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EXPRESSION OF RECOMBINANT HUMAN SERUM ALBUMIN IN *NICOTIANA TABACUM*

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ABSTRACT

Human serum albumin (HSA) is the most abundant protein in human blood plasma and also the most widely used protein by the pharmaceutical industry. Global demand for recombinant human serum albumin (rHSA) is increasing day by day due to the potential risks associated with plasma derived HSA and the limited availability of natural source. Though different expression systems are available for the production of rHSA, they all come up with a high price tag due to high cost of capitalization and production. Transgenic plants offer promising alternative for large scale, sustainable production of safe, functional, recombinant proteins of therapeutic and industrial importance. Therefore, as a preliminary step to produce human serum albumin in transgenic tobacco plants, HSA cDNA has been inserted into a plant expression vector pCAMBIA 1301 under the control of CaMV 35S promoter. The newly constructed vector pSBS.HSA was used to express HSA protein in *Nicotiana tabacum* using *Agrobacterium* mediated genetic transformation. Molecular confirmation of transformation was done through PCR analysis. Nucleotide sequencing was done to ensure that the 1.8 kb PCR fragment amplifying from transgenic tobacco is HSA itself. RT-PCR and SDS-PAGE confirmed that HSA is getting expressed in transgenic tobacco plants. Transgenic plants expressed recombinant HSA protein with a molecular mass of ~66 kDa. Our results demonstrate feasibility for efficient expression of human serum albumin in transgenic tobacco plants using the newly constructed vector pSBS.HSA.

Key words: Recombinant HSA. *Nicotiana tabacum*. *Agrobacterium*. Binary vector.

INTRODUCTION

Human serum albumin (HSA) is the most abundant protein in blood plasma with one litre of plasma containing about 60% HSA. There is about 42 ± 3.5 g of HSA per litre of human blood (1). It consists of a single non-glycosylated polypeptide chain of 585 amino acids forming a heart-shaped molecule with molecular weight of 66.5 kDa (2) and comprising three domains (3). Produced in the liver, HSA is largely responsible for maintaining normal osmolarity in the bloodstream but also functions as a carrier for numerous endogenous and exogenous compounds such as fatty acids, hormones, toxic metabolites, bile acids, amino acids, drugs, and metals (4). In addition to the osmotic function, HSA provides a high-capacity reservoir to stabilize the concentration of free ligands (5). HSA also functions as a major antioxidant in human body (6). Clinically, it is being used as a plasma expander. The administration of HSA is generally considered to be the gold standard for treating severe hypo-albuminemia and hemorrhagic shock (7-8). It is also used as a medical tool for the delivery of many biological products (9). Currently HSA is used in greater volumes than any other biopharmaceutical solution, with worldwide annual demand of ~500 tons (10).

Since 1940, human blood serves as the major source for HSA (11), but there are problems associated with the purity of HSA obtained from human blood plasma. There is an increasing public health concern with plasma-derived HSA (pHSA) with its potential risk for transmission of blood-derived infectious pathogens and viruses like Human Immunodeficiency Virus (HIV), Hepatitis C Virus (HCV), Hepatitis A Virus (HAV), variant Creutzfeldt-Jakob disease (vCJD) and West Nile virus (12). To eliminate the potential risk of viral contamination, regulatory agencies have encouraged pharmaceutical companies to use non–animal-derived sources for pharmaceutical production. Various expression systems have been used to produce rHSA, including Bacteria (13-14), yeast (15-17), transgenic animals (18-19), and transgenic plants (20-21). The development of plant based expression system is proving to be an efficient and economical method for the production of recombinant HSA (rHSA) as an alternative to pHSA.

Molecular farming is the production of pharmaceutically important and commercially valuable proteins in plants (22-23). Protein production in plants provides a number of advantages not found in other production platforms. A major advantage that all plant protein production systems have over cell culture systems (including bacteria, yeast, and mammalian cell culture) is the potential for significant reduction in cost production. It is estimated that

recombinant proteins can be produced in plants at 2–10% of the cost of microbial fermentation systems and at 0.1% of the cost of mammalian cell cultures, although this depends on the product yield (24). Plant-produced proteins are not susceptible to viral or prion contamination that can harm humans, as is always a concern with animal cell culture (25). Eukaryotic plants possess the chaperones and cellular machinery required to fold complex human proteins that bacteria and yeast may not be able to process properly (26). Most of the plant species are considered GRAS (generally regarded as safe) (27), meaning that if the protein can be expressed in a bioavailable form; purification steps could potentially be eliminated altogether. Plants have now established as versatile expression systems for many forms of different recombinant proteins.

Nicotiana tabacum for decades have served as a model organism in plant biology. It has made its scientific reputation due to the ease of gene transformation and genetic manipulation (28). The continuing popularity of tobacco reflects its status as a well established expression host for which robust transformation procedures and well-characterized regulatory elements for the control of transgene expression are available (29). Furthermore, its high biomass yields and rapid scalability make tobacco very suitable for commercial molecular farming. It is also a non-food, non feed crop, and so carries a reduced risk of transgenic material or recombinant proteins contaminating feed and human food chains (30).

One important aspect in the development of a transformation system has been the choice of promoter to drive expression of the transgene. One of the most widely used promoters in plant molecular biology is the CaMV 35S promoter. It drives strong and constitutive expression in most dicotyledonous and some monocotyledonous plants (31). The 35S promoter effectively puts its downstream gene outside virtually any regulatory control by the host genome and expresses the gene at approximately two to three orders of magnitude higher, thus allowing a strong positive selection. A number of plant transformation vectors, including the pCAMBIA series use the 35S promoter in their selectable marker genes (32-33). The pCAMBIA vectors have been widely used as carriers of genes-of-interest or for high throughput analysis (34-35).

In the present study, attempts were made to express rHSA in tobacco plant through *Agrobacterium* mediated genetic transformation. A new vector was constructed using pCAMBIA 1301, in which the gene of interest HSA is placed under the control of CaMV 35S promoter for a strong and constitutive expression.

MATERIALS AND METHODS

Plant material

Nicotiana tabacum L. Petite Havana strain (common tobacco) was used for the present study. Tobacco leaves used for the transformation experiments were obtained from *in vitro* grown plants. Media used was MS basal media (36) with different hormone and antibiotic concentrations. To raise *in vitro* plants, tobacco seeds were surface sterilized with 0.1% mercuric chloride and placed on sterile blotting sheet pre-wetted with MS solution. After two weeks, plantlets emerged directly from the seeds. Plantlets were removed and placed on to MS medium without any plant growth regulators for plant development. Co-cultivation medium was MS supplemented with 1.0 mg/l BA and 0.1mg /l NAA. Selection medium was MS supplemented with 1.0 mg/l BA and 0.1 mg /l NAA with 40 mg/l hygromycin and 500 mg/l cefotaxime.

Construction of the plant expression vector

The binary vector pLGMR.HSA harbouring HSA cDNA was kindly provided by Dr. E. Sunderasan, Research Officer, Biotechnology Unit, Rubber Research Institute of Malaysia (37). The gene of interest human serum albumin was excised from pLGMR.HSA vector and inserted in pCAMBIA 1301 T-DNA vector (M/S CAMBIA, Canberra, Australia) manipulating *Spe*I/*Nhe*I restriction sites. HSA cDNA was PCR amplified from pLGMR.HSA plasmid using Platinum® Pfx DNA Polymerase kit (M/S Invitrogen, USA) (F: 5'- GAC TAG TCA TGA AGT GGG TAA CCT TT -3', R: 5'-CTA GCT AGC TAA GCC TAA GGC AGC -3') (M/S Sigma, USA). The PCR amplified product and binary vector were subjected to double digestion using *Spe*I and *Nhe*I (M/S NEB, England). The digestion products were ligated using T4-DNA ligase (M/S NEB, England) and transformed to chemically competent *E. coli* (DH5α) cells. Colony PCR was used for the initial screening of transformants using HSA specific primers (HSA-F: 5'-TGA AGT GGG TAA CCT TTA TTT CC-3'and HSA-R: 5'-TAT AAG CCT AAG GCA GCT TGA C-3'). The PCR cocktail comprised of 100 μM dNTPs, 250 nM of each primer, 1.5 mM $MgCl_2$ and 0.5 U *Taq* DNA polymerase (M/S Bangalore Genei, India) with colony as the template. The PCR profile was: 10 minutes of denaturation at 94^{o}C for the degradation of cell wall and inactivation of nucleases followed by 36 cycles of amplification at 94^{o}C for 30 sec, 55^{o}C for 1 min, 72^{o}C for 2 min and final extension for 10 min at 72^{o}C. Plasmids were isolated and sequenced at M/S Scigenom, India, to check the orientation as well as fusion of HSA with reference to pCAMBIA 1301. Sequencing has been

carried out using a forward primer designed from the 3' region of HSA insert (HSA SF: 5'-CCT TGG TGA ACA GGC GAC CAT GC -3').

Transformation of *Agrobacterium tumefaciens* with the binary vector pSBS.HSA

Agrobacterium strains used for the present study was EHA105 (A *vir* helper, L,L-succinamopine type, harbors T-DNA deletion derivative of pTiBo542, a supervirulent type Ti plasmid, Rm^r)(38). The binary vector pSBS.HSA was transformed to EHA105 through freeze-thaw method (39). White colonies observed were screened through colony PCR to confirm the presence of binary vector pSBS.HSA using HSA specific primers mentioned earlier and *hpt*II specific primers (*hpt*-F: 5` CGA TTG CGT CGC ATC GAC 3`and *hpt*-R: 5`-CGT GCA CAG GGT GTC ACG-3`).

Genetic transformation of *Nicotiana tabacum*

The *Agrobacterium* strain EHA 105 transformed with the plasmid vector pSBS.HSA was used for the transformation of tobacco plants. Transformation was carried out using 4-5 week old *in vitro* grown tobacco plants following leaf disc transformation method (40). Leaf discs were placed on MS medium in the light for 2 days for pre-culture and then immersed in culture of *A. tumefaciens* for 10 min at 28°C with gentle shaking. Explants were removed from culture and co-cultivated with *Agrobacterium* for 2 days in dark on MS agar medium. After co-cultivation, leaf explants were incubated in MS medium containing 40 mg/l hygromycin (selection antibiotic) and 500 mg/l cefotaxime (to prevent bacterial overgrowth) at 22°C for selection. The developing shoots were excised and placed on fresh regeneration and selection medium for further enlargement of shoots. The shoots were then transferred to ½ MS medium without plant hormones for development of roots. Rooted transgenic plants were transferred to plastic cups containing soil and sand (1:1).

PCR screening of regenerated transgenic tobacco plants

The presence of HSA gene in the hygromycin resistant putative transgenic tobacco plants were confirmed by PCR. Genomic DNA was isolated from young leaves of tobacco plants (41) and used as template for PCR using primers specific to HSA and *hpt*II. Those plants with expected PCR products were used for further studies.

Cloning and sequencing of PCR amplified HSA fragment

To confirm that the 1.8 kb product amplifying from transgenic plant is HSA itself, the fragment was cloned and sequenced. Electrophoresis of PCR amplified products were carried

out in 1.0% agarose gel and the DNA bands were purified. PCR products were cloned using the pGEM®-T Easy vector system for sequencing (M/S Promega, USA), following the manufacturer's instructions. Transformants were primarily screened based on blue white screening and then by colony PCR of white colonies using HSA specific primers. PCR products were electrophoresed in 1.0% agarose gel. Colonies giving right amplification of insert DNA were selected and plasmid DNA was isolated from the recombinant white colonies. Plasmid sequencing was done at M/S Scigenom, India using M13 forward and M13 reverse primers. The nucleotide sequence analysis was carried out using bioinformatics tools like 'Blast N' programme (42) and 'clustal W' (43).

RNA isolation and RT-PCR

The presence of HSA transcripts in putatively transgenic tobacco plants was analyzed through RT-PCR. Total RNA was isolated from the leaves of transgenic as well as non-transgenic tobacco plants through LiCl precipitation method (44). All the reagents and utensils used for RNA isolation were treated with 0.1% DEPC to remove RNase contamination (45). The quality of the isolated RNA was checked on 0.8% agarose gel. The quantity of RNA obtained was determined spectrophotometrically. To remove contaminating genomic DNA, the RNA preparation (1µg) was subsequently incubated with RNase-free DNase (2 U/µg) (M/S NEB, England) for 15 min at 37°C. cDNA synthesis was carried out using ImpromII™ reverse transcriptase kit for cDNA synthesis (M/S Promega, USA). First strand cDNA synthesis was performed by reverse transcription with 1µg of total RNA using oligo-(dT) 12-18-mer primer and ImpromII RT enzyme. The total cDNA was used as template for the PCR amplification using HSA specific primers. The PCR temperature profile and components were the same as mentioned earlier, but the number of cycles was increased to 40. The amplified products were analyzed on 1% agarose gel.

Protein Isolation and SDS-PAGE

Total protein was isolated from the transgenic plants following phenol extraction method (46). Phenol treated protein in the sample was precipitated using 100 mM ammonium acetate in cold methanol. The precipitated protein was pelleted, washed with acetone and air dried. The air dried pellet was dissolved in solubilization buffer. The isolated protein was subjected to SDS PAGE on 10% acrylamide gel using the method described by Sambrook et al. (45).

RESULTS

Plant material

To raise *in vitro* plants, tobacco seeds were placed on sterile blotting sheet pre-wetted with MS solution. After two weeks, plantlets emerged directly from the seeds. Plantlets were removed and placed on to MS medium without any plant growth regulators. The leaves from fully developed plants were used for *Agrobacterium* mediated genetic transformation (Figure 1a & 1b).

Vector construction

HSA cDNA was cloned from binary vector pLGMR.HSA into pCAMBIA 1301 T-DNA vector after removing GUS reporter gene using restriction enzymes *Spe*I and *Nhe*I. HSA cDNA fragment was amplified with Pfx DNA polymerase enzyme at annealing temperature 55°C using the vector pLGMR.HSA as template. Sharp single band amplification of 1.8 kb was observed on 1% agarose gel (Figure 2). The amplified fragment and the vector were digested with *Spe*I and *Nhe*I restriction enzymes. Ligation of HSA cDNA and pCAMBIA 1301 plasmid was carried out so that the HSA cDNA fragment got inserted in place of the GUS reporter gene. The ligation mix was used for the transformation of an aliquot of chemically competent *E. coli* (DH5α) cells. Transformed colonies obtained on LB selection plate were selected through colony PCR amplification of 1.8 kb amplicon of HSA gene using specific primers.

Plasmids were isolated from the positive colonies and sequenced to check the orientation of the inserted HSA fragment and also to verify its fusion to the T-DNA region of pCAMBIA 1301. Up on sequencing it was confirmed that the HSA gene was successfully ligated to the binary vector pCAMBIA 1301 in right orientation and the cloning strategy was successful. The newly constructed plasmid vector was designated pSBS.HSA (Figure 3). The newly constructed plasmid vector pSBS.HSA was introduced into competent *Agrobacterium* strains by freeze thaw method. Transformed colonies obtained on the LB selection plate were screened by colony PCR using primers specific to HSA and *hpt*II (Figure 4). Positive colonies were selected for further transformation studies.

Genetic transformation of *Nicotiana tabacum*

The construct pSBS.HSA was introduced into tobacco through *Agrobacterium* mediated transformation of leaf discs. Co-cultivated leaf discs were selected on MS plates with 40 mg/l hygromycin and 500 mg/l cefotaxime. After 10-15 days, transgenic shoots were emerged

from the putatively transformed tissues. The non-transformed tissue got gradually bleached and finally turned dark. The different stages in the development of transgenic tobacco plants are shown in Figure 5. Shoots regenerated were subcultured and rooting was obtained on transfer in to ½ MS rooting medium. The rooted transgenic plants were transferred to soil and sand mixture (1:1) in paper cups (Figure 6).

Hygromycin resistant transgenic tobacco plants were screened by PCR. When PCR was carried out using primers specific for HSA gene sequence, a single band of 1.8 kb was amplified from the genomic DNA isolated from all the transgenic plants, but amplification was absent from DNA of non transgenic control plants (Figure 7). This confirms that HSA transgene is integrated to tobacco plants. For *hpt*II gene, a single band of 610 bp was amplified from transgenic plants (Figure 8). Amplification was not observed from DNA of non transformed tobacco tissues. Thus it was made sure that the plants growing on hygromycin containing medium harbour *hpt*II gene.

Cloning and sequencing of PCR amplified HSA fragment

For cloning, the PCR amplified 1.8 kb HSA band from the above PCR was excised from the gel and eluted using phenol-chloroform method. The purified PCR fragment was ligated in to pGEMT cloning vector. Putatively transformed colonies were observed on selection plate (50 mg/l ampicillin with X-gal) when 100 µl transformation mixture was plated. Blue/white screening was used for the initial screening of transformation. Five white colonies and one blue colony were randomly selected and subjected to colony PCR for further confirmation. Fragment of ~1.8 kb size was amplified when PCR was carried out with HSA specific primers, from all the white colonies selected, confirming the presence of the insert (Figure 9). No amplification was observed with the blue colony indicating that the amplification observed with the white colonies were due to the presence of inserts. Plasmids were isolated from positive colonies and electrophoresis was carried out in 1% agarose gel along with control plasmid without insert and found that the control plasmid moved ahead of the putatively recombinant plasmids. This result further confirmed the cloning event (Figure 10). Two plasmid samples were subjected to DNA sequencing. Sequencing reactions were carried with M13 forward and M13 reverse primers. Nucleotide sequence of the insert was deduced from sequence data obtained after forward and reverse sequencing reactions and was analyzed and confirmed as HSA sequence by comparing with the reported cDNA sequence

(NCBI Reference sequence: NM_000477.5) through BLAST and ClustalW analyses. It was confirmed that the 1.8 kb fragment amplifying from transgenic tobacco plant is HSA itself.

HSA expression analysis through RT-PCR

In order to check the presence of HSA gene transcripts in transgenic plants, RT-PCR was carried out. RNA isolated from transgenic as well as non-transgenic plants were without degradation (Figure 11). DNA contamination from the RNA was removed by DNase treatment. First strand cDNAs were synthesized using oligo-(dT) primers from the isolated RNA using the Improm-II^{TM} Reverse Transcription System. The total cDNA was subsequently used as template for the selective amplification of HSA cDNA using specific primers. A sharp single band amplicon of ~1.8 kb was observed from the cDNA of transgenic plants on electrophoresis in 1.0 % agarose gel (Figure 12). When cDNA from non-transgenic plant was used as template, no amplification was observed. This indicates that the HSA gene is getting expressed in transgenic tobacco plants.

Protein isolation and SDS-PAGE

Total protein was isolated from transgenic tobacco plants following phenol extraction method. Isolated protein was subjected to SDS-PAGE to check the integrity. The protein samples on 10% acrylamide gel showed good banding pattern without any degradation. Transgenic lines showed the presence of 66 kDa rHSA band, whereas that band was absent in the untransformed control line (Figure 13). This confirms the synthesis of HSA protein in transgenic tobacco plants.

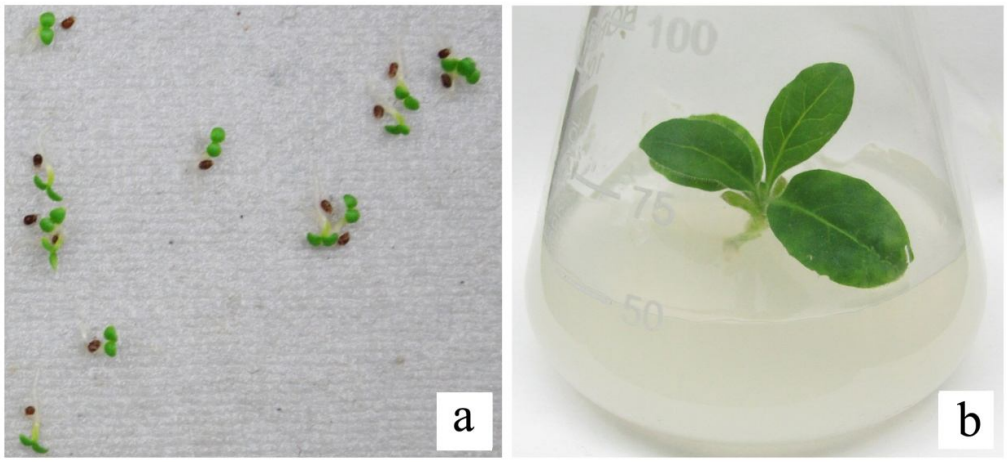

Figure 1 Tobacco plant development. a Tobacco seeds germinating in blotting sheet pre-wetted with MS solution. b Fully developed tobacco plant used for genetic transformation in MS-medium.

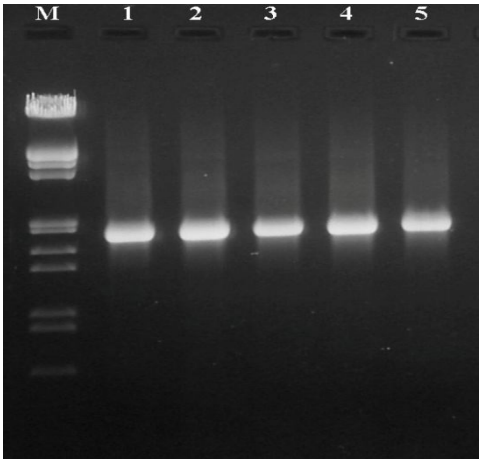

Figure 2 PCR amplification of HSA cDNA. *M*- λ marker-*Eco*RI *Hind*III double digest. *Lane 1-5* 1.8 kb amplicon of HSA cDNA from pLGMR.HSA plasmid

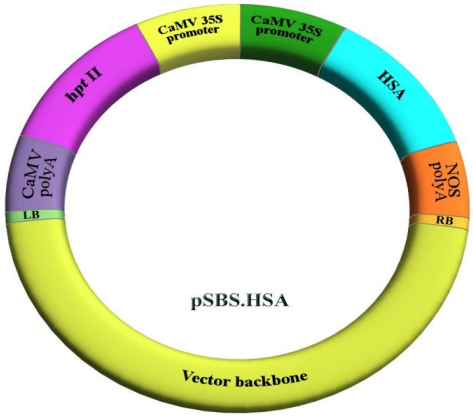

Figure 3 Schematic representation of the newly constructed binary vector pSBS.HSA.

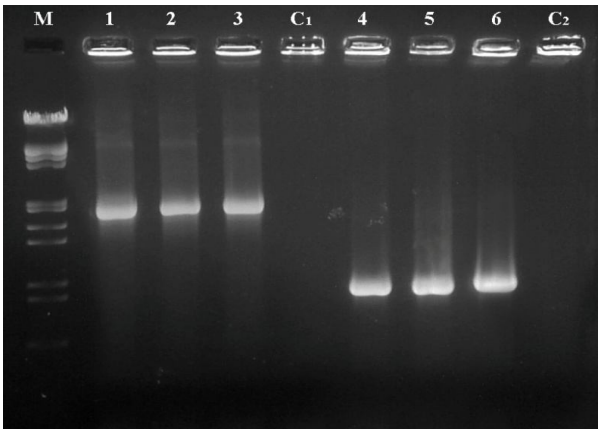

Figure 4: Colony PCR of *Agrobacterium*. *M*- λ marker-*Eco*RI *Hind*III double digest. *Lane 1-3* Recombinant colonies showing 1.8 kb amplicon of HSA. *C1*- control. *Lane 4-6* Recombinant colonies showing 610 bp amplicon of *hpt*II. *C2*- control.

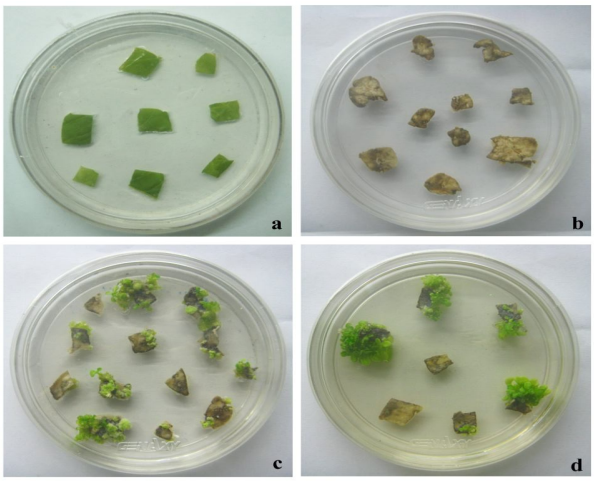

Figure 5 Different stages in the development of transgenic tobacco plantlets from leaf discs. a leaf discs kept for selection. b bleaching of tissues. c&d emergence transgenic plantlets and expiration of non-transformants.

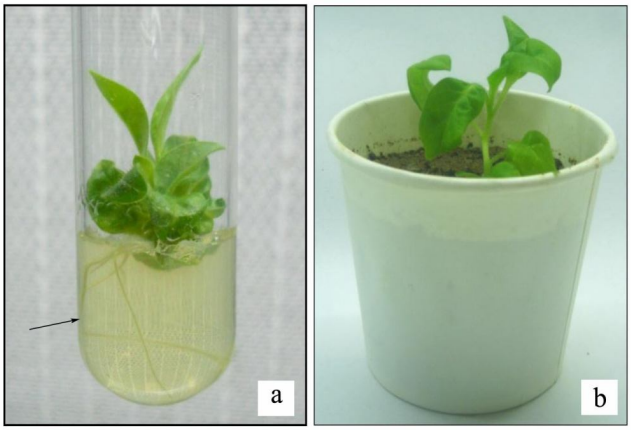

Figure 6 Transgenic tobacco plants. a transgenic tobacco plant showing rooting in ½ MS medium. b rooted transgenic tobacco plant in paper cup.

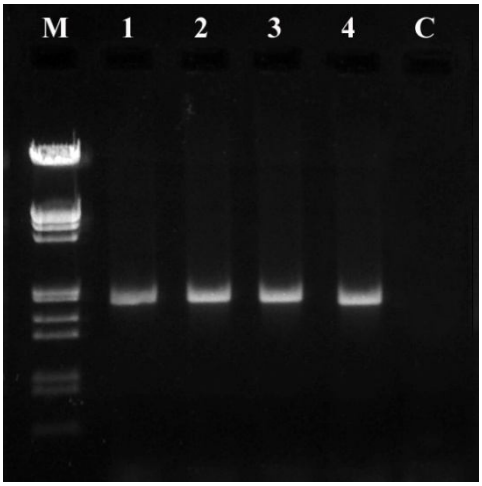

Figure 7 PCR amplification of HSA gene from transgenic tobacco plants. *M*- λ marker-*Eco*RI *Hind*III double digest. *Lane 1-4* 1.8 kb amplicon of HSA gene from transgenic tobacco plants. *C*- control.

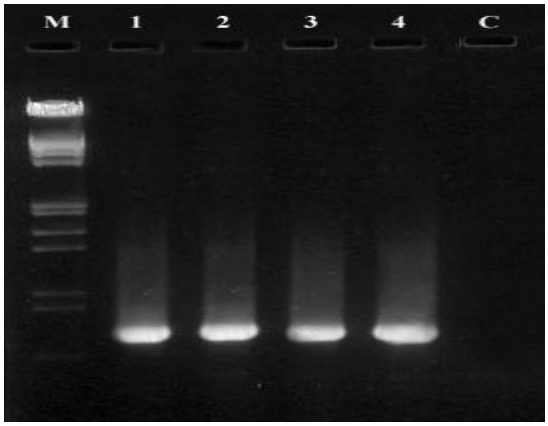

Figure 8 PCR amplification of *hpt*II gene from transgenic tobacco plants. *M*- λ marker-*Eco*RI *Hind*III double digest. *Lane 1-4* 610 bp amplicon of *hpt*II gene from transgenic tobacco plants. *C*- control.

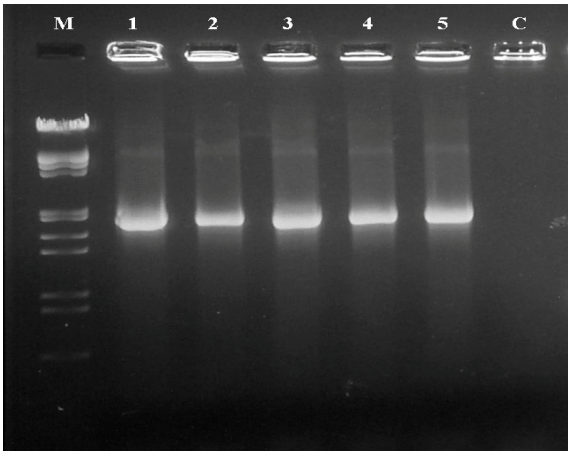

Figure 9 Colony PCR of transformed *E. coli* (DH5α). *M*- λ marker-*Eco*RI *Hind*III double digest. *Lane 1-5* PCR amplified 1.8 kb fragment of HSA gene from recombinant white colonies. *C*- control (blue colony).

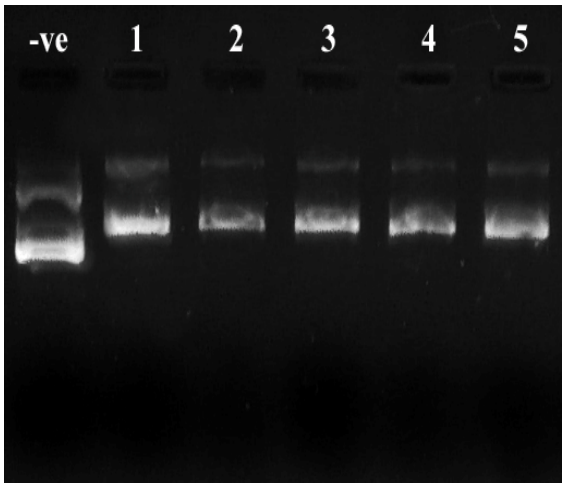

Figure 10 Plasmid isolation from recombinant colonies. *-ve* – Plasmid without insert. *Lane 1-5* recombinant plasmids with HSA insert

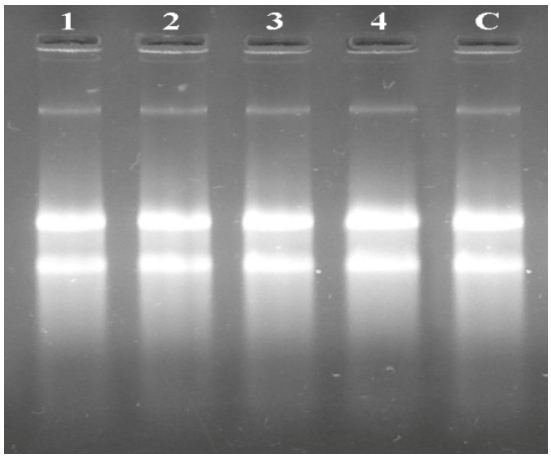

Figure 11 RNA isolated from tobacco plants. *Lane 1-3* RNA isolated from transgenic lines. *C*- Control

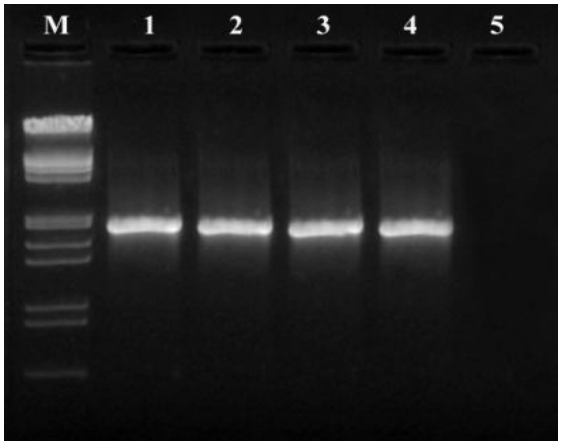

Figure 12 HSA expression analysis through RT-PCR. *M*- λ marker-*Eco*RI *Hind*III double digest. *Lane 1-4* RT-PCR amplified 1.8 kb fragment of HSA cDNA from transgenic plants. *Lane 5*- control.

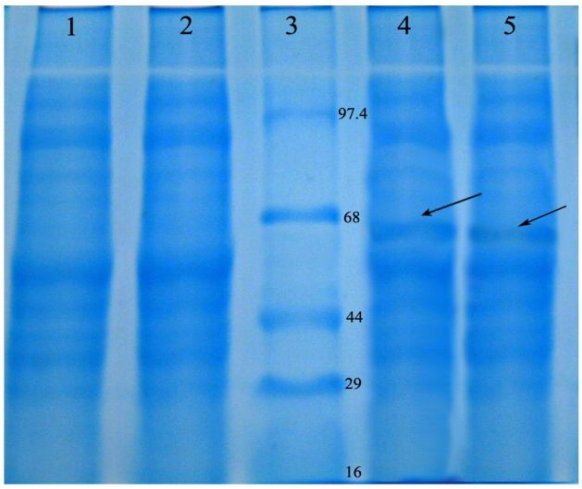

Figure 13 Total protein isolated from tobacco plants on SDS-PAGE. *Lane 1-2* Untransformed. *Lane 3* Protein molecular weight marker. *Lane 4-5* Transformed cells showing HSA expression.

DISCUSSION

Transgenic plants have emerged as a promising technology for the production of recombinant biopharmaceutical proteins. They offer a wide variety of advantages, and so their potential can be used as bioreactors for the production of therapeutic molecules.

In the present study, we generated transgenic tobacco plants expressing HSA protein through *Agrobacterium* mediated genetic transformation. Tobacco plants were selected as the experimental material as they were highly amenable to transformation studies. As a model plant, tobacco benefits from well-established gene transfer and regeneration methodologies. Majority of the discoveries in the field of plant cell, tissue culture have originated from the experimentation with tobacco plants (47). The co-cultivation of leaf discs with *Agrobacterium* can produce tobacco transformants with high quality and fertility. *Nicotiana tabacum* has served as the bioreactor for the production of wide variety of therapeutically important proteins (48-50). Studies have shown that rHSA can be produced in leaves of transgenic tobacco and potato and tobacco suspension cells (20). However, expression levels were, too low for most commercial applications being 0.02% of the total soluble protein.

One of the major obstacles to recombinant protein production in plants is the low level of protein expression. Different strategies - use of novel promoters, codon optimization, improvement of protein stability, targeting of recombinant proteins to intracellular compartments, and improvement of downstream processing technologies - have been used to improve production (51).

In the present study, we have developed a new vector pSBS.HSA which possess gene of interest HSA at T-DNA right border and *hpt*II gene as the selectable marker near left border both driven by CaMV 35S promoter. In dicot plants, CaMV35S is a suitable promoter because it is strong, constitutive and can cause high level of transgenic expression in leaves, fruits, tubers, roots and other organs (52). In a previous study, pBIN 19 vector was modified to create a new vector pBINPLUS with plant selectable marker located near the T-DNA's left border, a feature that ensures complete integration of the T-DNA sequences into the plant genome (53). The binary vector pART27 was designed by Gleave so as to have the *npt*II gene close to the left border to ensure that the selection marker is the last to be transferred into plants. This strategy ensures that all plants selected on kanamycin will have a complete T-DNA inclusive of the gene of interest (54). The vector pSBS.HSA was successfully transformed to tobacco plants and rHSA was expressed.

Presence of T-DNA region in transgenic plant was confirmed through molecular analysis. Hygromycin resistant transgenic tobacco plants were screened by PCR. When PCR was carried out using primers specific for HSA gene sequence, a single band of 1.8 kb was amplified from the genomic DNA isolated from all the transgenic plants and for *hpt*II gene a single band of 610 bp was amplified. It was confirmed that the 1.8 kb fragment amplifying from the transgenic tobacco plant is HSA itself by cloning and sequencing. Thus it was ensured that the T-DNA region has integrated into the genome of transgenic tobacco. RNA and protein analyses have demonstrated the expression of rHSA mRNA and protein in transgenic tobacco.

In conclusion, we have shown that rHSA can successfully be expressed in tobacco using the newly constructed vector pSBS.HSA. We anticipate that rHSA levels could be further increased by optimized cultivation conditions. The newly developed pSBS.HSA vector should result in higher productivity and increased applications of plant cultured cells for the production of high-value recombinant proteins.

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