhang et al., 1998; Tang et al., 1999). Tu

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et al. (2000) showed that the transgenic IR72 rice lines with the *Xa21* gene not only had a high level of resistance against BLB but also exhibited excellent field performance. Therefore, *Xa21* seems to be the best candidate gene so far to produce BLB-resistant transgenic plants (Bajaj and Mohanty, 2005).

The present study was therefore aimed at establishing genetically engineered resistance in basmati rice against BLB through transformation of the *Xa21* gene and evaluation of transgenic plants using local *Xoo* isolates.

2. Materials and methods

2.1. Transformation, selection and regeneration of transgenic plants

Two plasmids (Figure 1), pC822 (courtesy of Dr Pamela Ronald, UC Davis) and pZS1, having the *Xa21* and *hpt* genes, respectively, were used in a 3:1 mole ratio for cotransformation. Details of different media used for bombardment, selection, regeneration, and rooting of plants are given in Table 1. Mature embryos were excised from sterilized seeds soaked overnight. Embryos were arranged on the bombardment medium as shown in Figure 2a for 3–4 h and then bombarded following optimized conditions using 1.0- μ m gold particles at a helium pressure of 7500 kPa and target distance of 9 cm, while vacuum, macrocarrier flight distance, and rupture-disk to macrocarrier distance were kept constant at 685 mmHg, 8.0 mm, and 6.4 mm, respectively. Bombarded

embryos were kept on the same medium for 24 h before placing them on callus induction cum selection (CIS) medium. Embryos producing embryogenic hygromycin-resistant (*hyg^R*) calli were further transferred to fresh CIS medium every 15 days. The selected calli were transferred to preregeneration medium (PRM1) for 2 weeks and later to regeneration media (PRM2) to regenerate complete plants. Regenerated plants were established in soil and raised to maturity in a containment glass house.

2.2. Molecular analysis of putative BLB-resistant transgenic plants

Total DNA was isolated from fresh leaves using a modified CTAB method (Doyle and Doyle, 1990). Putative transgenic T₀ plants were first screened by PCR using *hpt*, *Xa21*, and RG100 specific primers (Table 2). The RG100 pair of primers can amplify a single band from any DNA extracted from rice and was used as an internal control. PCR was performed in 25 μ L of reaction mixture containing 50–100 ng of plant DNA, 40 ng of each of the primers, 2.5 μ L of 10X PCR buffer, 3.0 μ L of dNTPs (1 mM), 3.5 μ L of MgCl₂ (15 mM), 0.2 μ L of Taq polymerase (5 U/ μ L), and water to complete the volume. The reaction mixture was subjected to 1 cycle of 94 °C for 5 min followed by 35 cycles of 94 °C for 1 min, 60 °C for 1 min, and 72 °C for 3 min, with 1 cycle of 5 min at 72 °C to complete the reaction. Ten microliters of each amplified product was electrophoresed through 1% agarose gel containing 0.5 μ g/mL ethidium bromide at 100 V for 3–4 h. The size of the PCR products

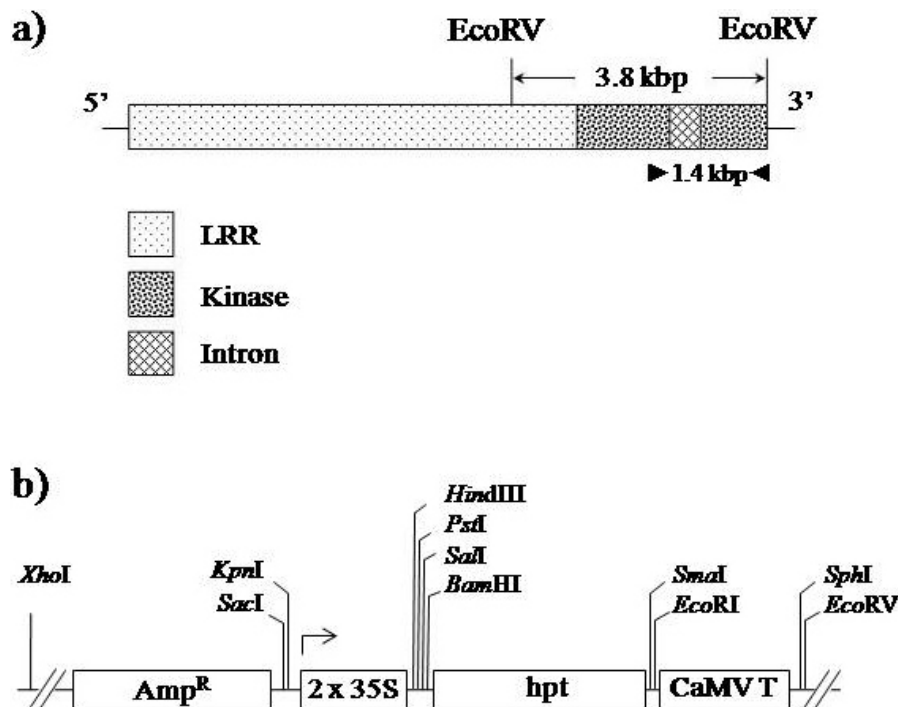


Figure 1. Partial maps of plasmids pC822 (a) and pZS1 (b).

Table 1. Composition of media used for bombardment, callus induction/selection, regeneration and rooting.

Bombardment medium	MS salts & vitamins supplemented with 2,4-D 2 mg L ⁻¹ ; sucrose 3%; Phytigel 0.3%; mannitol 0.25 M; pH 5.8.
Callus induction cum selection medium (CIS)	MS salts & vitamins supplemented with 2,4-D 2 mg L ⁻¹ ; sucrose 3%; Phytigel 0.25%; pH 5.8. Filtered sterilized hygromycin B 50 mg L ⁻¹ was added after autoclaving the medium.
Plant regeneration medium-1 (PRM-1)	MS salts & vitamins supplemented with kinetin 3 mg L ⁻¹ ; NAA 1 mg L ⁻¹ ; abscisic acid 10 mg L ⁻¹ ; maltose 3%; sorbitol 3%; Phytigel 0.25%.
Plant regeneration medium-2 (PRM-2)	Same as PRM-1 but without abscisic acid.
Rooting medium	Half-strength MS salts & vitamins supplemented with sucrose 2%; Phytigel 0.2%; pH adjusted to 5.8.

was visualized under UV light and photographs were taken using the Eagle Eye photo-documentation system (Stratagene, USA).

For Southern analysis, 10 µg of genomic DNA from T₀ lines and control plants was double-digested with *Hind*III and *Eco*RV, fractionated by gel electrophoresis, and transferred onto HybondTM-N⁺ membrane using an alkali transfer method following standard protocols as described by Sambrook et al. (1989). A 1.3-kbp probe consisting of the nick-translated ³²P-dCTP labeled *Hind*III-*Eco*RV fragment of the *Xa21* gene was used for hybridization. The filters were removed, wrapped in Saran wrap, and exposed to X-ray film for 2–3 days at –70 °C.

2.3. Progeny analysis

T₁ seeds were analyzed for the presence and expression of foreign genes. Ten seeds of each transgenic line along with the nontransformed control were surface-sterilized and plated on MS medium without growth regulators either without hygromycin B or containing 25 mg hygromycin B L⁻¹. Percentage of seed germination was recorded. Germinated plants were transferred to soil in the pots and grown to maturity inside the containment glasshouse. PCR was used to detect the presence of the *Xa21* gene in the T₁ plants.

2.4. Screening for BLB resistance

Two T₁ seedlings of each line were sown in pots at the three-leaf stage. The experiment was conducted in three replicates. Sixty-day-old *Xa21*-positive plants were used to screen for BLB resistance using two local isolates of *Xoo*, 1.2.1 and 3.2.2. The inoculum of both the isolates was prepared following methods described by Tu et al. (1998). The transgenic plants along with the controls were grown in a containment glasshouse at a temperature of 30 °C and 85% relative humidity (RH) during the day and 25 °C and 90% RH at night. Five to six leaves of each plant were inoculated at the maximum tillering stage with *Xoo* isolates using scissors dipped in the bacterial suspension by leaf-

clipping method (Kaufman et al., 1973). Plant reaction was scored after 14 days of inoculation. Disease reactions were characterized as resistant (R), moderately resistant (MR), or susceptible (S) based on leaf lesion length of 0–3 cm, 3–6 cm, or >6 cm, respectively.

2.5. Field performance of transgenic BLB-resistant lines

Limited field trials of some of the selected transgenic lines were conducted in three replicates following a randomized complete block design at the experimental area inside the premises of NIBGE, Faisalabad, Pakistan. Sixty-four *Xa21*-positive T₂ seedlings of each selected line along with the control were planted in plots measuring 1.8 × 1.8 m with plant to plant distance of 20 cm. Standard agronomic practices were used throughout the growing period. Data regarding different traits like days to flowering, plant height, number of fertile tillers per plant, grain length, and grain width were collected from 10 plants randomly selected from each of the replicated plots representing a line (transgenic/nontransformed control). Data regarding grain length and grain width were collected from 10 seeds of one plant each from the three replicated plots. Since no incidence of BLB was found during this season in Faisalabad, no data regarding BLB were recorded.

3. Results and discussion

3.1. Transformation of rice

In five different experiments, 884 mature embryos were bombarded and placed on selection medium. Most of the embryos turned brown and necrotic and only a few started producing calli from the scutellum tissues after 7–8 days of culture. Different steps of developing transgenic rice are shown in Figure 2. Twenty-one hyg^R calli were recovered with a transformation efficiency of 2.38%. The transformation efficiency for indica rice species is relatively low compared to the japonica rice species (Tie et al., 2012). While using an indica rice variety, TN1, Sivamani et al. (1996) recovered transgenic rice plants with an overall

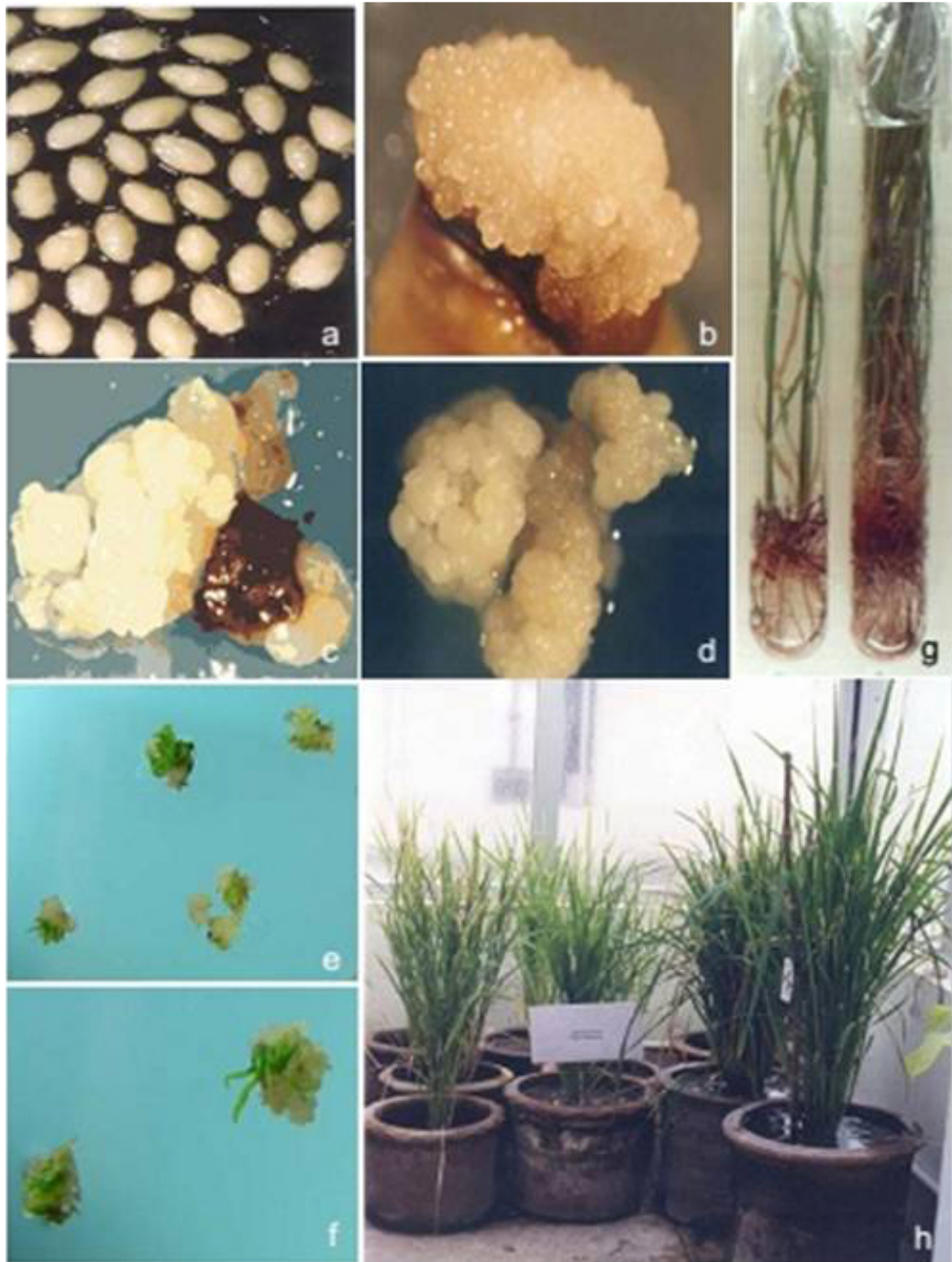


Figure 2. Different steps in the development of BLB resistant basmati rice: a) mature embryos arranged on bombardment medium 3–4 h prior to bombardment; b) hygromycin-resistant callus produced from bombarded embryo; c) vigorous proliferation of hygromycin-resistant callus on selection medium; d) induction of somatic embryos on hygromycin-resistant callus; e–f) plant regeneration from selected hyg^R calli; g) vigorous root formation in putative transgenic plants on root induction medium; h) regenerated plants established in soil and kept in containment glasshouse for growth, analysis, and seed collection.

transformation efficiency of 2%–4%. Martinez-Trujillo et al. (2003) obtained transgenic rice plants of an indica rice variety, Morelos A-92, with 4.6% efficiency. However,

much higher transformation efficiency (up to 22%) has been reported from japonica rice cultivar Taipie 309 (Chen et al., 1998).

Table 2. Sequence of PCR primers used for screening of putative transgenic plants.

Primer name	Sequence (5'-3')	Product size (kbp)
hpt-F hpt-R	AGAATCTCGTGCTTTCAGCTTCGA TCAAGACCAATGCGGAGCATATAC	0.9
Xa21-F Xa21-R	ATAGCAACTGATTGCTTGG CGATCGGTATAACAGCAAAAC	1.4
RG100-F RG100-R	GCTGGACGTGCCAAAGAGAG CGAACCACAGCCACAGCATG	0.7

3.2. Molecular analysis of transgenic plants

For molecular analysis of transgenic (T_0) plants, genomic DNA isolated from all 22 putative transgenic and control plants was subjected to PCR analysis using *hpt* and *Xa21* primers in independent reactions. Twenty-one plants amplified *hpt* and *Xa21* specific fragments while one plant line was found to be negative for both *hpt* and *Xa21* (not shown). Multiplex PCR was performed on PCR-positive lines from the first round of screening using *hpt*, *Xa21*, and RG100 specific primers. Figure 3 shows that all the putative transgenic plants amplified *hpt*, *Xa21*, and RG100 specific fragments. Use of the RG100 locus as an internal control to authenticate PCR results has been reported in a number of studies involving transgenic plant screening (Ghareyazie et al., 1997; Kiani et al., 2012).

Southern analysis (Figure 4) confirmed that the *Xa21* gene had been integrated in ten different plant lines except line S-3-03. Variation in the band intensity between the different lanes indicated the difference in number of

copies of the *Xa21* gene inserted in the plant genome. Some lines also showed rearrangements of the transgene before integration in the plant genome (Figure 4, lane 12). These observations are in agreement with reports that complex integration patterns can lead to transgenic loci consisting of two or more copies of the transgene (Takano et al., 1997). Integration is often associated with complex rearrangements including deletions, inversions, and duplication of the original inserted sequence (Zhu et al., 2010).

3.3. Screening of transgenic plants for hygromycin B and BLB resistance

Screening of T_1 plants for a functional hygromycin gene was done by a simple germination test. T_1 seeds from six selected lines along with nontransformed controls were placed on a modified MS medium either without hygromycin B or with 50 mg hygromycin B L⁻¹. A high percentage (83%–100%) of seeds of transgenic lines along with controls germinated on the medium with no

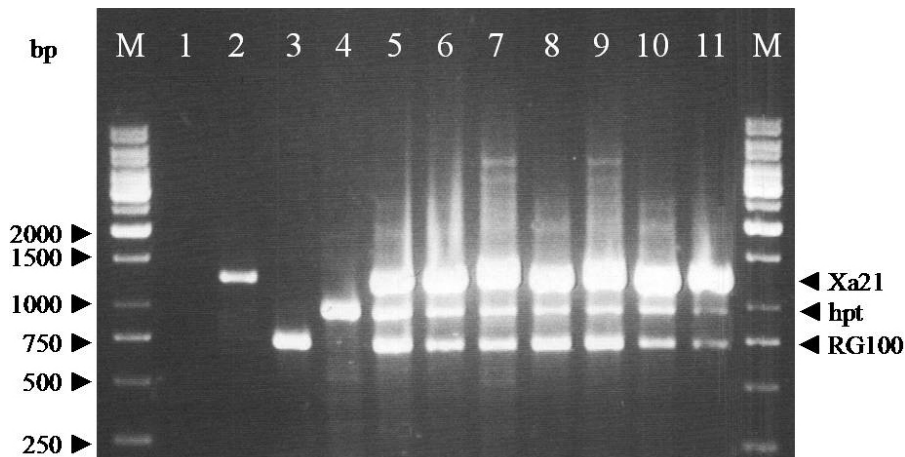


Figure 3. Agarose gel electrophoresis of multiplex PCR amplified products showing the amplification of *Xa21*, *hpt*, and RG100 locus from the genomic DNA isolated from different transgenic (T_0) lines transformed with *Xa21* and *hpt* genes. Lane M represents 1-kbp DNA ladder as molecular weight marker while lane 1 represents water control; lane 2, plasmid pC822; lane 3, DNA from a nontransformed plant (negative control); lane 4, plasmid pZS1; lanes 5–11, DNA from seven different putative transgenic (T_0) plants, respectively.



Figure 4. Southern analysis of 11 putative (T_0) transgenic lines transformed with *Xa21* gene. Lane 1, plasmid control (*Hind*III-*Eco*RV 1.3-kbp fragment); lane 2, DNA from a nontransformed plant; lanes 3–13, DNA from 11 different putative transgenic plants digested with *Hind*III-*Eco*RV, respectively.

hygromycin B, whereas on medium containing 50 mg hygromycin B L^{-1} , no germination was observed in any of the controls, while 86%–90% of seeds of the transgenic lines germinated, indicating that the *hpt* gene was functional in all these lines.

Results of screening of different transgenic rice lines and the nontransformed controls against two local isolates of *Xoo* are given in Table 3. All the control plants were found to be susceptible to both the isolates. Initial symptoms developed at the point of inoculation within 4–5 days in the form of leaf curling. In the resistant lines, the lesions progressed uniformly downward from the point of inoculation, whereas in the case of moderately resistant or susceptible plant lines, the lesions progressed more rapidly along the leaf margins. The transgenic plants showed a variable reaction to *Xoo* inoculation. Except line S-3-03, all transgenic lines produced significantly reduced lesion lengths compared to the controls in response to both *Xoo* isolates (Figure 5). In response to isolate 1.2.1, fourteen transgenic lines showed resistant reaction, five showed moderately resistant reactions, and two lines (S-3-03 and S-4-01) showed a susceptible reaction in contrast to the nontransformed control. In response to isolate 3.2.2, seventeen transgenic lines showed a resistant reaction, three lines exhibited a moderately resistant reaction, and only one line (S-3-03) was found to be susceptible. These results are in agreement with those of Tu et al. (1998), who developed *Xa21* transgenic IR72 lines and observed highly reduced lesions in transgenic BLB-resistant plants and an *Xa21* donor (IRBB21) compared to the significantly larger lesions in the control plants. Jiang et al. (2004) introduced *Xa21* and a fused Bt *cry1Ab/lac* into an elite indica cytoplasm male sterile (CMS) restorer line, Minghui 63. They demonstrated highly significant reduction in lesion lengths in the pyramided line Minghui 63/Bt & *Xa21* and in Minghui 63/*Xa21* compared to controls. Maruthasalam et al. (2007) pyramided the Pusa Basmati1 line with *chi11*, *tlp*, and *Xa21* and showed an enhanced resistance to both sheath blight and BLB. They showed that apart from the

reduction in the number of infection cushions, the leaves of transgenic plants also exhibited reduced lesion length with characteristic browning around the lesions, which clearly showed effective restriction of pathogenic invasion. These results indicated that *Xa21* conferred resistance in some of the transgenic T_1 plant lines. Recently Ellur et al. (2016) reported that basmati rice varieties (Pusa Basmati 1121 and Pusa Basmati 6) carrying two bacterial blight resistance genes, *xa13* and *Xa21*, were resistant to *Xoo* races of northwestern, southern, and eastern parts of India. These studies indicate that *Xa21* is one of the major genes conferring resistance against the BLB pathogen.

3.4. Field performance of transgenic lines

The transgenic lines were tested in the field on a limited scale. The results of the field experiments are given in Table 4. The transgenic lines exhibited variation in plant height, days to flowering, and number of fertile tillers per plant while no significant difference in grain yields was observed compared to the control plants. There was a significant reduction in plant height in all the transgenic lines compared to the control. The mean plant height recorded for the control plants was significantly higher ($P \leq 0.05$) compared to different transgenic lines. Similar results were also reported by other workers. Bashir et al. (2004) noticed significant reduction in plant height of the transgenic rice lines L-5, L-13, and L-6-411 compared to the controls. Kim et al. (2008) also observed highly significant reduction in plant height in insect-resistant transgenic lines of three Korean rice varieties. Three lines (S-1-02, S-2-04, and S-5-01) showed a significant increase in the number of fertile tillers compared to the controls while no significant increase or decrease in the number of fertile tillers per plant was observed in the other three lines. Shu et al. (2002) noticed tremendous variation in number of productive tillers in the T_0 and T_1 transgenic plants of nine different rice varieties. Table 4 shows that transgenic lines took a significantly longer time (8–12 days more) to flower compared to the nontransformed controls. Similar behavior of delayed

Table 3. BLB reactions of transgenic lines and their corresponding controls inoculated with two local *Xoo* isolates 1.2.1 (A) and 3.2.2. (B). Disease reactions were characterized as resistant (R), moderately resistant (MR), or susceptible (S) based on leaf lesion length of 0–3 cm, 3–6 cm, or >6 cm, respectively.

Line number	Reaction to isolate 1.2.1 (A)		Reaction to isolate 3.2.2 (B)	
	Lesion length (cm)	Reaction	Lesion length (cm)	Reaction
Control	17.82 ± 0.88 a	S	16.55 ± 1.08 a	S
S-1-01	0.92 ± 0.45 i	R	0.52 ± 0.25 g	R
S-1-02	0.53 ± 0.23 j	R	0.57 ± 0.23 g	R
S-1-03	2.72 ± 0.67 f	R	2.48 ± 0.48 d	R
S-1-04	1.98 ± 0.44 gh	R	1.35 ± 0.70 f	R
S-1-05	2.53 ± 0.50 fg	R	1.38 ± 0.56 f	R
S-1-06	3.12 ± 0.40 e	MR	1.27 ± 0.20 fg	R
S-1-07	0.67 ± 0.29 ij	R	0.43 ± 0.14 g	R
S-2-01	2.17 ± 0.54 g	R	1.27 ± 0.32 fg	R
S-2-02	3.72 ± 0.78 de	MR	1.93 ± 0.24 ef	R
S-2-03	5.65 ± 0.73 c	MR	3.28 ± 0.63 cd	MR
S-2-04	0.43 ± 0.14 j	R	0.50 ± 0.20 g	R
S-3-01	2.62 ± 0.49 f	R	2.27 ± 0.32 e	R
S-3-02	4.22 ± 0.68 d	MR	2.38 ± 0.52 de	R
S-3-03	16.18 ± 1.42 a	S	16.08 ± 0.85 a	S
S-3-04	1.28 ± 0.42 hi	R	0.98 ± 0.44 g	R
S-3-05	2.85 ± 0.29 f	R	2.10 ± 0.35 e	R
S-4-01	6.98 ± 1.25 b	S	4.80 ± 0.91 b	MR
S-4-02	2.87 ± 0.47 f	R	2.28 ± 0.51 e	R
S-4-03	4.27 ± 0.66 d	MR	3.62 ± 0.47 c	MR
S-5-01	0.73 ± 0.32 i	R	0.57 ± 0.29 g	R
S-5-02	3.00 ± 0.55 ef	R	2.70 ± 0.54 d	R

Means within a column followed by a common letter are not significantly different at the 5% level by DMRT.

Each value is the mean of three replicates.

± refers to standard deviation between the replicates.

flowering time has also been reported in several other studies (Tu et al., 2000; Bashir et al., 2004). There was no significant difference in the grain yield or other grain characteristics of the transgenic lines compared to the nontransformed controls. However, a few lines showed reduced grain width compared to the controls. Tu et al. (2000) observed that some of the transgenic IR72 lines having the *Xa21* gene gave excellent field performance and yield performance of one of the transgenic lines, T103-10, was comparable with that of control. Similarly, Datta et al. (2007) observed no significant variation in grain yields, 100-seed weight, and total biomass of the golden rice transgenic lines as compared to the control plants.

However, a few studies indicated that transgenic lines exhibited lower grain yields compared to their respective controls (Kim et al., 2008). These variations in phenotypic and agronomic characters may be a result of somaclonal variations, insertion mutagenesis, or transgene-induced endogenous gene silencing (Larkin and Scowcroft, 1981; Matzke et al., 1993; Kathuria et al., 2007). Field data of the subsequent generations, however, will help to determine the consistency of these characteristics in the next generations.

In conclusion, we have developed a simple procedure for the transformation of basmati rice varieties through particle bombardment and the resulting transgenic lines

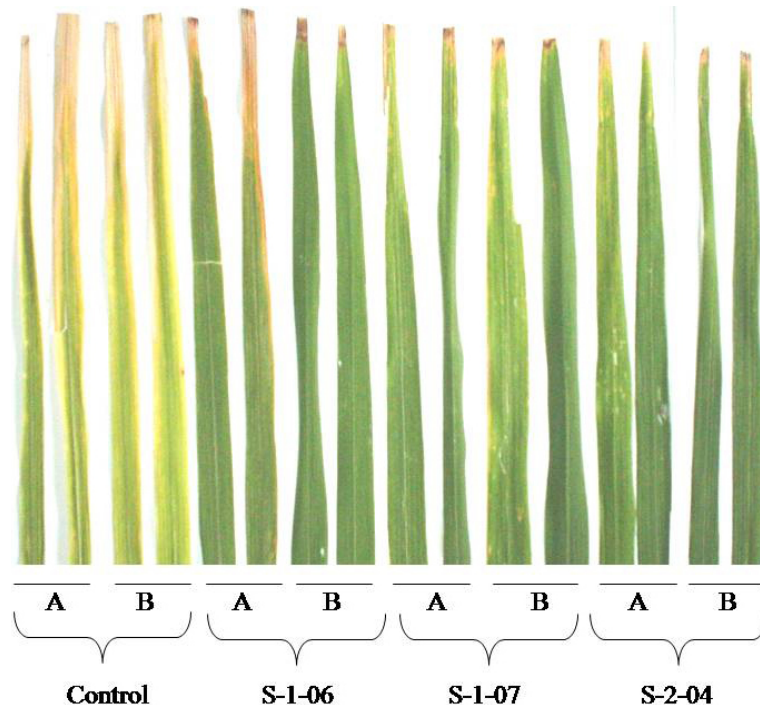


Figure 5. Screening of transgenic (T_1) lines against two local isolates of bacterial blight pathogen *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). Picture shows the screening of three transgenic lines along with the untransformed control. Each line is represented by a set of four leaves; of them, two leaves each were inoculated with local *Xoo* strains 1.2.1. (A) and 3.2.2 (B). Picture was taken after 14 days of *Xoo* inoculation.

Table 4. Field performance of transgenic (T_2) lines along with control plants.

Line	Plant height* (cm)	Number of fertile tillers per plant*	Days to flowering*	Grain yield per plant* (g)	Grain	
					Length (mm)†	Width (mm)†
Control	122.4 ± 4.34 a	21.4 ± 3.84 b	73.3 ± 1.26 c	21.94 ± 4.42 a	8.12 ± 0.36 a	1.70 ± 0.05 b
S-1-01	91.4 ± 2.31 b	25.2 ± 5.76 b	82.3 ± 0.50 a	25.82 ± 5.90 a	7.92 ± 0.39 a	1.69 ± 0.07 b
S-1-02	95.2 ± 3.83 b	28.8 ± 9.01 a	85.3 ± 0.96 ab	26.26 ± 10.2 a	7.87 ± 0.14 a	1.76 ± 0.05 ab
S-1-07	92.6 ± 10.0 b	20.4 ± 7.09 b	80.8 ± 3.30 bc	26.08 ± 14.3 a	8.00 ± 0.21 a	1.83 ± 0.13 a
S-2-04	84.0 ± 5.52 c	34.8 ± 6.83 a	83.3 ± 1.89 a	17.10 ± 4.05 ab	7.75 ± 0.30 ab	1.78 ± 0.10 a
S-3-04	87.6 ± 5.18 bc	26.0 ± 8.46 ab	82.8 ± 1.26 a	32.60 ± 16.38 a	7.83 ± 0.42 a	1.63 ± 0.16 bc
S-5-01	88.0 ± 3.08 b	26.2 ± 5.40 a	81.0 ± 2.45 b	20.92 ± 5.77 a	7.80 ± 0.32 a	1.70 ± 0.10 b

Means within a column followed by a common letter are not significantly different at the 5% level by DMRT.

*Based on data collected from 10 plants randomly selected from each plot representing a transgenic line/nontransformed control in a randomized complete block design having three replicates.

†Based on data collected from 10 seeds of plant representing a transgenic line/nontransformed control in a limited field trial having three replicates.

± refers to standard deviation between the replicates.

exhibited resistance against two local isolates of *Xoo*. Apart from this, the transgenic lines showed a significant reduction in plant height, which is an important development as yield losses due to lodging has been one of the major problems in basmati rice cultivation.

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