Int. J. Pharm. Investigation, 2021; 11(3) : 338-344

Original Article

Cloning and Expression of *Helicobacter pylori* ulcer Associated Gene - *iceA1* in Brinjal (*Solanum melongena* L.)

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ABSTRACT

Background: Plant biotechnology revolutionized the branch of plant sciences contributing to improving crop production. *Helicobacter pylori* was predominantly seen among all the sectors of the human population, closely stuck to stomach ulcers and cancer. Even though traditional vaccines are effective in preventing *H. pylori,* edible and non-edible parts of special transgenic plants were also being generated to express its antigens which could elicit an immune response after consuming. The induce by contact with epithelium (*iceA1*) protein of *H. pylori* is associated with virulence and stated as predisposing factor to stomach cancer. **Objectives:** This study intends to study the expression levels of *iceA1* gene in the brinjal plant. **Materials and Methods:** The present study, magnified the *iceA1* gene from *H. pylori strain* 26695 and transformed it into callus of brinjal (from leaf explants) through *Agrobacterium tumefacians* (EHA105). pBI121 vector was used to construct the plant expression vector, and the transgenic plants generated were further validated through gene amplification and protein expression studies. **Results:** Out of the 46 plants obtained from this study, five of them were found to be positive for the *iceA1* expression as seen on validation assays like gene amplification and protein expression studies. **Conclusion:** The study could beneficially produce a vaccine candidate against *H. pylori* from transgenic brinjal. In Addition, it could provide, valid scientific data which can further be used by researchers.

Key words: *Helicobacter pylori*, Transgenic brinjal, *iceA1*, *Agrobacterium,* Edible Vaccine.

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DOI: 10.5530/ijpi.2021.3.60

INTRODUCTION

Since its inception, plant biotechnology has always worked towards improving crop production and significantly leading to sustainability in the agriculture sector.[1] Vaccines, on the other hand, try to generate immune response, towards inactivated biological samples from bacteria or viruses forming a paramount revolution in the 19th century. To define, they are simply proteins, which bear resemblance to a pathogen, in an inactivated form stimulating the body's immune system in recognizing the foreign antigen at a later stage.[2]

A new form of vaccine has been shaped in the recent decades, to minimize the risk effects of earlier mentioned traditional vaccines. Such vaccines are mass-produced from genetically modified plants, which are artificially inserted with a disease-causing gene of pathogens (bacteria or virus), without altering the immunogenic capacity. These vaccines, called edible vaccines are found to switch on both systemic and mucosal response against a disease-causing pathogen.[2] The ever-increasing world's population along with living standards are forcing the biologists to implement novel agro-biotechnological methods wherein they can substantially generate crops.[3] that are of high yield producing diverse rich products like antibodies, therapeutic proteins, vaccines and many more secondary metabolites.[4] Gastric cancer is the 7th most prevalent cancer which accounts for 8.3% of global cancer mortality.[5]

This pathogenesis is due to the interaction of *Helicobacter pylori* with the environment and genetic factors. The pathogen invades, survives, colonizes and stimulates inflammation along the stomach mucosal layer causing severe evasion eventually leading to malignancy.[6] *Helicobacter pylori,* a flagellated, helical-shaped bacterium, is said to enhance its survival within the gastric environment. Such spiral form makes it capable to pierce through the gastric layer, colonizing the epithelium leading to aggressive invasiveness.[6]

H. pylori, was credited as class I carcinogen causing gastric cancer, by both the World Health Organization (WHO) and the International Agency for Research on Cancer.[7] Its infection among the human hosts leads to chronic gastritis causing peptic ulcer disease.[8] It also plays a critical role in the development of gastric adenocarcinoma mostly associated with stomach mucosa-associated lymphoid tissue (MALT) lymphoma, thereby adding to stomach carcinogenesis elevating the gastric cells to proliferate profoundly.[9]

Such infections are estimated to be 85–95% among the developing nations and accurately 30–50% within developed nations.[10] Bacteria enter the host either by oral-to-oral or fecal-to-oral routes. *H. pylori* adapts itself, within the gastric microenvironment, enabling it to survive even at low pH of 3.0.[11] *H. pylori* is said to enhance its virulence, by altering the host signaling pathways creating an inflammatory response

within the stomach mucosal layer. This causes the epigenetic outcomes within the stomach cells leading to gastric invasion and gastritis.[12] With increasing antibiotic resistance, the pathogen is prevailing and predominating causing acute to chronic gastritis which further leads to peptic ulcers and then gastric cancer.[13]

Many virulence factors are said to influence the pathogenesis of the bacteria along with the *iceA* (induce by contact with epithelium) gene. This gene attaches to the stomach epithelial layer invading the gastric mucosa. This is said to contain two forms (*iceA1* and *iceA2*) whose role in virulence,[14] is still controversial and unclear.[15] This *iceA1* form is stated to be connected with peptic ulcer disease,[16] and also being considered as a marker for peptic ulcer disease.[17] The bacteria transcribes this *iceA1* gene which aids in adhering to the epithelial cells, proposing its role in virulence,[18] Cheng *et al.*, 2007, were successful in generating plant expression vectors loaded with *Helicobacter* proteins like *cagA* and *ureB* which could express the fusion proteins against the strains.[19]

Traditional vaccine production though in need and urgency still is stalled due to the production cost and purification methods.[20] Moreover, plant pathogens and many secondary metabolites do interfere with the overall purification at an industrial level.[21] In recent decades, the plant expression vectors were considered and being studies to deliver recombinant proteins into the host in the name of vaccine antigens.[22] Such plant-derived therapeutic proteins are cost-effective in production with high quality. In addition, they are free from animal pathogens eligible for mass production.[23]

Gene expression studies, by blotting and real-time PCR methodologies, are predominating in recent years owing to their robustness and specificity.[24,25] Moreover, these methodologies are overlaid the microarray assays, with respect to expression levels.[26,27] Plant expression systems make the production of edible vaccines very easy from leaves, fruits and vegetables. Such oral-based delivery systems will definitely bypass the side effects of sterile administration methods of traditional vaccines. In addition, the purification process and old storage could be avoided which is unavoidable with the conventional vaccines.[28]

Brinjal, popularly called eggplant, is considered a common man's vegetable in India.[29] It is crowned as a common man's vegetable owing to its popularity in usage within the country.[30] It is also crowned as the 'King of Vegetables' in regard to its immense medicinal and pharmaceutical products.[31] It is seen commonly among the dishes of every household in India irrespective of their food preferences and income levels. It is having less calorific value with high nutritional benefits and water content. In addition, it is rich in fiber and minerals like calcium, phosphorus, folate. It also contains ample amounts of vitamin B and C.[32] Brinjal was now deeply buried into the Indian culture with numerous folk songs being tuned and played on the king which is humble among other vegetables.[33]

To trace the significant application of plants to produce *H. pylori* iceA1, this study expressed the clone of the *iceA1* gene from *H. pylori* strain 26695 in the brinjal plant, *Solanum melongena* L. *Cultivar* Arka Keshav. We have exemplified the expression of the iceA1 gene in brinjal plants, which could elicit an immune response against *H. pylori* strains. The results could pave paths to bring in possible vaccine candidates against *H. pylori* from the brinjal plant.

MATERIALS AND METHODS

Plant material and Experimentation

Brinjal (*Solanum melongena* L.*Cultivar* Arka Keshav) commonly called eggplant was collected from the Division of Vegetable Crops, IIHR, Bangalore. The experimentation was conducted with prior approval from the Institutional Biosafety Committee (IBSC) of JSS College, Mysore at Postgraduate Department of Biotechnology, JSS College, Mysuru, Genei India Pvt. Ltd., Bangalore and Department of Biotechnology and Crop Improvement, College of Horticulture, University of Horticulture Sciences, GKVK Post, Bangalore. Appropriate permission for the collection of plant or seed specimens has been obtained from the College of Horticulture, University of Horticulture Sciences, GKVK Post, Bangalore. The study was conducted in accordance with relevant international guidelines and legislation and all the experimental plants are discarded immediately after experimentation and before flowering as per the procedure.

Chemicals

Genomic DNA from *Helicobacter pylori* 26695 was bought from (ATCC® 700392DQ™) ATCC. All the chemicals used in the study were bought from Sigma Chemical Co and HiMedia and were of molecular grade and primers were ordered from Sigma Ltd, Bangalore. DNA ladders, *SacI* and *BamHI,* T4 DNA ligase were all bought from Genei Laboratories, Bangalore.

E. coli (DH5α) (F', *end A1 hsdR17* (rk^-mk^+) *glnV44 thi*-1 *recA1gyrA* (Nalr) *relA1*(*lacZY, AargF*) *U169deo R*Φ 80d*lac* (*lacZ*) M15 and PBI121 with T7 promoter was obtained from Genei Laboratories Pvt Ltd and Invitrogen Life Technologies, USA respectively. *Agrobacterium tumefaciens* strain *(*EHA105) was a gift from Department of Biotechnology, IIHR, Bangalore. The plant material used in our study was a callus transformed with *Agrobacterium tumefaciens* (EHA105), with PBI121 vector carrying the *iceA1* gene in the T-DNA [Figure 1].

Restriction sites of *BamHI* and *SacI* can be seen in the image. 35S is the promoter and 35S polyA is the adenylation signal for the *iceA1* gene. LB: left border; RB: Right border.

PCR amplification of *iceA1* gene

Gene amplification was done with *iceA1* forward and reverse specific primers (Table 1) which were designed based on the *H. pylori* sequence corresponding to ~519bp. The reaction mixture consisted of approximately 50ng of total gDNA as template, Hotstart Taq DNA polymerase (1U), dNTPs (0.2mM each), 1× PCR buffer, $MgCl_2$ (3mM) and primers (10pmol each) in a total reaction volume of 25µl. PCR parameters were initial denaturation at 94°C for 2min followed by 35 cycles (94°C, 40sec; 56°C 45sec; 72°C 1min) followed by a 15min final extension at 72°C. The PCR products were then resolved on 1.0% agarose gel and determined using a UV transilluminator. Genei clean purification system (Gel Extraction Kit, Genei) was used to purify the PCR products from the gel. Miniprep Kit (Genei) was used in isolating plasmid DNA with high yields (35µl) as instructed in the manual.

Cloning of *iceA1* gene into pBI121

In order to achieve the transgenics which could induce an immune r esponse among the patients of *H. pylori*, we cloned *iceA1* fragment (Partial) of ~519bp into bacterial strains, which was then amplified and purified followed by digestion with *BamHI* and *SacI* (37°C for 2hr). The plasmid used was also digested with the same restriction enzymes and the bands

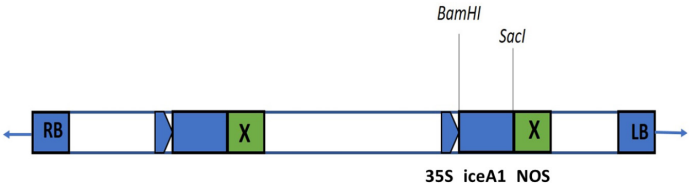

Figure 1: Schematic diagram of T-DNA of the pBI121 vector used in transforming brinjal callus through *Agrobacterium tumefaciens*.

obtained were excised and cleaned using the Genei gel extraction kit. The digested vector and *iceA1* product were then ligated (T4 DNA ligase) overnight at 16°C and then transformed into *E.coli* DH5α strain. Recombinant clones were screened and the digests were confirmed using the sequencing study (Eurofins, Bangalore).

Preparation of competent cells and Transformation

The *E. coli* cells were made competent by the calcium chloride method[34] and spread and incubated on an LB-agar plate without antibiotic at 37°C for 12 hr. A single isolated colony was inoculated into 10 ml of LB medium and grown till the optical density of 0.4-0.5 at 660nm (3- 4hr) was achieved. The cells were then chilled on ice for 30 min and harvested by centrifugation at 3000 rpm in SS 34 rotor (Sorvall refrigerated centrifuge, Evolution RC) at about 4°C for 15 min. The cell pellet obtained was then resuspended in a 30ml of filter-sterilized ice-cold acid salt buffer (100mM $CaCl_2$, 70mM $MnCl_2$ and 40mM sodium acetate, pH (5.2-5.5) and incubated for 45min on ice.

The cells were pelleted down at 3000rpm for 15min at 4°C and the pellet obtained was resuspended in 1/25th volume of culture in acid salt buffer containing 20% glycerol (cell pellet from 90 ml culture was suspended in 4.5 ml of buffer) and stored at -70°C until further use as 50µl aliquots. The competent DH5α *E. coli* cells were transformed with PBI121 vector containing *iceA1* gene by incubating them on ice for 30min followed by heat shock treatment at 37°C for 5min. Following which the transformed cells were plated on LB agar plates containing antibiotics (Kanamycin 20µg/ml) and incubated at 37°C. The bacterial colonies were further screened for PBI121 containing *iceA1* inserts.

Purification of the plasmid

Single isolated colonies were inoculated into 5ml of LB broth (with Kanamycin 20µg/ml) and incubated at 37°C for 12hr on a shaker incubator (120rpm). The vector was isolated using the alkaline lysis method.[35] In brief, overnight culture was centrifuged at 6000g for 5min at 4°C and the pellet obtained was resuspended in 200µl of prechilled solution I. 200µl of freshly prepared solution II was added to the tube and mixed gently by inverting upside down, followed by adding, 200µl of solution III. Contents are mixed gently and incubated on ice for 10min. Following incubation, the cell lysates were then centrifuged at 8000g for 10min at 4°C. The phenol: chloroform: iso-amylalcohol mixture (25:24:1; v/v) was added to the supernatant and mixed thoroughly. The contents were then centrifuged at 8000g for 10min at 4°C and added with an equal volume of pre-chilled isopropanol to precipitate the DNA. The DNA was then pelleted at 8000g for 10min at 4°C and resuspended in 10mM TE buffer for further use. The plasmid purity was checked on 0.8% agarose gel electrophoresis and the positive clones were confirmed by restriction digestion followed by DNA sequencing.

Callus induction

Brinjal seeds (pre-treated with 250ppm of GA3; Gibberellic acid) of variety Arka Keshav, were surface sterilized with sodium hypochlorite (6%) and water in a 1:1 ratio and rinsed with sterile distilled water for 3-4 times. About 20-25 seeds were then inoculated on Petri plates containing N6 callus induction medium (with 10,000mg/l Myoinositol, 200mg/l glycine, 50mg/l Thiamine HCl, 50mg/l Pyridoxine HCl, and 50mg/l of Nicotinic acid) of pH 5.75. 10µM of BAP and 1µM NAA were added to the medium after autoclaving the previously mentioned contents. 0.8% agar was used in solidifying the medium. The plates were then incubated at 25±2°C in dark. The calli obtained were removed from the germinating seeds and sub-cultured on a fresh callus induction medium. Median portions of cotyledonary leaves and transversely cut segments of hypocotyls were used as explants in the present study.[36]

Callus transformation with *Agrobacterium*

The calli obtained was transformed with *Agrobacterium* (EHA105) as per the protocol described by Patel *et al.*, 2013,[37] with slight modifications. The vectors were introduced into *Agrobacterium* through electroporaton. In brief, to about 50µl of cells, 5µl of plasmid DNA was added and mixed thouroughly. The contents were then transferred to a prechilled cuvette and electroporated. The *Agrobacterium tumefaciens* mobilized with vector inserts, were inoculated into 5ml of YEP (yeast-extract-mannitol medium; pH 7.0) medium (with 20mg/l Rifampicin and 50mg/l Kanamycin) and incubated at 28°C in a shaking incubator (200rpm) overnight. The overnight culture was transferred to 45ml of infection media (MS basal media with Thiamine HCl [1mg/l], Myoinositol [250mg/l], Casein hydrolysate [1.0g/l], Proline [690mg/l], Glucose [30g/l], 2,4-D [5.0mg/l] and Acetosyringone [200µM]) of pH 5.2 and incubated at 28°C in a shaker incubator (200rpm) for about 48hr until the OD_{600} reaches 0.5–0.6. Colony PCR of *Agrobacterium* was done to confirm the insert *iceA1*.

The embryogenic calli obtained was then infected with *Agrobacterium* by heat shock at 42°C for 3min followed by 12min incubation at room temperature. The calli was then blotted on to Whatman papers to remove excess bacterial suspension and then co-cultivated on co-cultivation media (media (MS basal media with Myoinositol [690mg/l], Casein hydrolysate [1.0g/l], Proline [1gm/l], maltose [0.5mg/l], 2,4-D [0.1mg/l], BAP [0.1mg/l] and Acetosyringone [200µM], Phytagel [3gm/l) of pH 5.8 for 2 days in dark at 25°C. After two days of cocultivation, the explants were transferred to the selection medium (media with 100mg/l of kanamycin) and carefully maintained on the selection medium for about 20 days with repeated subculturing for every 6 days on to fresh selection medium. After 3-4 weeks of selection, the calli growing on kanamycin alone were transferred to regeneration media (MS media with 2.0mg/l BAP, 30g/l Maltose, 3.0g/l Phytagel, 200mg/l Timentin and 50mg/l hygromycin) and then incubated in light at 25°C for about 3 weeks. The regenerating shoots were carefully excised and transferred to cocultivation media with kanamycin (25mg/1) and cefotaxime (250mg/l). The proliferating shoots were then transferred onto rooting medium (half-strength MS medium with 250mg/l cefotaxim along with IBA 5µM) and selection medium without kanamycin. On successful rooting, the plantlets were then subcultured into new bottles with a rooting medium and allowed to grow for 15 more days.

The primary transgenics about 8-10cm with the proper shoot and root were removed carefully and replanted into 6inch pots containing 50:50 peat-lite/sand in the greenhouse (26/22°C, 16hr light/ 8hr dark and high humidity). Once the plants were acclimatized, they were transferred to growth chambers (28/24°C, 16hr light/ 8hr dark) and given care and nutrients as per the standard management practice.

Isolation of total genomic DNA from leaves

A total of 46 plants were regenerated and from them, only five of them were found to be transgenic in nature which was confirmed with blotting and amplification stuides. Genomic DNA was isolated from the leaves by the CTAB method as described by Li, Z *et al.*, 2020.[38] In brief, about 100mg of leaf tissue was homogenized with 0.5ml of extraction buffer (2% CTAB, 1.4M NaCl, 20mM EDTA, 10mM Tris HCl; pH 8.0) containing 10µl of beta-mercaptoethanol. The contents were mixed gently and incubated for 30-40min at 65°C in a water bath, and added with equal volumes of chloroform: isoamyl alcohol (24:1) following incubation. The contents were then centrifuged at 5000rpm for 15min at room temperature and added with 1/10th volume of 5M NaCl and 0.8volumes of ice-cold isopropanol to precipitate the DNA. The DNA obtained was then pelleted at 8000rpm for 10min and washed with 70% ethanol. Following washing, the pellet was resuspended in 100µl of TE

buffer. 5µl of RNase (l0mg/ml) was added to the DNA and stored at -20° C following incubation at 37°C for 1 hr.

Primer design

Forward and Reverse primers used in our study (Table 1) were designed using the Primer 3 software (version 4.13) based on mRNA sequences deposited in GenBank.[39] The specificities of the primers were cross-checked by alignments with the original GenBank sequences using the standard nucleotide-nucleotide BLAST (blastn; provided online by NCBI).[40]

Protein extraction

The leaf samples (both transgenic and control) were ground with liquid N_2 in a mortar and pestle. About 0.2gm of tissue powder is resuspended in 2ml cold acetone and vortexed thoroughly for 30sec.[41] The pellet obtained was washed with acetone and centrifuged at 10000*g* for 3min at 4°C. It was transferred into a mortar and ground further to a fine powder with quartz sand and transferred into 2ml microfuge tubes and rinsed with ice-cold 10% TCA 3 to 4 times and added with cold 80% acetone. Following centrifugation, the pellet was used for extraction at room temperature.

The phenol extraction method was used in the study,[41] but with a slight modification. In brief, about 0.1gm of previously prepared powder was resuspended in 0.8ml phenol (Tris-buffered, pH 8.0) and 0.8ml of SDS buffer (sucrose 30%, SDS 2%, 0.1M Tris-HCl, pH 8.0, 5% 2-mercapto-ethanol). The contents are mixed thoroughly for 30sec and centrifuged at 10000g, 3min. The upper phenol phase was collected in fresh tubes, and added with 5volumes of cold methanol and ammonium acetate (0.1M) to precipitate the proteins. Proteins precipitated were then recovered by centrifuging at 10000*g* for 5min and washed twice with cold methanolic ammonium acetate and ice cold 80% acetone twice. The pellet thus obtained was dried and resuspended in 2-DE rehydration solution (8M urea, 4% CHAPS, 2% IPG buffer, 20mM dithiothreitol) and quantified (Genei, Bangalore) using bovine serum albumin as standard.

Soluble proteins extracted were separated on SDS– polyacrylamide gel electrophoresis (4.75% stacking and 12% resolving gel with 12% glycerol) and transferred onto Nylon membrane (Genei, Bangalore). The final concentration of the resolving buffer (Tris-HCl, pH 8.8) was modified to 0.75M, than the usual 0.375M. Protein samples were denatured at 95°C for 3min and resolved under constant 200V in a Bio-Rad mini-SDS PAGE gel apparatus.

RESULTS

PCR amplification and cloning of *iceA1* gene into pBI121

Gene amplification with *iceA1* primers showed positive amplification with amplicon at about 519bp. The bands confirm the *iceA1* gene amplification [Figure 2]. The purified vectors were run on agarose gel (1%) and the digests were found to yield two products releasing the *iceA1* gene product at approximately 519bp [Figure 3]. The nucleotide sequence [Figure 4] obtained was compared to GenBank sequences and found to have a "perfect" match (similarity, 95.98%) with sequences of their corresponding gene *iceA1* from GenBank (GenBank® accession no. LC420461) as performed using BLAST (version 2.7). This variation

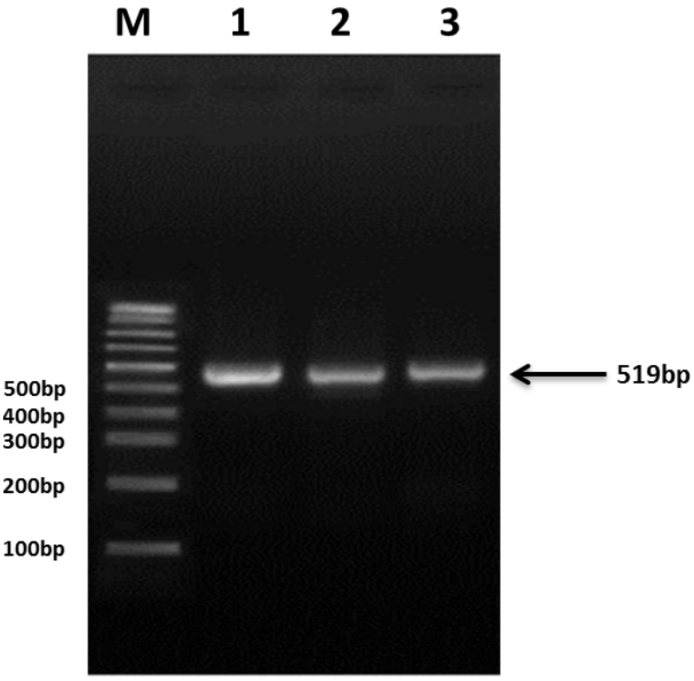

Figure 2: Image showing the *iceA1* gene amplification separated on 1% agarose gel.

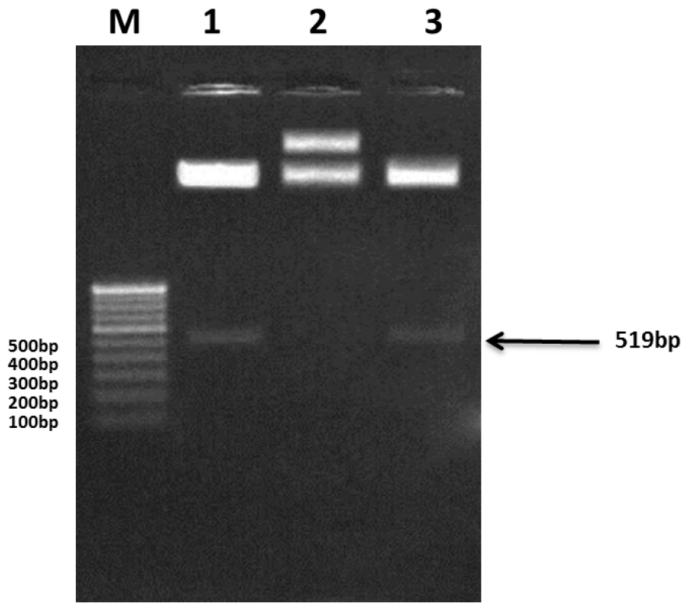

Figure 3: Restriction digestion of ligated pBI121-*iceA1* using *BamHI* and *SacI* on 1% agarose gel.
M Lane- 100bp ladder was used as a ladder. Lane 1 and 3; pBI121- *iceA1* digest (with *BamHI* and *SacI*) could be seen released iceA1 gene. Lane 2: pBI121-*iceA1* (undigested); Image as viewed on Gel Dock.

Table 1: Table showing the list of primers used in the study. FP: Forward primer, RP: Reverse primer. Tm: melting temperature.

Gene		Sequence	Length	Tm	GC%	Product length
iceA1	FP	TATCTGGATCCATGGAGTTTGATAAAGGGCAAACTC	36	76	41.7	519bp
	RP	ATATCGAGCTCCTATAAAGTAGTTTTTTGATGGT	40	67.4	32.4	

might be very common among the strains. Bacterial species of the same family are shown same colored box. Family names and their colors are shown on the right side of the Figures in italics. The right side of the tree contains the genus names [Figure 5]. The phylogenetic tree was based on aligning the 7 gene sequences of (16S rRNA, 23S rRNA, gyrB, pyrH, recA, rpoA and rpoD).

100bp ladder was used as a DNA ladder. Lane 1-3 were PCR products *iceA1* gene. M: Ladder (100bp). Image as viewed on Gel Dock.

Agrobacterium-mediated genetic transformation

A total of 46 independent brinjal plants were regenerated by *Agrobacterium* transformation [Figure 6]. Colony PCR of *Agrobacterium* confirmed the positive expression of *iceA1*. Bands at the size of 519bp could be seen in the agarose gel. They were further screened for the *iceA1* gene expression by PCR and by Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE). A PCR product of the expected size (519bp) was obtained from the genomic DNA of all the 5 transgenic lines, which advocates the positive expression of *iceA1* [Figure 7]. As expected, negative amplification was seen with no band in control samples (independent non-transgenic). No degree of non-specificity was observed, stating the purity of DNA and specificity of primers.

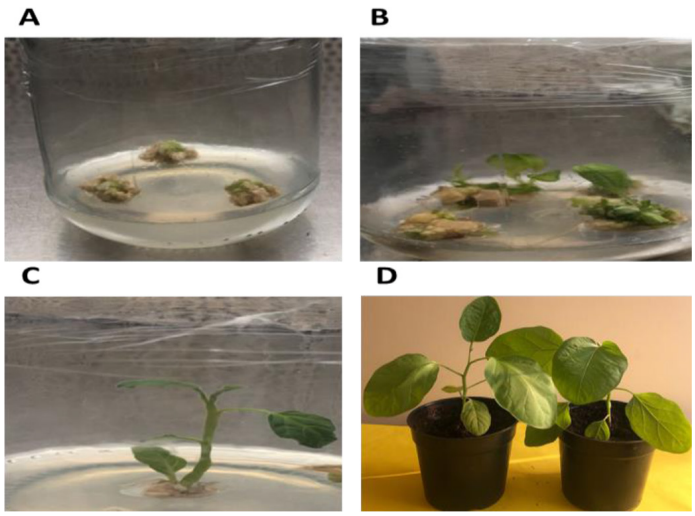

Figure 6: Photographs showing the development of transgenic brinjal cv Arka Keshav following Agrobacterium transformation,
A: Callus initiation, B, C: Regeneration of shoot on selection media, D: growth of plantlets.

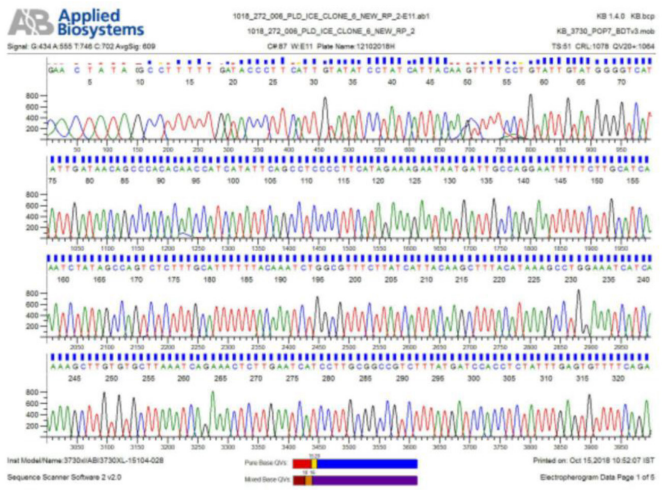

Figure 4: Chromatogram of the released *iceA1* gene of insert purified from the gel in the previous section.
Sequencing was done with both forward and reverse *primer.*

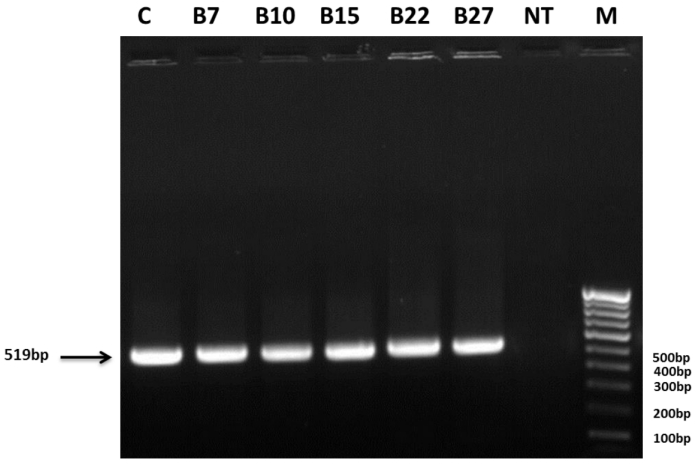

Figure 7: Image showing the PCR amplification of *iceA1* gene from gDNA isolated from the leaves of both transgenic and control.
PCR products were run on 1% agarose gel. 100bp ladder was used as a molecular marker. Lane NT: Non-transgenic plant; Lane B7, B10, B15, B22 and B27: transgenic plants. Lane 7: Lane C: positive control pBI121-*iceA1* positive control

Figure 5: Phylogenetic tree showing the relationship of the nearest family members with the query sequence 315171.
Our query sequence was *iceA1* gene from *H.pylori* (26695) ATCC 700392.

Protein expression

As seen from the protein electrophoresis, intense bands at about 20KDa were observed in all the transgenic lines (B7, B10, B15, B22 and B27). No band was seen in case of non transgenic or control line [Figure 8]. This confirms the expression of *iceA1* protein within the leaves of transgenic plants.

DISCUSSION

Producing therapeutic proteins within plants was reported previously using a wide variety of expression systems, though most of them solved the very purpose partially.[42] Leaves, vegetables and fruits of plants are used to produce, recombinant therapeutics like vaccines which are cheaper than conventional vaccines.[43] *Helicobacter pylori* is one of

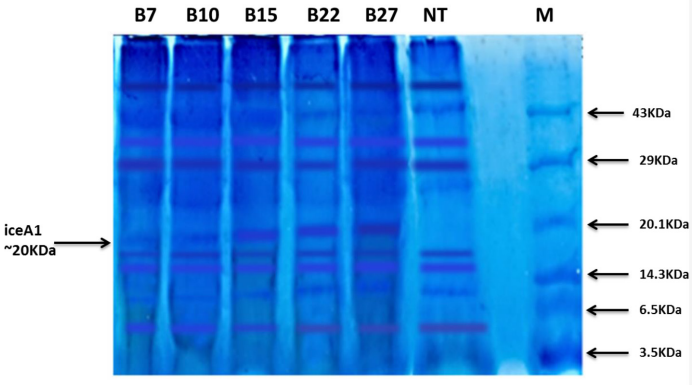

Figure 8: SDS-PAGE gel showing the protein bands of the transgenic and control plants separated.
B7, B10, B15, B22 and B27 are the transgenic plants. NT: Non transgenic. M: Protein ladder. Intense bands were seen at ~20KDa.

the most predominant bacterial strains, which was reported to affect more than 40–50% of humans worldwide,[44] causing peptic ulcers and gastric cancer. *H. pylori*, a gram-negative strain colonizes the stomach lining causing chronic gastritis and ulcers, which are prone to develop into adenocarcinoma and lymphoma.[45,46] Vaccines are inactivated protein suspensions from bacteria or viruses, to elicit the immune response against deadly diseases.[2] They help the body to detect the foreign antigen through the immune system. In recent decades, plant-based vaccines are being produced in amounts, to not only reduce the harmful effects of traditional vaccines but also to minimize the cost.[47,48] These genetically altered vaccines from the plants are inserted into plant tissues without even affecting the immunogenicity.[49]

iceA1 antigen gene was cloned into a binary vector pBI121 with selectable marker kanamycin resistance gene and transformed into brinjal callus by *Agrobacterium*. Out of the 46 plants, five of them were found to be transgenic and was confirmed from the gene amplification studies. The protein expression was also confirmed through SDS-PAGE wherein an intense band of approximately 20KDa was seen in transgenic lines only. The outputs of our experiments recommend that the *iceA1* transgenic brinjal can further be studied to use as a vaccine candidate against *H. pylori*.

In the present years, many studies are being done to generate edible vaccines from ready-to-eat fruits and vegetables, to follow the oral route of administration.[2] Edible vaccines are mostly generated when the desired transgene is inserted into the selected plant cell. In addition, these vaccines are more cheap and safe than other conventional vaccines available in the market.[50] To conclude, we report the successful integration of the transgene into the nuclear genome of the brinjal plants, with no limitations. These studies of ours confirm that this methodology could be well used to express several valuable proteins. Further we plan to confirm the expression of proteins by quantitative real time PCR and protein expression studies using western blot.

ACKNOWLEDGEMENT

The authors acknowledge the supports from JSS Research Foundation, JSS Technical Institutions Campus, Mysore; Genei Laboratories Pvt Ltd, Bangalore; Department of Biotechnology and Crop Improvement, Postgraduate Centre, College of Horticulture, University of Horticulture Sciences Campus, GKVK, Bangalore and Postgraduate Department of Biotechnology, JSS College, Mysore.

CONFLICT OF INTEREST

The authors declare no potential conflicts of interest.

ABBREVIATIONS

WHO: World Health Organization; **MALT:** Mucosa Associated Lymphoid Tissue; **PCR:** Polymerase chain reaction; **TE buffer:** Tris EDTA buffer; **HCl:** Hydrochloric acid; **BAP:** 6-Benzyl Amino Purine; **NAA:** Naphthalene acetic acid; **SDS-PAGE:** Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis.

Author contributions

MJM, BYSK and RB conceived and designed the study, MJM and RB performed the experiments, MJM, BYSK, NH, RB, BF and SP analyzed the data, MJM and SP drafted the manuscript and all the authors reviewed the manuscript.

Consent for publication

All authors consent to publish this work.

Ethics approval

IBSC Registration number: JSSC111220191080.

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Article History: Submission Date : 25-08-2021; Revised Date : 10-09-2021; Acceptance Date : 10-09-2021.
Cite this article: Mehran MJ, Kumar BYS, Haraprasad N, Barzigar R, Fakrudin B, Paul S. Cloning and Expression of *Helicobacter pylori* ulcer Associated Gene - *iceA1* in Brinjal (*Solanum melongena* L.). Int. J. Pharm. Investigation. 2021;11(3):338-44.