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Original Article

Expression of *Helicobacter pylori* CagA in *Solanum melongena* L-A Cost Effective Strategy for Vaccine Development

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ABSTRACT

Background: *Helicobacter pylori* is implicated in several severe gastrointestinal disorders, including gastric cancer, affecting a significant global population. This study aims to exploit plant biotechnology for vaccine development by engineering brinjal (*Solanum melongena* L.) to express *H. pylori*'s cytotoxin-associated gene A (*cagA*) antigen. Utilizing transgenic plants as an innovative strategy not only mitigates the high costs associated with traditional vaccine production but also leverages their capacity to induce a mucosal immune response. **Materials and Methods:** We used the brinjal variety 'Arka Keshav' for transformation. The *cagA* gene from *H. pylori* strain 26695 was cloned into the pBI121 vector and transferred into brinjal using *Agrobacterium tumefaciens*-mediated transformation. Transgenic expression was verified through PCR, quantitative real-time PCR (qPCR), Western blotting and ELISA. Immunohistochemistry was used to assess the localization of the *cagA* protein within the plant tissues. **Results:** Cloning and amplification confirmed the insertion of the *cagA* gene approximately ~1700 bp in size. Transgenic brinjal lines were successfully generated, with distinct expression levels of *cagA* observed. ELISA and Western blot analyses indicated significant *cagA* protein expression, particularly in lines B11 and B17, which showed the highest antigen concentrations. The consistency of mRNA and protein expression validated the effectiveness of the transgenic approach. **Conclusion:** The study demonstrates the feasibility of using genetically modified brinjal as a platform for producing edible vaccines against *H. pylori*. This approach not only presents a cost-effective alternative to traditional vaccines but also offers potential for enhancing accessibility in regions burdened by gastric diseases associated with *H. pylori*. Future research should focus on optimizing expression systems and evaluating the clinical efficacy of these plant-based vaccines.

Keywords: *Helicobacter pylori*, Transgenic brinjal, *cagA antigen*, Edible vaccines.

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INTRODUCTION

The majority of gastric cancer instances worldwide are linked to *Helicobacter pylori (H.pylori)*,[1-4] hence it was classified as a Group I carcinogen by the International Agency for Research on Cancer (IARC), a division of the World Health Organization (WHO).[5,6] *H. pylori* is known to be a highly infectious pathogen causing persistent and chronic gastric conditions,[7] affecting more than half of the global population.[5] Although, in milder conditions, *H. pylori* can be effectively managed with antibiotics, there is a need for effective vaccine development as a way forward, considering the fact that it is now battling with antibiotic resistance.[8,9] Cytotoxin associated gene-A (Cag A) protein with a molecular weight of 120-145 kDa is known to enhance bacterial virulence there by leading to gastric cancer.[10] This protein gets secreted into epithelial cells and goes through tyrosine phosphorylation pathway thereby triggering the uncontrolled cell division leading to cancer.[11] This protein supposedly works on intercellular tight junctions there by altering cellular morphology.[12,13] Research has

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shown that *cagA* influences these junctions independent of its phosphorylation status, playing a vital role in drawing both the tight junction-associated protein, Zona Occludens-1 (ZO-1) and the Junctional Adhesion Molecule (JAM), to areas of bacterial interaction.[14] Additionally, studies involving immunoprecipitation have indicated that E-cadherin engages with both the wild type and phosphorylation-resistant forms of *cagA*, disrupting the interaction between E-cadherin and β-catenin, leading to β-catenin's accumulation within the nucleus and cytoplasm.[12] Plant expression vectors harboring *Helicobacter* proteins such as *cagA* and *ureB* have have been shown to express themselves as fusion proteins against *H. pylori*.[15] Vaccination remains the most robust method for managing *H. pylori* infections,[16] with the urease subunit B (*ureB*) gene proving more effective than urease itself as a protective antigen. Nevertheless, the cost of producing new vaccines remains high, primarily because downstream processing and purification of recombinant proteins constitute the majority of total production costs.[17] Complexities due to plant pathogens, phenolics and other secondary metabolites further complicate industrial-level purification.[18] Recombinant proteins derived from plants present a more economical manufacturing alternative, offering high-quality products free from animal-derived contaminants. The creation of new vaccines and pharmaceuticals through molecular farming is especially crucial in developing countries.[19] Notably, plant systems can generate edible vaccines in forms like leafy vegetables, fruits, or other produce, facilitating oral administration that eliminates the need for traditional purification, reduces injection-associated risks, lowers healthcare worker dependency and removes the need for cold storage.[20] This oral delivery mechanism not only enhances both systemic and mucosal immunity but also utilizes the natural bioencapsulation of plant cell walls to shield proteins from digestive breakdown.[21] Advancements in plant biotechnology, such as the development of novel synthetic promoters, have greatly enhanced the precision of gene expression in transgenic plants,[22] driving forward both agricultural innovation and the field of plant-made pharmaceuticals.[23] The global rise in population and increasing consumer demand propel forward the genetic modification of plants to improve yields, enhance stress tolerance and enable the production of valuable biological products like vaccines and therapeutic proteins.[24,25] Brinjal, or eggplant, widely recognized as a staple vegetable,[26] In many low-income regions, it is employed in traditional Ayurvedic practices to manage diabetes, hypertension and obesity. Brinjal, belonging to the Solanaceae family, thrives globally and is particularly receptive to tissue culture techniques, showing robust growth from leaf, cotyledon and hypocotyl explants. It is also effectively transformed using Agrobacterium with both cointegrate and binary vectors.[27] Although model plants such as carrot, Arabidopsis, rice and tomato are frequently utilized for gene expression studies and developing pharmaceuticals,[28] brinjal remains underexplored in the field of genetic engineering and molecular farming. Limited studies have focused on its capacity to produce pharmaceuticals,[29,30] presenting a unique opportunity for expansion in this area. Consequently, our study has utilized the brinjal cultivar 'Arka Keshav' to host the cagA gene from *H. pylori* strain 26695. We have successfully demonstrated the gene's expression within brinjal, indicating its potential utility in creating plant-based vaccines. This promising initial result encourages further exploration into the use of brinjal for developing accessible and cost-effective vaccines against *H. pylori*, particularly beneficial in regions where traditional vaccine production and storage are logistically challenging.

MATERIALS AND METHODS

Plant Material and Experimental Methods

For our experiments, we used the brinjal variety *Solanum melongena* L. cv. 'Arka Keshav,' approved by the Institutional Biosafety Committee (IBSC) of JSS College. The genomic DNA for *H. pylori* strain 26695 was obtained from ATCC (ATCC® 700392DQTM). We sourced a biotin-conjugated *anti-cagA H. pylori* antibody (HSN/SAC: 38220090) was acquired from Thermo Fisher Scientific. The rest of the molecular biology grade chemicals and reagents were procured from Sigma Chemical Co., HiMedia and Qualigens. The primers used were procured from Sigma Ltd., Bangalore. Essential laboratory supplies including agarose, bromophenol blue, Ethidium Bromide (EtBr), Luria-Bertani (LB) media, glucose, Tris-saturated phenol, molecular markers, *SacI and BamHI* restriction enzymes, T4 DNA ligase, as well as plastic and glassware were provided by Genei Laboratories, Bangalore and Tarson Pvt. Ltd., India.

The *E. coli* strain DH5α U169deo RΦ 80dlac (*lacZ*) M15 was sourced from Genei Laboratories Pvt. Ltd., India. The transformation vector pBI121, which includes the 35S promoter, was supplied by Invitrogen Life Technologies, USA. The *Agrobacterium tumefaciens* strain EHA105, critical for our genetic engineering work, was supplied by the Department of Biotechnology at IIHR, Bengaluru, India. This specific strain of A. tumefaciens, carrying the pBI121 vector with the *cagA* gene integrated within its T-DNA region, was utilized to transform callus tissues derived from brinjal, as illustrated in [Figure 1].

Preparation of Competent Cells

The process of preparing competent *E. coli* cells followed the calcium chloride method as detailed by Chang *et al.* (2017).[31] *E. coli* cells were initially spread on LB agar devoid of antibiotics and then placed in an incubator at 37°C for a period of 12 hr. Subsequently, a solitary colony was cultivated in 10 mL of LB medium at the same temperature until the Optical Density (OD) at 660 nm reached between 0.4 and 0.5, which typically took 3-4 hr. The cultures were then cooled rapidly by placing them on ice for half an hour and centrifuged at a lower temperature of 4°C for 15 min using a Sorvall refrigerated centrifuge with an SS 34 rotor. The resulting cell pellets were immersed in 30 mL of a chilled,

filtered acid salt solution consisting of 100 mM CaCl2, 70 mM MnCl2 and 40 mM sodium acetate, with a pH adjusted to between 5.2 and 5.5 and kept on ice for another 45 min. Afterward, these cells were resuspended in a 20% glycerol solution, reducing their volume to 1/25th of the original. This concentrated suspension, typically around 4.5 mL from a starting culture of 90 mL, was portioned into 50 µL aliquots and kept at -70ºC.

To initiate the transformation process, the competent DH5α *E. coli* cells were first placed on ice for duration of 30 min. Subsequently they were exposed to a short period of heat at 37ºC for 5 min to facilitate DNA uptake. These prepared cells were then transformed with the pBI121 vector containing the cagA gene. Post-transformation, the cells were plated on LB agar plates enhanced with 20 µg/mL kanamycin and incubated at 37ºC for 12 hr, allowing selection of colonies that had successfully integrated the vector.

Isolation of plasmid genome

To isolate plasmid DNA, we began by cultivating an individual bacterial colony in 5 mL of LB medium comprising antibiotics, incubating it at 37ºC for 12 hr with agitation at 120 rpm. The process of DNA extraction was performed using the alkaline lysis technique.[32] following incubation, the cells were centrifuged at 6000 g for 5 min at 4ºC to form a compact cell pellet. This pellet was then reconstituted in 200 µL of cold Solution I. Subsequently, 200 µL of recently made Solution II was introduced and the mixture was carefully inverted to mix. To further the lysis, 200 µL of Solution III was introduced, thoroughly stirred and the tube was subsequently chilled on ice for 10 min. To aid in phase separation, the lysate was centrifuged at 8000 g for 10 min at 4ºC. The upper liquid layer was subsequently placed in a fresh container and combined with an equal quantity of phenol:chloroform:isoamyl alcohol (25:24:1 v/v) prior to undergoing centrifugation under identical conditions to enhance phase separation. DNA was separated by adding a similar amount of cold isopropanol and the resulted solid was recovered by additional centrifugation at 8000 g for 10 min at 4ºC. The resulting DNA pellet was cleaned, air- dried and redissolved in 10 mM TE buffer for storage. The integrity and quality of the isolated plasmid DNA were evaluated using electrophoresis using a 0.8% agarose gel. Verification of the insertion sequences within the plasmids was accomplished through restriction enzyme analysis and subsequent DNA sequencing, ensuring the clones were appropriate for continued experimental use.

Table 1: Table showing the list of primers used in the study. RT: Real time primers; FP: Forward primer, RP: Reverse primer. Tm: melting temperature. Actin was used as house keeping gene.

Gene		Sequence	Length	Tm	GC%	Product length
cagA	FP	ATATCAGGATCCATGGATTTACTTGATGAAAGGGGT	36	74.9	38.9	~1700bp
	RP	TATCGAGAGCTCTTATTTTTGAGCTTGTTGAGCCAATTGC	40	78.1	40	
cagA (RT)	FP	ATTGTATGCGGGCAATGGTG	20	67.6	50	151bp
	RP	CCCTTTCTCACCACCTGCTA	20	64.1	55	
Actin (RT)	FP	TCAGGTGTCCAGAGGTGTTGTA	22	64.5	50	150bp
	RP	ATGGTTGTGCCTCCTGAAAGTA	22	64.5	45.4	

Figure 1: Diagram of the Construct for Agrobacterium-Mediated Transformation.

This figure illustrates the genetic construct employed for brinjal transformation, derived from pBI121. It highlights the insertion sites for the restriction enzymes BamHI and SacI. The diagram includes the 35S promoter, crucial for initiating transcription and the 35S polyA signal, responsible for adenylation of the cagA gene. Also depicted are the Left (LB) and Right (RB) margins of the T-DNA region, integral to successful plant transformation.

Amplification of genomic DNA

To amplify the *cagA* gene, primers were specifically designed to target a ~1700 base pair segment of the *H. pylori* cagA sequence (see Table 1). The reaction for the PCR was prepared in a 25 µL size, including approximately 50 ng of genomic DNA, 1 unit of Hotstart *Taq DNA* polymerase, 0.2 mM of each dNTP, 1x PCR buffer, 3 mM $MgCl_2$ and 10 pmol of each primer. DNA was amplified using 35 cycles after initial denaturation at 94°C for 10 min. Each cycle was defined by denaturation at 94°C for 30 sec, annealing 55°C for 30 sec, elongation at 72°C for 2 min and extension at 72°C for 10 min as per specified method.[33-35] Results were visualized in 1% agarose gel and DNA fragments were isolated and cleaned from agarose gel using Genie gel extraction kit using manufacturers protocol. In addition, we isolated high-quality plasmid DNA using the Genei Miniprep Kit, resulting in 35 µL of purified plasmid.[36,37]

Insertion and cloning

Both the *cagA* gene fragment and the pBI121 vector were digested with *BamHI* and *SacI* enzymes at 37°C for 2 hr to prepare them for ligation. Post-digestion, the DNA fragments were isolated via gel electrophoresis, excised and then purified via the Genei Gel Extraction Kit. Subsequently, the *cagA* gene was ligated into the prepared vector at a temperature of 16°C for the duration of one night utilizing T4 DNA ligase. The process of preparing competent *E. coli* cells followed the calcium chloride method as detailed by Chang *et al.* (2017).[31] *E. coli* cells were initially spread on LB agar devoid of antibiotics and then placed in an incubator at 37°C for a period of 12 hr. Subsequently, a solitary colony was cultivated in 10 mL of LB medium at the same temperature until the Optical Density (OD) at 660 nm reached between 0.4 and 0.5, which typically took 3-4 hr. The cultures were then cooled rapidly by placing them on ice for half an hour and centrifuged at a lower temperature of 4°C for 15 min using a Sorvall refrigerated centrifuge with an SS 34 rotor. The resulting cell pellets were immersed in 30 mL of a chilled, filtered acid salt solution consisting of 100 mM CaCl2, 70 mM $MnCl_2$ and 40 mM sodium acetate, with a pH adjusted to between 5.2 and 5.5 and kept on ice for another 45 min. Afterward, these cells were resuspended in a 20% glycerol solution, reducing their volume to 1/25th of the original. This concentrated suspension, typically around 4.5 mL from a starting culture of 90 mL, was portioned into 50 µL aliquots and kept at -70°C.

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Callus induction and agrobacterium mediated transformation

The initiation of the callus formation process involved growing an individual colony was cultivated in 5 mL of LB medium along

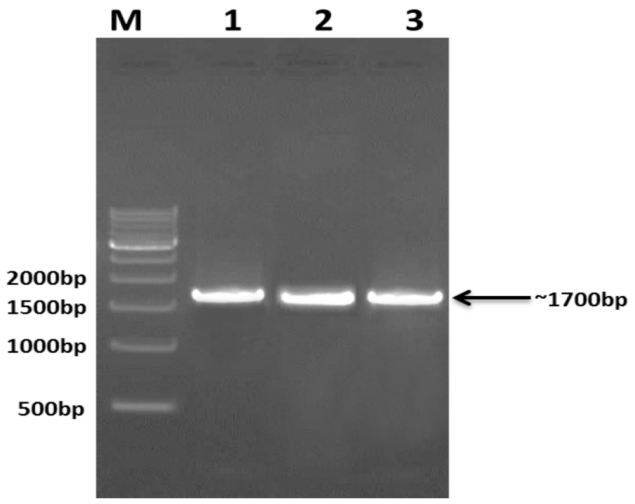

Figure 2: PCR Amplification Results for cagA Gene from H. pylori 26695.

This figure displays the PCR amplification results for the cagA gene, visualized on a 1% agarose gel exposed to UV light within a gel documentation system. The lanes are annotated as follows: lanes 1, 2 and 3 show the amplified cagA gene product, while the Molecular weight ladder (marked as M) serves as a size reference.

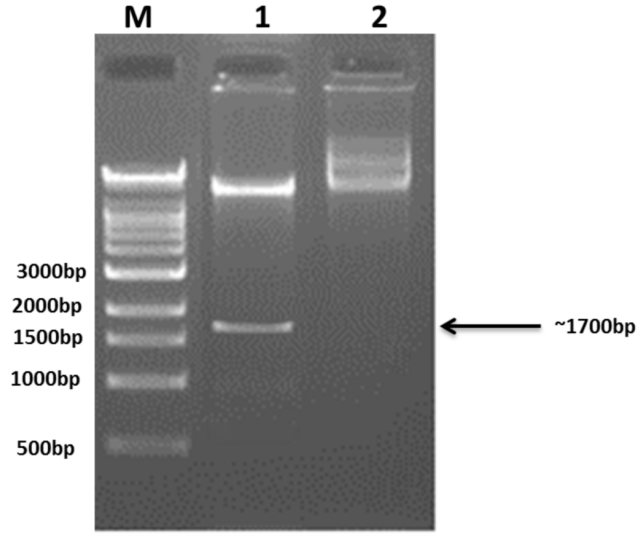

Figure 3: Restriction digestion of ligated pBI121-cagA.

Presented in this figure is the restriction digestion analysis of the pBI121-cagA construct using BamHI and SacI enzymes, visualized on a 1% agarose gel. Lane descriptions are as follows: Lane 1 shows the cagA gene released from the pBI121-cagA construct by BamHI and SacI digestion, Lane 2 exhibits the undigested pBI121-cagA construct and the Molecular-weight ladder (M) is in the adjacent lane for size comparison.

with antibiotics at a steady temperature of 37°C for 12 hr, using a shaker operating at 120 rpm. DNA was then isolated through the alkaline lysis technique.[32] After the overnight growth, cells were rapidly centrifuged at 6000 g for 5 min in a 4°C environment to form a compact pellet. Subsequently, the pellet was reconstituted in 200 µL of Solution I that was kept at a low temperature of 4°C. To this, 200 µL of freshly mixed Solution II was added and the container was flipped several times for thorough mixing. Next, another 200 µL of Solution III was introduced, mixed smoothly and the entire sample was cooled on ice for a duration of 10 min. After the cooling period, the lysate underwent centrifugation at 8000 g for 10 min at a temperature of 4°C to facilitate the separation of different phases. The resultant clear layer was extracted and combined with an equal amount of phenol:chloroform:isoamyl alcohol (25:24:1 v/v), followed by additional centrifugation at 8000 g for 10 min at 4°C. The clarified liquid was then combined with an equal volume of cold isopropanol to induce precipitation of the DNA, which was collected by subsequent centrifugation at the same conditions. The precipitated DNA pellet was cleaned, dried by exposure to air and reconstituted in 10 mM TE buffer. The plasmid DNA was evaluated for its purity by on a 0.8% agarose gel. The precise insertion and sequence integrity within the plasmids were established through restriction enzyme digestion followed by DNA sequencing, validating their suitability for further experimental use.

Adapting the procedure outlined by Patel *et al.* (2013), we transformed eggplant-derived calli using *Agrobacterium tumefaciens* strain EHA105.[43] For this process, 5 µL of plasmid

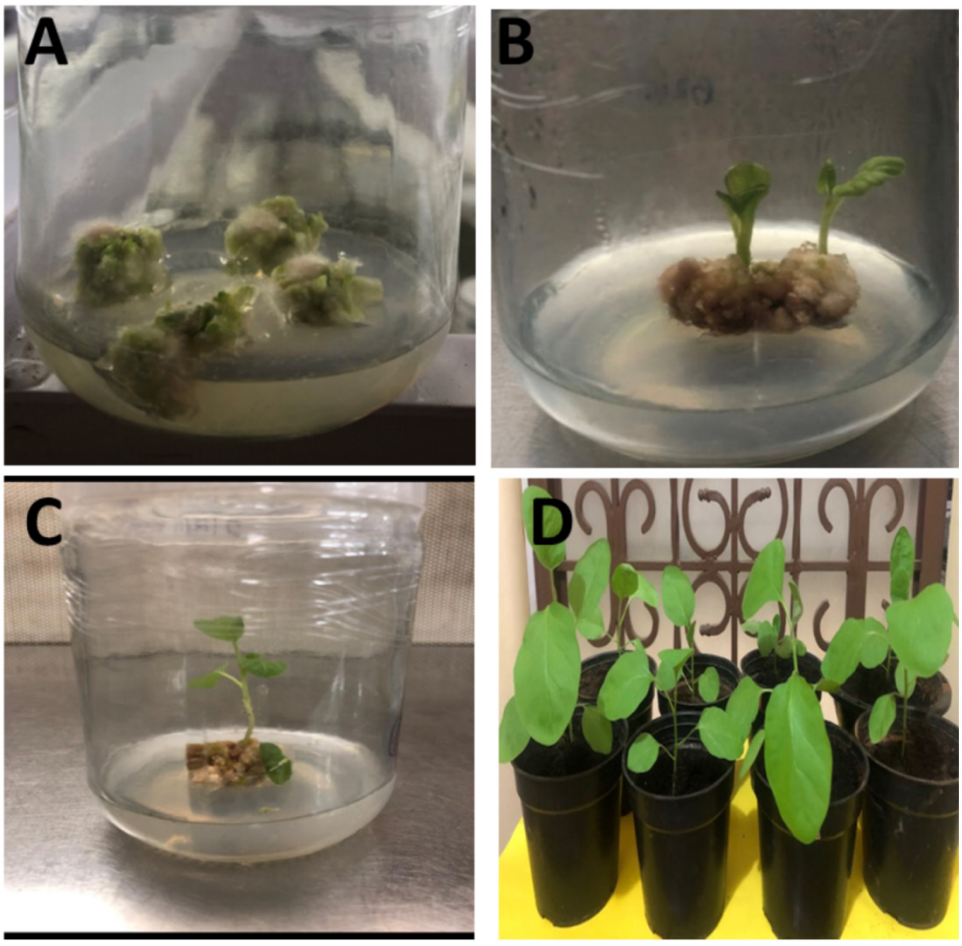

Figure 4: Development of Transgenic Brinjal Plants Through transformation mediated by *Agrobacterium*.

This figure delineates the progressive stages involved in developing transgenic brinjal cv Arka Keshav through *Agrobacterium*-mediated transformation. Panel shows the initial phase of callus development from leaf discs transformed by *Agrobacterium* and placed on selective medium. Panels B and C depict the growth of kanamycin-resistant transgenic shoots, which are now rooting in MS medium. Finally, Panel D shows the successful cultivation of transgenic brinjal plants, fully grown and potted in soil. This series visually represents each critical phase in the transformation and cultivation process, demonstrating the effective adaptation of the plants to their transgenic traits.

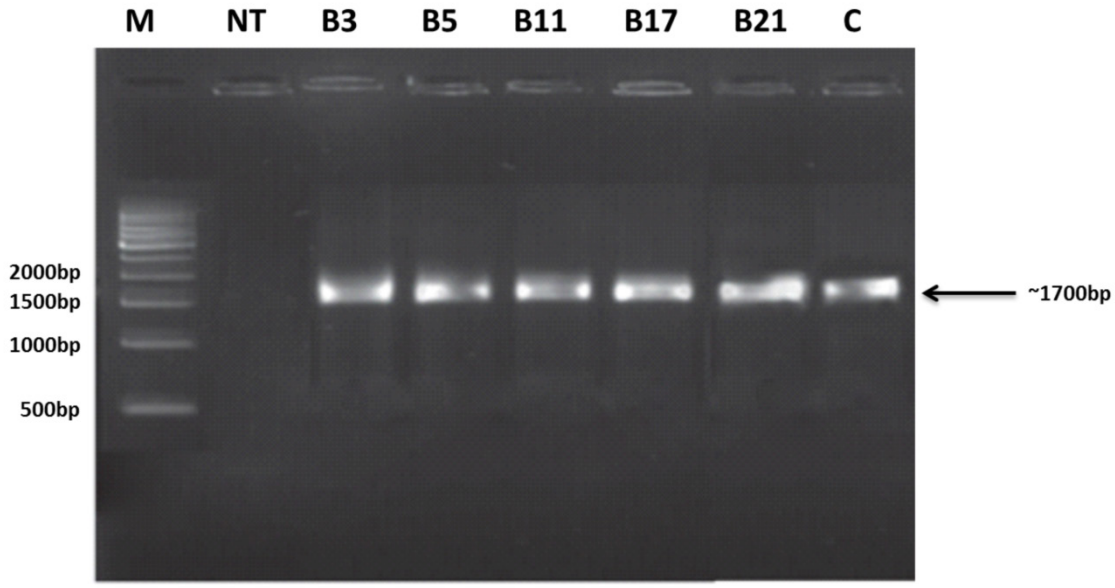

Figure 5: PCR Detection of *cagA* Gene in Transgenic Brinjal Plants.

This figure showcases the PCR results that confirm the presence of the *cagA* gene in transgenic brinjal plants. These plants' leaf samples were used to extract DNA, which was then amplified using primers made especially for the *cagA* gene. The layout of the gel electrophoresis is detailed as follows: a Molecular-weight ladder is labeled as (M), representing the size markers. DNA from a Non-Transformed plant is indicated by (NT), serving as a negative control. Samples from pBI121-*cagA* transformed plants are displayed in lanes labeled B3, B5, B11, B17 and B21, showing the successful incorporation of the gene. The lane marked (C) contains the pBI121-*cagA* plasmid, which acts as a positive control, ensuring the PCR process functioned correctly.

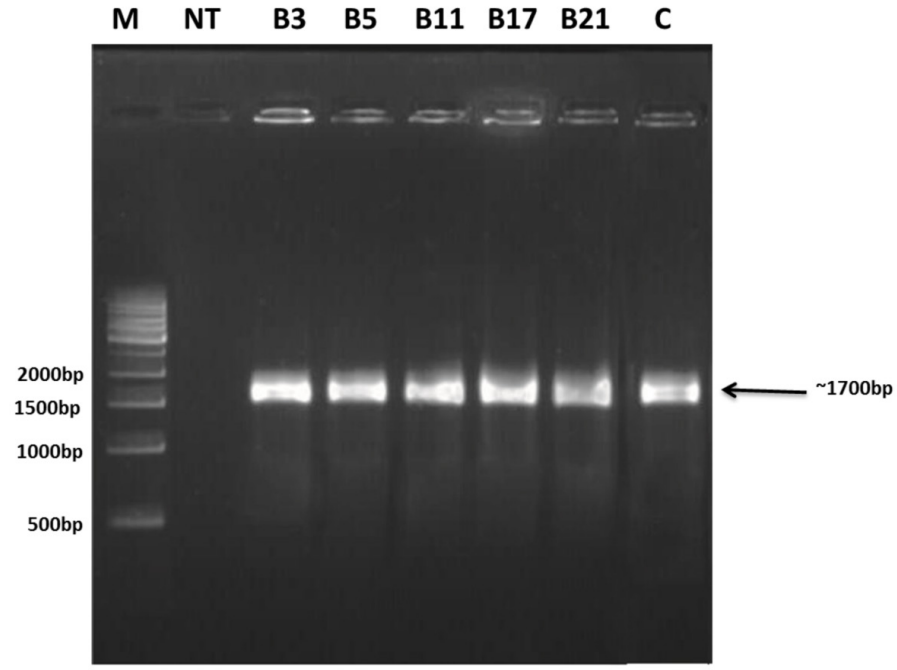

Figure 6: RT-PCR Amplification Results from Transgenic Plant RNA.

The RT-PCR findings from transgenic plants' total RNA, which were amplified using primers aimed at roughly ~1700 bp segments of the *cagA* gene, are shown in this figure. The electrophoresis gel includes various lanes as follows: a molecular-weight ladder (M) that provides size references for the amplified fragments, an RT-PCR product from the cDNA of a Non-Transformed plant (NT) serving as a n control for comparison and RT-PCR products from the cDNA of transformed plants displayed in lanes labelled B3, B5, B11, B17 and B21, which indicate successful gene expression. Additionally, the lane marked (C) contains the pBI121-*cagA* plasmid, utilized as a control for validation to confirm the specificity and efficacy of the amplification. This visualization highlights the consistent presence and activity of the *cagA* gene in the transgenic brinjal plants.

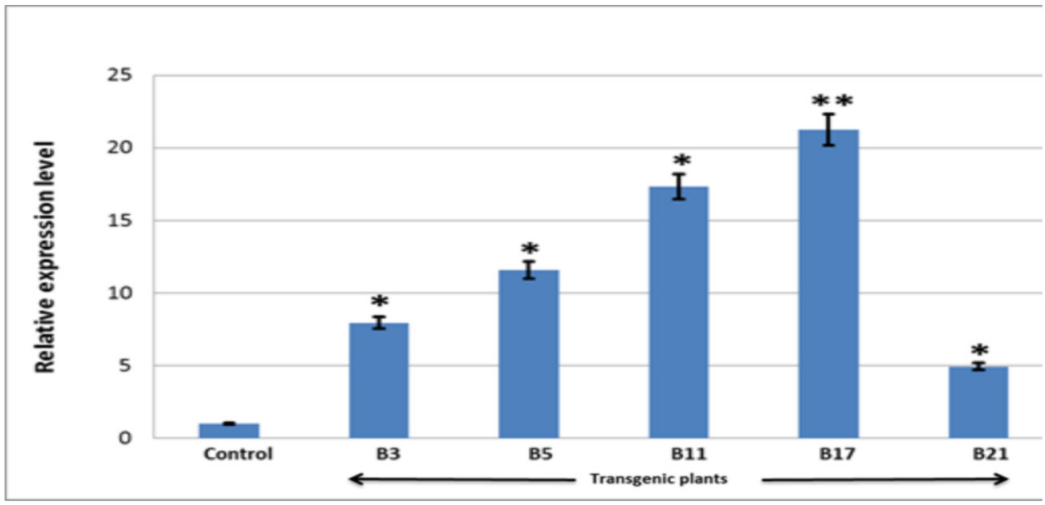

Figure 7: Relative Expression Levels of the *cagA* Gene in Transgenic Brinjal Plants.

This chart displays the comparative expression levels of the cagA gene in transgenic brinjal plants compared to control plants, whose expression has been normalized to 1 and 100%. The data are presented as the average of duplicate measurements, expressed as value±Standard Deviation (SD). Asterisks signify the statistical significance levels, with '**' indicating high significance and '*' indicating significance at the 5% level.

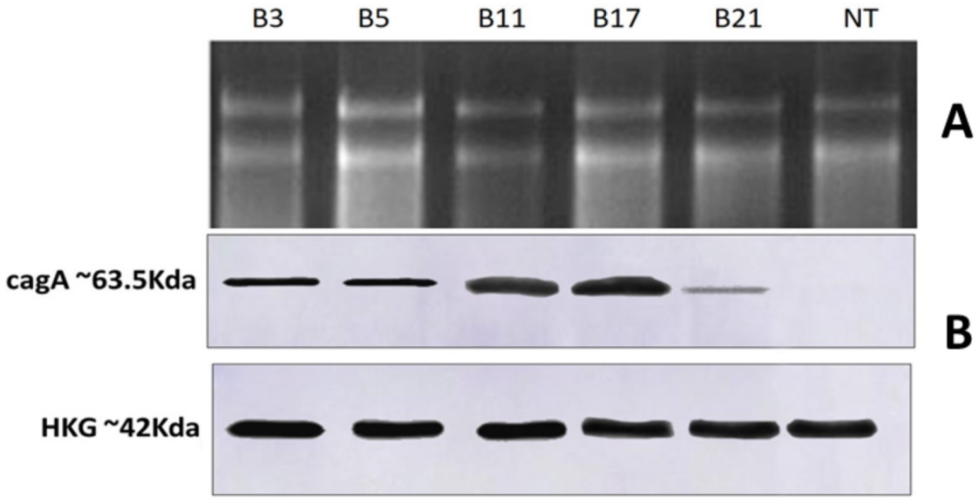

Figure 8: RNA Quality and Protein Expression Analysis in Transgenic Brinjals.

(A) This panel displays RNA extracted from leaf samples of transgenic and control (NT) brinjal plants, visualized on a 2% agarose gel to assess RNA integrity. (B) Western blot analysis showing that, in contrast to a Non-Transgenic control (NT), the cagA protein is present in independent transgenic brinjal lines (B3, B5, B11, B17 and B21). Beta-actin is included as a Housekeeping Protein (HKP). The cagA protein bands are apparent at approximately 63.5KDa, with Beta Actin appearing at around 42KDa.

DNA was combined with 45 µL of *Agrobacterium* cells and the resulting mixture was moved to a cooled electroporation cuvette for electroporation. Following this, the transformed vectors were cultured in 5 mL of YEP medium (yeast-extract-mannitol, pH 7.0) containing 20 mg/L Rifampicin and 50 mg/L Kanamycin and left overnight at 28°C on a rotary shaker set at 200 rpm. Subsequently, the culture was moved to 45 mL of infection medium (enhanced MS basal medium with Thiamine HCl, Myoinositol, Casein hydrolysate, Proline, Glucose, 2,4-D and Acetosyringone) at pH 5.2 and continued incubation at a temperature of 28°C. The insertion of the *cagA* gene into *Agrobacterium* was confirmed using colony PCR. During the infection stage, the embryogenic calli were exposed to a 3 min heat shock at 42°C, followed by a 12 min cooling at room temperature. Residual bacteria were removed using Whatman filter papers and the calli were then placed in dark conditions on co-cultivation media at 25°C for two days. This medium consisted of MS basal medium enriched with Myoinositol, Casein hydrolysate, Proline, Maltose, 2,4-D and

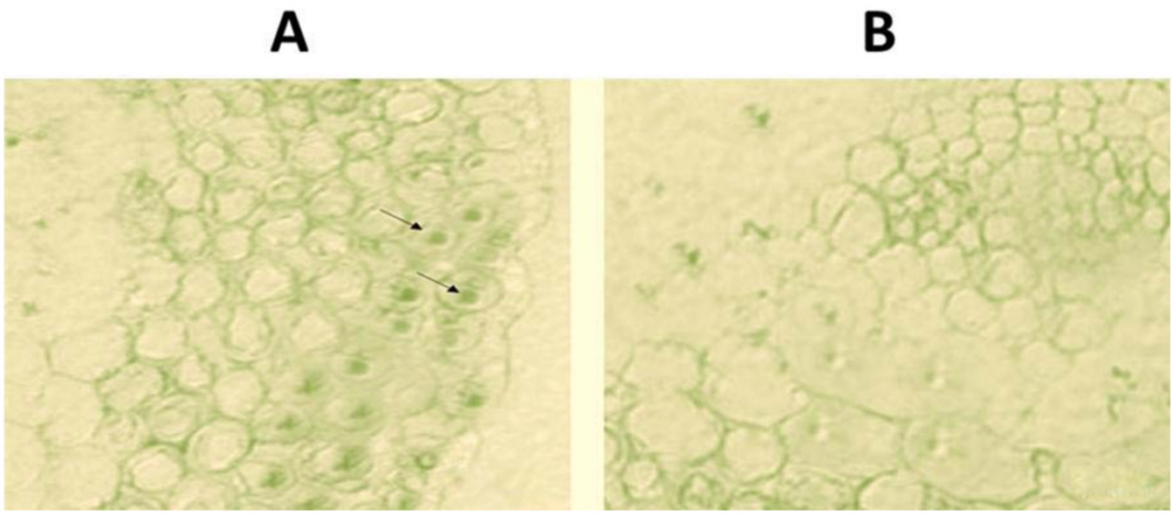

Figure 9: *cagA* Protein Localization in Brinjal Leaves.

This figure presents microscopic pictures displaying the *cagA* protein's distribution in brinjal leaves. Panel (A) displays the presence of the *cagA* protein in transgenic plants, indicated by arrows, while panel (B) shows leaves from non-transgenic plants that do not express the *cagA* protein. The study does not include negative controls, such as samples without antibodies or those treated with PBS. Three separate leaf samples and three distinct cross-sections are used to create the photos and the scale bars show 30 µm.

BAP. following co-culture, the explants were shifted to selecting media comprising 100 mg/L Kanamycin, with media changes every six days for approximately 20 days. The successful calli were then moved to regeneration media that included BAP, Maltose, Phytagel, Timentin and Hygromycin and were maintained under light conditions at 25°C for 3-4 weeks. Regenerated shoots initially developed on media containing Kanamycin and Cefotaxime, before being transitioned to a rooting medium comprising half-strength MS medium with Cefotaxime and IBA, but without Kanamycin to enhance root formation. The plantlets were subsequently transferred to fresh rooting media for an additional 15 days. Finally, the primary transgenic plants were acclimatized in a greenhouse designed for specific temperature and light conditions prior to potting in a mixture of peat-lite and sand. They were then maintained under controlled conditions in a culture room.

Genomic DNA extraction from plant leaves

Genomic DNA was extracted from leaf tissue using CTAB method as per the protocol.[32] Approximately 100 mg of finely ground leaf material was treated with 0.5 mL of a CTAB buffer. This buffer included 2% CTAB, 1.4 M NaCl, 20 mM EDTA, 10 mM Tris-HCl (pH 8.0) and 10 µL of beta-mercaptoethanol. Samples were mixed and incubated in a waterbath at 65°C for 30 min. Following incubation, a same amount of chloroform:isoamyl alcohol (24:1) was added which was then centrifuged at 5000 rpm for 15 min at ambient temperature. The upper layer containing water was collected and combined with 1/10th volume of 5 M NaCl. DNA was precipitated using cold isopropanol. Precipitated sample was then centrifuged at 8000 rpm for 10 min and purified using 70% ethanol solution. After Centrifugation, DNA pellet had dried and dissolved in 100 µL of TE buffer. In order to eliminate any remaining RNA, a solution containing 5 µL of RNase A (10 mg/mL) was introduced and then incubated at 37°C for 1 hr. The DNA that had been purified was thereafter stored at a temperature of -20°C for future analytical use.

PCR amplification of *cagA*

CagA mRNA expression was analyzed using RT PCR and western blotting with RNA isolated from leaves of both transgenic plant and wild variety. The primers for these amplifications (listed in Table 1) were generated with the Primer 3 tool (version 4.13), designed to align with mRNA sequences recorded in GenBank (45). We confirmed the specificity of these primers with the BLASTN algorithm[38-41] to ensure accurate targeting within the gene. Each primer pair was designed to span identical exons, facilitating the amplification of both genomic DNA and cDNA from our samples. To exclude any potential DNA contamination the sample were treated with DNase. The effectiveness of these primers in amplifying cDNA was substantiated through standard RT- PCR procedures and subsequent gel electrophoresis, ensuring the integrity and specificity of our PCR assays.[42-44]

RNA isolation and cDNA synthesis

Following the transformation of brinjal plants, leaves were frozen using liquid nitrogen and preserved at -80°C for later analysis. To extract RNA, the frozen leaves were finely ground with liquid nitrogen with a pestle and mortar. RNA was isolated from 100mg of this leaf powder using RNeasy plant mini kit by Genei laboratories pvt ltd using manufacturers protocol. Agarose gel electrophoresis was performed to ensure the RNA extracts were free from genomic DNA contamination [Figure S1]. Using

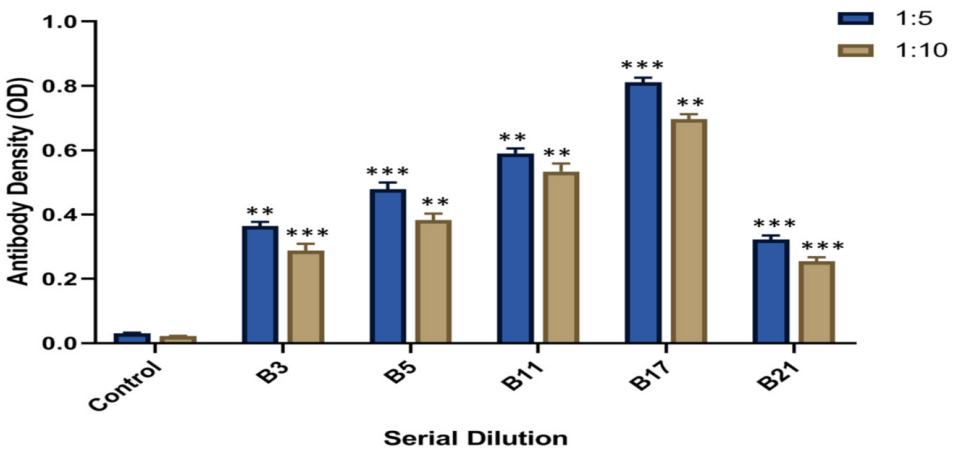

Figure 10: ELISA Data for *cagA* Antigen in Transgenic Brinjals.

This ELISA reader-generated graph displays the *cagA* antigen levels in transgenic brinjals at 1/5 and 1/10 dilution ratios relative to the control plant. The control plant showed no indication at all. The averaged values are presented as mean±SD based on triplicate samples. Significant differences are indicated by triple asterisks ('***'), while significance at the 1% level is indicated by double asterisks ('**').

a Nanodrop® spectrophotometer, the purity and concentration of RNA were measured; an OD260 of 1.0 indicates an RNA concentration of 40 µg/mL.[45-48] The cDNA was synthesized from approximately 2 µg of the extracted RNA, which equates to about 1.02 µL given the RNA concentration, using the M-MuLV RT-PCR Kit as per Ramalho *et al.* (2004) protocols.[49] In order to synthesized the RNA, random primers and 1 µL of RT enzyme were combined. The mixture was then incubated for 10 min at 25°C and 45 min at 70°C. Afterwards, this cDNA was subjected to RTPCR and amplified fragments were visualized on 1.2% agarose gel as per the protocol.[50]

Evaluation of Quantitative Real-Time PCR

Following the manufacturer's instructions, we used Bio-Rad's SYBR Green PCR Master Mix to perform RT-PCR analyses on the generated cDNA samples. Table 1 lists the precise oligonucleotide primers that were utilized.[51] 10 pmol/L of each individual primer, 2.5 µL of 10x PCR buffer with SYBR Green, 2.5 µL of 10 mM dNTPs, 10.4 µL of PCR-grade water, 0.1 µL of 5U *Taq polymerase* from Genei and 2 µL of the target cDNA made up the 25 µL total volume of the qPCR reaction mixtures. This setup was designed to enable simultaneous amplification of the *cagA* target gene and the Actin reference gene.[52-55] The denaturation phase of the PCR cycle was carried out at 94°C for 10 min, followed by 35 cycles of 30 sec denaturation at 94°C, 30 sec of annealing at 55°C and 30 sec extension of 72°C. Final extension was carried out at 72°C for 10 min. To maintain precision and minimize contamination, samples were processed in duplicate and included non-template controls. Data obtained from the qPCR were analyzed using CFX Maestro Software. The ΔΔCT approach was utilized to calculate the differences in gene expression by employing the 2^-ΔΔCT calculation algorithm.[56-58] An unpaired t-test was utilized for statistical analyses, with a significance level of ≤0.05 for *p*-values.[59,60]

Protein Isolation and Western Blot Assessment

Using a mortar and pestle, the leaves were ground into a fine powder with liquid nitrogen in order to isolate proteins from the leaves of both genetically modified and wild-type brinjal plants. After being ground into a powder, 0.2 g of this leaf material was suspended in 2 mL of cold acetone and vortexed for 30 sec.[61] The suspension was centrifuged at 10,000 g for 3 min at 4°C. Pellet was rinsed using acetone before grinding it again using quartz sand. The ground pellet was then washed with 10% TCA, followed by ice cold acetone (80%). Post wash pellet was dried at room temperature and protein was extracted from the pellet using phenol method.[61] 100 ug of pellet was suspended in 800ul of TBP and 800ul of SFS buffer containing 30% sucrose, 2% SDS, 0.1M Tris (pH 8) and 5% 2-mercaptoethanol. The mixture was thoroughly mixed before centrifuging at 10,000 rpm for 3 min. Proteins precipitated by adding five volumes of cold methanol with 0.1 M ammonium acetate after the upper phenolic layer was removed. The proteins were then separated using a further centrifugation at 10,000 rpm for 5 min. They were subsequently cleaned using 80% acetone and methanolic ammonium acetate. Pellet was dried and dissolved in a 2-DE rehydration solution that contained 20 mM dithiothreitol, 4% CHAPS, 2% IPG buffer and 8 M urea. Using a protein test kit with bovine serum albumin as the standard. For western blot analysis the separated proteins were loaded onto 12% SDS- PAGE with a 4.75% stacking gel. Proteins were denatured in sample buffer and electrophoresis was carried out at 200V. after the electrophoresis process, the proteins were transferred onto nylon membranes. These membranes were subsequently treated with a 3% bovine serum albumin in TBST buffer to prevent any nonspecific binding. The membranes

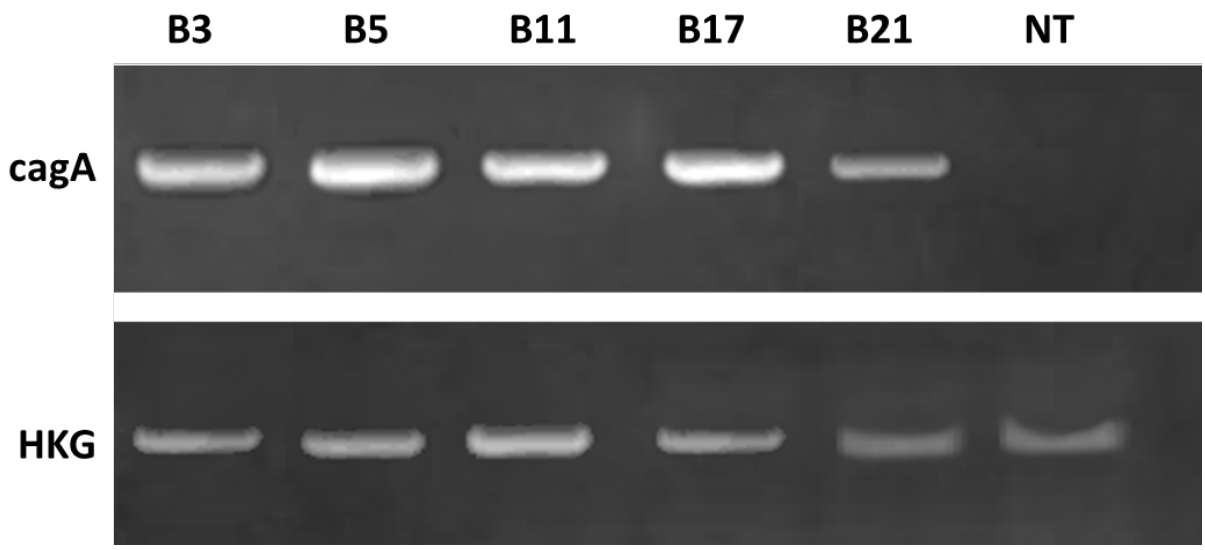

Figure S1: Evaluation of *cagA* gene expression in all five transgenic brinjal lines.

were treated with a secondary biotin-conjugated anti-*cagA Helicobacter pylori* antibody from Thermo Fisher, diluted at 1:500 and incubated for about an hour at room temperature. Detection was achieved using Streptavidin at a dilution of 1:1000 and signals were visualized using a Western blot detection kit. Beta-actin was used as the loading control.

Immunohistochemistry assay

Leaf sections from both genetically modified and non-modified plants were prepared for immunohistochemical analysis using the Technovit 7100 Embedding Kit (Heraeus Kulzer, Wehrheim, Germany), following adapted procedures from Hama *et al.* 2022.[62] Transverse sections, 5 mm thick, were precisely sliced and mounted on slides using a microtome. To deactivate endogenous peroxidase activity, these sections were treated with 3% hydrogen peroxide for 15 min at ambient temperature. Sections were washed and blocked using 10% Bovine Serum Albumin (BSA) to reduce any non-specific interactions. The blocked sections were incubated overnight at 4°C with a biotin-conjugated primary anti-*cagA H. pylori* antibody from Thermo Fisher (dilution 1:2000). Incubated sections were then thoroughly washed with PBS and incubated in secondary antibody (biotin labelled goat anti rat IgG at 1:500 dilution). the slides were rinsed with distilled water and observed under olympus IX-70 microscope.

ELISA

For the ELISA test, extracts from *cagA* gene-modified brinjal plants were diluted in a sodium bicarbonate buffer (pH 9.8) at 1:5 and 1:10 ratios. These diluted samples were then utilized to coat the wells of an ELISA plate, aiming for antigen concentrations of 5 mg/mL per well.[63,64] The plates were placed in an incubator of 37°C for 2 hr. Post incubation, the plates were rinsed three times with a washing buffer that contained 0.1% Tween 20 in PBS, each wash lasting 5 min. The wells were subsequently blocked with 2% skim milk for 90 min to prevent non-specific bindings. This step was followed by another set of three washes using the Tween 20-PBS wash buffer. Biotin conjugated primary antibody was added to the antigen coated wells at 1:500 concentration and incubated for 1 hr at room temperature. Following the period of antibody incubation, the plates were again Washed to remove any unbound primary antibodies. A 1:10,000 dilution of Streptavidin-HRP conjugate was added to each well, incubate for 30 min, followed by the addition of 0.5 M HCl to stop the reaction. The absorbance at 450 nm measured using a Bio-Rad microplate reader. Protein extracts from unmodified brinjal leaves served as the negative controls. All steps were performed in triplicate to ensure accuracy and consistency in the results.

RESULTS

Cloning and Amplification of the *cagA* Gene into pBI121

The PCR process with *cagA*-specific primers effectively produced an amplicon approximately ~1700 bp in size, evidenced by the bands visible in [Figure 2]. Additional analysis using agarose gel electrophoresis of the plasmids following enzymatic digestion identified two distinct fragments [Figure 3], with one matching the expected size of the *cagA* gene at about ~1700 bp. Sequence analysis of these fragments was performed and the findings were assessed against sequences available in the GenBank database. The BLAST alignment tool, showed that our sequence high degree of similarity, with 97.93% match to existing *cagA* gene sequences listed in GenBank (GenBank® accession no. CP026326.1), highlighting the genetic diversity within different strains of this gene.

Agrobacterium Genetic Transformation in Brinjal

Agrobacterium transformation successfully regenerated 52 distinct brinjal plants, as illustrated in [Figure 4]. The incorporation of the

cagA gene within these genetically modified plants was verified through colony PCR on the *Agrobacterium* cultures, displaying bands approximately ~1700 bp in size on the gel, aligning with the expected size of the *cagA* gene. To further confirm the expression of *cagA*, both PCR amplification and immunoblot analysis were utilized. Genomic DNA from all five transgenic lines [Figure 5] and cDNA from these lines produced a PCR product consistent with the anticipated ~1700 bp size [Figure 6], affirming active gene expression. Conversely, control samples comprised of genomic DNA and cDNA from non-transgenic plants showed no bands, as anticipated, confirming the absence of *cagA* amplification. The lack of non-specific bands in these tests indicated that the DNA was highly pure and specifically targeted the intended gene.

Real-Time PCR Analysis of *cagA* Gene Expression The transcription levels of the *cagA* gene across various samples were analysed by running the RT PCR amplicons on a 2% agarose gel. The Housekeeping Gene (HKG) exhibited a uniform expression pattern, evident at approximately 150 bp in all the samples tested. Importantly, *cagA* gene expression was detected in all five evaluated samples, as shown in [Figure S1]. Although gene expression predominantly occurred around 151bp, variations in expression intensity were observed among samples B3, B5, B11 and B17 compared to B21. Notably, samples B11 and B17 demonstrated significant overexpression with levels recorded at 17.3302±0.42466 and 21.2610±0.416804 respectively, whereas sample B5 exhibited a lower expression level at 11.59±0.284 [Figure 7]. Statistical analysis showed a significant F-value of 0.003**, indicating non-uniform variance among the samples. The p-value of 0.009** at a 5% significance level led to the rejection of the null hypothesis, indicating statistically significant variations in expression levels between the transgenic brinjal plants and their non-transgenic counterparts. Further, paired t-tests performed in SPSS identified significant differences in gene expression between the transgenic and control plants across all comparisons: B3 *cagA* – Control (p = 0.025*), B5 *cagA* – Control (p = 0.012*), B11 *cagA* – Control (p = 0.012*), B17 *cagA* – Control (p = 0.009**) and B21 *cagA* – Control (p = 0.041*). The rejection of the null hypothesis confirms significant distinctions in the expression levels between transgenic samples B3, B5, B11, B17 and B21 compared to the controls [Figure 7].

Analysis of *cagA* Protein Expression in Genetically Engineered Brinjal. The primary objective of this study was to generate *cagA* proteins in plants that have been genetically altered. Through Western blot analysis, significant expression of the *cagA* protein was detected in the transgenic brinjal plants, exhibiting a molecular weight of around 63.5 KDa [Figure 8]. On the other hand, protein extracts from non-transgenic or control plants showed no bands at this molecular weight. The genetically engineered brinjal varieties B3, B5, B11, B17 and B21 displayed a strong positive correlation between the levels of *cagA* mRNA and protein expression. Specifically, samples B11 and B17 exhibited notably higher *cagA* protein levels compared to the other tested samples, corroborating the RT-PCR findings and demonstrating consistency between mRNA and protein expression levels. Furthermore, bands indicative of beta-actin, which served as a reference for sample loading control, were consistently observed at a molecular weight of 42 KDa [Figure 8].

Analysis of the *cagA* protein using immunohistochemistry in brinjal leaves Immunohistochemical tests were conducted to assess the localization of *cagA* protein within the leaves of genetically modified and unmodified brinjal plants. In the non-transgenic samples, *cagA* protein was nearly undetectable. However, in the transgenic samples, clear staining indicating the presence of *cagA* protein was noted in the parenchymal cells under the leaf epidermal layers. While the epidermal layer showed minimal specific staining, there was substantial accumulation of the protein in the parenchymal tissues. Notably, the protein was predominantly localized in the cytoplasm, rather than the cell walls. This investigation is possibly the first to document such findings and it draws comparisons with the work of Qing Gu *et al.* (2006),[65] who demonstrated the expression of the full-length *ureB* gene in transgenic rice, triggering an antigen-based response against *H. pylori*. The results of our immunohistochemical analysis align with those described by Qing Gu *et al.*, but with a focus on the *cagA* protein, as illustrated in [Figure 9].

ELISA Results for Transgenic Brinjal Plants

The Enzyme-Linked Immunosorbent Assay (ELISA) demonstrated that the transgenic brinjal plants were capable of producing significant levels of the *cagA* protein. The concentrations of antibodies in these samples were quantitatively assessed and depicted in [Figure 10]. Notably, among the transgenic lines, samples B11 and B17 exhibited the highest concentrations of *cagA* antigen at dilutions of 1:5 and 1:10. Statistical evaluations confirmed the significance of these results at the 1% level, corroborating the findings from both RT- PCR and Western blot analyses. In contrast, the antigen was not detectable in the control samples from non-transgenic plants. The strong correlation observed between the RT-PCR and ELISA data highlights a consistent positive relationship between the *cagA* mRNA and protein levels in the transgenic lines. This coherence across various testing methods affirms the accuracy of the results and highlights the effective expression of the *cagA* gene in these genetically engineered brinjal plants.

DISCUSSION

Recent advancements in plant biotechnology have catalyzed the production of recombinant proteins, including vaccines, using various plant-based expression systems.[66,67] Despite these technological strides, several challenges persist that hinder the broader implementation of plants as bioreactors for producing recombinant biopharmaceuticals. One significant barrier is

enhancing the cost-effectiveness of this approach. *H. pylori*, a bacterium affecting approximately 40-50% of the global population,[1] is often linked to several gastrointestinal diseases such as ulcers and various cancers, including those of the stomach and intestine. Additionally, it is linked to a heightened risk of developing adenocarcinoma and lymphoma.[68] Vaccines are vital for enhancing immunity to specific pathogens and are key in controlling disease outbreaks. Traditional vaccines, which are proteins that simulate the pathogen, are typically created through processes that either heat-kill or attenuate the pathogenic strains.[69] They train the immune system to recognize and effectively respond to foreign antigens. Plant-based vaccines offer a promising alternative by potentially lowering production costs and simplifying the production process compared to traditional vaccines.[70,71] Utilizing transgenic plants to produce biopharmaceutical proteins represents a strategic approach to addressing the costs and other limitations tied to conventional vaccine production systems.[72] These vaccines involve genetically modifying plants to carry pathogen-specific genes, preserving their immunogenic properties.[73] In our research, we successfully introduced and expressed the *cagA* protein in brinjal plants, marking a first for this species. This result aligns with earlier studies in which rice expressed the *H. pylori* with approximately 30 regenerated plants showing accumulation of the *ureB* antigen as verified by PCR and blotting techniques.[65]The increasing prevalence of antibiotic- resistant strains of bacteria such as *H. pylori* underscores the critical need for developing new vaccines.[74] This pathogen not only maintains high infection rates worldwide but has also shown a growing resistance to multiple antibiotics in both developing and developed nations.[75] These challenges emphasize the need for cost-effective vaccine development strategies. While eukaryotic systems can often produce proteins inexpensively and effectively, most prokaryotic systems do not perform essential post-translational modifications, making them less suitable for vaccine production.[76] In our experiments, we engineered the *cagA* gene into the pBI121 binary vector, with kanamycin resistance marker and introduced it into brinjal calluses using an *Agrobacterium*-mediated transformation technique. Five of the 52 examined plants exhibited transgenic traits, as shown by the *cagA* gene product's detection. The presence of the *cagA* gene in these plants, at both the RNA and protein levels, was verified using RT-PCR and Western blot analysis, suggesting that *cagA* transgenic brinjal could serve as an edible vaccine candidate against *H. pylori*. The concept of edible vaccination is emerging as a compelling solution for human and animal health applications. Although these vaccines face challenges such as public acceptance of their effectiveness and safety, they represent a cost-effective and potentially safer alternative to conventional vaccines.[77] The findings of this research contribute to the expanding field of plant-based vaccine research, providing new insights and methodologies for developing affordable and safe vaccines against prevalent bacterial infections like those caused by *H. pylori* and paving the way for further advancements in the realm of edible vaccines.

CONCLUSION

Our study demonstrates the successful genetic modification of brinjal (*Solanum melongena* L.) to produce the *cagA* protein from *H.pylori*, highlighting its potential as a novel edible vaccine candidate. The incorporation of the *cagA* gene into the brinjal genome was confirmed through rigorous molecular techniques including PCR, Western blot analysis and ELISA, establishing the consistent expression of the antigen at both the transcriptional and protein levels. The transgenic brinjal lines showed varied but significant expression of the *cagA* protein, with lines B11 and B17 exhibiting particularly high levels, which correlated positively with the immunogenic response as evidenced in immunohistochemical analyses. These findings suggest that these plants can effectively elicit an immune response, a key characteristic for potential vaccine candidates. Furthermore, this research underscores the advantages of using plants as biofactories for vaccine production, offering a cost-effective, safe and scalable alternative to traditional methods. Plant-based vaccines, like the one developed in this study, could significantly reduce the logistical and financial burdens associated with conventional vaccine production and distribution, particularly in low-resource settings. The successful expression of the *cagA* protein in brinjal not only provides a basis for further research into edible vaccines against *H. pylori* but also contributes to the broader field of molecular farming. Future studies should focus on the clinical efficacy and safety of these transgenic plants, optimizing expression systems and scaling up production to fully realize their potential in combating *H. pylori* infections globally. This study paves the way for advancing plant-based pharmaceutical technology, offering promising insights into the development of new, effective and accessible strategies for disease prevention, especially in regions most burdened by gastric diseases linked to *H. pylori*.

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AVAILABILITY OF DATA AND MATERIAL

The data and supplementary materials supporting this publication can be obtained from the corresponding author upon reasonable request.

CONSENT FOR PUBLICATION

All listed authors have approved the manuscript and agree with its submission for publication.

ETHICS APPROVAL

This research received approval from the Institutional Biosafety Committee (IBSC) at JSS College, Mysore, located at the Postgraduate Department of Biotechnology, JSS College, Mysuru, Genei India Pvt. Ltd., Bangalore and the Department of Biotechnology and Crop Improvement, College of Horticulture, University of Horticultural Sciences, GKVK Post, Bangalore (IBSC Registration number: JSSC111220191080). Necessary permissions for collecting plant or seed specimens were secured from the College of Horticulture, University of Horticultural Sciences, GKVK Post, Bangalore.

CONFLICT OF INTEREST

The authors report no conflicts of interest relevant to this publication.

ABBREVIATIONS

H. pylori: *Helicobacter pylori;* **cagA:** Cytotoxin-associated gene A; **PCR:** Polymerase Chain Reaction; **qPCR:** Quantitative Polymerase Chain Reaction; **ELISA:** Enzyme-Linked; Immunosorbent Assay; **DNA:** Deoxyribonucleic Acid; **RNA:** Ribonucleic Acid; **SDS:** Sodium Dodecyl Sulfate; **TCA:** Trichloroacetic Acid; **PBS:** Phosphate Buffered Saline; **BSA:** Bovine Serum Albumin.

SUMMARY

This study explores the innovative use of genetically engineered brinjal (*Solanum melongena* L.) as a biofactory for producing an edible vaccine against *Helicobacter pylori*, targeting the bacterium's cytotoxin-associated gene A (*cagA*) antigen. By integrating the *cagA* gene into brinjal using *Agrobacterium*-mediated transformation, we achieved stable expression, which was confirmed through PCR, qPCR and Western blot analyses. Notable results included significant antigen production in specific transgenic lines, with strong immunogenic potential demonstrated by ELISA and immunohistochemical staining. These findings highlight the potential of plant-based vaccines as a cost-effective and accessible approach for combating *H. pylori* infections, particularly in low-resource settings. This research paves the way for further development of edible vaccines, which could significantly impact global health by providing an alternative to traditional vaccine production methods.

REFERENCES

1. Nešić D, Buti L, Lu X, Stebbins CE. Structure of the Helicobacter pylori CagA oncoprotein bound to the human tumor suppressor ASPP2. Proceedings of the National Academy of Sciences. 2014;111(4):1562-7.
2. Fallone CA, Chiba N, Zanten SV, Fischbach L, Gisbert JP, Hunt RH. The Toronto consensus for the treatment of Helicobacter pylori infection in adults. Gastroenterology. 2016;151(1):14.
3. Josephson M, Skole K. The Houston Consensus Conference on Testing for Helicobacter pylori infection. Clinical Gastroenterology and Hepatology. 2018;16(12):2004-5.
4. Turabi KS, Deshmukh A, Paul S, Swami D, Siddiqui S, Kumar U. Drug repurposing-an emerging strategy in cancer therapeutics. Naunyn-Schmiedeberg's Archives of Pharmacology. 2022;395(10):1139-58.
5. Hooi JK, Lai WY, Ng WK, Suen MM, Underwood FE, Tanyingoh D. Global prevalence of Helicobacter pylori infection: systematic review and meta-analysis. Gastroenterology. 2017;153(2):420-9.
6. Chatterjee A, Paul S, Bisht B, Bhattacharya S, Sivasubramaniam S, Paul MK. Advances in targeting the WNT/β-catenin signaling pathway in cancer. Drug Discovery Today. 2021.
7. Ohnishi N, Yuasa H, Tanaka S, Sawa H, Miura M, Matsui A. Transgenic expression of Helicobacter pylori CagA induces gastrointestinal and hematopoietic neoplasms in mouse. Proceedings of the National Academy of Sciences. 2008;105(3):1003-8.
8. Barzigar R, Kb HARAPRASADN, Mehran M. Challenges and recent developments associated with vaccine antigens production against Helicobacter pylori. Int J App Pharm. 2020;12(4):45-50.
9. Graham DY, Shiotani A. New concepts of resistance in the treatment of Helicobacter pylori infections. Nature Clinical Practice Gastroenterology and Hepatology. 2008;5(6):321-31.
10. Barzigar R, Haraprasad N, Kumar BYS, Mehran MJ. Cloning and Expression of Vacuolating Cytotoxin A (VacA) Antigenic Protein in *Nicotiana benthamiana* Leaves a Potential Source of the Vaccine against Helicobacter pylori. International Journal of Pharmaceutical Investigation. 2021;11(1).
11. Backert S, Clyne M, Tegtmeyer N. Molecular mechanisms of gastric epithelial cell adhesion and injection of CagA by Helicobacter pylori. Cell Communication and Signaling. 2011;9(1):1-11.
12. Murata-Kamiya N, Kurashima Y, Teishikata Y, Yamahashi Y, Saito Y, Higashi H. Helicobacter pylori CagA interacts with E-cadherin and deregulates the β-catenin signal that promotes intestinal transdifferentiation in gastric epithelial cells. Oncogene. 2007;26(32):4617-26.
13. Oliveira MJ, Costa AM, Costa AC, Ferreira RM, Sampaio P, Machado JC. CagA associates with c-Met, E-cadherin and p120-catenin in a multiproteic complex that suppresses *Helicobacter pylori*–induced cell-invasive phenotype. The Journal of infectious diseases. 2009;200(5):745-55.
14. Bagnoli F, Buti L, Tompkins L, Covacci A, Amieva MR. *Helicobacter pylori* CagA induces a transition from polarized to invasive phenotypes in MDCK cells. Proceedings of the National Academy of Sciences. 2005;102(45):16339-44.
15. Cheng C, Chen Z, Zhu C. Construction of plant expression vectors with fusion gene of Helicobacter pylori cagA, ureB and ctb and its genetic transformation in tobacco. Wei sheng wu xue bao= Acta microbiologica Sinica. 2007;47(1):29-33.
16. Pan X, Ke H, Niu X, Li S, Lv J, Pan L. Protection against Helicobacter pylori infection in BALB/c mouse model by oral administration of multivalent epitope-based vaccine of cholera toxin B subunit-HUUC. Frontiers in immunology. 2018;9(1003).
17. Sabalza M, Christou P, Capell T. Recombinant plant-derived pharmaceutical proteins: current technical and economic bottlenecks. Biotechnology letters. 2014;36(12):2367-79.
18. Abdoli-Nasab M, Jalali-Javaran M, Cusidó RM, Palazón J, Baghizadeh A, Alizadeh H. Expression of the truncated tissue plasminogen activator (K2S) gene in tobacco chloroplast. Molecular biology reports. 2013;40(10):5749-58.
19. Soria-Guerra RE, Rosales-Mendoza S, Márquez-Mercado C, López-Revilla R, Castillo-Collazo R, Alpuche-Solís ÁG. Transgenic tomatoes express an antigenic polypeptide containing epitopes of the diphtheria, pertussis and tetanus exotoxins, encoded by a synthetic gene. Plant cell reports. 2007;26(7):961-8.
20. Streatfield SJ, Howard JA. Plant production systems for vaccines. Expert review of Vaccines. 2003;2(6):763-75.
21. Tiwari S, Verma PC, Singh PK, Tuli R. Plants as bioreactors for the production of vaccine antigens. Biotechnology advances. 2009;27(4):449-67.
22. Ali S, Kim WC. A fruitful decade using synthetic promoters in the improvement of transgenic plants. Frontiers in plant science. 2019;10(1433).
23. Hahne G, Horn M, Reski R. Plant biotechnology in support of the Millennium Goals: Springer; 2011 2011.
24. Kashima K, Yuki Y, Mejima M, Kurokawa S, Suzuki Y, Minakawa S. Good manufacturing practices production of a purification-free oral cholera vaccine expressed in transgenic rice plants. Plant cell reports. 2016;35(3):667-79.
25. Madanala R, Gupta V, Pandey AK, Srivastava S, Pandey V, Singh PK, *et al.* Tobacco chloroplasts as bioreactors for the production of recombinant superoxide dismutase in plants, an industrially useful enzyme. Plant molecular biology reporter. 2015;33(4):1107-15.
26. Wei Q, Du L, Wang W, Hu T, Hu H, Wang J. Comparative transcriptome analysis in eggplant reveals selection trends during eggplant domestication. International journal of genomics. 2019; 2019.
27. Iannacone R, Grieco PD, Cellini F. Specific sequence modifications of a cry3B endotoxin gene result in high levels of expression and insect resistance. Plant molecular biology. 1997;34(3):485-96.

28. Saini DK, Kaushik P. Visiting eggplant from a biotechnological perspective: A review. Scientia Horticulturae. 2019;253:327-40.
29. Mehran MJ, Kumar BYS, Haraprasad N, Barzigar R, Paul S. Cloning and Expression of *Helicobacter pylori* ulcer Associated Gene-iceA1 in Brinjal (*Solanum melongena* L. International Journal of Pharmaceutical Investigation. 2021;11(3):338-44.
30. Mehran MJ, Kumar BYS, Haraprasad N, Barzigar R, Fakrudin B, Paul S. Evaluation of iceA1 Gene Expression of *Helicobacter pylori* Risk Factor of Gastric Cancer in Transgenic Brinjal. Indian Journal of Pharmaceutical Education and Research. 2021;55(4):1197-206.
31. Chang AY, Chau V, Landas JA, Pang Y. Preparation of calcium competent Escherichia coli and heat-shock transformation. JEMI methods. 2017;1(22-25).
32. Sambrook J, Russell DW. Preparation of plasmid DNA by alkaline lysis with SDS: minipreparation. Cold Spring Harbor Protocols. 2006;2006(1):4084.
33. Paul S, Heckmann LH, Sørensen JG, Holmstrup M, Arumugaperumal A, Sivasubramaniam S. Transcriptome sequencing, de novo assembly and annotation of the freeze tolerant earthworm, *Dendrobaena octaedra*. Gene Reports. 2018;13:180-91.
34. Paul S, Sudalai Mani DK, Sandhya SS, Subburathinam B, Vijithkumar V, Vaithilingaraja A, *et al.* Identification, tissue specific expression analysis and functional characterization of arrestin gene (ARRDC) in the earthworm Eudrilus eugeniae: a molecular hypothesis behind worm photoreception. Molecular Biology Reports.2022: 1-12.
35. Sad RRK, Saini R, Kumari P, Roy P, Paul S. Impacts on dietary habits and health of Indian population during COVID-19 lockdown. Public Health Review: International Journal of Public Health Research. 2020;7(6):38-50.
36. Hippo signaling pathway has a critical role in Zika virus replication and in the pathogenesis of neuroinflammation. The American journal of pathology. 2020;190(4):844-61.
37. Arumugaperumal A, Paul S, Lathakumari S, Balasubramani R, Sivasubramaniam S. The draft genome of a new Verminephrobacter eiseniae strain: a nephridial symbiont of earthworms. Annals of microbiology. 2020;70:1-18.
38. Alaguponniah S, Krishna DV, Paul S, Christyraj JRSS, Nallaperumal K, Sivasubramaniam S. Finding of novel telomeric repeats and their distribution in the human genome. Genomics. 2020;112(5):3565-70.
39. Chinnadurai L, Eswaramoorthy T, Paramachandran A, Paul S, Rathy R, Arumugaperumal A. Draft genome sequence of *Escherichia coli* phage CMSTMSU, isolated from shrimp farm effluent water. Microbiology Resource Announcements. 2018;7(14).
40. Kumavath R, Paul S, Pavithran H, Paul MK, Ghosh P, Barh D, *et al.* Emergence of Cardiac Glycosides as Potential Drugs: Current and Future Scope for Cancer Therapeutics. Biomolecules. 2021;11(9).
41. Miao X, Kumar RR, Shen Q, Wang Z, Zhao Q, Singh J. Phytoremediation for Co-contaminated Soils of Cadmium and Polychlorinated Biphenyls Using the Ornamental Plant *Tagetes patula* L. Bulletin of Environmental Contamination and Toxicology.2022: 1-7.
42. Bleve G, Rizzotti L, Dellaglio F, Torriani S. Development of reverse transcription (RT)-PCR and real-time RT-PCR assays for rapid detection and quantification of viable yeasts and molds contaminating yogurts and pasteurized food products. Applied and environmental microbiology. 2003;69(7):4116-22.
43. Rana I, Kataria S, Tan TL, Hajam EY, Kashyap DK, Saha D. Mindin is essential for cutaneous fibrogenesis in a new mouse model of systemic sclerosis. bioRxiv.2022(2022): 26-477822.
44. Rana I, Kataria S, Tan TL, Hajam EY, Kashyap DK, Saha D. MINDIN (SPONDIN-2) IS ESSENTIAL FOR CUTANEOUS FIBROGENESIS IN A MOUSE MODEL OF SYSTEMIC SCLEROSIS. Journal of Investigative Dermatology. 2022;143(5):699-710.
45. Ponesakki V, Paul S, Mani DKS, Rajendiran V, Kanniah P, Sivasubramaniam S. Annotation of nerve cord transcriptome in earthworm Eisenia fetida. Genomics data. 2017;14:91-105.
46. Paul S, Arumugaperumal A, Rathy R, Ponesakki V, Arunachalam P, Sivasubramaniam S. Data on genome annotation and analysis of earthworm Eisenia fetida. Data in brief. 2018;20:525-34.
47. Rathy R, Paul S, Ponesakki V, Kanniah P, Muthu SP, Arumugaperumal A. Data on genome sequencing, analysis and annotation of a pathogenic Bacillus cereus 062011msu. Data in Brief. 2018;17:15-23.
48. Mo Q, Bolideei M, Rong SJ, Luo JH, Yang CL, Lu WY, *et al.* GSK2334470 attenuates high salt-exacerbated rheumatoid arthritis progression by restoring Th17/Treg homeostasis. iScience. 2024;27(6):109798.
49. Ramalho AS, Beck S, Farinha CM, Clarke LA, Heda GD, Steiner B. Methods for RNA extraction, cDNA preparation and analysis of CFTR transcripts. Journal of Cystic Fibrosis. 2004;3:11-5.
50. Green MR, Sambrook J. Amplification of cDNA generated by reverse transcription of mRNA: two-step reverse transcription-polymerase chain reaction (RT-PCR. Cold Spring Harbor Protocols. 2019;2019(5):095190.
51. Schmidt GW, Delaney SK. Stable internal reference genes for normalization of real-time RT-PCR in tobacco (*Nicotiana tabacum*) during development and abiotic stress. Molecular Genetics and Genomics. 2010;283(3):233-41.
52. Expósito-Rodríguez M, Borges AA, Borges-Pérez A, Pérez JA. Selection of internal control genes for quantitative real-time RT-PCR studies during tomato development process. BMC plant biology. 2008;8(1):1-12.
53. Paul S, Balakrishnan S, Arumugaperumal A, Ejjsb T, D.H. Genome sequencing, Analysis and Characterization of Baculovirus Infecting the Caterpillar, Spilosoma Obliqua Walker (Arctiidae)(Insecta: Lepidoptera) from India. J Virol Antivir Res. 2021;5(2).
54. Ponesakki V, Syamala SS, Paul S, Arumugaperumal A, Selvakumar P, Vijayan V, *et al.* ACTIVATION OF MOUSE EAR LOBE TISSUE REGENERATION BY METABOLITES OF EARTHWORM. J Exp Zool. 2023;26(1):1191-205.
55. Paul S, Arumugaperumal A, Balakrishnan S, Jebasingh EJ, Thangapandi SB, Devi HS, *et al.* Pathogenicity, structure and genome analysis of the granulovirus infecting the caterpillars of *Pieris brassicae* Linn.(Insecta: Lepidoptera: Pieridae). Journal of Experimental Zoology India. 2023;26(1).
56. Tatta ER, Paul S, Kumavath R. Transcriptome Analysis revealed the Synergism of Novel Rhodethrin inhibition on Biofilm architecture, Antibiotic Resistance and Quorum sensing in Enterococcus faecalis. Gene. 2023;871(147436).
57. Paul S, Balakrishnan S, Arumugaperumal A, Lathakumari S, Syamala SS, Arumugaswami V, *et al.* The transcriptome of anterior regeneration in earthworm Eudrilus eugeniae. Molecular Biology Reports. 2021;48:259-83.
58. Hedayati M, Garousi B, Rezaei Z, Nazerian Y, Yassaghi Y, Tavasol A, *et al.* Identifying SCC Lesions Capable of Spontaneous Regression by Using Immunohistochemistry: A Systematic Review and Meta-Analysis. Dermatol Pract Concept. 2024;14(2).
59. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. methods. 2001;25(4):402-8.
60. Paul S, Balakrishnan S, Arumugaperumal A, Lathakumari S, Syamala SS, Vijayan V. Importance of clitellar tissue in the regeneration ability of earthworm Eudrilus eugeniae. Functional and Integrative Genomics.2022: 1-32.
61. Wang W, Scali M, Vignani R, Spadafora A, Sensi E, Mazzuca S, *et al.* Protein extraction for two-dimensional electrophoresis from olive leaf, a plant tissue containing high levels of interfering compounds. Electrophoresis. 2003;24(14):2369-75.
62. H OH, G A, R B, H L, M D. Immunohistochemical diagnosis of human infectious diseases: a review. Diagnostic Pathology. 2022;17(1).
63. Barzigar R, Mehran MJ, Haraprasad N, Kumar BYS, Fakrudin B. Transient recombinant expression of highly immunogenic CagA, VacA and NapA in *Nicotiana benthamiana*. Biotechnology Reports. 2022;33:e00699.
64. Nayeri FD, Anbuhi MH. Transient expression of etanercept therapeutic protein in tobacco (*Nicotiana tabacum* L. International journal of biological macromolecules. 2019;130:483-90.
65. Gu Q, Han N, Liu J, Zhu M. Expression of Helicobacter pylori urease subunit B gene in transgenic rice. Biotechnology letters. 2006;28(20):1661-6.
66. Mehran MJ, Zendehbad SH, Malla S. Cloning and expression of a partial UreA antigen for the production of vaccine against helicobacter pylori, the risk factor for gastric cancer. Asian J Pharm Clin Res. 2014;7(1):111-7.
67. Mehran MJ, Zendehbad SH, Malla S. Free radical scavenging and antioxidant potential activity of cassava plants. Asian J Pharm Clin Res. 2014;7(1):66-70.
68. Lyon F. IARC monographs on the evaluation of carcinogenic risks to humans. Some industrial chemicals. 1994;60:389-433.
69. Kurup VM, Thomas J. Edible vaccines: promises and challenges. Molecular biotechnology. 2020;62(2):79-90.
70. Ma Y, Lee CJ, Park JS. Strategies for Optimizing the Production of Proteins and Peptides with Multiple Disulfide Bonds. Antibiotics. 2020;9(9).
71. Zendehbad SH, Mehran MJ, Malla S. Flavonoids and phenolic content in wheat grass plant (*Triticum aestivum*. Asian J Pharm Clin Res. 2014;7(4):184-7.
72. Yao J, Weng Y, Dickey A, Wang KY. Plants as factories for human pharmaceuticals: applications and challenges. International journal of molecular sciences. 2015;16(12):28549-65.
73. Stern AM, Markel H. The history of vaccines and immunization: familiar patterns, new challenges. Health affairs. 2005;24(3):611-21.
74. C-y Y, S-y C, G-c D. Transgenic peanut (*Arachis hypogaea* L.) expressing the urease subunit B gene of Helicobacter pylori. Current microbiology. 2011;63(4):387-91.
75. Schillberg S, Fischer R, Emans N. Molecular farming of recombinant antibodies in plants. Cellular and Molecular Life Sciences CMLS. 2003;60(3):433-45.
76. Giddings G, Allison G, Brooks D, Carter A. Transgenic plants as factories for biopharmaceuticals. Nature biotechnology. 2000;18(11):1151-5.
77. Kalbina I, Engstrand L andersson S, Strid Å. Expression of Helicobacter pylori TonB protein in transgenic Arabidopsis thaliana: toward production of vaccine antigens in plants. Helicobacter. 2010;15(5):430-7.

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