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JAPONICA RICE CULTIVAR 'BEGAMI' (JP-5) FROM NORTHERN PAKISTAN EXHIBITS *OsSWEET11*-MEDIATED SUSCEPTIBILITY TO HIMALAYAN FOOTHILL STRAINS OF *XANTHOMONAS ORYZAE* PV. *ORYZAE*

IRFAN ULLAH[1†], MUDASSAR NAWAZ KHAN[1†], MUHAMMAD ZAKRIA[2], HAMIDA MAHMOOD[3], ZAHEER ABBAS[4], HAMID ALI[1], ASIF IQBAL[5], FAISAL NOUROZ[3], TARIQ MAHMOOD[5]*, AYAZ AHMAD[6]* AND SAJID ALI[7]

[1]Department of Biotechnology and Genetic Engineering, Hazara University Mansehra, Pakistan
[2]Crop Disease Research Institute, NARC, Islamabad, Pakistan
[3]Department of Bioinformatics, Hazara University Mansehra, Pakistan
[4]National Institute for Genomics and Biotechnology, NARC, Islamabad, Pakistan
[5]Department of Agriculture, Hazara University Mansehra, Pakistan
[6]Department of Biotechnology, Abdul Wali Khan University Mardan, Mardan, Pakistan
[7]Department of Horticulture and Life Science, Yeungnam University, Gyeongsan, Republic of Korea
[†]These authors contributed equally to this work

*Corresponding author's email: tmahmood74@hu.edu.pk ; ahdayazb5@awkum.edu.pk

Abstract

Bacterial blight (BB) of rice is caused by bacterium *Xanthomonas oryzae* pv *oryzae* (Xoo), giving significant yield loss to the crop. The pathogen injects its Transcription Activation-Like Effectors (TALEs) into host cells, where the TALEs bind to the effector binding elements (EBEs) in host gene promoters, inducing susceptibility (*S*) or resistance (*R*) genes, thus causing disease or resistance. A major class of Xoo-induced *S* genes in rice encodes cellular sucrose transporters, referred to as the *OsSWEET* genes family. Three *OsSWEET* genes—*OsSWEET11*, *OsSWEET13*, and *OsSWEET14*—are distinctly induced by different Xoo strains from diverse geographical regions of the world, via their cognate TALEs, to cause BB in the field. Begami (JP-5) is a farmer-maintained BB-susceptible Japonica-type rice landrace from northern Pakistan, locally known as Begami and Garhla in different regions. However, the *OsSWEET* gene induced by Pakistani Xoo strains in Begami rice is not known. In the current study, *OsSWEET11* was identified as specifically induced by KP-3, a representative Xoo strain from the Himalayan foothills, in Begami. Further, promoter analysis of *OsSWEET11* in the variety identified intact EBE sequence specific to TALE PthXo1 of Xoo. The study suggests that Begami offers *OsSWEET11* as a major *S* gene target for BB disease. The study further suggests that KP-3 harbors PthXo1, a cognate TALE of *OsSWEET11*, as its major virulence effector for the disease. Characterizing Xoo strains, and rice cultivars resistant or susceptible to these strains, is a fundamental step to understanding the Xoo-rice coevolution and developing race-specific resistance strategies in a specific region. The present study provides a baseline to develop a resistant Begami cultivar either by breeding or targeted editing of *OsSWEET11* promoter.

Key words: Bacterial Blight; Effector binding element (EBE); TAL effector; Expression analysis

Introduction

Bacterial blight (BB) disease of rice, caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo) bacterium, adversely affects both yield and quality of rice (Aftab *et al.*, 2022), causing 20–40% yield loss at tillering and 50% loss at early developmental stage (Jiang *et al.*, 2019; Ullah *et al.*, 2022). The disease causes wilting and necrosis in leaves, impairs photosynthesis, and depending on nature of cultivar, environmental conditions, and the timing of pathogen exposure, ultimately reduces grain yield (Shi *et al.*, 2015; Pan *et al.*, 2023). Using BB-resistant rice cultivars is the most effective, cost-efficient, and eco-friendly approach to control BB (Ali *et al.*, 2016). The approach, however, requires understanding the disease/resistance mechanism between the pathogen and its host.

Xoo bacterium enters the rice plant through wounds and natural openings, multiplies, and blocks the host xylem. It injects its special kinds of type-3 effectors, known as Transcription Activation-Like Effectors (TALEs) into host cells, which travel to the nucleus and induce the cognate host susceptibility (*S*) or resistance (*R*) genes in a TALEs-specific manner. TALEs bind to the corresponding DNA motifs, known as effector binding elements (EBEs), in the promoters of *S* or *R* genes, and result into either susceptibility or resistance symptoms (Yang *et al.*, 2006). Except North American strains, TALEs are present across all Xoo strains of the world regions and vary significantly in number and diversity along the regions. Asian Xoo strains, for example, harbor 10–20 TALEs, as represented by TALEs PthXo1, AvrXa7, PthXo3, PthXo2, and PthXo2-like, while African strains possess 9 conserved TALEs, as denoted by TALEs TalC, TalF and Tal5 (Streubel *et al.*, 2013; Li *et al.*, 2019; Oliva *et al.*, 2019).

Major *S* genes targeted by major Xoo TALEs in rice fields around the world are members of the *OsSWEET*

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genes family and include *OsSWEET11*, *OsSWEET13*, and *OsSWEET14*. The former two are specifically induced by the TALEs PthXo1 and PthXo2/PthXo2-like, respectively, while the latter is activated by multiple TALEs-AvrXa7, PthXo3, TalC, and Tal5-from geographically distinct Xoo populations worldwide, which either bind to distinct BEs or overlap portions of these EBEs to induce *OsSWEET14* (Yang & White, 2004; Yang *et al*., 2006; Antony *et al*., 2010; Streubel *et al*., 2013; Zhou *et al*., 2015). Furthermore, other *OsSWEET* genes, such as *OsSWEET12*, *OsSWEET15*, and *OsSWEET11b* have been experimentally implicated in BB, however, neither their cognate TALEs are known nor has their expression been detected in the field during BB occurrence (Streubel *et al.*, 2013).

Understanding host/pathogen genetics is vital in plant-pathogen interactions as it helps uncover the mechanisms underlying susceptibility and resistance. The co-evolutionary pattern of TALEs and their cognate *S* or *R* genes in rice is important relative to geographical adaptation of a particular cultivar. The strategy helps identify the *S/R* gene induced by the Xoo strains of a particular geography and accordingly, deploy varieties resistant to those strains. Given the selection pressure in which TALEs and their cognate *S/R* genes coevolve from extant geographic strains, the strategy further helps anticipate the risk of emerging and highly aggressive Xoo strains (Schandry *et al.*, 2018). Over 30 Xoo races have been identified globally, each with distinct geographic characteristics and identification systems. The Philippines classifies 10 races, represented by PXO61, PXO86, PXO79, PXO71, PXO112, PXO99, PXO145, PXO280, PXO330, and PXO341. Japanese races are represented by T7174, T7147, T7133, H75373, H75304, and H8584, while Chinese races are represented by YN18, YN1, GD414, Zhe173, ScYc-b, YN7, YN11, FuJ, and YN24 (Adhikari *et al.*, 1999; Yang & White, 2004; Mishra *et al.*, 2013; Kuang *et al.*, 2017). Altogether, these races harbor major TALEs, including but not limited to PthXo1, PthXo2, PthXo3 and AvrXa7, and induce major *S* genes *OsSWEET11*, *OsSWEET13*, and *OsSWEET14* in a gene-for-gene specific manner.

Research into *OsSWEET* genes and the Xoo strains that induce them will facilitate the precise interventions needed to enhance BB resistance (Oliva *et al.*, 2019; Xu *et al.*, 2019). *OsSWEET11* is of particular importance as it is otherwise a vital protein required for rice fertility and seed setting in florescence but hijacked by Xoo in the leaves. Begami (JP-5) is farmer-maintained but BB-susceptible Japonica-type rice landrace adapted to the northern Pakistan, locally known as Begami and Garhla in different regions. Hereafter, the term 'Begami' is used to refer to this landrace. A representative Himalayan foothills Xoo strain, KP-3, has been shown to induce *OsSWEET11* in the BB-susceptible international check rice varieties (Shah *et al.*, 2025). However, how Begami cultivar responds to KP-3 infection in terms of *OsSWEET* genes expression is not known. This is the first report to document the induction of *OsSWEET11* in Begami by KP-3 and correlate the induction to TALE PthXo1 possibly present in KP-3, and PthXo1-specific EBE present in the promoter of *OsSWEET11* in the cultivar.

Materials and Methods

Plant material: Six cultivated varieties of rice were provided by National Agriculture Research Center Islamabad, Pakistan (Table 1). All the rice varieties were grown in the Agriculture field of Hazara University Mansehra.

Table 1. Details of the rice samples used in the study, including their laboratory codes and corresponding rice varieties.

S. No.	Lab/No.	Variety
1.	C-1	IR-24
2.	C-2	Begami S-88
3.	C-3	Begami-152
4.	C-4	Begami/ Garhla/JP-5
5	C-5	Basmati-385
6	C-6	Super Basmati

Collection and isolation of Xoo strains: Rice leaves exhibiting typical BB symptoms—water-soaked lesions along the leaf veins progressing into yellowish-brown necrotic streaks—were collected from naturally infected rice fields in the Mansehra region, Khyber Pakhtunkhwa Province, Pakistan. The samples were enclosed in a mailing envelope, tagged, and promptly conveyed to the laboratory for subsequent processing. Infected leaves were washed thoroughly with sterile water to remove dust and surface contaminants, then air-dried under a laminar flow hood. For surface sterilization, four 1.5 mL Eppendorf tubes containing sterile distilled water and one tube containing 70% ethanol were prepared. Leaf segments (2-4 cm) were cut using sterile scissors, immersed in 70% ethanol for 30 seconds using sterile forceps, and then sequentially rinsed in each of the four sterile distilled water tubes for 15 seconds each to remove residual ethanol. Following surface sterilization, the leaf segments were macerated in 700 µL of autoclave distilled water using autoclave pipette tips and kept at room temperature (18–25°C) for 1 hour to facilitate the release of bacterial cells into the suspension. A sterile wire loop was immersed in the suspension and subsequently streaked onto nutrient agar plates. The plates underwent incubation at a temperature of 28°C for a duration of 3 to 4 days. Colonies displaying typical morphological characteristics of Xoo-smooth, round, mucoid, and golden-yellow-were selected and re-streaked on fresh NA plates to obtain pure single-colony cultures. These were further incubated at 28°C for 3 days to ensure purity and maintained for downstream analyses. Pure single colonies were preserved in 50% glycerol stocks and stored at −80°C freezer for long-term use.

Re-isolation of Xoo strain from glycerol stocks: The Xoo strains (PXO99 and KP-3) previously isolated and preserved were revived from glycerol stock. A disinfected wire loop was inserted into the Eppendorf tubes containing glycerol stock and then streaked onto a petri dish with nutrient agar media. The petri dishes were incubated for four days at 28°C.

DNA extraction from the Xoo bacteria for PCR analysis: Thermo Scientific's Gene JET Genomic DNA Purification Kit (K0721) was used to extract the DNA. Two loops of bacterial culture were transferred to 1.5mL tubes with 180µl digestion buffer, vortexed, and incubated with 20µL Proteinase K. The samples were lysed at 56ºC for 2 hours,

then treated with 20µl RNAase A for 10 minutes. After adding 200 µL lysis buffer, the mixture was gently shacked, followed by 400µl of 50% ethanol. The lysate was subjected to a Gene JET column and centrifuged at 600xg for 2 minutes. After discarding the flow-through, the column was washed with 600 µL of wash buffer I at 800 x g for 2 minutes and then 600 µL of wash buffer II at 12,500 x g for 3 minutes. A fresh tube was prepared with the column, to which 200µL of elution buffer was added for DNA elution. The mixture was kept at room temperature for ten minutes and subsequently centrifuged at 8000xg for one minute. The purified DNA was maintained at -20°C for 24 hours prior to subsequent applications.

Specific primers JLXooF/R for Xoo were designed based on polymorphic regions of the putative glycosyltransferase gene (Table 2). A 25µl PCR master mix was prepared using 2x Green master mix (Thermo Scientific™), with 1µl of genomic DNA. The PCR amplification was conducted using MiniAmp Plus, Thermal Cycler (under the following parameters: an initial denaturation at 96ºC for 6 minutes; 38 cycles consisting of 95ºC for 60 seconds, 59ºC for 40 seconds, and 72ºC for 30 seconds; concluding with a final extension at 72ºC for 9 minutes. The PCR products were electrophoresed on a 2% agarose gel in 1X TAE buffer, stained with ethidium bromide (10 µg/ml), and visualized under UV illumination.

Koch's postulates: Inoculum was prepared by streaking stored pure cultures on nutrient agar plates for two days and suspending the pure mass of the strains in sterile distilled water at the concentration of 10^8 colony forming units (CFU) per ml. Leaves of the one-month-old check varieties were clip-inoculated, following BB-like lesion was measured (Kauffman *et al.,* 1973).

Inoculation and RNA extraction: The rice seedlings, aged 15 days, were inoculated with Xoo suspension using a needleless syringe. After 24 hours of inoculation, leaves were cut and immediately frozen in liquid nitrogen. Leaves from each sample were processed into a fine powder using a pre-chilled mortar and pestle in liquid nitrogen. The resulting powder was then transferred to a 1.5 ml RNase-free tube containing 800 µL of TRIzol reagent and 200 µL BP solution (1% PVP and 2% β-ME). The mixture underwent thorough shaking and was subsequently incubated at room temperature for a duration of 15 minutes. Approximately 300 µL of chloroform was added to the mixture. The mixture was shaken for 1 minute and then incubated for 30 minutes at 25°C. Following incubation, centrifugation was performed at 13,500 g for 20 minutes at 4°C. The aqueous phase (600 µL) was transferred to new tubes, and RNA was precipitated using 600 µL of ice-cold isopropanol overnight at -20°C. This was followed by centrifugation at 13,500 g for 15 minutes at 4°C. The pellet underwent a washing procedure using 800 µL of 80% ethanol, followed by centrifugation at 12,500 g for a duration of four minutes at a temperature of 4°C. The RNA pellet underwent drying at room temperature and was then dissolved in 65 µl of deionized water, and incubation at -28°C for overnight. The isolated RNA was stored at -20°C for future applications.

Qualitative analysis of RNA: The assessment of RNA quality was conducted through agarose gel electrophoresis. A gel was made by mixing 1 g of agarose powder with 50 ml of 1X TAE buffer, heated till boiling and then chilling it to 60°C before adding ethidium bromide. 5 µL RNA samples were meticulously introduced into the wells. The gel was run at a voltage of 80 V until the RNA had migrated to about three-quarters of the total gel length. The RNA bands were analyzed using UV light facilitated by a trans-illuminator.

cDNA preparations: Isolated RNA underwent cDNA synthesis utilizing the "Thermo Scientific. #K1622" reagent, adhering to the manufacturer's protocol. The kit comprised RevertAid reverse transcriptase for the synthesis of extended cDNA strands, an RNAase inhibitor to safeguard RNA integrity, and an RT enhancer to eliminate DNA contamination. The mixture included oligo dT primers along with random hexamers. The reaction mixture underwent incubation at 42°C for a duration of 1 hour, subsequently subjected to 5 minutes at 85°C. For future analysis, the cDNA samples were stored at a temperature of -20ºC.

Qualitative analysis of cDNA: The confirmation of cDNA synthesis was achieved through standard PCR employing the housekeeping gene sucrose phosphate synthase (SPS) alongside its designated primers, as detailed in Table 2 (Zafar *et al*., 2020). The ultimate PCR product underwent separation on a 1.5% agarose gel and was subsequently documented under UV light utilizing a trans-illuminator.

Semi-quantitative PCR analysis of *OsSWEET11*: The expression of *OsSWEET11* in Begami rice upon inoculation with Xoo was analyzed via semi-quantitative PCR using *OsSWEET11*-specific primers (Table 2). The PCR reaction mixture, totaling 25 µL, comprised 0.75 µL Taq polymerase (Thermo Fisher), 2.5 µL $MgCl_2$, 0.75 µL of 10 mM dNTPs, 2.5 µL 10X buffer, 0.5 µL each of forward and reverse primers (0.1 pmol), 4 µL cDNA (30 ng), and 14.5 µL ddH2O. A MiniAmp Plus thermal cycler was used to carry out the optimal reaction. The conditions that were used were as follows: an initial denaturation at 95ºC for five minutes, followed by 34 cycles of 94ºC for fifty seconds, 54ºC for forty seconds, and 72ºC for one minute, and finally a final extension at 72ºC for seven minutes. After the PCR products were amplified, they were resolved on a 2% agarose gel and then observed using a trans-illuminator while there was UV light present.

DNA extraction from the leaves of Begami Rice: Fresh Begami rice leaves were put in 2 mL Eppendorf tubes with 800 µL of 2x CTAB buffer (300 mM NaCl, 50 mM Tris-HCl, pH 8.0, 25 mM EDTA, and 2% CTAB). The samples underwent incubation at 62°C for 2 hours, were subsequently crushed using sterile steel needles, and were then incubated overnight at the same temperature. On the following day, 800 µL of Chloroform: Isoamyl alcohol (24:1) was introduced, and the mixture was incubated for 45 minutes at 25°C. The samples underwent centrifugation at 13,500 rpm for 20 minutes at 4°C, after which 600 µL of the clear supernatant was transferred to new tubes. An equal volume of 2-propanol was introduced, and the samples were incubated at -20°C for a duration of overnight. On the following day, samples underwent centrifugation at 13,500 rpm for 15 minutes, after which

the supernatant was discarded. The DNA pellet was washed three times with 75% ethanol, air-dried, and then dissolved in 80 µL of TE buffer. The DNA was analyzed for quality using a 1% agarose gel stained with ethidium bromide. The DNA concentration was quantified using a spectrophotometer and subsequently adjusted to 40 ng/µL for further applications.

PCR Amplification of the Promoter Region of *OsSWEET11* Gene: The promoter region of *OsSWEET11* was amplified utilizing specific primers (8NKpI-F5 and 8N3G-R1, Table 2) in a 25 µL reaction mixture. The mixture included 1 µL genomic DNA (40 ng), 0.5 µL primers forward and reverse (0.1 pmol), 0.5 µL dNTPs (10 mM), 0.75 µL Taq DNA polymerase, 2 µL $MgCl_2$, and 1X Taq buffer. The PCR was performed on a MiniAmp Plus thermal cycler under precise conditions: an initial denaturation at 95°C for 5 minutes, followed by 38 cycles of 94°C for 1 minute, 53°C for 50 seconds, and 72°C for 2 minutes, and a final extension at 72°C for 10 minutes. The products were resolved on a 2% agarose gel using 1X TAE buffer, then stained with ethidium bromide. Visualization was performed under UV light, and the samples were subsequently dispatched to Macrogen, Korea, for sequencing.

Data Analysis: The experiment utilized a randomized complete block design featuring three replications. The analysis of data was conducted utilizing the Tukey HSD test via Statistix v8.1 software, establishing statistical significance at $p<0.001$. Disease incidence was determined by calculating the percentage of leaf area impacted by lesions, employing the formula:

$$\text{Disease incidence (\%)} = \frac{\text{Leaf lesion length}}{\text{Total leaf length}} \times 100$$

Sequence analysis: The promoter sequences obtained from Macrogen were analyzed using bioinformatics tools. The presence of specific regulatory elements, including EBEs and TATA boxes, was identified.

Results

Collection, identification and confirmation of Xoo strains: Rice leaves exhibiting typical BB symptoms such as water-soaked lesions extending along the leaf veins and turning into yellowish-brown necrotic streaks were collected from infected rice fields at the foothills of Himalayas in the Mansehra region, Khyber Pakhtunkhwa province, Pakistan (Fig. 1A). The yellow, mucoid, and circular colonies- characteristic features of Xoo, were sub-cultured for pure colonies (Fig. 1B) and stored in 50% glycerol stock at -80°C for further analysis. High quality genomic DNA of selected Xoo was set at approximately 50ng/ul concentration in Elution Buffer (Fig. 1C). Using Xoo-specific primers, JLXooF/R, a 230bp fragment was amplified in Xoo-like strains (Fig. 1D).

A representative local Himalayan foothills Xoo strain, KP-3 and a tester Xoo strain, PXO99 were used to check the response of the local rice varieties, including locally adopted Begami cultivar. One month old rice cultivars were used for pathogenicity test and clip-inoculation was done for inoculation, followed by BB-like lesions measurement. The Xoo strain KP-3 and PXO99 were highly virulent on international check rice variety IR-24, and Begami-88, Begami-152, Begami, Super Basmati, and Basmati 385 (Tables 3, Fig. 2). Water was used as zero strain control (mock inoculation) which did not produce any symptoms (Fig. 2). Both PXO99 and KP-3 isolates induced similar lesion length in Begami rice.

Table 2. Primers sequences used in present study.

S. No.	Primers name	Sequences (5' to 3')	Purpose	References
1.	JL-Xoo-F JL-Xoo-R	CCTCTATGAGTCGGGAGCTG ACACCGTGATGCAATGAAGA	Xoo specific	Lu *et al.*, 2014
2.	SPS-RT- F SPS-RT- R	GCCATGGATTACATATGGCAAGA ATCTGTTTACTCGTCAAGTGTCATCTC	Semi-quantitative PCR	Zafar *et al.*, 2020
3.	SWEET11- RT-F SWEET11- RT-R	GGGATTTCTGGCTAGTTTCT CGAGGTAGAGGACGATGTAG	Semi-quantitative PCR	Eom *et al.*, 2019
4.	8NKpI-F5 8N3G-R1	TGAGTGGTCATACGTGTCATATTG CATTGCTACTGGTGATGAAGGT	Promoter sequencing	Oliva *et al.*, 2019

Table 3. Tukey HSD test for lesion length and lesion percentage produced by Xoo strains KP-3 and PXO99, and zero strain (water control/mock inoculation).

Genotypes	Lesions length			Percent lesions length		
	KP-3	PXO99	Zero strain	KP-3	PXO99	Zero strain
IR-24	5.433^{C}	6.6667^{C}	0.100	72.716^{B}	87.104^{A}	1.170^{B}
Begami S-88	6.8000^{BC}	8.9500^{BC}	0.100	91.966^{A}	93.273^{A}	1.405^{A}
Begami-152	12.500^{A}	16.500^{A}	0.100	94.953^{A}	89.028^{A}	0.800^{CD}
Begami/Garhla/JP-5	12.833^{A}	10.417^{BC}	0.100	89.317^{AB}	83.469^{A}	0.687^{D}
Basmati-385	10.833^{A}	11.833^{AB}	0.100	89.466^{AB}	91.462^{A}	0.819^{CD}
Super Basmati	10.333^{AB}	10.750^{BC}	0.100	83.788^{AB}	92.599^{A}	0.994^{BC}
Minimums	5.433	6.6667	0.100	72.716	83.469	0.687
Maximums	12.500	16.500	0.100	94.953	93.273	1.405
%CV	22.48	24.00	-	10.79	11.62	12.83
SE	1.27	1.497	-	5.4884	5.9896	0.0722
P	0.0000	0.0000	0.0000	0.0044	0.6794	0.0000

Values followed by the same letter within a column are statistically similar at the 5% significance level ($p<0.05$)

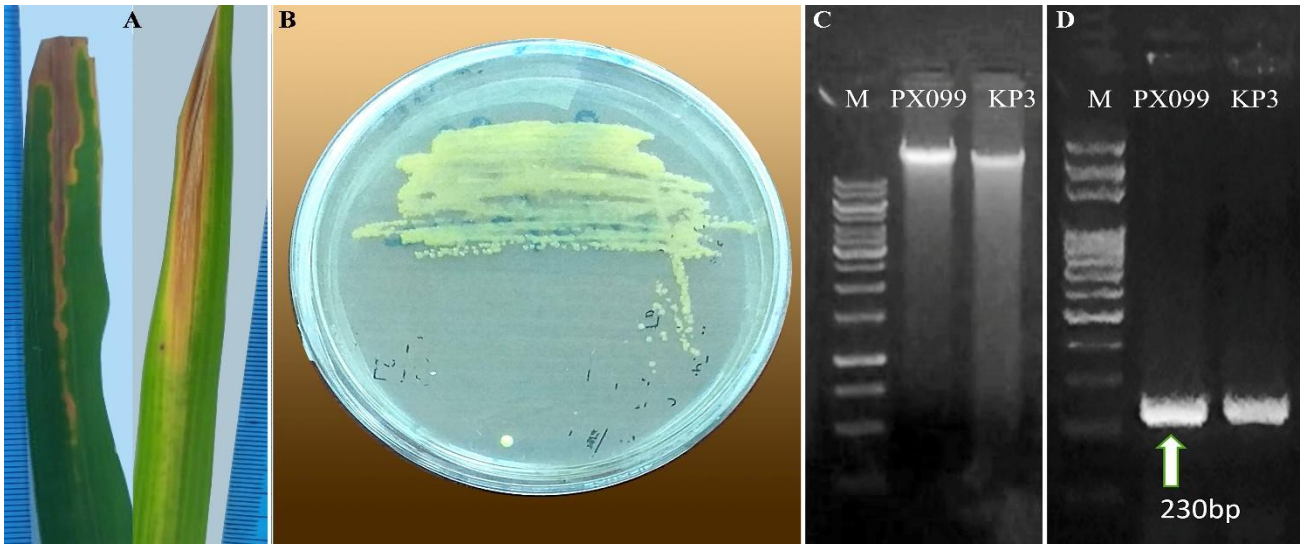

Fig. 1. Collection, isolation, and identification of Xoo strains. **A,** Infected leaves of rice collected from Himalayan foothills. **B,** *Xanthomonas oryzae* pv. *oryzae* grown on solid nutrient media. **C,** Genomic DNA isolated from Bacterial colonies. **D,** Confirmation of Xoo isolates using specific primer (M=100bp DNA ladder).

Fig. 2. Confirmation of pathogenicity by the representative the KP-3 strain. The KP-3 strain, along with reference strain PXO99 and a negative control (sterile water/mock), was inoculated into the leaf tips. After 15 days, BB lesions developed on leaves inoculated with both strains, confirming the pathogenicity of KP-3. No symptoms appeared in the control.

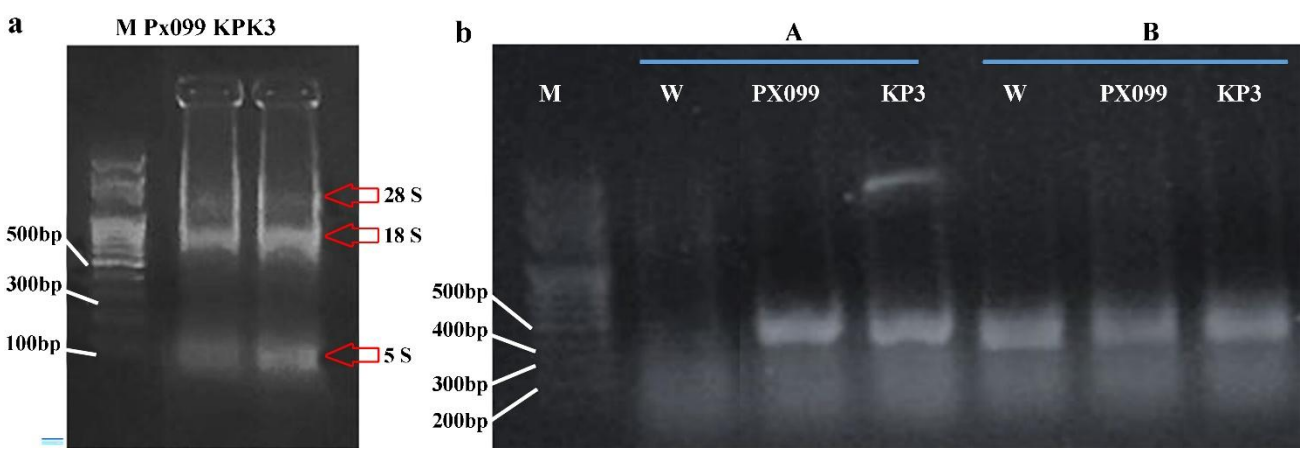

Fig. 3. RNA expression analysis of *OsSWEET11* gene in response to KP-3 strain. (a) Two-week-old rice seedlings were inoculated with approximately 10^8 CFU/ml of KP-3 and check PXO99 strains. RNA was extracted at 24h after the inoculation and run on 2% agarose gel. (b) Semi quantitative PCR for *OsSWEET11* on the cDNA was performed at 35 cycles. Panel A is amplification with *OsSWEET11*-specific primers, while panel B is amplification with internal control SPS gene primers.

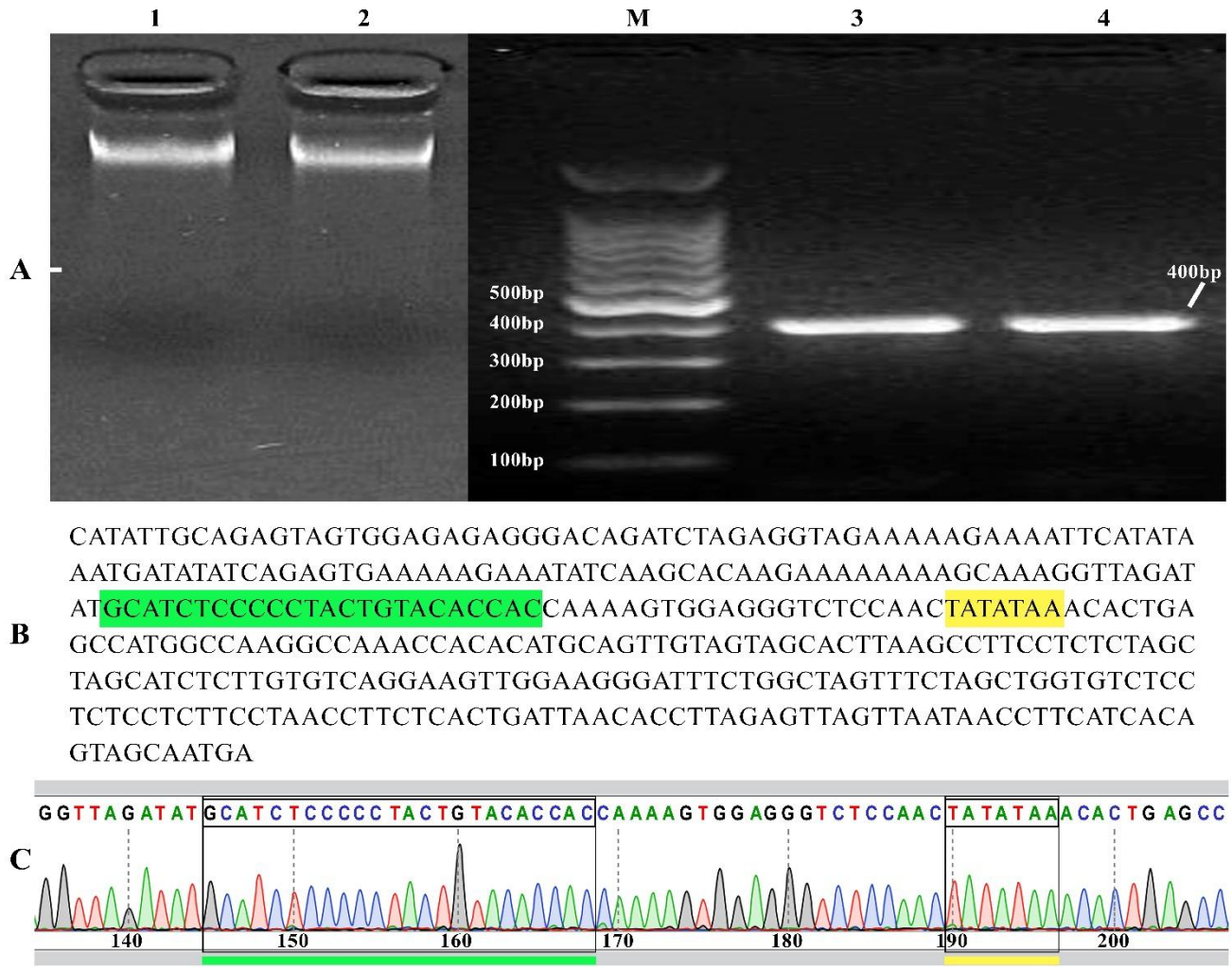

Fig. 4. Promoter analysis of the *OsSWEET11* in Begami cultivar. **A)** Gel electrophoresis results of Genomic DNA isolated from fresh leaves of the cultivar (1, 2). PCR amplification of the *OsSWEET11* promoter (3, 4). M= 100bp DNA leader**. B)** Upstream sequences of *OsSWEET11* promoter. EBE for PthXo1, highlighted in green, and TATA box is highlighted yellow. **C)**, Chromatograms of the Os*SWEET11* EBE for PthXo1 is shaded green circle, while TATA box is present on 190th base position in the promoter.

Expression analysis of *OsSWEET11* gene: Extracted RNA was of sound quality (Fig. 3a). Semi-quantitative PCR analysis revealed that the *OsSWEET11* gene was induced in Begami rice leaves upon inoculation with KP-3 strain. The Xoo strain PXO99 was used as a positive check strain for *OsSWEET11* induction. The loading control SPS gene gave a normalized estimate of equal amount of initial cDNA as a template for the PCR analysis (Fig. 3b).

TALE PthXo1-specific EBE identification in *OsSWEET11* promoter in Begami cultivar: Genomic DNA having clear and intact band on 1% agarose gel was isolated from fresh leaves of Begami rice (Fig. 4A, Lanes 1, 2). To validate *OsSWEET11* as an *S* gene induced by KP-3, possibly via its PthXo1-like TALE, and to know if PthXo1-specific EBE is present in the *OsSWEET11* promoter, a 400 bp fragment from the promoter was amplified in Begami using 8NKPI primers (Fig. 4A, Lanes 3, 4) for Sanger DNA sequencing. Sequencing confirmed that the *OsSWEET11* promoter in Begami carries the PthXo1-specific EBE—located between −80 and −56 bp upstream of the transcription start site and immediately upstream of the TATA box—showing 100% identity with the Nipponbare/Japonica reference (Fig. 4B, C).

Discussion

The PCR-based identification of Xoo strain yielded a specific 230 bp band, confirming the specificity of the JLXooF/R primers used (Lu *et al.*, 2014; Ullah *et al.*, 2024). This finding is consistent with previous studies that have demonstrated similar specificity and sensitivity in detecting Xoo using targeted PCR approaches (Bangratz *et al.*, 2020; Ejaz *et al.*, 2022; Ullah *et al.*, 2024). The ability to efficiently identify Xoo through PCR offers a reliable diagnostic tool for early detection and management of BB in rice, a critical measure for improving rice yields.

OsSWEET11 is well known to be induced by PthXo1 in several international check rice cultivars, including IR24 and Nipponbare, by tester Xoo strains (Chu *et al.*, 2006; Yang *et al.*, 2006; Zaka *et al.*, 2018). Recently, *OsSWEET11* was also induced by KP-3 in the international check IR24 and Kitaake rice cultivars (Shah *et al.*, 2025), however, its expression in local varieties/landraces was not known. In the current study, the induction of *OsSWEET11* in Begami by the KP-3 indicates that Begami offers *OsSWEET11* as major *S* allele for BB disease. This is the first report of rice–Xoo coevolution in the ecosystem of the Himalayan foothills in which *OsSWEET11* is induced in the local Begami rice by the local Xoo strain, KP-3.

This observation suggests that KP-3 likely harbors PthXo1 or a PthXo1-like TALE as a major virulence effector. A recent study by Shah *et al*., (2025) supports this possibility; however, definitive evidence for the presence of PthXo1 in KP-3 is still lacking and will require full genome sequencing. In addition, PXO99, a reference Xoo strain carrying PthXo1 as a major TALE, also induced *OsSWEET11* in the Begami cultivar. Together, these findings strengthen the likelihood that KP-3 possesses PthXo1 or a functionally related TALE, although direct confirmation will require characterization of the TALE repertoire of KP-3.

Xoo utilizes this virulence mechanism by hijacking *OsSWEET11*, a gene normally involved in pollen development and seed filling, to facilitate BB in rice (Yang *et al.*, 2006). Except for that *OsSWEET11*, as a transmembrane protein, secretes the sugar from inside the cell out to where Xoo resides, the detailed signal transduction susceptibility pathway driven by *OsSWEET11* is not known. The loss-of-susceptibility by the loss of non-expression of *OsSWEET11* impairs the pathogen's ability to access host sugars, thus curtailing on pathogen's multiplication and disease progression (Yang *et al.*, 2006; White & Yang, 2009; Oliva & Quibod, 2017). It appears that *OsSWEET11* has limited normal physiological function in leaves until the heading stage, as Ma *et al.*, (2017) reported its negligible expression in 6-week-old leaf blades.

TALEs have been identified in diverse Xoo strains such as PXO99 and PXO79 (Philippines), SK2-3 (Thailand), IX-280 (India), and NXO-260 (Nepal), which all target *OsSWEET11* via PthXo1 or its homologs (Hopkins, 1993; Ochiai *et al.*, 2005; Bogdanove *et al.*, 2011; Zhou *et al.*, 2015; Oliva *et al.*, 2019). KP-3 strain induced *OsSWEET11* not only in Begami (current study) but also in other susceptible rice cultivars (Shah *et al.*, 2025), suggesting that KP-3 secretes PthXo1-like effector. The induction of *OsSWEET11* by these geographically diverse Xoo strains highlights the converged role of PthXo1-like TALEs in virulence. Further, induction of *OsSWEET11* by KP-3 in Begami positions KP-3 as a newly identified member, originating from the Himalayan foothills, among a group of globally distributed Xoo strains that relies on PthXo1 or its homolog to support BB infection. Furthermore, the current study findings demonstrate that Begami landrace—grown and consumed in Northern Pakistan— harbors the susceptible allele of *Os8N3* gene (*OsSWEET11 or Xa13*). These results align with earlier research emphasizing the central role of *OsSWEET11* in BB susceptibility and reinforce its importance as a target for developing BB-resistant rice cultivars (Chen *et al.*, 2010; Streubel *et al.*, 2013).

In this study, the promoter region of the *OsSWEET11* in Begami cultivar was sequenced and identified to possess PthXo1-specific susceptible EBE allele. The finding perfectly corresponds with susceptibility allele, *Xa13*, also known as *OsSWEET11*, to cause BB of rice. In contrast, resistant rice varieties such as IRBB13 carry the recessive homozygous allele, *xa13/xa13*, which harbors natural nucleotide polymorphisms in its EBE, thus disrupting its PthXo-binding specificity. This results into non-binding of PthXo1 to the EBE, consequently into non-expression of *OsSWEET11*-a condition called recessive resistance (Chu *et al.*, 2006; Römer *et al.*, 2010). Previous studies have shown that rice plants homozygous for *xa13* are symptomless when inoculated with Xoo strains that solely rely on PthXo1, indicating that *xa13* is a recessive resistance allele (Antony *et al.*, 2010; Oliva *et al.*, 2019). Therefore, the presence of an intact EBE for PthXo1 in Begami cultivar confirms its susceptible status to the Himalayan foothills strains of Xoo.

Conclusion

The susceptibility of local Begami cultivar to the KP-3 and PXO99 strains is linked to the expression of *OsSWEET11* in the cultivar by these strains. The finding aligns with previous research highlighting the critical role of *OsSWEET11* in BB susceptibility and underscores the importance of targeting this gene for developing resistant rice varieties (Chen *et al.*, 2010; Streubel *et al.*, 2013). Breeding of Begami with IRBB13 (*xa13/xa13*) or any other germplasm/cultivar of choice with *xa13/xa13* recessive homozygous allele will be an appropriate approach for attaining BB-resistance in Begami cultivar. Alternatively, targeted genome editing of the *OsSWEET11* promoter to disrupt the PthXo1-specific EBE will offer a promising and rapid development of resistant Begami cultivar without altering its other agronomic traits.

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