IMAGE CLASSIFICATION THROUGH NEURO EVOLUTION ALGORITHM

Preeti Lata Sahu[1], Ms. Aradhana Singh[2], Mr.Khomlal Sinha[3]

[1]M.Tech.Scholar, [2]Assistant Professor, [3]Sr. Assistant Professor in, Dept.of Computer Science & Engg. CSIT, Durg, Chhattisgarh (India)

ABSTRACT

During these decades, with the advancement of internet and the availability of picture catching gadgets, the span of digital image accumulation is expanding quickly. The bulk of data kept in computer files and databases is increasing at challenging rate; improved image classification technologies have become mandatory. Neuro- evolution classification technique is one of the process that can be use to improve the classification effectiveness. The inputs to the Neuro-evolution classification system were classified by extracting features of each image. The proposed method utilized the extracted features of images to classify it into different classes. Our objective is to analyze the proposed method and assessed the performance of these classification methods in terms of different performance measures such as accuracy, precision and computational speed.

Keywords: Computational Speed; Accuracy; Image Classification; Neuro-Evolution Classification Technique; Precision.

I. INTRODUCTION

As more and more images are captured in electronic form the need for programs which can find objects of interest in a database of images is increasing. For example, it may be necessary to find all JPEG images in a database. The classical approach to problems of this kind is to examine the objects of interest and determine a set of features, such as entropy, mean, standard deviation, skewness and correlation of image, and format, compression, file size, color type and format signature of file and write programs to extract the features. Learning approaches under investigation include neural network and differential evolution algorithms.

Data mining is a powerful new technology with great potential to help companies focus on the most important information in their data warehouses. Data mining tools predict future trends and behaviors, allowing businesses to make proactive, knowledge-driven decisions. The automated, probable analyses offered by data mining move beyond the analyses of past events provided by nostalgic tools typical of decision support systems. It can answer business questions that traditionally were too time consuming to resolve. Image mining is a tool and a methodology for data mining in picture-archiving systems. It is an interdisciplinary field that handles the mining of information, image data association or additional patterns not unambiguously stored in the images. It utilizes methods from computer vision, image processing, image retrieval, data mining, machine learning, database and artificial intelligence.

We proposed a Neuro-evolution classification technique for data mining. The inputs to the Neuro-evolution classification system were classified by extracting features of each image. The proposed method utilized the extracted features of images to classify it into different classes. Our objective is to analyze the proposed method and assessed the performance of these classification methods in terms of different performance measures such as accuracy, precision and computational speed.

II. LITERATURE REVIEW

There have been a large number of studies which have examined the impact of neural network and evolution algorithm on image classification performance. Kharat, Kailash (2012) concluded two approaches for Brain Tumor Detection based on artificial neural networks. The networks were categorized into feed-forward neural networks and Back propagation neural Network. The purpose is to develop tools for discriminating malignant tumors from benign ones assisting decision making in clinical diagnosis.

C.Bhuvaneswar (2014) stated a novel fusion based feature extraction and feature selection is done by genetic algorithm that selects the top ranked features and classification is done through J48, KNN, MLP NN classifiers to classify the lung CT dataset. The algorithm has been designed based on the concept of texture and pixel co-efficient features. This method effectively works well for the detection of lung diseases with high sensitivity, specificity and accuracy. Results show that MLP NN classifier with median absolute deviation techniques and genetic algorithm for feature selection yields better results.

Shi. Yujing (2012) used texture features and the method of improved multi-class support vector classifier to classify images, effectively avoiding the partial bias caused by match fails, and offset the lack of color feature extraction. This paper combined GLCM texture feature extraction methods, used Minimal Hyper-Sphere Contains methods to classify 400 images of Corel image database and tested in public data set UCI, proved its feasibility. This plays an important role in the classification and retrieval of education image resource in the future.

III METHODOLOGY

As more and more images are captured in electronic form the need for programs which can find objects of interest in a database of images is increasing. For example, it may be necessary to find all JPEG images in a database. The classical approach to problems of this kind is to examine the objects of interest and determine a set of features, such as entropy, mean, standard deviation, skewness and correlation of image, and format, compression, file size, colour type and format signature of file and write programs to extract the features. Learning approaches under investigation include neural network and differential evolution algorithms.

We proposed a Neuro-evolution classification technique for data mining. The inputs to the Neuro-evolution classification system were classified by extracting features of each image. The proposed method utilized the extracted features of images to classify it into different classes. Our objective is to analyze the proposed method and assessed the performance of these classification methods in terms of different performance measures such as accuracy, precision and computational speed.

3.1 Neural Network

The first proposal to use an artificial neuron appeared in 1943, but computer usage of neural networks did not actually until the 1980s. Neural Networks (NN), often referred to as artificial neural network (ANN) working of the human brain. Neural network is information processing systems that consist of a graph representing the processing system as well as various algorithms that access that graph. As with human brain, the NN consist of many connected processing elements. The NN, then, is structured as a directed graph with many node (processing elements) and arcs (interconnections) between them. The nodes in the graph are like individual neurons, while the arcs are their interconnections. Each of these processing elements functions independently from the others and uses only local data (input and output to the node) to direct its processing. This feature facilitates the use of NNs in a distributed and/or parallel environment.

The NN can be viewed as a directed graph with source (input), sink (output) and internal (hidden) nodes. The input node exist in a input layer, while the output node exist in an output layer. The hidden nodes exist over one or more hidden layers. To perform the image mining task, a tuple is input through the input nodes and the output node determines what the prediction is.

3.2 Differential Evolution

Differential Evolution (DE) is a method that optimizes a problem by iteratively trying to improve a candidate solution with regard to a given measure of quality. Such methods are commonly known as metaheuristics as they make few or no assumptions about the problem being optimized and can search very large spaces of candidate solutions.

DE optimizes a problem by maintaining a population of candidate solutions and creating new candidate solutions by combining existing ones according to its simple formulae, and then keeping whichever candidate solution has the best score or fitness on the optimization problem at hand. In this way the optimization problem is treated as a black box that merely provides a measure of quality given a candidate solution and the gradient is therefore not needed.

A basic variant of the DE algorithm works by having a population of candidate solutions (called agents). These agents are moved around in the search-space by using simple mathematical formulae to combine the positions of existing agents from the population. If the new position of an agent is an improvement it is accepted and forms part of the population, otherwise the new position is simply discarded. The process is repeated and by doing so it is hoped, but not guaranteed, that a satisfactory solution will eventually be discovered.

Differential evolution algorithm has four steps:

1) Initialization It define the upper and lower bound of each parameter.
2) MutationDifferential evolution optimizes a problem by maintaining a population of candidate solution. It expand the search space.
3) Recombination It create new candidate solution by combining existing ones.
4) SelectionCandidate solution which has the best score or fitness on the optimization problem is selected.

Neuro-evolution, or neuro-evolution, is a form of machine learning that uses evolutionary algorithms to train artificial neural networks. It is most commonly applied in artificial life, computer games, and evolutionary robotics. A main benefit is that neuro-evolution can be applied more widely than supervised learning algorithms, which require a syllabus of correct input-output pairs. In contrast, neuro-evolution requires only a measure of a

network's performance at a task. For example, the outcome of a game (i.e. whether one player won or lost) can be easily measured without providing labeled examples of desired strategies.

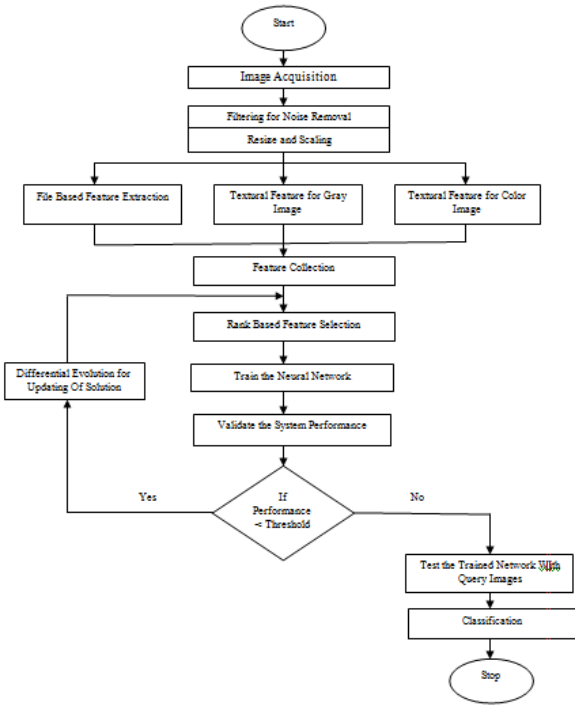


Fig.1. Flow Diagram of Proposed Methodology

IV EXPECTED RESULT

The image classification has been implemented with the help of two algorithm applied successively: Artificial Neural Network and Differential Evolution Algorithm. This section present results and the parameters influences on these results.

V. CONCLUSION

As a conclusion, we have taken on our objective which is to analyse the proposed Neuro-Evolution classifier based on different performance measures like accuracy, precision and computational speed. These classifiers are tested with n data sets. The results suggest that the neuro-evolution classifiers studied and analyzed, the proposed neuro-evolution classifier has the potential to significantly improve the conventional classification methods for use in Data Mining research field. These classification methods, described here are powerful and effective.

REFERENCES

[1] C. Bhuvaneswari," A new fusion model for classification of the lung diseases using genetic algorithm", 1110-8665 _ 2014 Production and hosting by Elsevier B.V. on behalf of Faculty of Computers and Information, Cairo University (2014).

[2] S.Haykin, " Neural networks: a comprehensive foundation". 2nd ed. Prentice Hall; p. 253–77 (1998).

[3] Kharat and D.Kailash. "Brain Tumor Classification Using Neural Network Based Methods", International Journal of Computer Science and Informatics ISSN (PRINT): 2231 –5292, Vol-1, Iss-4 (2012).

[4] Shi. Yujinga, " Image Classification of Education Resources Based of Texture Features", International Workshop on Information and Electronics Engineering (IWIEE) (2012).

[5] Shweta Kharya,"Using Data Mining Techniques for Diagnosis And Prognosis of Cancer Disease", international Journal of Computer Science, Engineering and Information Technology (IJCSEIT), Vol.2, No.2 (2012).

[6] Saima Anwar Lashari, "Performance Comparison of Musical Instrument Family Classification Using Soft Set", International Journal of Artificial Intelligence and Expert Systems (IJAE), Vol.3, (2012).

BIOGRAPHICAL NOTES

Preeti Lata Sahu is presently pursuing M. Tech. final year in Computer Science and Engineering Department from C.S.I.T , Durg ,Chhattisgarh, India.

Ms Aradhana Singh is working as a Assistant Professor in Computer Science and Engineering Department, C.S.I.T ,Durg , Chhattisgarh.

Mr Khomlal Sinha is working as a Sr. Assistant Professor in Computer Science and Engineering Department, C.S.I.T ,Durg , Chhattisgarh.

SURVEY OF REGION OF INTEREST AND ITS APPLICATIVE UTILITY IN VARIOUS AREAS OF MEDICAL FIELD

Rushabh R Shah[1], Rutvi R Shah[2], Dr.Priyanka Sharma[3]

[1,2] Research Scholar, GTU , Nirma University, (India)

[2]Guide Raksha Shakti University, (India)

ABSTRACT

Recently the medical field has started using images and storing them for future reference also for most of the disease. So there is an increase in taking number of images for different disease. Also for a single person more number of images are taken from different angles. So there are different issues related with taking images and storing them for later viewing or analysis. Also in that images there would be only certain region which will be of importance and the other region will be of no interest. So the concept of finding region of interest from the images and storing images such that the region of interest in images would not be compressed and other part in the image would be compressed. Due to this there will be saving in the storage space also. So in this paper we try to survey different medical field where this type of concept will be used and then try to compare from different paper how useful the above mentioned concept is.

Keywords: Image Processing, Medical field, Region of Interest (ROI), Video Processing

I INTRODUCTION

Medical field is nowadays using computer operated software / hardware for diagnosis of various disease or to analyse various parts of body. For that they are taking various images of the body parts in terms of X-rays or Ultra sonic etc. So image processing is gaining much importance in the field of medical. Doctors rely on the images captured by various devices and then view the image and according to the image taken give the diagnosis. Often after giving the diagnosis they store such images for either near future use or for records purpose. Now if they are going to store images of each and every patient that they are going to diagnose then at the end of the day it would result into bulkier storage and after certain months even GBs of hard disk would be full. Then they are left with either going for new storage option or otherwise store the images in such a way that they occupy less space or gaining freedom of storing more numbers then. Now if the images are stored in compressed form then there is chance of loss of vital information in the image which may lead to wrong diagnosis. So Region of interest is an important concept where the doctor can save space and at the same time store image without losing the vital information. So in this paper we will be analysing the areas where region of interest can be applied in the medical domain. The outcomes of this survey can be an aid to researchers in this field.

1.1 Different Areas where ROI technique can be applied in medical domain

a. Respiration rate (RR) is one of the important vital signs used for clinical monitoring of neonates in intensive care units. Detection of the chest-and-abdomen region of the neonate is crucial to determining the respiration rate accurately [1]. As the skin of neonates is very fragile, it is preferred to have monitoring systems with minimal contact. Hence, recently several methods of contact free monitoring of vital signs using video cameras have been proposed. Monitoring of vital signs aids in proper diagnosis and provides early indications of hidden clinical abnormalities. Neonatal Intensive Care Units (NICU) commonly monitors the respiration rate (RR), the heart-rate (HR), and the blood oxygen saturation (SPO2) of neonates [4]. Most monitoring systems acquire vital signs using electrodes or contact sensors positioned suitably at various points on the body. It is desirable to have monitoring systems for neonates that have minimal or no contact with the neonate since the electrodes/sensors may often cause discomfort to the neonate in the form of shearing of the skin, scars, irritation, and etc. [5]. Due to this reason, contact free monitoring of vital signs has recently been achieved by means of a video camera positioned suitably in the vicinity of the baby [6], [7]. Here we can identify the ROI, which for this purpose can be Chest-and-abdomen region.

This region must be demarcated from the remaining body parts of the neonate (e.g., head, hands, legs) and constantly monitor only this ROI region in order to compute the RR from the video stream.

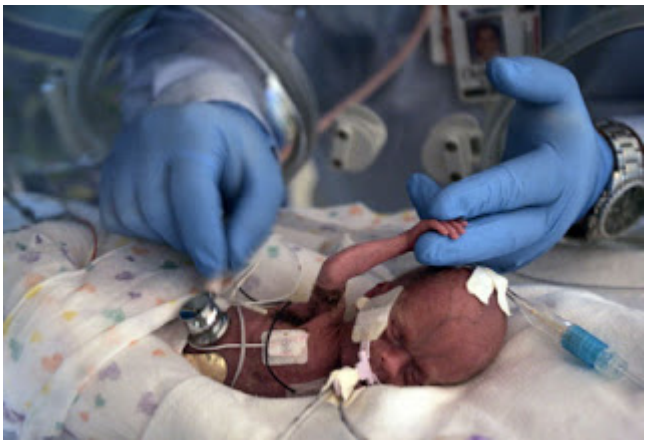


Fig 1 Neonatal Intensive Care Unit

Image URL: http://3.bp.blogspot.com/hUxQKflTkLM/TY9Kys4ewDI/AAAAAAAAAG8/nzgYjOO8s_8/s320/R_sun1.jpg

b. Human spine is a multifunctional structure of human body consisting of bones, joints, ligaments, and muscles which all undergo a process of change with the age. A sudden change in these features either naturally or through injury can lead to some serious medical conditions which puts huge burden on health services and economy. While aging is inevitable, the effect of aging on different areas of spine is of clinical significance. So any image taken of spine can be of importance in finding ROI from the image according to need.

c. Dry eye is a symptomatic disease that affects the activities of daily living, adversely impacting important task such as computer use, driving and others. For automatic image processing methodology to be applied to this, there are two clinical test : analysis of lipid pattern and tear film breakup time test. In tear film breakup time test experts usually analyze the bottom part of the iris ignoring certain areas such as sclera, eyelids etc and focusing on the area where the tear has higher contrast. This will lead to the fist step which consist of preprocessing the images to extract the region of interest.[8]

d. For the diagnosis in the gynecology field different diagnostic capabilities have brought certain advancement. Different methodology includes 3D ultrasound, Ultrasound Contrast Media and Harmonic imaging in its various forms and its derivatives have been there. Image segmentation is commonly used to define tissue and fluid boundaries, either by manual or semi automated methods. Segmentation and classification process separate region of interest from surrounding tissue or acoustic noise that prevents visualization of anatomic structures. Several different methods are there defined for detection of surface boundaries by image segmentation. Once the boundaries are defined a particular region of interest can be analyzed for quantitative volume measurements. [9]

e. This concept of Region of interest can be evaluated for the tumor tracking accuracy also. Although conventional wisdom states that the ROI reconstruction requires a priori information of a small region inside the ROI and 180° scanning without angular truncation for accurate image reconstruction, the computer simulation showed that the ROI reconstruction method without any a priori information had equivalent accuracy in terms of tumor tracking compared with the case reconstructing the entire region. [3]

1.2 Algorithm how one can proceed for selecting ROI

By seeing the above applications the need for selecting ROI is getting more prominent and for that the proposed algorithm would be as follow.

a. Firstly some data set is need for training purpose to train computer with some images of similar types.
b. Second step would be to generate some feature vector based on region of interest part of the training images.
c. Some test images are to be provided based on the training set of images and the applications should be able to automatically identify the region of interest part from the images.
d. Then after selecting the ROI automatically the system should be trained to segment that ROI part and compress the rest of the image.
e. After that ROI part should be merged with the compressed image and then it should be stored in the system.

By following the above steps our applications would be able to select ROI and then store the images in such a manner that the size of the image is reduced.

II CONCLUSION

So we have here surveyed different medical field be it orthopaedic field, genecology field, paediatric field etc where Region of Interest concept can be applied and can be helpful to the Doctors / practitioners to give the analysis and can help them to save more data then they were able to save previously. Future work can be to select different field and show the comparisons how the space reduction can actually happen by our proposed technique.

REFERENCES

[1] Venkitaraman, A., & Makkapati, V. V. (2015, January). Motion-based segmentation of chest and abdomen region of neonates from videos. In*Advances in Pattern Recognition (ICAPR), 2015 Eighth International Conference on* (pp. 1-5). IEEE.

[2] Halter, R., Hartov, A., Poplack, S., diFlorio-Alexander, R., Wells, W., Rosenkranz, K., ... & Paulsen, K. (2015). Real-time electrical impedance variations in women with and without breast cancer.

[3] Tashima, H., Yoshida, E., Shinaji, T., Haneishi, H., Ito, H., & Yamaya, T. (2013, October). Monte Carlo simulation of region-of-interest reconstruction for real-time tumor tracking by OpenPET. In *Nuclear Science Symposium and Medical Imaging Conference (NSS/MIC), 2013 IEEE* (pp. 1-3). IEEE.

[4] M. R. Neuman, Neonatal Monitoring. John Wiley and Sons, Inc., 2006.

[5] D. Teichmann, C. Bruser, B. Eilebrecht, A. Abbas, N. Blanik, and S. Leonhardt, "Non-contact monitoring techniques - principles and applications," in Engineering in Medicine and Biology Society (EMBC), Annual Intl. Conf. IEEE, 2012., 2012, pp. 1302–1305.

[6] M.-Z. Poh, D. J. McDuff, and R. W. Picard, "Advancements in non contact, multiparameter. physiological measurements using a webcam," IEEE Trans. Biomedical. Eng., vol. 58, no. 1, pp. 7–114, Jan. 2011.

[7] ——, "Non-contact, automated cardiac pulse measurements using video imaging and blind source separation," Opt. Expr., vol. 18, pp. 10 762– 10 774, May 2010.

[8] Image Analysis and Modeling in Opthamology By Eddie Y. K. Ng, U. Rajendra Acharya, Jasjit S. Suri, Aurelio Campilho February 11, 2014 by CRC Press ISBN 9781466559301

[9] Ultrasound and Color Doppler in Obstetrics, Gynecology and Infertility by Prof. R Rajan , Indian Academy of human reproduction, Kerala, India

EFFICIENT DIGITAL CIRCUITS BASED ON CNTFET

Kalpita S. Mane[1], P. P. Narwade[2]

[1]Department of E&TC, MGMCET, Kamothe, (India)
[2]Assistant Professor, Department of E&TC, MGMCET, Kamothe, (India)

ABSTRACT

This paper deals with basic logic circuits using the carbon nanotube field effect transistor (CNTFET). Due to various beneficial properties of carbon nanotubes, these circuits are more efficient than Metal Oxide Field Effect Transistor (MOSFETs). The parameters like power, delay and power delay product of CNTFET based circuits are compared with that of CMOS technologies. Both the simulations are performed in HSPICE software. The CNTFET based circuits provides more power reduction, delay reduction, and Power Delay Product (PDP) reduction when compared with CMOS technologies.

Keywords: Carbon nanotube (CNT), CNT field-effect transistor (CNTFET), Metal Oxide Semiconductor Field Effect Transistor (MOSFETs).

I INTRODUCTION

As per Moore's law, the number of transistors that are placed in an integrated circuit increases exponentially by almost doubling every two years. Continuously reducing the size of transistor i.e. scaling of MOSFET technology has been carried continuously in order to meet the density and sustain the IC predicted by Moore's law. Since in year 2006, the gate length of a MOSFET device has entered the deep submicron/nano regime at 65-nm feature size. Now-a-days, 45-nm technology is a reality used, and in the near future 32-nm has been predicted to be the feature size [1]. As the physical gate length is reduced to below 65-nm, several device-level effects, such as short channel effect, large parametric variations and exponential increase in leakage current, have substantially affected the I-V characteristics of traditional MOSFETs. This results in major concerns for scaling down the feature size of these devices. To meet the challenges of nano scale CMOS, and achieve similar performance like CMOS, CNTFET is explored. This CNTFET consists of utilizing new circuit techniques together with alternative technologies to replace conventional silicon and the current MOSFET-based technology [2].

1.1 Carbon Nanotubes (CNTs)

Carbon is the 4th most abundant element in the Universe by mass after (Hydrogen Helium and Oxygen) having atomic number of 6. It forms almost 10 million pure organic compounds with any other element. Carbon Nanotubes are long, thin cylinders of carbon, and were discovered in 1991 by Sumio Iijima of NEC Crop in Tokyo [2]. He proposed the new type of carbon structure which was needle like tubes of diameter varying from 4-30 nm. By survey of applications regarding to the transistors the channel of traditional MOSFET will replace by CNT. These are large that are unique for their size, shape, and remarkable physical properties. Carbon Nanotubes (CNTs) have attraction of researchers worldwide in recent years because of their small dimensions and unique architecture properties. For passive or active elements in post-CMOS, Nano-electronics carbon Nanotubes is the best replacement device [3].

1.2 Carbon Nanotube Field Effect Transistor (CNTFET)

In the era of Nano scale, Carbon Nanotube Field Effect Transistor (CNTFET) is a promising device for future integrated circuits because of its tremendous properties like ballistic electron transport, high carrier mobility. In 1998, the first carbon nanotube field-effect transistors (CNTFETs) were reported. Which is one of the most promising alternatives to the MOSFET is the CNTFET. The most important and significant attribute of CNTFET is its spectacular ability in current carrying or current driving, and experiments have shown that CNTFET is the best for this purpose. CNTFET can operate five times faster than CMOS in the best case without any extra power overhead [3].

CNFETs are one of the molecular devices that avoid most fundamental silicon transistor restriction and have ballistic or near ballistic transport in their channel. Therefore a semiconductor carbon nanotube seems to be appropriate to be used as the channel of field effect transistors. Applied voltage to the gate can control the electrical conductance of the CNT by changing electron density in the channel. By using appropriate diameter suitable threshold voltage for CNFET can be achieved.

The threshold voltage of the CNFET is proportional to the inverse of the diameter of CNT and can be expressed as:

$$V_{th} = \frac{0.42}{D_{CNT}(nm)} \quad \text{(i)}$$

For a CNT with *(n, m)* as chirality and *a=0.249* as lattice (that is carbon to carbon atom distance) the diameter is:

$$D_{CNT} = \frac{a\sqrt{n^2+nm^2+m^2}}{\pi} \quad \text{(ii)}$$

As mentioned above, CNTs are used in CNFETs as channel and depending on the connections between source and drain with channel (CNTs) there are two main CNFETs. It works on the principle of direct tunneling through a Schottky barrier at the source–channel junction; therefore, these transistors are called Schottky Barrier CNFET (SB-CNFET). SB-CNFET shows ambipolar behavior and constrains usage of these transistors in conventional CMOS-like logic families. Schottky barrier restricts the trans-conductance in the ON state, and thus Ion/Ioff ratio becomes rather low. Second device is MOSFET-like CNFET which is doped in un-gated portions and has similar behaviour to CMOS transistors and it presents unipolar behaviour. The semiconductor junction will eliminate schottky barrier and therefore there is higher ON current unlike SB-CNFETs. Other advantages of MOSFET-like CNFETs are high on-off ratio and their scalability compared to their schottky barrier counterparts. In this paper we utilized MOSFET-like CNFETs for designing the logic gates [1].

II SPICE COMPATIBLE MODEL FOR CNTFET

CNTs are used in the channel region of the CNTFET. Different types of CNTFET have been demonstrated in the literature. There are mainly two types of CNTFET: Schottky barrier CNTFET (SB-CNTFET) and MOSFET-like CNTFET as shown Fig. 1. In SB-CNTFET the channel is made of intrinsic semiconducting CNT and direct contacts of the metal with the semiconducting nanotubes are made for source and drain regions. The device works on the principle of direct tunneling through the Schottky barrier (SB) at the source-channel junction thus, the trans-conductance of the device is controlled by the gate voltage.

In MOSFET-like CNTFET doped CNTs are used for the source and drain regions and channel is made of intrinsic semiconducting CNT. A tunable CNTFET with electrical doping is also proposed. It works on the principle of barrier-height modulation by the application of gate potential.

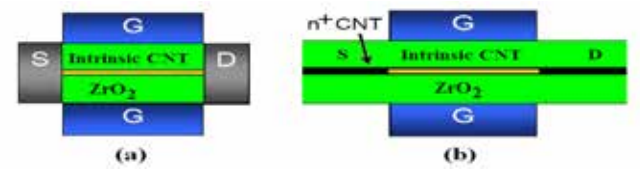


Fig. 1. Different types of CNTFETs: (a) Schottky barrier (SB) CNTFET (b) MOSFET-like CNTFET [4].

All the below circuits are simulated using Synopsys HSPICE 2008 simulator tool with SPICE model for CNTFET at 32nm technology. The CNTFET standard model has been designed for unipolar, MOSFET-like CNTFET device in which each transistor may have one or more CNTs [5]-[12]. The parameters of the CNTFET model and their values, with brief descriptions are shown in below Table I.

Table I: CNTFET Model Parameters

Parameter	Description	Value
Lch	Physical Channel Length	32 nm
Lgeff	Mean free Path in instrinsic CNT Channel	100 nm
Lss	Length of doped CNT source-side extension region	32 nm
Ldd	Length of doped CNT drain-side extension region	32 nm
Kgate=Kox	Electric constant of high K top gate electric material	16
Hox=Tox	thickness of high K top gate electric material	4 nm
Csub=Cb	Coupling Capacitance between channel region and substrate	40 pF/m
CNT pitch	Region of CNT tube placed	20 nm

III ANALYSIS OF LOGIC GATES

Different digital logic gates such as INVERTER, NAND, NOR, OR and combinational circuits have been designed using CNTFET. These logic gates/circuit are simulated by using HSPICE software. After simulation, the

circuits have analyzed for their average power, delay and PDP (Power delay product). In Fig.2, the schematic design and simulation of combinational circuit is shown.

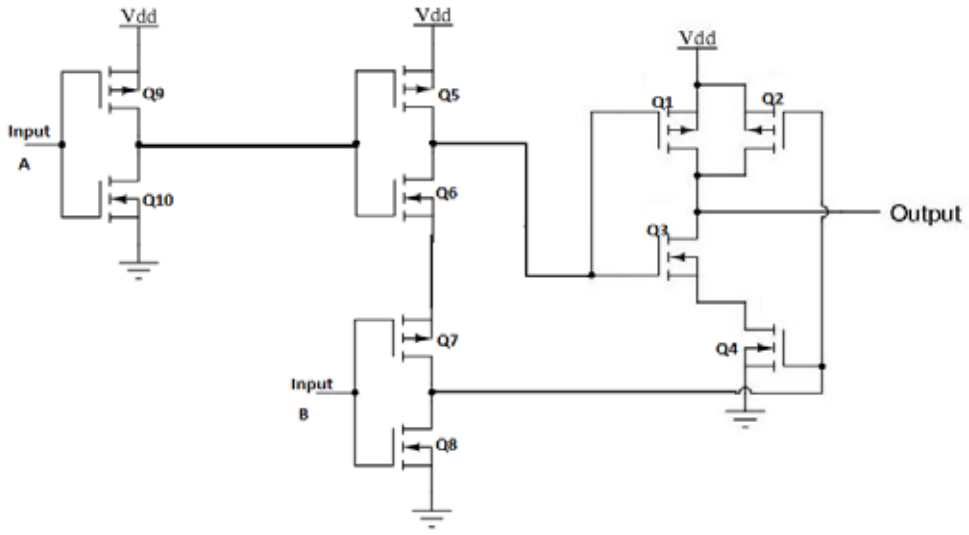


Fig. 2 Design of Combination Circuit Using OR and NOT Gate

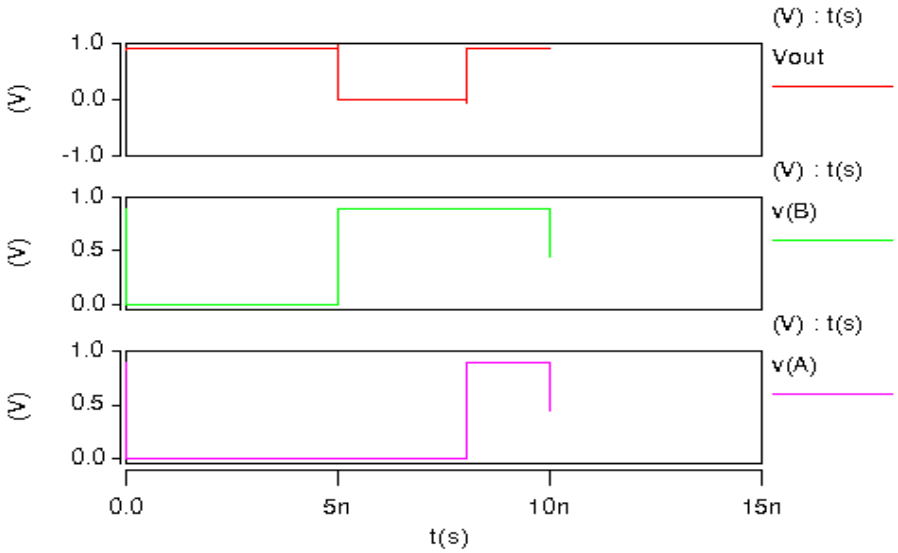


Fig. 3 Transient Response Using CNTFET

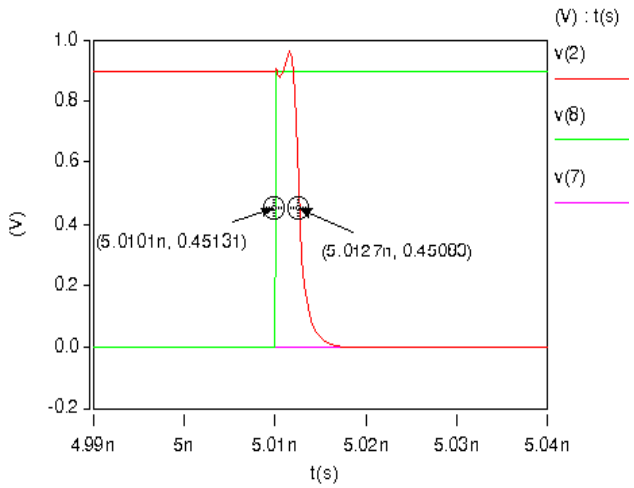


Fig.4 Transient Response Using CNTFET Showing Delay

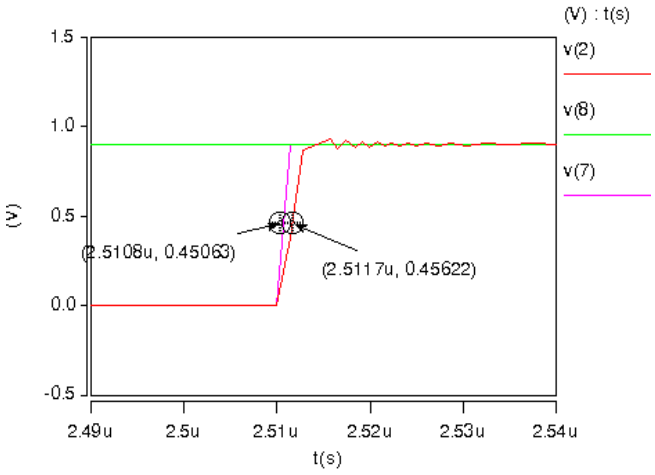


Fig. 5 Transient Response Using MOSFET Showing Delay

The design shown in Fig. 2 is obtained for Output=Y= Σm (0, 1, 3), i.e. $Y = \overline{A} + B$. In this way CNTFET inverter, NAND, NOR and OR gates have been simulated in HSPICE software for CNTFET model of 32nm technology. The performance parameter viz. power, delay and power delay product (PDP) have been calculated for these circuits and also for CMOS circuit design.

IV RESULTS

The circuit shown in Fig. 2 is verified using HSPICE software. The results obtained for power dissipation (P), Delay (D), and Power delay product (PDP) are tabulated in Table II and III. The result shows significant reduction in power dissipation, delay and hence power delay product.

TABLE II: Performance Analysis of CMOS logic gates at VDD=0.9V

CMOS			
Designs using logic gates	**P (J/Sec)**	**D (Sec)**	**PDP (J)**
INVERTER	9.9528E-07	4.4E-09	43.7923E-16
NAND	3.8244E-05	6.2E-09	23.7113E-14
NOR	8.0468E-07	2.1E-09	16.8982E-16
OR	1.8877E-6	6E-09	11.3262E-13
Combinational Circuit ($Y = \overline{A} + B$)	7.3781E-07	2.6E-09	19.1831E-14

TABLE III: Performance Analysis of CNTFET logic gates at VDD=0.9V

CNTFET			
Designs using logic gates	**P (J/Sec)**	**D (Sec)**	**PDP (J)**
INVERTER	4.0243E-09	4E-13	16.0972E-22
NAND	5.4986E-09	1.6E-12	8.7977E-21
NOR	8.9529E-09	10E-12	89.529E-22
OR	1.5163E-07	7E-12	10.6141E-19
Combinational Circuit ($Y = \overline{A} + B$)	1.1770E-08	3.1E-12	3.6487E-20

V CONCLUSION

This paper compares CNTFET based logic circuits with CMOS based logic circuits using HSPICE software. Simulations are carried at room temperature with voltage 0.9V and performances are studied by using parameters like power, delay, and PDP. The result shows that CNTFET based circuit design gives good performance like fast output response, less average power dissipation, and improved transient response than CMOS based circuits designs.

REFERENCES

[1] Aparna Anand, S.R.P. Sinha, "Performance Evaluation of Logic Gates Based On Carbon Nanotube Field Effect Transistor", International Journal of Recent Technology and Engineering (IJRTE), ISSN: 2277-3878, vol. 2, no.5, pp. 120-125, Nov. 2013.

[2] Deepak V. Gohil, Mr.Yogesh D. Parmar, " Hspice Simulation of D Latch and Double Edge Triggered Flip-Flop Using CNTFET " , *IJEDR* , vol. 2, no. 2 , ISSN: 2321-9939 , pp. 2734-2739, Feb. 2014.

[3] Radha Tapiawala, Rahul Kashyap," Design of Universal Logic Gates based on CNTFET for Binary and Ternary Logic", International Journal of Engineering Research & Technology (IJERT), ISSN: 2278-0181, vol. 3, no. 6, pp. 604-609, Jun. 2014.

[4] Subhajit Das, Sandip Bhattacharya, Debaprasad Das, "Design of Digital Logic Circuits using Carbon Nanotube Field Effect Transistors", International Journal of Soft Computing and Engineering (IJSCE), ISSN: 2231-2307, vol.1, no.6, pp.173-178, Dec. 2011.

[5] P. A. GowriSankar , K. Udhayakumar, " A novel carbon nano tubes field effect transistor based arithmatic computing circuit for low -power analog signal processing application", *The 7th INTER-ENG 2013*, pp. 154-162, Jun. 2014.

[6] Sanjeet Kumar Sinha, SaurabhChoudhury, " CNTFET based Logic Circuits: A Brief Review", *International Journal of Emerging Technology and Advanced Engineering*, ISSN 2250-2459, vol.2, no.4, pp. 500-504, Apr. 2012.

[7] RonakZarhoun, Mohammad HosseinMoaiyeri, Samira ShirinabadiFarahani, and KeivanNavi, "An Efficient 5-Input Exclusive-OR Circuit Based on Carbon Nanotube FETs", *ETRI Journal*, vol. 36, no. 1, pp. 89-98, Feb. 2014.

[8] Roberto Marani & Anna Gina Perri, "Simulation of cntfet digital circuits: a verilog-a implementation ", *IJRRAS*, vol. 11 no.1, pp. 74-81, Apr. 2012.

[9] Ashkan Khatir1 , Shaghayegh Abdolahzadegan2 ,ImanMahmoudi, "High speed multiple valued logic full adder using carbon nano tube field effect transistor", *International Journal of VLSI design & Communication Systems (VLSICS)* , vol.2, no.1, pp. 1-9, Mar 2011.

[10] AliakbarRezaei, Mehdi Masoudi, FazelSharifi, KeivanNavi, " A Novel High Speed Full Adder Cell based on Carbon Nanotube FET (CNFET)", *International Journal of Emerging Sciences 4(2)*, ISSN: 2222-4254, pp. 64-74, Jun. 2014.

[11] AparnaAnand, S.R.P. Sinha , "Performance Evaluation of Logic Gates Based On Carbon Nanotube Field Effect Transistor", International Journal of Recent Technology and Engineering (IJRTE), ISSN: 2277-3878, vol. 2, no.5, pp.120-125, Nov. 2013.

[12] Sameer Prabhu, Dr. NishaSarwade, "Application of CNTFET as Logic Gates and its implementation using HSPICE", International Journal of Modern Engineering Research (IJMER) , , ISSN: 2249-6645, vol. 3, no. 6, pp. 3646-3648, Nov. - Dec. 2013.

Biographical Notes

Ms. Kalpita S. Mane is presently pursuing ME final year in Electronics & Telecommunications Engineering Department from MGMCET, Kamothe, Navi-Mumbai, India.

Mr. P. P. Narwade is working as a Assistant Professor in Electronics & Telecommunications Engineering Department from MGMCET, Kamothe, Navi-Mumbai, India.

HIGH-LEVEL EXPRESSION AND PURIFICATION OF A THERAPEUTIC RECOMBINANT SERINE PROTEASE INHIBITOR FROM TRANSGENIC TOMATO PLANTS

Shweta Jha[1], Indraneel Sanyal[2] and Devindra Vijay Amla[3]

[1] *Centre of Advanced Studies in Botany, J. N. Vyas University, Jodhpur-342001, (India)*
[2,3] *CSIR- National Botanical Research Institute, Lucknow-226001, (India)*

ABSTRACT

Human a_1-proteinase inhibitor (a_1-PI) is the most abundant serine protease inhibitor in blood and heterologous expression of recombinant a_1-PI has great potential for therapeutic applications. Present study is focused to express and purify functional recombinant α_1-PI protein from transgenic tomato plants. Human α_1-PI gene was designed and codon-optimized according to dicot plant preferences and the significance of flanking regulatory sequences was documented for higher expression in plants. In addition, response of protein accumulation site on yield, biological activity and in planta stability was analyzed via protein targeting to different subcellular locations. Modified gene encoding recombinant α_1-PI was employed for Agrobacterium-mediated transformation of tomato. Maximum accumulation of recombinant α_1-PI was achieved from 1.5 to 3.2% of TSP by retention in ER lumen with highest biological activity for elastase inhibition. Recombinant a_1-PI was purified from transgenic tomato plants with high yield, homogeneity and biological activity by immunoaffinity chromatography. The purified protein appeared as a single band of ~50 kDa on SDS-PAGE. Results of mass spectrometry revealed the identity and structural integrity of the purified protein comparable to native serum α_1-PI. Our data suggested significance of transgenic plants to use as bioreactors for the production of stable and biologically active recombinant therapeutic proteins.

Keywords :Human a_1-proteinase Inhibitor; Protein Purification; Recombinant Protein Expression; Serine Protease Inhibitor; Transgenic Tomato Plants

I INTRODUCTION

Human α_1-proteinase inhibitor (α_1-PI), also known as α_1-antitrypsin (AAT), is the most abundant serine protease inhibitor (SERPIN) in human plasma. While α_1-PI inhibits a wide range of serine proteases, its main physiological role is to control the proteolytic activity of neutrophil elastase and maintain protease-antiprotease homeostasis in the lungs [1]. Its deficiency is either due to genetic disorders or heavy smoking, which results into development of various diseases, including pulmonary emphysema, cystic fibrosis, hepatic carcinoma, rheumatoid arthritis, psoriasis and dermatitis [2]. Currently, plasma-derived human α_1-PI is the only available option for treatment of pulmonary emphysema by augmentation therapy, which appears to be insufficient to meet the anticipated clinical demand, and

also carries the potential risk of contamination with blood-borne pathogens. In this context, efforts to develop recombinant α_1-PI, as an alternative to the plasma-derived protein, have been reported [3, 4, 5, 6, 7]. Large-scale production of safe and biologically active recombinant α_1-PI has been exploited in several alternative hosts like *E. coli*, yeast, insect cells, CHO cells, transgenic animals, rice and tobacco cell suspension cultures for therapeutic applications [3, 8, 9, 10]. The recombinant α_1-PI protein expressed in *E. coli* and yeast cells was either non-glycosylated or aberrantly glycosylated resulting in significantly decreased stability and biological activity [11, 12, 13, 14], whereas recombinant human α_1-PI expressed in milk of transgenic mice, goat and sheep were found to be associated with animal native α_1-PI and α_1-antichymotrypsin as major immunogenic impurities, and contamination by animal-borne pathogens [15]. Plants provide an attractive expression platform for overexpression of recombinant proteins due to advantages of simple growth requirements, product safety, unlimited scalability, cost-efficacy and complete post-translational modifications [16]. The potential of 'molecular pharming', using transgenic plants as 'bioreactors' to produce therapeutic proteins has been demonstrated [17]. Expression and purification of functional recombinant human α_1-PI from rice cell cultures using *Amy3D* sugar-regulated gene cassette [8, 18], or from leaves of transgenic tobacco [19, 20] or tobacco suspension cultures [9, 10] using chemically-inducible virus amplicon system, and from transgenic tobacco chloroplasts [21] have been demonstrated. However, yield and quality of recombinant α_1-PI protein was low and poor or the protein was unglycosylated that severely restricted its therapeutic application.

Considering the importance of recombinant α_1-PI as possible alternative to serum-derived α_1-PI protein for therapeutic application, the present work has been developed with the objective of production ofstable and biologically active recombinant α_1-PI protein in transgenic tomato (*Solanum lycopersicum* var. PED) plants for possible use in therapeutic applications.

II MATERIALS AND METHODS

2.1 Plant expression vectors and transformation of tomato plants

The 1,182 bp sequence of α_1-PI gene (GenBank accession no. **EF638826**) was codon-optimized and designed for optimum expression in dicot plants. The native transit peptide sequence of human α_1-PI was substituted with 90 bp modified PR1a signal peptide sequence of tobacco in the pPWK vector for targeting of the recombinant protein to apoplast, while PR1a in conjunction with KDEL motif at 3' end was used to develop pPAK vector for ER retention of the protein (Fig. 1, 2a). The 114 bp modified transit peptide sequence of sweet potato sporamine with N-terminus propeptide (SPS-NTPP) without 3' KDEL motif was used to develop pSWK vector for vacuolar targeting, while modified gene without any flanking sequences either on 5' or 3' end was used to develop pWSP vector for cytosolic accumulation of the protein (Fig. 2a). *Agrobacterium tumefaciens* strain LBA4404 was transformed with these four vectors independently and used for nuclear transformation of tomato (*Solanum lycopersicum* var. PED) using leaf-disc method with some specific modifications [22]. The kanamycin-resistant T_0 plantlets were developed under culture room conditions and then transferred to glasshouse for growth, flower development and seed setting.

2.2 Molecular characterization of transgenic tomato plants

Genomic DNA was isolated from 100 mg of young leaf tissues of transgenic tomato plants using GenElute plant genomic DNA miniprep kit according to manufacturer's instructions (Sigma, USA). PCR analysis was performed using α_1-PI gene-specific primers, α_1-PI(F) 5'-GAAGATCCTCAAGGAGATGCTGC-3' and α_1-PI(R) 5'-CTTCTGAGTAGGGTTAACCACCTT-3' and Southern hybridization was performed using α [^{32}P] dCTP labelled α_1-PI gene probe for detection of α_1-PI gene [23]. Total RNA was isolated from 100 mg fresh leaves of transgenic tomato plants in 1 ml TRI-reagent for analysis of α_1-PI transcript. The first strand of cDNA was synthesized with enhanced Avian HS RT-PCR kit according to manufacturer's instructions (Sigma, USA). The α_1-PI transcript was detected by RT-PCR using 100 ng of cDNA template and α_1-PI gene-specific primers.The tomato β-actin gene (Tom 52: GenBank accession no. **U60482**) was used as endogenous control and 194 bp fragment was amplified using the forward primer 5'-GCTGGATTTGCTGGAGATGATGC-3' and reverse primer 5'-TCCATGTCATCCCAATTGCTAAC-3'.

2.3 Quantitative estimation of recombinant α_1-PI protein

Cell free plant extracts were prepared from leaves of 12-week old transgenic tomato plants and quantification of recombinant α_1-PI protein in the crude extracts or purified protein samples was performed by direct antigen coating-enzyme linked immunosorbent assay (DAC-ELISA) using the commercial anti-human α_1-PI antibody (Sigma, USA) as described earlier [24]. Expression levels were quantified on a linear standard curve plotted with pure human serum α_1-PI protein (Sigma, USA).

2.4 Western immunoblotting

A 50 µg of cell free plant extract or 100 ng of purified recombinant α_1-PI protein sample was fractionated on 12% SDS-PAGE followed by staining with silver salts according to the standard procedure [23]. Electrophoresed protein samples were transferred onto Immobilon polyvinylidenedifluoride (PVDF) membrane (Millipore, USA) for Western blotting using the commercial anti-human α_1-PI antibodies (Sigma, USA).

2.5 Biological activity of recombinant α_1-PI protein for elastase inhibition

The biological activity of recombinant α_1-PI in cell free plant extracts and of purified protein samples was determined by residual porcine pancreatic elastase (PPE) inhibition activity assay using N-succinyl-Ala-Ala-Ala-*p*-nitroanilide (Sigma, USA) as chromogenic substrate, as described earlier [7]. Pure human serum α_1-PI was used as a standard to quantify the biologically active recombinant α_1-PI.

2.6 Purification of recombinant α_1-PI from transgenic tomato plants

A 100 g fresh leaf tissue from the promising transgenic tomato plants developed with different vectors and showing maximum expression was homogenized in liquid nitrogen and resuspended in five volumes of homogenization buffer (20 mM Tris-HCl, pH 7.4, 150 mM NaCl, 10 mM EDTA, 14 mM b-ME and 0.05% Triton X-100). The crude homogenate was filtered through nylon mesh, centrifuged at 12,000 rpm for 20 min and soluble proteins in the supernatant were precipitated by adding increasing amounts of ammonium sulphate upto 50% saturation. The

remaining proteins in the supernatant were precipitated with 50-95% saturation of ammonium sulphate, resuspended, dialyzed against equilibration buffer (0.1 M Tris-HCl, pH 8.0, 0.5 M NaCl) and filtered through 0.22 µm syringe filter (Whatman, CA) prior to loading onto immunoaffinity column prepared by coupling of rabbit anti α_1-PI antibody to CNBr-activated Sepharose 4B matrix (Sigma, USA). The column was pre-equilibrated and the clarified protein samples were loaded onto the antibody column at a flow rate of 0.5 ml min^{-1}. The column was washed with equilibration buffer until A_{280} of the effluent reached to zero. The bound antigen was eluted with 0.1 M Na_2CO_3, pH 11.2, 0.5 M NaCl and immediately neutralized with 1 M Tris-HCl, pH 6.8. The eluted fractions containing α_1-PI protein were pooled, dialyzed against storage buffer (50 mM Tris-HCl, pH 7.5, 75 mM NaCl), concentrated and used for further biochemical investigations.

2.7 Mass spectrometric analysis

Protein spots were excised from SDS-PAGE gel and 'in-gel' digested with trypsin [25]. Identification and characterization of purified protein sample was performed with 4800 MALDI-TOF/TOF mass spectrometer (Applied Biosystems, USA) in reflector positive ion mode respectively, as described earlier [14].

III RESULTS

3.1 Construction of plant expression vectors and a_1-PI gene cloning

The cDNA sequence of human a_1-PI gene (GenBank accession no. X01683) was designed *in silico* to display codon usage patterns of abundantly expressed dicot plant genes to achieve high-level expression of recombinant a_1-PI protein in dicot plants (Fig. 1a). In modified a_1-PI gene, 52% of the native human protein codons (205 out of total 394) were replaced with dicot-preferred codons according to CUTG (Codon Usages Tabulated from Gene Bank) website (http://www.kazusa.or.jp/codon/) with substitution of about 281 favoured nucleotides. The final G+C content in the modified a_1-PI gene was decreased to 45.8% in contrast to 51.2% in native human a_1-PI gene, to complement with the overall G+C content of dicot plant genes. Several potential factors responsible for lowexpression level of protein were identified and eliminated, such as polyadenylation signals, mRNA instability sequences, RNA polymerase II termination signals and potential splicing sites in the coding region of the a_1-PI gene. A 5' untranslated region (UTR) of Alfalfa mosaic virus (AMV) and optimum translation initiation context (TAAACAATGG) was also incorporated upstream of the signal peptide sequences for proper initiation of translation (Fig. 1b).Different signal peptide sequences like signal sequence of tobaccopathogenesis related protein (PR1a) gene and sweet potato sporamine-A (SPS-NTPP) gene were incorporated at 5'end of the modified a_1-PI gene for apoplast and vacuolar targeting respectively. For accumulation of the mature a_1-PI protein into the endoplasmic reticulum (ER) of the plant cell, a 12 nucleotide sequence (AAAGATGAACTG) for KDEL (Lys-Asp-Glu-Leu) amino acids as ER-retention signal was incorporated at 3' end of the modified a_1-PI gene (Fig. 1b, 2a). The *in silico*designed and modified a_1-PI gene was synthesized in three parts by PCR-based gene synthesis (PGS) approach using 24 to 55 bp long overlapping oligonucleotides (Fig. 1a).

The modified a_1-PI gene was expressed under the control of CaMV35S double enhancer (PECaMV35S) constitutive promoter along with 38 bp AMV 5'-UTR and various 5' and 3' flanking regulatory sequences. The gene constructs were subsequently cloned into backbone of binary vector pBIN19, mobilized into competent *Agrobacterium tumefaciens* strain LBA4404 *via* electroporation, and used for nuclear transformation of tomato for development of stable transgenic lines. The various binary vectors harbouring the modified a_1-PI gene were characterized by restriction digestion of recombinant vector DNA with *Bam*HI and *Eco*RI, followed by Southern hybridization with radiolabeled *Xho*I/*Kpn*I fragment of a_1-PI gene as the probe, which showed the expected DNA fragment of 1.5 kb (Fig. 2b). PCR amplification with specific primers for modified a_1-PI gene and *npt*II gene showed amplified fragment of 1,182 bp full-length modified a_1-PI gene and 678 bp internal fragment of *npt*II gene, respectively (Fig. 2b).

3.2 Genetic transformation of tomato

Agrobacterium tumefaciens strain LBA4404 harbouring different chimeric plasmid constructs was used for genetic transformation of tomato (*Solanum lycopersicum*) variety Pusa Early Dwarf (PED) using leaf-disc method. Vegetative leaf discs of tomato were used as explants for co-cultivation with *Agrobacterium*, resulting in overall transformation frequency between 12-14%. Antibiotic selection on kanamycin based on *npt*II gene was used for screening of putative transformed plantlets. Different stages of tomato regeneration following transformation and selection of plantlets on kanamycin supplemented medium are shown in Fig. 3. The putative T_0 transformants were developed under culture room condition and then transferred to contained transgenic house for growth to maturity and seed setting. Constitutive expression of heterologous recombinant a_1-PI in transgenic tomato plants did not show any morphological alternations. All the transgenic plants were phenotypically normal, healthy and fertile.

3.3 Molecular characterization of stable transgenic tomato plants

Several independent primary transformants of tomato were screened and verified for integration and expression of recombinant a_1-PI gene following kanamycin selection. The integration of a_1-PI transgene in plant genomic DNA was confirmed by PCR and Southern hybridization. Results showed the amplification of anticipated fragment of 1,182 bp for a_1-PI gene and 678 bp for *npt*II gene with specific set of primers (Fig. 4a and b);however, no such amplification was observed in untransformed control plant under identical assay conditions. Copy number of the transgene was detected by Southern hybridization with radiolabeled *Xho*I/*Kpn*I fragment of a_1-PI gene used as the probe. Most of the transgenic plants showed the presence of single copy of a_1-PI transgene (Fig. 4c). The size of DNA fragments showing hybridization to radiolabeled probe were variable and quite larger than positive control of 1,326 bp of full-length modified a_1-PI gene, reflecting random integration of a_1-PI transgene in the genome of transgenic plants.The presence of full-length stable transcripts for a_1-PI gene in transgenic plants was demonstrated by RT-PCR with a_1-PI gene specific primers, which revealed amplification of expected fragments of 1,182 bp similar to positive control (Fig. 4d).

3.4 Analysis of inheritance pattern of recombinant a_1-PI gene in transgenic population

Independent T_0 transgenic plants developed with different vectors expressing high-level of recombinant a_1-PI protein were self-pollinated for detailed investigation of inheritance and segregation of transgene. T_1 seeds of highly expressing transgenic tomato plants were germinated on antibiotic-supplemented medium (Fig. 5a) and observed for segregation pattern of *npt*II gene. The results of chi-square analysis based on kanamycin resistance trait showed that most of the plants reflected typical 3:1 Mendelian segregation ratio in T_1 generation (Table 1). The chi-square values (χ^2) for T_1 progeny were found to be statistically significant at 5% level of significance and 1 degree of freedom, except for few plants like PAK 25, SWK 8, and PWK 6, which showed higher χ^2 values (Table 1). On the basis of segregation pattern and performance of T_0 plants, four stable promising transgenic lines expressing higher levels of a_1-PI viz., PAK 31, SWK 1, PWK 26 and WSP 14 were selected and grown further for purification and biochemical characterization of the recombinant a_1-PI protein.

3.5 Expression level and biological activity of plant-derived recombinant a_1-PI protein

Qualitative and quantitative estimations of the recombinant a_1-PI protein expressed in T_1 transgenic plants were monitored by DAC-ELISA, residual PPE activity assay for elastase inhibition and Western immunoblotting. Significant variation in the final yield and accumulation of recombinant a_1-PI protein targeted to different subcellular location was observed in leaves of transgenic tomato plants developed with various gene constructs. Maximum yield of recombinant a_1-PI protein upto 3.05±0.89% of TSP was documented in ER-targeted transgenic line followed by vacuole and apoplast targeted lines which showed average expression level upto 1.89 ± 0.65 % and 1.40 ± 0.48 %, while cytosol targeted protein showed very low expression level (Fig. 5b). The biologically active recombinant a_1-PI protein was in correspondence with the level of total a_1-PI protein in all the four transgenic lines. The composite data is shown in fig. 5b. Cell-free extracts of untransformed control plants did not show any inhibition for elastase activity. Western immunoblot analysis of the protein in crude extract showed the expression of ~50 kDa recombinant a_1-PI protein in transgenic lines as compared to ~52 kDa of purified human serum a_1-PI protein (Fig. 5c). Differences in molecular weights between human serum a_1-PI and plant expressed recombinant a_1-PI protein might be due to differential glycosylation pattern in these two systems.

3.6 Purification of recombinant a_1-PI from transgenic tomato plants

T_1 Transgenic plants expressing high levels of recombinant a_1-PI protein were selected and crude cell free extracts of total protein were prepared from 100 g leaf tissues by homogenization in extraction buffer with liquid nitrogen. The soluble proteins in the crude extract were precipitated by adding increasing amounts of ammonium sulphate. Precipitated fractions were re-suspended and analyzed by DAC-ELISA for quantitative estimation of α_1-PI protein. Results showed maximum precipitation of recombinant a_1-PI between 50-95% saturated fractions of ammonium sulphate (Fig. 6a). High quality purification of recombinant a_1-PI from transgenic tomato plants was achieved by subsequent immunoaffinity chromatography using anti-a_1-PI antibody affinity column. Positive fractions from ammonium sulphate precipitation were pooled, dialyzed and loaded onto pre-equilibrated CNBr-activated Sepharose 4B column having immobilized polyclonal rabbit anti-a_1-PI antibodies. The bound protein was eluted at high pH

with 0.1 M sodium carbonate (pH 11.2) and eluted fractions were immediately neutralized with Tris.HCl (pH 6.8) to prevent the loss of biological activity. The fractions containing recombinant a_1-PI were pooled, dialyzed, concentrated and analyzed by SDS-PAGE and Western immunoblotting to confirm the molecular mass, integrity and purity of the eluted protein (Fig. 6b and c). The results had demonstrated effective purification of recombinant a_1-PI to ~99% homogeneity with high average yield and biological activity, as evident by a single band of ~50kDa on SDS-PAGE (Fig. 6b).

3.7 Recombinant a_1-PI protein analysis by MALDI-TOF/TOF

Analysis of peptide fragments generated by 'in-gel' trypsin digestion of purified recombinant a_1-PI protein by MALDI-TOF MS (peptide mass fingerprinting) and MS/MS (peptide sequencing) in reflector positive ion mode showed high resemblance with native human a_1-PI (Fig. 6d). The resulting spectrum was searched using Mascot search engine and the purified protein was significantly identified as a_1-PI with a MOWSE score of 212 at p <0.05 with high sequence coverage. These results confirmed the identity of the purified product as recombinant a_1-PI.

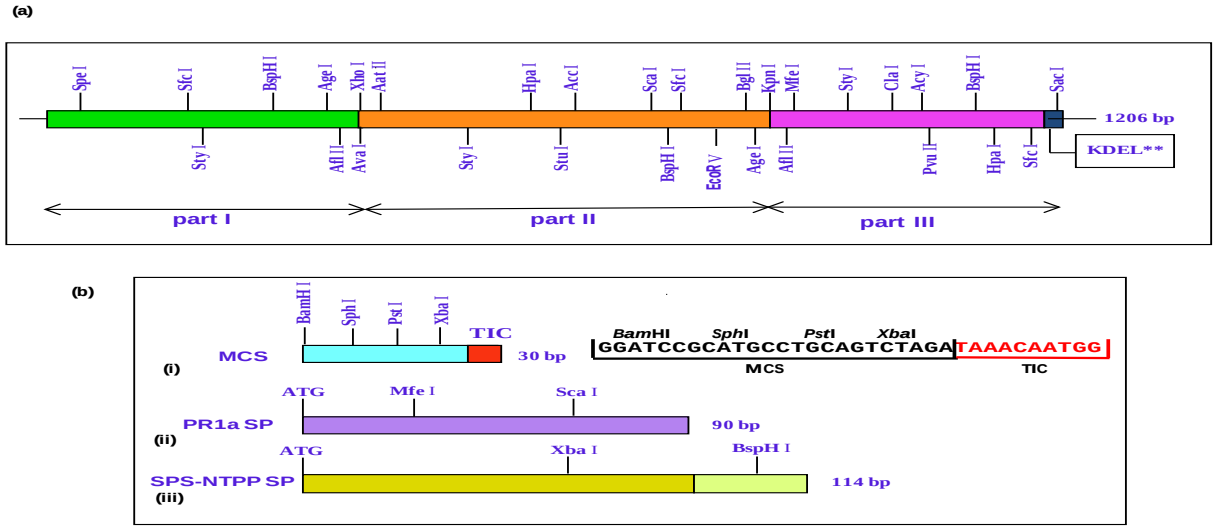


Fig. 1. Restriction architect of modified a_1-PI gene and signal peptide sequences. (a) Modified a_1-PI gene of 1206 bp divided into part I, II and III (shown with different colours) containing 25 unique restriction sites at distance of about every 50 bases.(b) (i) multiple cloning site (MCS) of 4 restriction sites with translation initiation context (TIC); (ii) 90 bp PR1a signal peptide sequence of tobacco;(iii) 114 bpsporamineA signal peptide sequence with N-terminal propeptide of sweet potato (SPS-NTPP).KDEL- ER retention signal followed by two stop codons, SP-signal peptide.**

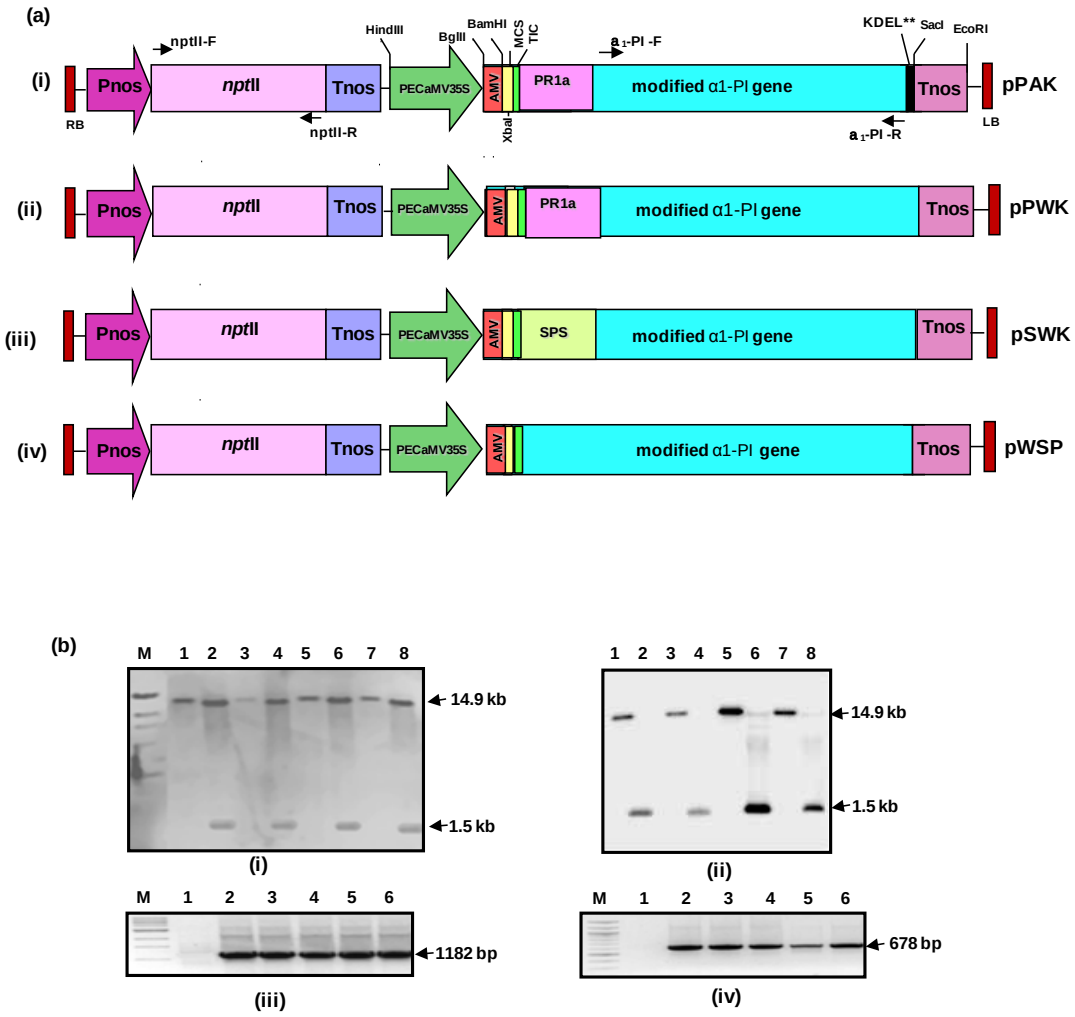


Fig. 2. Binary vector constructs of modified a_1-PI gene. (a) Schematic representation of the T-DNA region of modified α_1-PI gene constructs for sorting of protein to different subcellular locations; (i) endoplasmic reticulum (pPAK); (ii) apoplast (pPWK); (iii) vacuole (pSWK) and (iv) cytosol (pWSP). (b) Molecular characterization of a_1-PI gene constructs by (i) restriction digestion; lane M, λ-*Hin*dIII marker; lane 1, 3, 5, 7, different binary vectors of modified a_1-PI gene digested with *Bam*HI; lane 2, 4, 6, 8, with *Bam*HI/*Eco*RI; (ii) Southern blot of gel (i) hybridized with *Xho*I/*Kpn*I digested 500bp a_1-PI fragment as radiolabeled probe; (iii) PCR amplification with a_1-PI specific primers; lane M, 1 kb ladder; lane 1, negative control; lane 2-5, clones of binary vectors; lane 8, pUC19 cloning vector as positive control; (iv) PCR amplification with *npt*II specific primers; lane M, 100 bp ladder; lane 1-6, as in (iii).

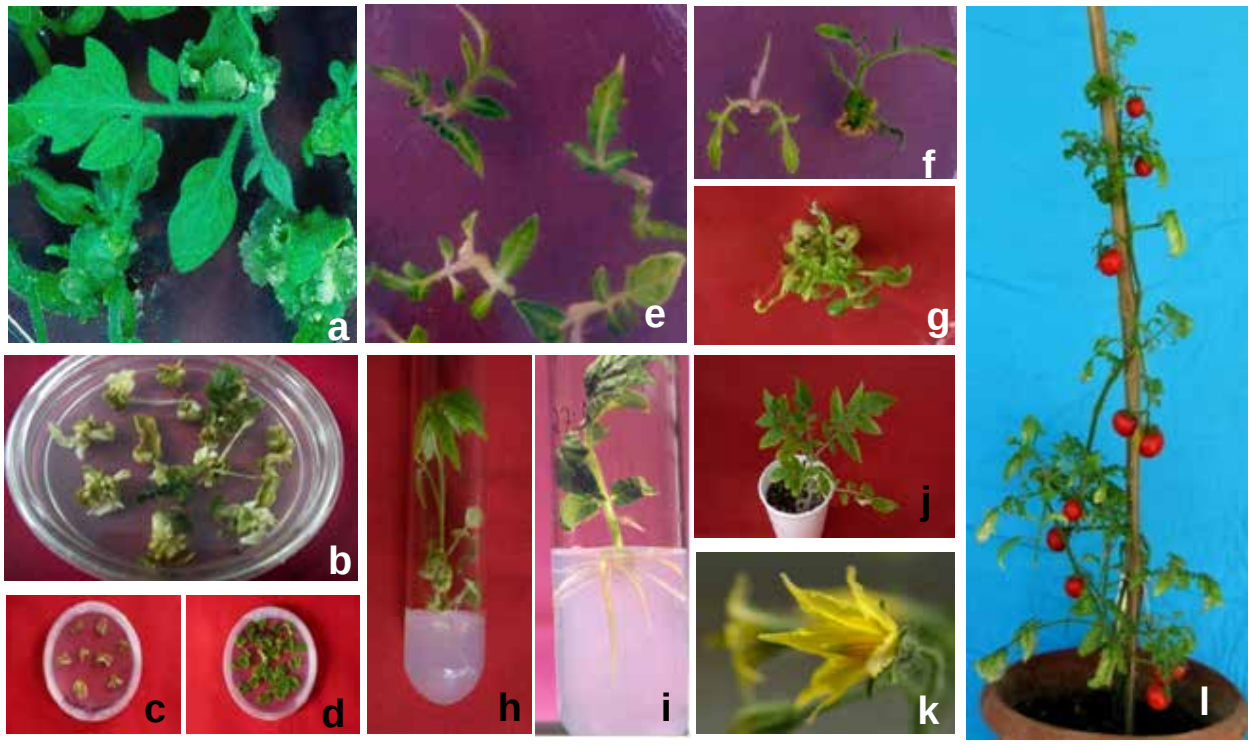


Fig. 3. *Agrobacterium*-mediated transformation of tomato and selection of transformants. (a) Vegetative leaf explants. (b) Shoot regeneration 3 weeks after culture on shoot induction medium (SIM) with 50 mg/l kanamycin. (c-d) Selection on kanamycin supplemented medium; (c) untransformed explants and (d) *Agrobacterium*-cocultivated transformed explants. (e-g) Stages during selection and screening showing non-transformed escapes, chimeric and transformed regenerating shoots. (h) Elongation of *in vitro* regenerated shoot. (i) Rooting. (j) Hardening. (k) Flower development in tissue culture developed plants in glasshouse; (l) Fertile transgenic tomato plant in glasshouse.

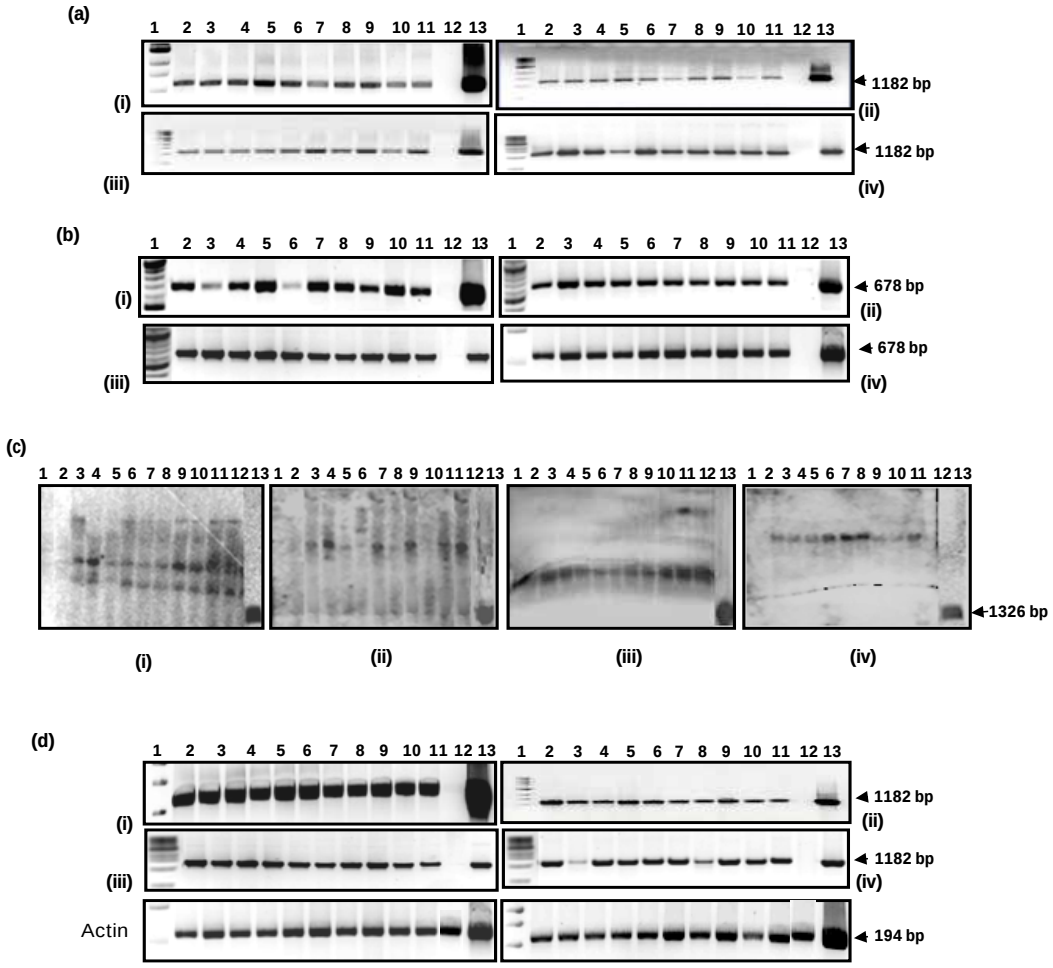


Fig. 4. Molecular characterization of T_0 transgenic plants of tomato. Confirmation of transgene integration by PCR and Southern hybridization with genomic DNA isolated from plants developed with constructs (i) pPAK, (ii) pPWK, (iii)pSWKand (iv) pWSP. PCR amplification of (a) 1182 bpa_1-PI gene and (b) 678 bp fragment of *npt*II gene;lane 1, 1 kb DNA ladder (a) or 100 bp ladder (b); lane 2-11, T_0 transgenic plants; lane 12, negative control- untransformed control plant; lane 13, positive control- corresponding binary vector. (c) Southern blot hybridized with a_1-PI specific radiolabeled probe; lane 1, ׀-*Hin*dIII marker; lane 2, negative control- untransformed control plant; lane 3-12, transgenic plans; lane 13, positive control- full length modified a_1-PI gene.(d) Detection and quantitation of a_1-PI transcript in T_0 transgenic plants of tomato by RT-PCR amplification with RNA isolated from plants developed with constructs (i) pPAK, (ii) pPWK, (iii) pSWKand (iv) pWSP; lane 1, 1 kb DNA ladder; lane 2-11, T_0 transgenic plants; lane 12, negative control- untransformed control plant; lane 13, positive control. Tomato b-actin gene (lower panel) was taken as endogenous control.

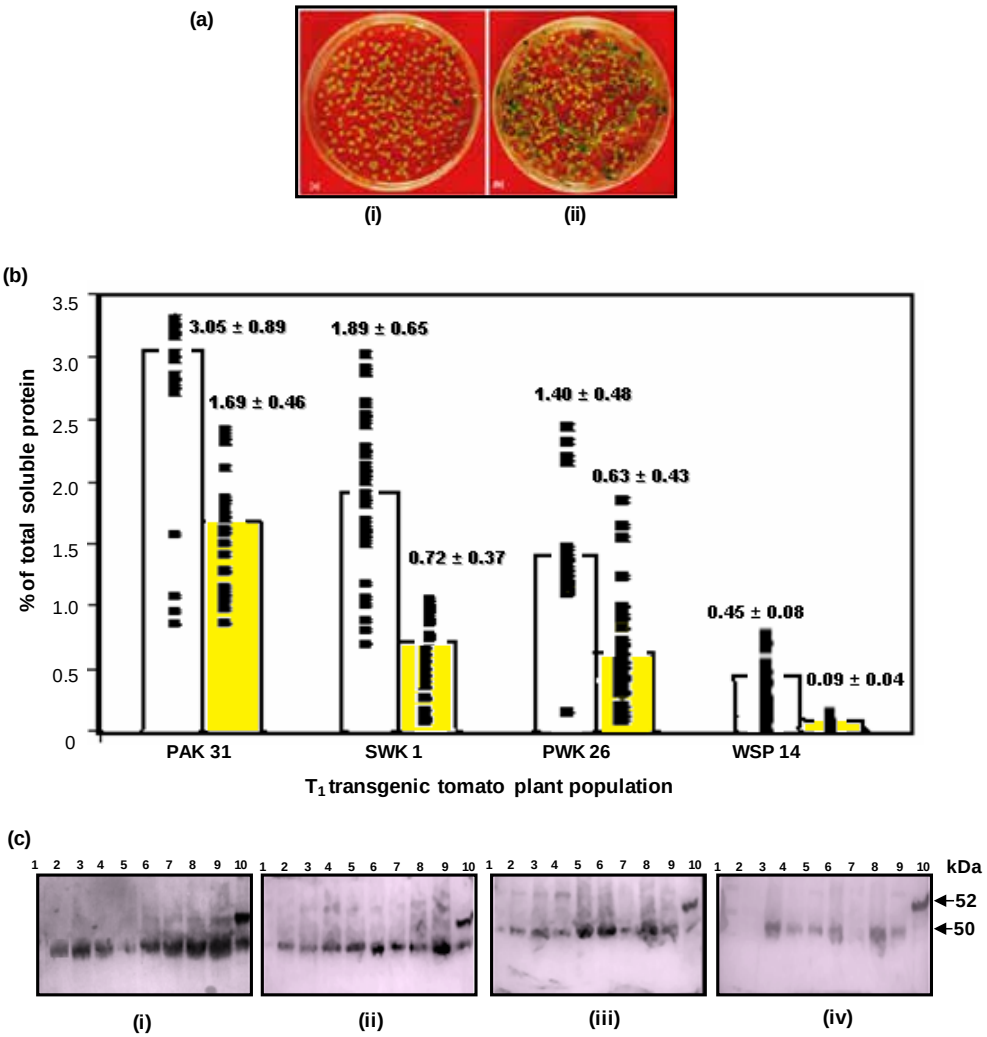


Fig. 5. (a) Germination of seeds on MS medium supplemented with kanamycin (100 mg l^{-1}); (i) Seeds from untransformed control plants; (ii) T_1 seeds obtained from T_0 transgenic plants. (b) Accumulation of recombinant α_1-PI protein in T_1 population of transgenic plants by quantitative DAC-ELISA (▭) and corresponding residual PPE activity assay (▬) for recombinant α_1-PI protein expressed in T_1 population of highly expressing transgenic lines PAK 31 (ER), SWK 1 (vacuole), PWK 26 (apoplast) and WSP 14 (cytosol) respectively. Average quantity of total recombinant α_1-PI protein and its corresponding biological activity is shown as % of TSP ± standard deviation on top of histogram bars. (c) Western immunoblot analysis with crude protein extract; lane 1, untransformed control plant; lane 2-9, transgenic plants expressing recombinant a_1-PI protein targeted to ER (i), vacuole (ii), apoplast (iii) and cytosol (iv); lane 10, purified human serum a_1-PI. The numbers on the right indicate the size of the a_1-PI protein.

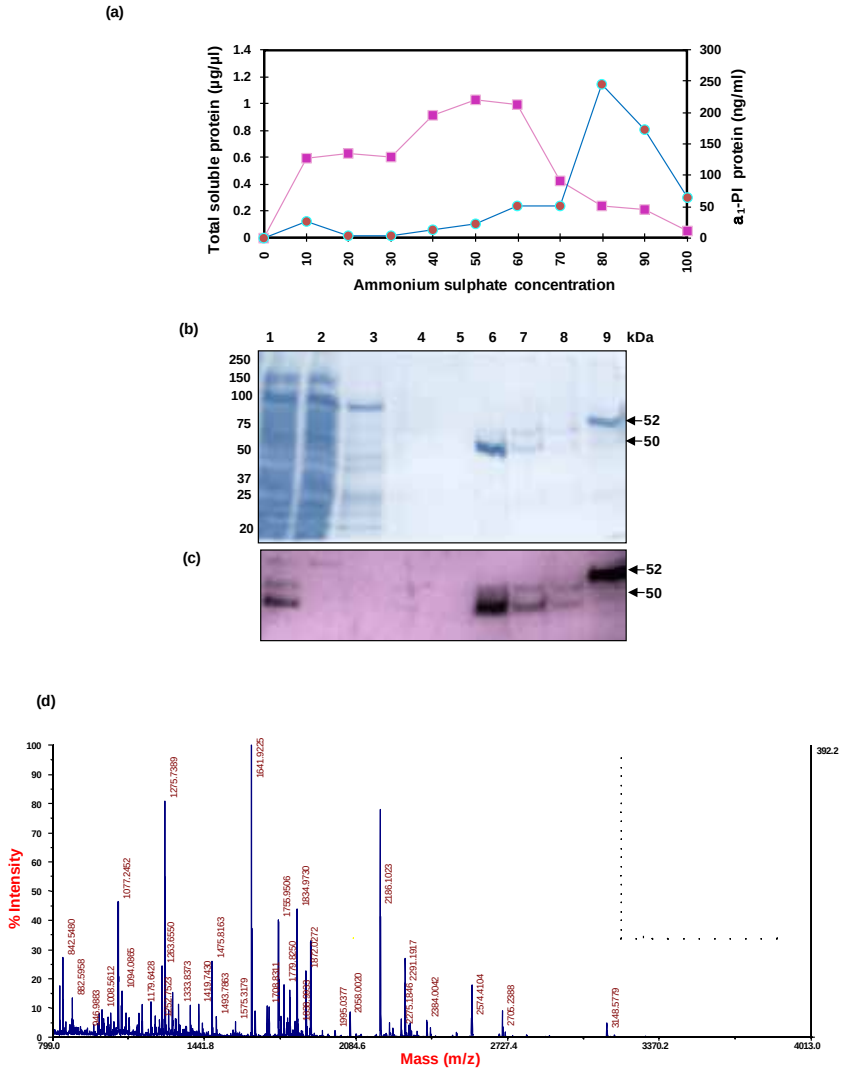


Fig. 6. Purification of recombinant α_1-PI protein from transgenic tomato plants by immunoaffinity chromatography. (a) Ammonium sulphate precipitation of crude protein extract showing amounts of precipitated total soluble protein (■) and recombinant a_1-PI protein (·) at different salt saturations. (b) SDS-PAGE analysis; lane 1, protein sample loaded onto anti-α_1-PI antibody column after ammonium sulphate fractionation at 50-95% saturation; lane 2, flow through; lane 3, 4, 5, wash; lane 6, 7,8, pooled elution fractions eluted with high pH sodium carbonate (pH 11.2); lane 9, pure human serum α_1-PI protein as positive control. (c) Western immunoblotting of the gel (b) with a_1-PI specific antibodies. (d) Identification and characterization of purifiedrecombinant a_1-PI protein by MALDI-TOF/TOF. The observed MS spectrum (peptide mass fingerprint) of the tryptic digests of recombinant a_1-PI protein was obtained in reflector positive ion mode.

Table 1. Segregation analysis of kanamycin resistance gene (*npt*II) in T_1 progeny of transgenic tomato plants.

T_0 transgenic plant ID	Response of seeds on kanamycin selection medium			χ^2 value[a]
	Total	Kan^r	Kan^s	
PAK 13	58	38	20	2.782
PAK 15	28	20	8	0.191
PAK 24	63	46	17	0.132
PAK 25	23	13	10	4.188
PAK 27	41	32	9	0.203
PAK 31	33	24	9	0.091
SWK 1	56	41	15	0.095
SWK 8	46	25	21	10.46
SWK 12	56	36	20	3.43
SWK 23	16	12	4	0.000
SWK 30	38	28	10	0.035
PWK 6	48	29	19	5.44
PWK 16	83	57	26	1.77
PWK 26	32	21	11	1.5
PWK 29	68	45	23	2.816
PWK 34	23	20	3	1.753
WSP 3	18	10	8	3.6
WSP 14	35	27	8	0.086
WSP 29	91	70	21	0.180
WSP 33	39	28	11	0.214
Negative Control	50	0	50	-

[a] $\chi 2$ value at 5 % level of significance = 3.84

IV DISCUSSION

Plant cell cultures and whole plants are currently being investigated as an alternative to microbial, mammalian cell cultures and animals for production of recombinant proteins, including human therapeutic molecules [17, 26]. The major advantages of transgenic plant cells as compared to bacteria, yeast and insect cell lines are post-translational modifications of recombinant proteins, including their glycosylation being similar to those in mammalian cells, agricultural-scale sustainable production and minimal risk of contamination by mammalian pathogens. Stable transgenic plants may offer an alternative approach for the production of safe, stable and biologically active recombinant a_1-PI protein. Low expression of heterologous foreign genes in plants results from codon bias [27], mRNA instability, premature polyadenylation [28] and instability of the expressed recombinant protein encoded by the native transgene.The level of expression and stability of recombinant protein in plants can be influenced by

several other factors like transgene copy number, chromosomal location, *cis*-regulatory elements and final location for accumulation of the protein in plant cells or tissues [29, 30]. Several strategies have been applied to increase yield of recombinant protein in plants including modified flanking sequences, use of specific subcellular targeting signals and development of downstream processing techniques [16, 31, 32]. Protein targeting to specific subcellular compartments is a key factor determining the in-planta stability and yield of the recombinant proteins owing to the biochemical environments of the compartments, available space for protein storage and their protease complement [33]. The sorting of foreign proteins into different subcellular organelles such as ER, apoplast, vacuole or cytosol using the appropriate signal peptides has shown strong impact on protein accumulation and final yield [34, 35, 36, 37]. In this study, recombinant α_1-PI was targeted to cytosol, ER, apoplast and vacuoles to evaluate accumulation, biological activity and stability of the protein in each compartment. The average yield of recombinant α_1-PI protein in leaves of transgenic lines developed with different vectors ranged between 1.55 to 3.2% of TSP except for cytosol targeted protein where significantly lower yield was obtained. This may be attributed to the negative redox potential of cytosolic milieu, which is unfavorable for disulfide bond formation and correct folding, absence of proper glycosylation and the action of ubiquitin-proteasome proteolytic pathway [38]. Maximum sequestration and accumulation of active α_1-PI protein was achieved with substitution of PR1a signal sequence and KDEL as reported earlier for other heterologous proteins in plants [39, 40, 41]. ER lumen provides a large space for accumulation of foreign proteins, relatively protective oxidizing environment favorable for disulfide bond formation, molecular chaperones for correct protein folding and low abundance of proteolytic enzymes. In order to enhance translational efficiency, a viral leader sequence in tandem combination of translation initiation context was introduced at the upstream of the modified gene [14]. The lytic vacuoles in leaves have high proteolytic content and acidic environment; therefore, they are not considered as a suitable destination for recombinant proteins [42].

It is well known that plasma derived α_1-PI inhibits neutrophil elastase with the formation of stable complex in an equimolar ratio [43]. Results from porcine pancreatic elastase inhibitory activity assay clearly demonstrated the formation of stable complex of recombinant α_1-PI with elastase and inhibition of elastase activity similar to that of plasma-derived a_1-PI. The genetic analysis of T_1 population of transgenic plants has shown inheritance and segregation of transgene in a typical Mendelian pattern, except for some T_0 plants. This may be due to random integration and some possible rearrangement of the transgene during the integration in the primary transformants.

Earlier expression and secretion of recombinant α_1-PI protein was demonstrated in genetically transformed rice cell suspension cultures [8, 18] but the C-terminal region of the protein was truncated that resulted in a lower biological activity and yield. Although recombinant a_1-PI was expressed with very high expression levels in transgenic tobacco chloroplasts [21], the protein was unglycosylated and less stable. Plesha *et al*. [20] and Huang *et al*. [10] had also transiently expressed the recombinant a_1-PI in tobacco leaves or suspension using chemically-inducible cucumber mosaic virus amplicon system, but observed micro-heterogeneity in the expressed protein. The recombinant α_1-PI protein expressed in rice and tobacco suspension cultures showed several bands of different molecular weights that reacted with rabbit anti- α_1-PI antibody and the amount of active α_1-PI was only 10–20% of the total α_1-PI protein expressed [10]. Such micro-heterogeneity in the size of the recombinant α_1-PI protein expressed in tomato plants was not observed and only a single band of around ~50 kDa was cross-reacted with anti-

α_1-PI antibody. This advocates the use of stable transgenic plants for sustainable expression of human therapeutic proteins, including α_1-PI, in preference to production by plant suspension culture or transient systems where expression is significantly affected by several factors and needs extensive optimization for competitive cost effective production [44].

Isolation and purification of recombinant protein from plants is a difficult task, owing to the complexity of the plant system compared to bacterial or yeast systems. The purification of recombinant a_1-PI from transgenic rice cell cultures had been described earlier by [8], but they could not achieve pure homogenous preparation of the protein. Their procedure involved a series of complex and cumbersome chromatography steps and several columns resulting in loss of the target protein in each step beside the increased manufacturing cost. In the present study, immunoaffinity chromatography in combination with ammonium sulphate precipitation was employed to obtain the purified recombinant a_1-PI from leaves of transgenic tomato plants. It is well established that affinity chromatography is a powerful purification method, which takes advantage of highly specific binding affinity of the target protein with an immobilized ligand that reduces the number of purification steps and increases the purity of the isolated product [45]. This technique allowed the purification of recombinant a_1-PI protein in one simple, rapid, convenient and cost-effective step unlike those employed earlier [8]. The eluted protein showed the electrophoretic mobility and other properties similar to those of the reference human serum a_1-PI, suggesting no major physicochemical changes occurred during the purification procedure. Moreover, the a_1-PI protein purified from immunoaffinity column showed higher yield, purity, homogeneity and specific activity as evident from SDS-PAGE,Western immunoblotting and mass spectrometric analysis.The results showed a single band of anticipated molecular mass (~50 kDa) for plant-expressed a_1-PI in comparison to serum purified a_1-PI (~52 kDa) reflecting differential patterns of glycosylation in both systems, particularly in the terminal galactosylation and sialylation [46]. Taken together, this method has the potential to be scaled up for obtaining purified homogenous preparations of recombinant a_1-PI protein from transgenic plants.

V CONCLUSION

This study has shown the feasibility to express and purifya clinically important human serine protease inhibitor, a_1-PI in transgenic tomato plants from the modified and codon-optimized gene. The significance of different 5' and 3' regulatory sequences flanking the modified gene, and protein sorting to different subcellular compartments for higher yield and stability have also demonstrated. Studies are now required to assess the *in vivo* stability and pharmacokinetic behaviour of the recombinant a_1-PI expressed in plants, and possibility of engineering the protein for humanized glycosylation for therapeutic applications [47].

VI ACKNOWLEDGEMENTS

We are grateful to Director, CSIR-NBRI, Lucknow for providing infrastructural support. We thankfully acknowledge Council of Scientific and Industrial Research (C.S.I.R.), New Delhi, India for providing funds and senior research fellowships to SJ. This work was carried out under the CSIR-NBRI In-house Project OLP 0031.

REFERENCES

[1] J. Travis, Structure, function, and control of neutrophil proteinases, *American Journal of Medicine, 84, 1988, 37-42.*

[2] C.A. Blank, and M. Brantley, Clinical features and molecular characteristics of α-1-antitrypsin deficiency, *Annals of Allergy, 72, 1994, 105-121.*

[3] E. Karnaukhova, Y. Ophir, and B. Golding, Recombinant human alpha-1 proteinase inhibitor: towards therapeutic use, *Amino Acids, 30, 2006, 317-332.*

[4] S. Jha, S. Agarwal, I. Sanyal, V.Jain, and D.V. Amla, Over-expression of human serum alpha-1-protease inhibitor (α_1-PI) in alternate hosts for therapeutic applications, *Journal of Surgical Sciences, 1(1), 2010, 31-37.*

[5] S. Jha, S. Agarwal, I. Sanyal, and D.V. Amla, Differential subcellular targeting of recombinant human α_1-proteinase inhibitor influences yield, biological activity and *in planta* stability of the protein in transgenic tomato plants, *Plant Science 196, 2012, 53-66.*

[6] S. Mishra, S. Jha, R. Singh, S. Chaudhary, I. Sanyal, and D.V. Amla, Transgenic chickpea expressing recombinant human α_1-proteinase inhibitor (α_1-PI) driven by a seed-specific promoter from the common bean *Phaseolus vulgaris* (L.), *Plant Cell Tissue & Organ Culture, 115, 2013, 23-33.*

[7] S. Jha, I. Sanyal, and D.V. Amla, Single amino acid substitutions in recombinant plant-derived human α_1-proteinase inhibitor confer enhanced stability and functional efficacy, *Biochimica et Biophysica Acta, 1840, 2014, 416-427.*

[8] J. Huang, T.D. Sutliff, L. Wu, S. Nandi, K. Benge, M. Terashima, A.H. Ralston, W. Drohan, N. Huang, and R.L. Rodriguez, Expression and purification of functional human α-1-antitrypsin from cultured plant cells, *Biotechnology Progress 17, 2001, 126-133.*

[9] T.-K. Huang, M.A. Plesha, B.W. Falk, A.M. Dandekar, and K.A. McDonald, Bioreactor strategies for improving production yield and functionally of a recombinant human protein in transgenic tobacco cell cultures, *Biotechnology and Bioengineering, 102, 2009, 508-520.*

[10] T.-K. Huang, M.A. Plesha, K.A. McDonald, Semicontinuous bioreactor production of a recombinant human therapeutic protein using a chemically inducible viral amplicon expression system in transgenic plant cell suspension cultures, *Biotechnology and Bioengineering, 106, 2010, 408-421.*

[11] R. Bischoff, D. Speck, P. Lepage, L. Delatre, C. Ledoux, S.W. Brown, and C. Roitsch, Purification and biochemical characterization of recombinant α-1-antitrypsin variants expressed in *Escherichia coli*, *Biochemistry, 30, 1991, 3464-3472.*

[12] K.-S. Kwon, M. Song, and M.-H. Yu, Purification and characterization of α-1-antitrypsin secreted by recombinant yeast *Saccharomyces diastaticus, Journal of Biotechnology, 42, 1995, 191-195.*

[13] I.M. Tamer, and Y. Chisti, Production and recovery of recombinant protease inhibitor α-l-antitrypsin, *Enzyme Microbial Technology, 29, 2001, 611-620.*

[14] S. Agarwal, S. Jha, I. Sanyal, D.V. Amla, Expression and purification of recombinant human α_1-proteinase inhibitor and its single amino acid substituted variants in *Escherichia coli* for enhanced stability and biological activity, *Journal of Biotechnology, 147, 2010, 64-72.*

[15] L.T. Spencer, J.E. Humphries, and M.L. Brantly, Antibody response to aerosolized transgenic human alpha1-antitrypsin, *The New England Journal of Medicine, 352, 2005, 2030-2031*.

[16] S.J. Streatfield, Approaches to achieve high-level heterologous protein production in plants, *Plant Biotechnology Journal, 5, 2007, 2-15*.

[17] S.R. Karg, and P.T. Kallio, The production of biopharmaceuticals in plant systems, *Biotechnology Advances, 27, 2009, 879-894*.

[18] M. Terashima, Y. Murai, M. Kawanura, S. Nakanishi, T. Stoltz, L. Chen, W. Drohan, R.L. Rodriguez, and S. Katoh, Production of functional human α-1-antitrypsin by plant cell culture, *Applied Microbiology and Biotechnology, 52, 1999, 516-523*.

[19] M.R. Sudarshana, M.A. Plesha, S.L. Uratsu, B.W. Falk, A.M. Dandekar, T.-K. Huang, and K.A. McDonald, A chemically inducible cucumber mosaic virus amplicon system for expression of heterologous proteins in plant tissues, *Plant Biotechnology Journal, 4, 2006, 551-559*.

[20] M.A. Plesha, T.-K. Huang, A.M. Dandekar, B.W. Falk, and K.A. McDonald, High-level transient production of a heterologous protein in plants by optimizing induction of a chemically inducible viral amplicon expression system, *Biotechnology Progress, 23, 2007, 1277-1285*.

[21] M. Nadai, J. Bally, M. Vitel, C. Job, G. Tissot, J. Botterman, and M. Dubald, High-level expression of active human alpha-1-antitrypsin in transgenic tobacco chloroplasts, *Transgenic Research, 18, 2009, 173-178*.

[22] S. McCormick, J. Niedermeyer, A. Branson, H. Horsch, R. Fraley, Leaf disc transformation of cultivated tomato (*L. esculentum*) using *Agrobacterium tumefaciens*, *Plant Cell Report, 5, 1986, 81-84.*

[23] J. Sambrook, and D.W. Russell, *Molecular cloning: a laboratory manual* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 2001).

[24] S. Agarwal, S. Jha, I. Sanyal, D.V. Amla, Effect of point mutations in translation initiation context on the expression of recombinant human a_1-proteinase inhibitor in transgenic tomato plants, *Plant Cell Report, 28, 2009, 1791-1798.*

[25] A. Shevchenko, H. Tomas, J. Havlis, J.V. Olsen, M. Mann, In-gel digestion for mass spectrometric characterization of proteins and proteomes, *Nature Protocol, 1, 2006, 2856-2860.*

[26] J.K. Ma, P.M.W. Drake, and P. Christou, The production of recombinant pharmaceutical proteins in plants, *Nature Reviews Genetics, 4, 2003, 794-805*.

[27] C. Gustafsson, S. Govindarajan, and J. Minshull, Codon bias and heterologous protein expression, *Trends in Biotechnology, 22, 2004, 346-353*.

[28] P. Jarvis, F. Belzile, and C. Dean, Insufficient and incorrect processing of the Ac transposase transcript in iae1 and wild-type *Arabidopsis thaliana, Plant Journal, 11, 1997, 921-931*.

[29] R.M. Twyman, E. Stoger, S. Schillberg, P. Christou, and R. Fischer, Molecular farming in plants: host systems and expression technology, *Trends in Biotechnology, 21, 570-578, 2003*.

[30] P.M. Doran, Foreign protein degradation and instability in plants and tissue cultures, *Trends in Biotechnology, 24, 2006, 426-432*.

[31] T.J. Menkhaus, Y. Bai, C. Zhang, Z.L. Nikolv, and C.E. Glatz, Considerations for the recovery of recombinant proteins from plants, *Biotechnology Progress, 20, 2004, 1001-1014*.

[32] M. Benchabane, C. Goulet, D. Rivard, L. Faye, V. Gomord, and D. Michaud, Preventing unintended proteolysis in plant protein biofactories, *Plant Biotechnology Journal 6, 2008, 633-648*.

[33] J. Callis, Regulation of protein degradation, *Plant Cell, 7, 1995, 845-857*.

[34] N.S. Outchkourov, B. Rogelj, B. Strukelj, and M.A. Jongsma, Expression of sea anemone equistatin in potato: effect of plant proteases on heterologous protein production, *Plant Physiology, 133, 2003, 379-390*.

[35] M. Gils, R. Kandzia, S. Marillonnet, V. Klimyuk, and Y. Gleba, High-yield production of authentic human growth hormone using a plant virus-based expression system, *Plant Biotechnology Journal, 3, 2005, 613-620*.

[36] J. Yang, L.A. Barr, S.R. Fahnestock, and Z.B. Liu, High yield recombinant silk-like protein production in transgenic plants through protein targeting, *Transgenic Research, 14, 2005, 313-324*.

[37] S. Petruccelli, M.S. Otegui, F. Lareu, O.T. Dinh, A.C. Fitchette, A. Circosta, A KDEL-tagged monoclonal antibody is efficiently retained in the endoplasmic reticulum in leaves, but is both partially secreted and stored to protein storage vacuoles in seed, *Plant Biotechnology Journal, 4, 2006, 511-527*.

[38] R.D. Vierstra, The ubiquitin/26S proteasome pathway, the complex last chapter in the life of many plant proteins, *Trends in Plant Science, 2003, 135-142*.

[39] A. Schouten, J. Roosien, F.A. van Engelen, G.A. de Jong, A.W. Borst-Vrenssen, J.F. Zilverentant, D. Bosch, W.J. Stiekema, F.J. Gommers, A. Schots, and J. Bakker, The C-terminal KDEL sequence increases the expression level of a single-chain antibody designed to be targeted to both the cytosol and the secretory pathway in transgenic tobacco, *Plant Molecular Biology, 30, 1996, 781-793*.

[40] T.-J. Kang, N.-H. Loc, M.-O. Jang, and M.-S. Yang, Modification in cholera toxin B subunit coding sequence to enhance expression in plants, *Molecular Breeding, 13, 2004, 143-153*.

[41] D. Li, , J.O. Leary, Y. Huang, N.P.A. Huner, A.M. Jevnikar, and S. Ma, Expression of cholera toxin B subunit and the B chain of human insulin as a fusion protein in transgenic tobacco plants, *Plant Cell Report, 25, 2006, 417-424*.

[42] C. Goulet, and D. Michaud, *Degradation and stabilization of recombinant proteins in plants* (In: Floriculture, Ornamental and Plant Biotechnology, Vol. IV (Taxeira de Silva, J., ed.), 35-40. London: Global Science Books, 2006).

[43] J. Travis, M. Owen, P. George, R. Carrell, S. Rosenberg, R.A. Hallewell, and P.J. Barr, Isolation and properties of recombinant DNA produced variants of human α-l-proteinase inhibitor, *Journal of Biological Chemistry, 260, 1985, 4384-4389*.

[44] A.K. Sharma, and M.K. Sharma, Plants as bioreactors: recent developments and emerging opportunities, *Biotechnology Advances, 27, 2009, 811-832*.

[45] M. Sugiura, S. Hayakawa, T. Adachi, Y. Ito, K. Hirano, and S. Sawaki, A simple one-step purification of human α_1-proteinase inhibitor by immunoadsorbent column chromatography, *Journal of Biochemical and Biophysical Methods, 5, 1981, 243-249.*

[46] E. Stoger, J.K. Ma, R. Fischer, and P. Christou, Sowing the seeds of success: pharmaceutical proteins from plants, *Current Opinion in Biotechnology, 16, 2005, 167-173.*

[47] A. Castilho, R. Strasser, J. Stadlmann, J. Grass, J. Jez, P. Gattinger, R. Kunert, H. Quendler, M. Pabst, R. Leonard, F. Altmann, and H. Steinkellner, *In planta* protein sialylation through overexpression of the

respective mammalian pathway, *Journal of Biological Chemistry, 285, 2010, 15923-15930.*

A NOVELONTOLOGY BASED FRAMEWORK FORMAPPING RESEARCH PAPER TITLES TO TOPIC DOMAINS

Swaraj K P[1] , Dr. Manjula D[2], Adhithyan V[3], Manish Kumar L[4], Hemnath K B[5]

[1,2,3,4,5] *Department of Computer Science and Engineering, College of Engineering, Guindy Anna University (India)*

ABSTRACT

Domain based classifications of research articles published is a laborious task which is usually done manually.Because of the enormous amount of journal articles published in various domains, an automated mapping technique is the need of the hour. In this article, afast novel ontology based approach is proposed to automatically map research articles to their domains. This will help to quickly ascertain the domain of each research article and also to unravel the trending topics and the number of articles published in each domain in a particular time span. . Last 3 years of DBLP bibliographic dataset is used in this study. Hadoop map reduce technique is used to speed up the keyword extraction and subsequent ontology mapping process.Experiment studies revealed that the proposed ontology based research article to topic mapping technique framework is accurate, efficient and scalable.

Keywords: Trend detection, Topic detection, Classification, DBLP, Search, Ranking

I. INTRODUCTION

The field of computer science has been evolving and this fact is underscored by the ever increasing sub domains in the Computer Science and Engineering domain.Wide range of research and academic activities are occurring in each of these sub domains on a daily basis. This undeniable fact is emphasized by a countless numbers of technological advancements and discoveries in this domain.A large number of research articles are published online relating to these day to day developments in this fascinating field. Downloading majority of these articles and classifying it into domains and sub domains is a herculean and gruelling task. Currently, there is no well-known system to detect domain topics from research paper titles. This paper proposes a suitable framework for the same.

DBLP is a bibliographic dataset which provides metadata about computer science publicationsin xml format, hosted at University of Trier, in Germany.This bibliographic dataset is utilized by researchers world-wide for getting info about the range of articles published in various topics in computer science under various categories such as articles, journals, conference proceedings and so on. [6].This dataset spans a wide range of computer science topics such as Computer Networks, Image Processing, Graphics, Artificial Intelligence, Databases, Data Mining, Cloud computing, Information Extraction and so forth and has meta data of more than 2.5 million articles which can be further classified into several sub-topics, sub-sub-topics and so on. This xml dataset has lots of information which entices researchers to deeply delve into it to unravel interesting statistics from it. For

example, analysis of dataset for a particular time interval, say most recent 12 months reveals that; there will be set of certain topics in computer science which will have more significant number of publications depicting its academic interest than other sub domains. Certain topics will be showing emergent trends with steadily increasing number of articles.The major problem with this dataset is that it doesn't provide mapping of article titles to various topics in computer science discipline .Hence to find the relevant topic of a particular research article is very time consuming task. Since the data set is of very huge size manually classifying is almost impossible. The novel framework proposed in this article is an accurate solution to this problem. It employs computer science domain ontology along with support ontology to classify research paper titles in dynamically updated bibliographic dataset into respective topics which greatly enhances the accuracy and efficiency. ACM Computer Classification System Domain ontology which is well-known domain ontology has been used in this framework. This ontology is mainly used as an information model encompassing various hierarchical relationships between topics in computer science domain for this mapping.

Map Reduce is anopen source software framework to quickly process bigsemi structured and unstructured data in parallel across a distributed cluster of commodity computersystems. Map reduce libraries have been made available for wide range of platforms taking into consideration its huge popularity across users.The distributed cluster is made possible by hadoop framework which is an open source project of Apache Foundation.. The proposed framework utilizes hadoop architecture and map-reduce algorithms to greatly enhance the speed while detecting keywords and phrases from titles for quickly mapping titles to ontology topics.

The rest of the paper is organized as follows. Section 2 highlightsthe related works. Section 3 describes in detail on the novel framework used for the mapping of article titles based on domain ontology Section 4 discusses the various map reduce algorithms used. Section 5 analyses the experimental results and Section 6 concludes with future research directions.

II. RELATED WORK

Keywords are generally used for classification. Closed frequent keyword set [2] is generally usedinidentifying topics rather than maximal frequent keyword-set. The semantic information present in titles is preserved for accurate mapping to domain ontology. Clusteringand classifying documents based on frequent itemsets [3] has been studied in the algorithms FTC and HFTC [4] and the Apriori-based algorithm [5]. All of these works consider the documents in vector space model. Hence documents are represented as bags of words and then find frequent itemsets. The major lacuna of bag of words techniques or vector space model is that the relative ordering of terms cannot be maintained. As a result of this the semantic information which is present is lost.In this proposed framework, phrases from the titles of the research papers are extracted first and frequent substrings are derived as frequent keyword-sets,maintaining the underlying semantics which will help to accurately map titles to topics in domain ontology.

III. PROPOSED SYSTEM

The steps for proposed framework are listed below.

1. DATASET used is DBLP. At first DOM parser is used to extract titles from xml file
2. These titles are then stored in a file.
3. Phrases and keywords are then extracted from file using map reduce and generic methods.

4. ACM classification ontology 2012(topic-subtopic relation) is used as domain ontology. We are considering only certain topics in this study. Hence a part of this ontology iscreated using Protégé software and stored in owl format.
5. Then the keywords are mapped with titles[1].
6. The titles are then mapped with ACM classification ontology using the semantics preserved n-gram keywords.
7. sparql query with apache-jenaapi is used in java to map titles to topics.

The build system has following features:

1. Domain ontology is used to map tiles to topics.
2. Semantics of keywords in titles is preserved to accurately map titles to domain topics with high precision and accuracy

[1]*(map reduce is used to analyze performance metrics of normal approach with map-reduce based approach)*

The proposed system has the following phases:

1. Pre-processing-This phase includes title extraction, phrase detection, keyword formation using map-reduce and without map-reduce from xml dataset,
2. Mapping of n-gram keyword in titles to ontology.

3.1 Pre-processing

3.1.1 Title Extraction

DBLP is a computer science Bibliography dataset which contains the metadata of over 2.8 million publications. It is actually a collection of metadata of articlespublished by many authors in several thousands of journals or conferences. It is an xml file having a root tag and various sub tags which contains all bibliographic records. The popular DOM parser was used to extract titles and year from xml dblp dataset. The extracted titles and year will be indexed and stored in file. Also, the xml dataset was processed using map-reduce framework for improving and comparing performance metrics.

3.1.2 Title Extraction – Map Reduce

Map-reduce framework processes large dataset in parallel and efficient manner. Mapper function will take the input file and a set of titles is given as output based on year. The output of mapper function is stored in a file. Map function will allocate the number of map tasks based on the number of input records in the xml records.

XML Configuration	**Title Extraction using Map Reduce**
1. *Specify Input format class* 2. *Define start tag and end tag to be parsed* 3. *Specify i/p path and o/p path* 4. *Specify all required classes*	**Input to Map Function:** DBLP xml dataset 1) *Create a Java - DOM XML parser* 2) *Get elements by tag name* 3) *For each node:* i) *retrieve Titles based on the year*

3.1.3 Phrase detection and Keyword formation

From each title, phrases are figured out. A phrase can be defined as a substring present between two stopwords which will be meaningful.Around 114 Standard English stopwords have been used to derive phrases. A keyword

is a substring of phraseand is derived in such a way that the underlying semantics is preserved.The keywords both 1-gram and bi-gramextracted will be associated with title in file.Similar to that of Title Extraction,Map-reduce is used to extract phrases and keywords.

Phrase Extraction using Map Reduce	**Keyword set Formation using Map Reduce**
Input to Map Function: Extracted Titles and Stop words *Declare hash map<string, integer>* *Declare string temp, phrase* *Read stop words and store it in hash map* *for each title:* *Iterate until a space* *Store iterated word in temp* *Check whether temp is in hash map* *if temp is in hash map* *eliminate temp* *store phrase* *else* *phrase += temp* *temp = “”*	**Input to Map Function:** Extracted Phrases *Declare string keyword* *for each phrases:* *separate phrases by space* *for each word:* *keyword = word* *store keyword*

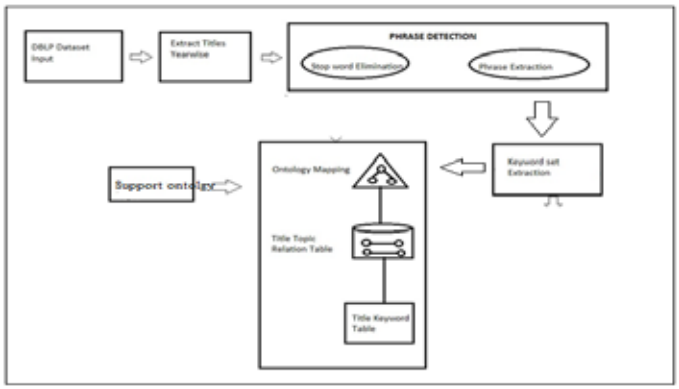


Figure.1 Architecture of proposed system

Example:

Title: DDBJ new system and service refactoring.

Year: 2012

Phrases: DDBJ new system, service refactoring

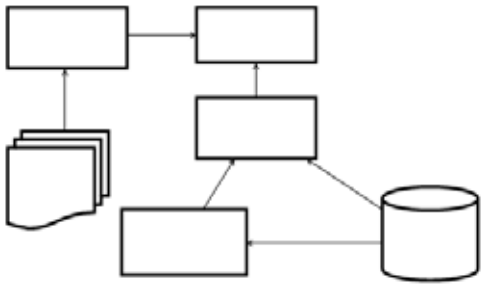
Keywords (one gram): DDBJ, new, system, service,refactoring

Keywords (two gram): DDBJ new, new system, system service, system refactoring.

Thus each keyword extracted will have potential to identify a topic or subtopic.

3.1.4 Title Keywords to Ontology Mapping

Using apache jenaapi with java, sparql query can be used to query ontology owl file.Asparqlquery is used to obtain the classification hierarchy and the result is stored in database. After storing of ontology, keywords of the titles are mapped with topic and subtopics of ontology based on a ranking function.



Procedure 1:

Keyword (both onegram and twogram) to paper mapping

map = all frequent keywords from database

for each title in database: keywords[] = get corresponding keyword of title index = index no of title from databasefor each keyword: if keyword found in map append(keyword, title)

IV. PERFORMANCE

In the pre-processing stage we have used 6 node hadoop cluster and map reduce algorithms along with normal java based approach up to keyword formation for calculating performance metrics.

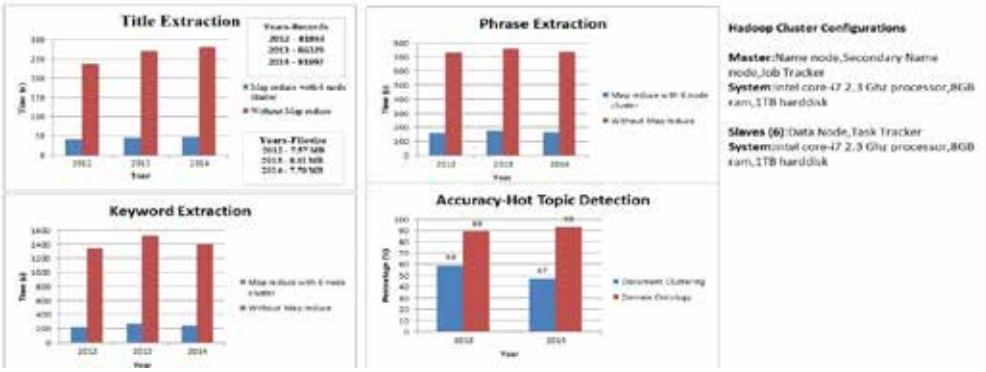


Figure.4 Performance during title extraction, phrase and keyword extraction

V. CONCLUSION

In this work we focused on a novel ontology based approach to quickly identify the domain topic of a research article based on domain ontology. We pre-processed the titles from DBLP dataset using hadoop map reduce and converted it into keywords which are then used to map titles to ontology. ACM ontology helps to accurately map a title with a topic. This framework aids budding researchers inquickly finding domain topic of a research article. This work can be extended to find trending topics, subtopics and retrieve paper titles under the researcher's area of interest with high precision.

REFERENCES

[1]. Kumar Shubankar, AdityaPratap Singh, VikramPudi ,"A FrequentKeyword-Set Based Algorithm for Topic Modeling and Clustering of Research Papers", IEEE ICDM ,2013

[2]. N. Pasquier, Y. Bastide, R. Taoull, and L. Lakhal, "Efficient Mining of Association Rules Using Closed Itemset Lattices," Information Systems, 1999

[3] R. Agarwal, and R. Srikant, "Fast Algorithms for Mining Association Rules," Proceedings of the 20th VLDB Conference, 1994.

[4] F. Beil, M. Ester, and X. Xu, "Frequent Term-Based Text Clustering," Proceeding of the 8th International Conference on Knowledge Discovery and Data Mining (KDD), 2002.

[5] S. M. Krishna, and S. D. Bhavani, "An Efficient Approach for Text Clustering Based on Frequent Itemsets," European Journal of Scientific Research, 2010.

[6] Ley M.: The DBLP Computer Science Bibliography:Evolution, Research Issues, Perspectives. In: Proceedings of the 9th International Symposium on String Processing and Information Retrieval, Springer-Verlag London, UK, 1–10.(2002)

A REVIEW ON ENHANCEMENT OF AN IMAGE SING IMAGE DEHAZING AND FILTERING TECHNIQUES

Vinkey Sahu[1], Manjusha Singh[2]

[1]*Department of Computer Science Engineering, C.S.I.T, Durg, Chhattisgarh (India)*

[2] *Asst. Professor, Department of Computer Science Engineering, C.S.I.T, Durg, Chhattisgarh (India)*

ABSTRACT

Images are often degraded by the atmospheric haze, a phenomenon due to the particles in the air get scatter light. Haze induces a loss of contrast, its visual effect is blurring of distant object. Here in this paper an approach to remove the effect of the haze and noise from the input hazy images is being presented. Here two different techniques for enhancing the quality of an image are being presented. First one is the Single Image Dehazing and second one is the filtering techniques. Both the methods treats haze and noise separately, i.e. image dehazing is used for removing the haziness and filtering techniques is used for removal of noise and sharpness enhancement.

Keywords - Dehazing, Filtering, Dark Channel Prior, Guided Joint Bilateral Filter.

I. INTRODUCTION

Image enhancement is the process of enhancing the features of a digitally stored image by manipulating the image with the software. The principle objective of enhancement is to process an image so that the result is more suitable then the original image for specific application. It accentuates or sharpens image features such as edges, boundaries or contrast to make a graphic display more helpful for display and analysis. The enhancement doesn't increase the inherent information content of the data, but it increases the dynamic range of the chosen features so that they can be detected easily. The greatest difficulty in image enhancement is quantifying the criterion for enhancement and therefore a large number of image enhancement techniques are empirical and require interactive procedures to obtain satisfactory results.

This papers deals with the enhancement of the foggy images by eliminating the fogginess and noise from that image. Here two different techniques are being used for improving the quality and visibility of the foggy images. They are as follows:

1.1 Image Dehazing

Image captured in foggy weather condition often suffers from the bad visibility. Whenever images are captured outdoors, haze tends to adversely impact the quality of the background. More specifically weather and other atmospheric phenomena such as haze greatly reduce the visibility of the distant regions in images of outdoor scenes. Haze tends to adversely affect the quality of the image resulting in the poor visibility, low contrast etc. Haze along with the fog and clouds are limiting factors for visual range in the atmosphere and heavily reduce contrast in the scenes.

Image dehazing is a highly interdisciplinary challenge involving optical physics as well as computer vision and computer graphics. Haze along with the fog and clouds are limiting factors for visual range in the atmosphere and heavily reduce the contrast in the scenes. The principle objective of the image dehazing is improvement of the visibility and recovery of the colors, as if imaging is done in clear conditions. The term dehazing mean to produce an image of a scene that does not contain haze effects, although the source of that image originally comprised haze.

Manipulating a digital image to remove the effect of the haze is termed as image dehazing. In order to solve such problem image dehazing is applied. Image dehazing is the process of eliminating the haziness from an image that is shot under either in foggy weather condition or any other obstacles in the air that destroy the clarity of the image. Manipulating a digital image to remove the effect of the haze is termed as image dehazing. The main aim here is to find a way to separate the haze content from the actual image content and then subtract that haze part from the image to end up with an original clear image.

1.2 Image Filtering

Images are often corrupted by the random variations in the intensity, illumination or have poor contrast. Image filtering is the process of enhancing or modifying the quality of the image by transforming the pixel intensity values to reveal certain characteristics in image. Image processing operations implemented with filtering includes smoothening, sharpening, edge enhancement and template detection.

Filtering is a key step in digital image processing and analysis. It is mainly used for amplification or attenuation of some frequencies depending on the nature of the application. Filtering can either be performed in the spatial domain or in a transformed domain. The selection of the filtering method, filtering domain, and the filter parameters are often driven by the properties of the underlying image. Filtering is generally used for image enhancement. Filtering can also be used for analysis.

II. EASE OF USE

The method implemented in this research paper will provide a proper solution for the haze and noise removal. Image dehazing techniques is used for separating the haziness, along with that filtering mechanism is also being used for enhancing the sharpness and elimination of the noise from that image. It will reduce the effect of the haze caused by the scattering of the light. It will be capable of providing a color correction through the airlight estimate and restoration of the color especially for the distant object will be possible.

III. METHODOLOGY

3.1 Using Guided Joint Bilateral Filter

Xiao in [1] proposed a new fastest method for single image based on filtering. The basic idea is to compute an accurate atmospheric viel that is not only smoother, but also respect with the depth information of the underlying image. Firstly an initial atmospheric scattering light is obtain through median filtering, then refinement is done by using guided joint bilateral filtering to generate a new atmospheric viel which removes the abundant textures information and recovers the depth edge information. Finally the scene radiance using the atmospheric attenuation model is being solved. This method is able to generate a better dehazing effect atdistant scene and places where depth changes abruptly. Furthermore this method can be performed in parallel, therefore

it can further be accelerated using GPU(Graphical User Interface), which makes the method applicable for real-time system. Figure below depicts the result obtain using the guided joint bilateral filter.

(a) **(b)**

In the above fig-1, (a) represents the input image whereas (b) represents the output.

3.2 Denoising and Dehazing

Images of the outdoor scene often contain degradation due to haze, resulting in the contrast reduction and color fading. For many reasons one may need to remove these effects. Haze removal is a difficult problem due to the inherent ambiguity between the haze and the underlying scene. Furthermore all images contain some noise due to sensor error that can be in the haze removal process if ignored.

In this paper [2] Matlin proposed two methods, both for removing haze and noise from an image. Two effective methods are being used for the final scene radiance recovery. The first approach is to denoise an image prior to dehazing. This approach is the adaption of the existing techniques, the dark channel prior, for estimating haze from a single hazy image. This serial approach essentially treats haze and noise separately. Second approach is proposed simultaneously to denoise and dehaze using an iterative, adaptive, non parametric regression methods. Finally the experimental results of both the methods are being compared. Result shows that when the noise level is precisely known, simply denoising prior to dehazing performs well. When the noise level is not given, latent error from either “under” denoising or “over” denoising can be amplified, and in this situation, the iterative approach yields the superior results. The quality of this approach is sensitive to inexact levels of noise. The proposed iterative method proved to be more robust, offering visually comparable results with other methods when the noise level is known and better preserving results when the noise level is estimated. Figure below depicts the example of the proposed methodology.

(a) **(b)** **(c)** **(d)**

In the above fig-2, (a) represents the input image, (b) represents direct Dehazing, (c) represents Denoise + Dehazed & (d) represents proposed method.

3.3 Dehazing: Combining PMB and NPMB Methods.

Zhang in [3] proposed a research algorithm for single image dehazing combining Physics Model Based (PMB) and Non Physics Model Based (NPMB) methods. Firstly based on a newly presented haze-free image prior - Dark Channel Prior and a common haze imaging model, for a single input image. The Dark Channel is used to calculate the atmospheric light. Secondly, Retinex algorithm is being constructed based on the two bilateral filters, which is applied to the brightness of the input hazy image, in order to obtain the new and enhanced brightness image and then get the anti-brightness image of the new brightness image. After that we obtain the transmission through an adaptive median filter. Finally the scene radiance is obtained through the atmospheric scattering model. The success of the physics model based method lies on the prior or assumption. In the non physical model, some algorithms are able to meet the real time requirements, but its performance is not ideal. Therefore it is necessary to combine these two methods, in order to achieve better results. A large number of experiments show that the proposed algorithm has significant effect on dehazing and has better performance in image quality and computational time.

(a) **(b)**

In The Above Fig-3, (A) Represents The Input Image (B) Represents The Output By Combining The PMB &NPMB Methods.

3.4 Dark Channel Prior

Kaiming He in [4] proposed that the dark channel prior method is based on the prior assumption which is basically used for the single image dehazing process. This dark channel prior method is based on the statistics approach of the outdoor haze free images. It has been observed that in most of the regions which do not covered the sky; at that region some pixels are having very low value in at least one color (RGB) of the channel. These pixels are known as dark pixels. In hazy images the intensity of the dark pixels in the colored channel is basically contributed by the airlight. These dark pixels are used to estimate the haze transmission. Thus finally after estimating the transmission map for each pixel, combining it with the haze imaging model and soft matting technique to recover a high quality haze free image.

(a) **(b)**

(c) **(d)**

In The Above Fig-4, (A) Represents The Input Image, (B) Represents The Estimated Transmission Map, (C) Represents The Refined Transmission Map And (D) Represents The Output.

V. CONCLUSION

In this paper we have addressed the problem of simultaneously removing the haze and noise from a single image. Here two different techniques for enhancing the quality of an image are being presented. First one is the Single Image Dehazing and second one is the filtering techniques. Both the methods treats haze and noise separately, i.e. image dehazing is used for removing the haziness and filtering techniques is used for removal of noise and sharpness enhancement.

REFERENCES

Journal Papers

[1] Xiao, C., & Gan, J. (2012). " *Fast image dehazing using guided joint bilateral filter*". *The Visual* Computer, *28(6-8), 713-721*.

[2] Matlin, E., & Milanfar, P. (2012, February). "*Removal of haze and noise from a single image*". In IS&T/SPIE Electronic Imaging (*pp. 82960T-82960T*). International Society for Optics and Photonics.

[3] Zhang, H., Liu, Q., YANG, F., & WU, Y. (2013). "*Single Image Dehazing Combining Physics Model based and Non-physics Model based Methods*" .Journal of Computational Information Systems, *9(4), 1623-1631.*

[4] He, K., Sun, J., & Tang, X. (2011). "*Single image haze removal using dark channel prior*". Pattern Analysis and Machine Intelligence, IEEE Transactions on, *33(12):2341-2353.*

[5] Fattal, R. (2008, August). "*Single image dehazing*". In ACM Transactions on Graphics *(TOG) (Vol. 27, No. 3, p. 72).* ACM.

Books

[1] Rafael C.Gonzalez and Richad E.Woods(2006) "*Digital Image Processing*", second edition , Pearson edition.

BIOGRAPHICAL NOTES

Ms. Vinkey Sahu is presently pursuing M. Tech. final year in Computer Science Engineering Department from C.S.I.T, Durg ,Chhattisgarh, India.

Mrs. Manjusha Singh is working as a Assistant Professor in Computer Science Engineering Department, C.S.I.T, Durg, Chhattisgarh, India.

SPACC – SECURING PERSONAL HEALTH CARE RECORDS USING ABE IN CLOUD COMPUTING

Sunder V[1], Radha.N[2]

[1]PG Scholar, [2]Asst. Professor, Dept. Of CSE, Mahendra Engineering College, (India)

ABSTRACT

Personal Health Record (PHR) is an emerging patient-centric model of health information exchange, which is now being outsourced to third party, such as cloud providers. However, this affects the privacy concern of any individual, since personal health information could be exposed to third party servers where there is a chance for getting accessed by unauthorized parties too. So to assure privacy for patients' control over access to their own PHRs, the best method is to encrypt the PHRs before outsourcing. For this, a novel patient-centric framework is proposed here. With the aid of a semi-trusted server, data access controls to PHRs are stored. Attribute Based Encryption (ABE) techniques is leveraged to encrypt each patient's PHR file to achieve fine-grained and scalable data access control for PHRs. Enabling dynamic modification of access policies or file attributes are also proposed here. On-demand user/attribute revocations are supported along with break-glass access under emergency scenarios.

I. INTRODUCTION

The main goal of this framework is to provide secure patient-centric PHR access and efficient key management at the same time. The key idea is to divide the system into multiple security domains (namely, public domains (PUDs) and personal domains (PSDs)) according to the different users data access requirements. The PUDs consist of users who make access based on their professional roles, such as doctors, nurses and medical researchers. In practice, a PUD can be mapped to an independent sector in the society, such as the health care, government or insurance sector. For each PSD, its users are personally associated with a data owner (such as family members or close friends), and they make accesses to PHRs based on access rights assigned by the owner.

1.1 What is E-Health cloud?

Modern information technology is increasingly used in healthcare with the goal to improve and enhance medical services and to reduce costs. In this context, the outsourcing of computation and storage resources to general IT providers (cloud computing) has become very appealing. E-health clouds over new possibilities, such as easy and ubiquitous access to medical data, and opportunities for new business models. However, they also bear new risks and raise challenges with respect to security and privacy aspects.

1.2 Model of the E-Health Cloud

In the past, health care providers (such as the family doctor) have stored medical records of their patients on paper locally. This allowed a controlled environment with easy management of data privacy and security:

keeping the paper records in a locked cabin at the doctor's practice. Even the increasing use of personal computers and modern information technology in medical institutions allowed for a moderate effort to manage privacy and confidentiality of individual medical records. This was due to the decentralized and locally managed infrastructure of each institution.

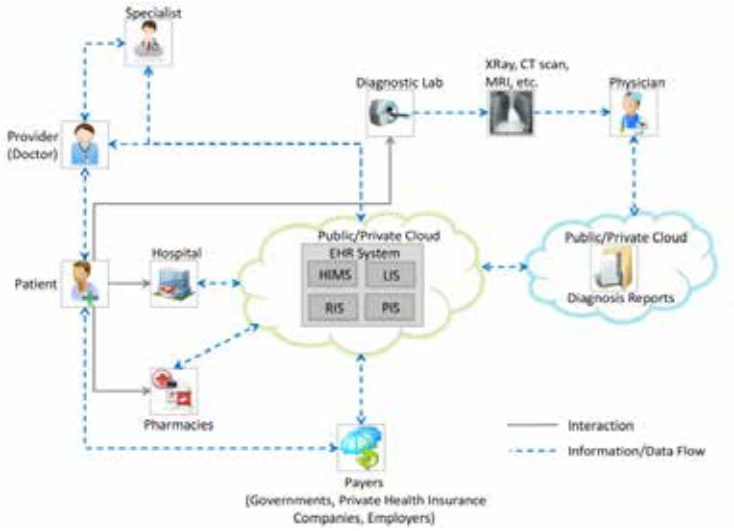


Fig 1: A simple model of e-health cloud

1.3 Advanced E-Health Cloud Infrastructure

In contrast to PHRs, which are managed by the patients, Electronic Health Records (EHR) are managed by health professionals only. In most countries this involves different legal requirements and a clear distinction between PHRs and EHRs. As a result, infrastructures that involve EHRs are usually more complex than the simple e-health cloud model. The general requirement in this model is still the functional and semantic interoperability of the data stored in EHRs. The EHRs are created, maintained, and managed by health care providers, and can be shared (via the central EHR server in the cloud) with other health professionals. But storing and processing EHRs is not the only service that can be outsourced to the cloud. The health care providers can use billing services that manage their billing and accounting with the health insurances of the patients. This is a typical scenario that can be found in practice: Many doctors outsource the billing to third party providers. Those billing services accumulate the billing of several patients for different health insurances, but also for various health care providers at the same time. As a consequence, privacy becomes an even more important aspect in this model because health insurances or billing services should not be able to access private details of EHRs.

To protect the EHR data, smartcards are typically used to:

(1) Authenticate health professionals and patients,

(2) sign EHR documents to provide authenticity,

(3) Encrypt the EHR data before they are stored in the cloud, and

(4) Authorize the access to EHR data.

Data and services of the e-health cloud can only be accessed with special interface connections to the telematics infrastructure boundary. This interface connection is typically a special hardware device that establishes secure network connections via a Virtual Private Network (VPN) to the e-health data centers. Due to the increased privacy requirements, many countries define standards and specifications for national e-health infrastructures that include technical means for security and privacy. However, existing security concepts in e-health

concentrate on controlling access to data (e.g., smartcard-based access control to web-based PHRs and EHRs), protection of data transfer (encryption for confidentiality, digital signatures for integrity and authenticity), and network security (firewalls, VPNs). The latter focuses on the separation of different networks, e.g., administrative networks of health insurances from EHR servers and from other applications. However, little care is taken on what happens after access to data is allowed, i.e., how data is processed and stored on end-user client platforms. Viruses or Trojan horse programs can corrupt data and eavesdrop on patients' records, violating both legal and individual privacy requirements.

II. SYSTEM STUDY

E-health provides various advantages in the current scenario.

The existing method of handling PHR is using hardcopies or non secure way of storing as softcopy.

- Due to the high cost of building and maintain specialized data centers, many PHR services are outsourced to or provided by third-party service providers ,for example ,Microsoft Health Vault.
- The main concern is about whether the patients could actually control the sharing of their sensitive Personal health information (PHI), especially when they are stored on a third-party server which people may not fully trust.

The proposed solution for this problem is use patient centric store of health record. Using attribute based encryption, store the data very securely. And allow different attribute to access the data if needed.

- Extensive analytical and experimental results are presented which shows
- Data confidentiality
- On Demand revocation
- Write access control
- Scalability and usability

III. SYSTEM ARCHITECTURE

The purpose of the design is to plan the solution of a problem specified by the requirement document. The design of the system is the most critical factor affecting the quality of the software and has a major impact on the later phases, particularly testing and maintenance.

System design aims to identify the modules that should be in the system, the specifications of these modules and their interactions to produce the desired results. Since the system is a user interface, a good user interface design is critical to the success of a system. An interface that is difficult to use will result in a high level of user errors.

While designing user interface we must take into account the physical and mental capabilities of the people who use the software. The main constraints are:

- User familiarity
- Consistency
- Recoverability
- User guidance

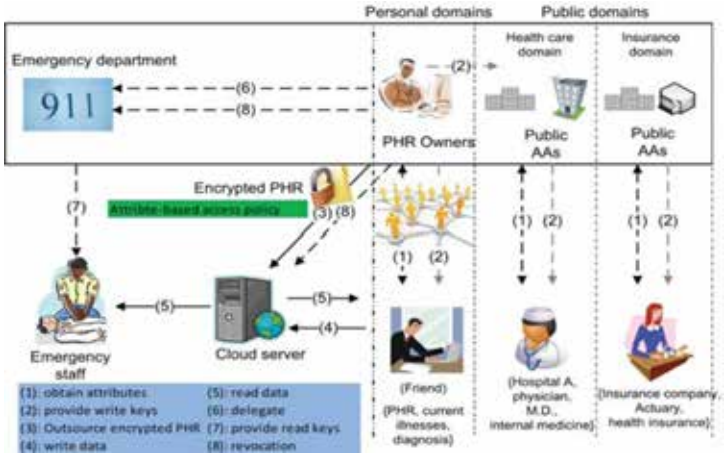


Fig. 2: SPACC - System Architecture

3.1 Modules

The different modules implemented in our application are:

· PHR Owner module

· Attribute based Access Policy Module

· Data confidentiality Module

3.1.1 PHR Owner Module

The PHR owner is the patient who desire to store health details securely. In this module the PHR owner register in the website of the application. He will get a secure username and password. This module generates a private key and public key for the patient. The PHR owner can decide his friends or relatives or insurance agency as his attributes and can send the access key. He can upload files in the cloud space and as desired he can download the file. And can make an emergency case to access the data.

3.1.2 Attribute Based Access Policy Module

Different attribute are the doctor, friend, relative and the insurance agent. The patient can set the attributes. And patient can send the username and password for the different attributes. The attribute doctor can access the files of the patient from cloud space. He can perform the read and write operations. But other attribute can only read the files. They cannot edit the files.

3.1.3 Data Confidentiality Module

The confidentiality of data is assured by attribute based encryption algorithm. Attribute based encryption is one of the latest encryption method. It is a randomized encryption method. A randomized secure key is generated based on one attribute of the patient. This key cannot be predicted by anyone else. Based on this key, the files are encrypted and stored in the cloud space. The decryption key is generated only for the authorized attributes.

IV. CONCLUSION

Personal health record (PHR) is an emerging patient-centric model of health information exchange, which is stored at a third party, Microsoft's cloud system. Here wide privacy concerns are considered since personal health information could be exposed to those third party servers and to unauthorized parties. To assure the patients' control over access to their own PHRs, the attribute based encryption method is used for the PHRs before outsourcing. The application addresses the unique challenges brought by multiple PHR owners and users,

in that greatly reduce the complexity of key management while enhance the privacy guarantees compared with previous works. Since ABE is used to encrypt the PHR data, so that patients can allow access not only by personal users, but also various users from public domains with different professional roles, qualifications and affiliations. To fully realize the patient centric concept, patients shall have complete control of their own privacy through encrypting their PHR files to allow fine-grained access. Here single trusted authority (TA) system is used to assure patient centric control.

V. FUTURE WORKS

The work could be extended to wider areas where we could easily manage a large group of medical related activities provided by many public organizations. It includes items such as commercial health plans, centers for Medicare and Medicaid services, state Medicaid programs etc. Managing these tasks is really a big challenge as it is extremely complex. Educating users regarding the potential use and need of such approaches and making them familiar with the technology is yet another main challenge as, GUI has to be made more and more simpler.

VI. ACKNOWLEDGEMENT

We express our sincere gratitude to Dr. S.Kannan,, the honourable Head of the Department of Computer Science and Engineering, MEC, for his constant guidance and support throughout our work. We also express our deepest sense of gratitude to all the anonymous persons who has inv olved in our works directly or indirectly, for their valuable support, advice and involvement, which was a major source of inspiration and encouragement. We are also thankful to the editor in chief, the associate editors and anonymous referees for their comments.

BIBLIOGRAPHY

[1] Ming Li, Member, IEEE, Shucheng Yu, Member, IEEE, Yao Zheng, Student Member, IEEE, Kui Ren, Senior Member, IEEE, and Wenjing Lou, Senior Member, IEEE, Scalable and Secure Sharing of Personal Health Records in Cloud Computing Using Attribute-Based Encryption

[2] Roger S Pressman, Software Engineering- A practitioners approach,Fifth Edition, McGraw-Hill publication.

[3] Ramez Elmasri And Shamkant Navathe,Fundamentals of Database, Sixth Edition, Pearson Education.

[4] Simon Robinson, Christian Nagel, Karli Watson, Jayglynn, Morgan Skinner and Bill Evjen- “Professional C# ”- Third Edition, Wiley India Publication.

[5] H. Lo¨ hr, A.-R. Sadeghi, and M. Winandy, Securing the E-Health Cloud,Proc. First ACM Int’l Health Informatics Symp. (IHI ’10),pp. 220-229, 2010.

[6] M. Li, S. Yu, K. Ren, and W. Lou, Securing Personal Health Records in Cloud Computing: Patient-Centric and Fine-Grained Data Access Control in Multi-Owner Settings, Proc. Sixth Int’l ICST Conf. Security and Privacy in Comm. Networks (SecureComm ’10),pp. 89-106, Sept. 2010.

DESIGN OF AGENT BASED SYSTEM FOR MONITORING AND CONTROLLING SLA IN CLOUD ENVIRONMENT

Sarwan Singh[1], Manish Arora[2]

[1,2] *National Institute of Electronics and Information Technology, Chandigarh, (India)*

ABSTRACT

Cloud Computing is mainly used as utility where cloud user hires services on pay-per usage mode. Cloud Provider provides services and Cloud User avails services. The services are provided on mutually agreed terms and conditions. Service Level Agreement (SLA) is an agreement between Cloud Service Provider and Cloud User, describing terms and conditions of services being offered. The SLA has both functional and non-functional requirements and has multiple levels. In order to maintain the synergy of Cloud user, the resource allocation has to be strictly monitored and controlled. Any deviation of SLA parameters has to be immediately highlighted as it affects both Cloud user and Cloud Provider. This paper presents the design of Agent Based Monitoring and Controlling multi level SLA in Cloud Environment. The agent monitors functional SLA parameters contiguously and compares against the agreed SLA parameters. It also generates alerts in case of any deviation. The proposed design will help both Cloud Provider and Cloud User in controlling agreed levels of SLA parameters.

Keywords: Agent, Cloud Computing, Service Level Agreements (SLA), SLA Parameters, Monitoring & Controlling SLA Agent

I INTRODUCTION

Cloud Computing model enables user to have on-demand access to resources from available pool of resources. The Cloud provider insures the reliable services to Cloud user. The demand of resources to accommodate exponential growth of ICT has made Computing as fifth essential utility after water, electricity, gas and telephony [1]. Cloud computing is an on-demand computing where cloud users are provided with pool of hardware and/or software resources and these resources can be used in pay-per-use basis [2]. Cloud computing raises many issues related to service contracts in implementation and deployment stages both at Hardware and Software levels. These issues further relates to service contracts management and compliance checking. By managing these issues motivation/interest of Cloud user can be maintained in cloud services.

To ensure reliable service, the Cloud provider monitors and controls the set of Terms and Conditions agreed with Cloud user. Agreed Terms and Conditions are mentioned in Service Level Agreements (SLA). It is considered as the most important element that provides some degree of assurance to both Cloud users and Cloud providers in the Cloud paradigm. The SLA defines the scope of usage and provision of resources. Cloud provider needs SLA to define the trust and quality of services they provide to users as well as an agree framework for costs and charges. This makes monitoring and controlling SLA the most important from Cloud

provider side. The Terms and Conditions are mostly part of resources that to provide to Cloud user like RAM, computation time etc. The resources are provided from a common pool.

SLA plays a vital role in enforcing Quality of Service (QoS). The issues related to SLA in Cloud computing are about service availability, response time, performance, security etc. Thus a dynamic and volatile agent based system is required to monitor the terms of SLA and act timely to provide QoS in Cloud and ensures trust of Cloud users. This motivated to frame and design an agent based system for monitoring and controlling SLA and has been described in the paper. The paper has been organized into six sections; section II reviews the background and related work, section III briefs the architecture of proposed system, section IV details multilevel SLA, section V describes the design of proposed Agent based system for monitoring and controlling SLA followed by conclusion and scope for future work in section VI.

II BACKGROUND AND RELATED WORK

Service Level Agreement (SLA) is a document that includes a description of agreed services, service level parameters, guarantees, actions and remedies for all cases of violations. Thus SLA is an important agreement of negotiations between Cloud service provider and Cloud user. The SLA is very important to determine the resource/services availability, reliability, scalability, security, etc. Furthermore, SLA is a legal document which describes the way in which services will be made available and framework for service charges.

SLA compliance has been implemented and validated in research projects of French ANR SemEUsE and European Celtic SERVERY cooperative [3] [4] [5]. The three layered model comprises of Service Monitoring (top level), Data Collector (middle layer) and Core Monitoring (lowest level) is used. Core Monitoring takes data from low level indicators i.e. from hardware and passes data to Data Collector layer which filters and processes data received and send it to Service Monitoring layer in the appropriate format [6]. This ensures the services are provided to Cloud users. SLA parameters are classified into functional and non-functional requirements. In order to fulfil the need of Cloud user, both functional and non-functional requirements of Cloud services are satisfied. Non-functional requirements like availability, scalability, cost calculation method, configuration of service are used in monitoring SLA.

The agent based three layered model is used for Monitoring and Controlling SLA in Cloud Environment [7]. The agents are considered to be autonomous entities, such as software programs or robots [8]. The agents interact with each other for a selfish or cooperative goal. In other words agents can share a common interest or they can pursue their own interests. Agent must be able to change its behaviour based on changes occurring in its environment. Agent should be reactive, autonomic, collaborative in behaviour, adaptive, etc. In the three-layered architecture, lowest layer comes in the action as soon as Cloud user logs in and virtual machine is created/initiated. The Monitoring and Controlling Agent (MCA) as a Terms Collector (TC) gathers the SLA terms from Cloud service provider whenever new cloud user is registered. MCA as a Term Monitor (TM) gathers information from Cloud environment from time to time to maintain QoS being provided by the Cloud service provider. The information collected as TC i.e. SLA's agreed upon and data gathered as TM i.e. the SLA's being provided, are compared and any violations are projected as alerts to Cloud users/provider.

2.1 Proposed Model of Agent Based Monitoring and Controlling Multilevel SLA

Based on the review and background of related work, various SLA parameters currently used in ensuring Quality of Service (QoS) in Cloud environment, an agent based Monitoring and Controlling Multilevel SLA (MACSLA) has been modelled. Figure 1 shows architecture of proposed model. The Monitoring and

Controlling Agent (MCA), as Term Collector (TC) gathers the agreed SLA parameters and their desired level from the database. The database stores information like profile of Cloud user and SLA parameters and their desired level. It also collects current SLA parameters and their level from running virtual machine as Term Monitor (TM). MCA then compares both the levels. On finding variation in the levels of SLA parameter, agreed and provided, alerts are sent to Cloud user as well as Cloud provider.

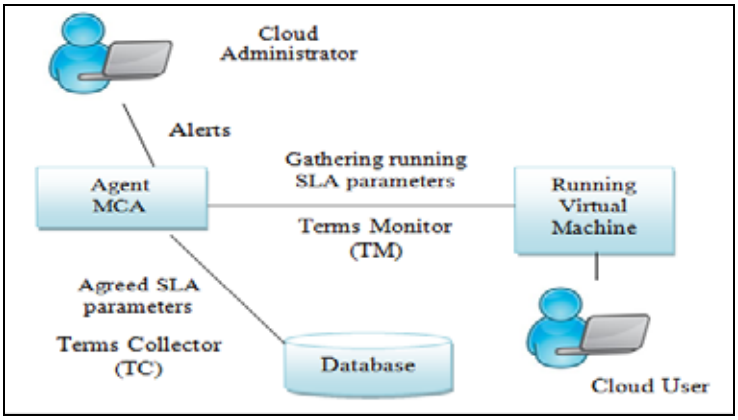


Figure 1: Proposed Architecture Of Agent Based MACSLA

This will help Cloud provider to ensure QoS as well as increase resources if required and provisioned in SLA. Cloud user will also have monitoring mechanism to avail services. The MCA monitors and controls the agree SLA parameters continuously so long as services are provided.

IV MULTILEVEL SLA

Various research-works on SLA parameters have been studied and identified in detail. The identified parameters are organized into three levels. Level 1 (L1) contains are basic SLA parameters being negotiated between Cloud service provider and Cloud User. Level 2 (L2) classifies these parameters into three groups: Infrastructure as a Service (IaaS), Platform as a Services (PaaS), Software as a Service (SaaS).

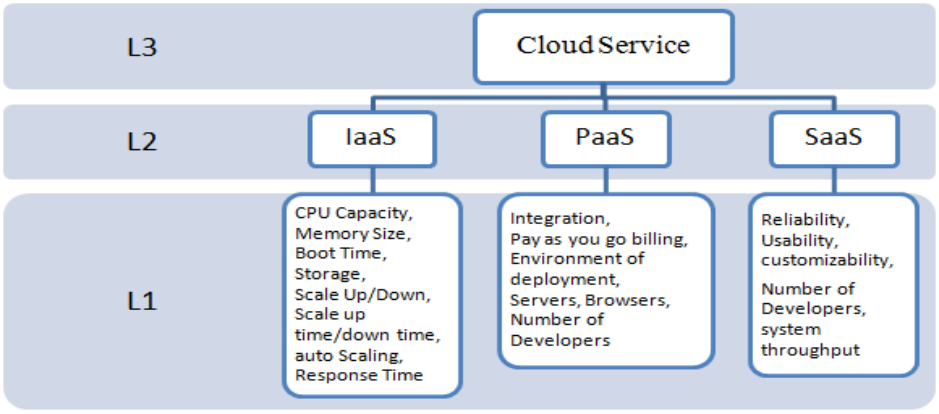


Figure 2: Multilevel SLA parameters

IaaS parameters are number of CPU cores, Memory size, booting time required to start Virtual Machine (VM), storage size, Scale up/down (maximum/minimum number of VMs for one user), Scale Up time/down time(time to increase/decrease a specific number of VMs), Auto Scaling i.e. user can scale up/down or not, Response time to complete and receive the process. PaaS parameters are Integration (Integration between services and other platforms), Pay as you go billing (charging based on resources or time of service), number of Developers using platform simultaneously etc. SaaS parameters are Reliability (ability to keep system operational in most of

time), usability (easy built-in user interfaces), customizability (flexible to use with different type of users), number of users using software simultaneously, and system throughput (system response speed). All these parameters at L2 are further grouped into higher level i.e. L3 to know the overall QoS and named Cloud Service Parameter. The three levels and parameters at each level are shown in figure 2.

V DESIGN OF MACSLA

Based on model described in previous section, MACSLA agent has been designed. The designing includes Database Design, Agent Design and Process Design.

5.1. Database Design

The purpose of database is to store information on Cloud user, the agreed parameters and desired levels. The design of MACSLA has been shown in figure 3 as Entity Relation Diagram (ERD). It has four entities: user, virtual machines, running SLA and SLA. SLA contains SLA parameters and their desired levels. Virtual Machine contains parameters on which it has been created and provided to Cloud user on demand. Running SLA contains information on resources being used by virtual machine provided to Cloud user. User contains profile of Cloud user. User can have multiple agreed SLA parameters. User can demand machine for use. The responsibility of agent MCA is to store resource information continuously and compares with agreed SLA. On finding any deviation, MCA will send alerts. The tables have been designed based on Entity Relationship diagram in MySQL.

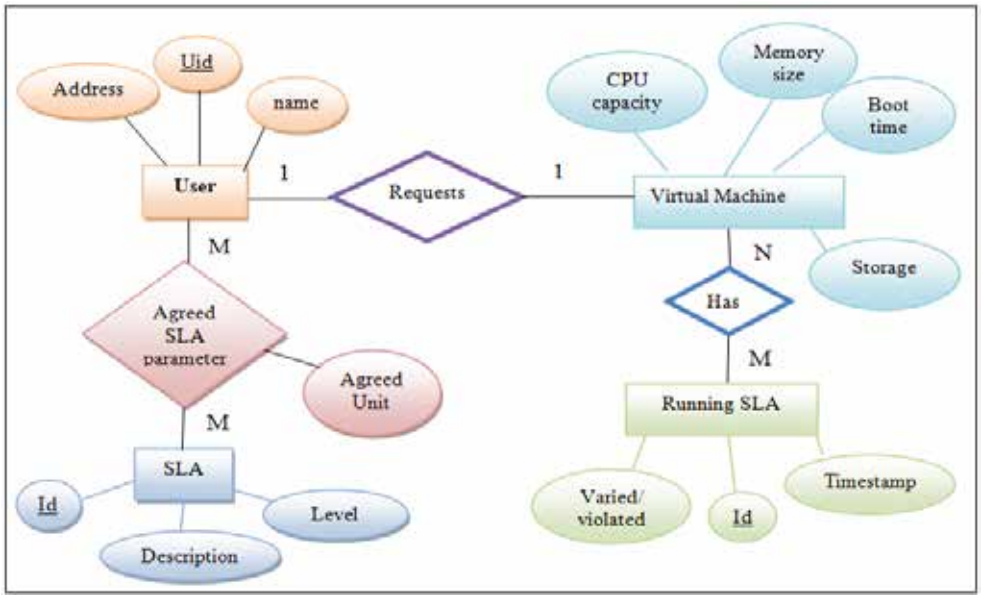


Figure 3: Entity Relationship Diagram

5.2. Agent Design

The roles and responsibilities of agent MCA has been identified and described in Figure 4. MCA agent has two major roles to play: Term Collector (TC) and Term Monitor (TM). The responsibilities of each role have been given below.

- As Term Collector, MCA gathers agreed SLA parameters from the Cloud Service Provider whenever a new resource has been allocated.

- As Term Monitor, MCA continuously monitors the current SLA parameters for the resource being used and compares with the agreed SLA parameters.

MCA performs three tasks. These are:

- Keeps strict vigil on new upcoming resource i.e. creation/initialization of Virtual Machine, Cloud user activating or booting new/existing VM.
- Monitoring/retrieving agreed SLA parameters.
- Gathering SLA parameters of VM in action or running VM.

It takes input as agreed SLA parameters, SLA's currently being provided to Virtual Machine and compares the parameter values and generate alerts as an outcome.

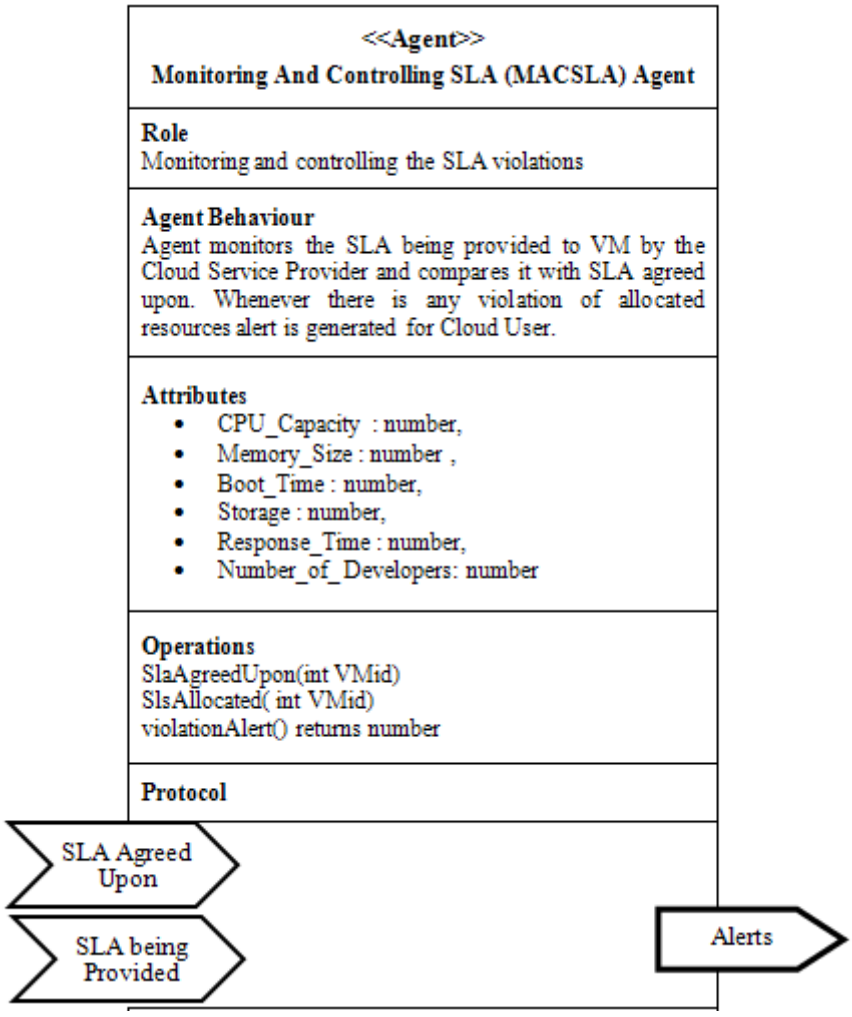


Figure 4: Description of MACSLA Agent

5.3. Process Design

The Activity Diagram shown in figure 5 describes flow of various activities being carried out by MAC agent. Agent comes into action when Cloud Service provider starts allocating resources to Cloud users. Agent looks for running virtual machine (VM) it gathers agreed SLA parameters and running level of SLA parameters. It then compares these values and on finding any deviation, alert is generated and reported to Cloud user and Cloud provider.

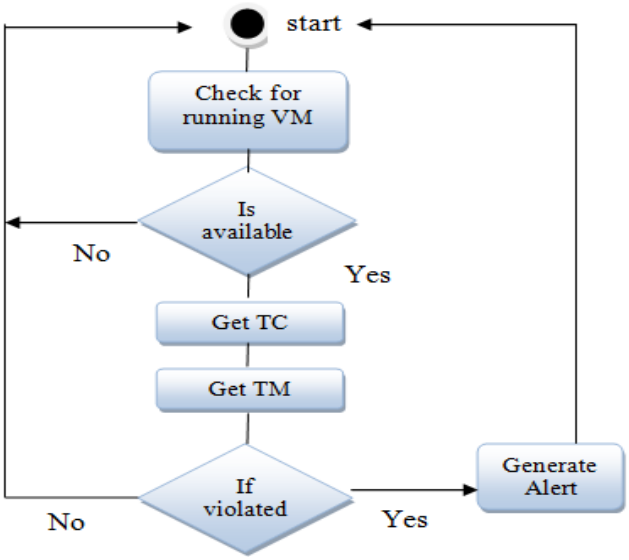


Figure 5: MACSLA Activity Diagram

VI CONCLUSION AND SCOPE FOR FUTURE WORK

The proposed design of Agent based Monitoring And Controlling SLA (MACSLA) will ensure quality of services in terms of maintaining agree levels of SLA in Cloud Environment. The agent, MCA, dynamically generates alerts to in case of deviation and sends to both Cloud provider and Cloud user. The functional SLA parameters used by agent are also identified as hierarchy of three levels. The designed three levels SLA will help in quantifying quality of services at individual parameter level, service level and Cloud provider level. The scope for future work includes: implementation of design of MACSLA in Cloudsim environment based on Agent technology, verification and validation of tasks performed by agent.

REFERENCES

[1] Rajkumar Buyya, Chee Shin Yeo, Srikumar Venugopal, James Broberg, and Ivona Brandie, "Cloud Computing and Emerging IT Platforms: Vision, Hype, and Reality for Delivering Computing As the 5th Utility", Future Generation Computer System, Volume 25 Issue 6, pp. 599-616, June, 2009

[2] Nawsher Khan, Ahmad Noraziah, Mustafa Mat Deris, and Elrasheed I. Ismail "Cloud Computing : Comparison of Various Features", International Conference DEIS 2011, CCIS 194, pp. 243-254, 2011

[3] Antonin Chazalet, "Service Level Agreements Compliance Checking in the Cloud Computing – Architectural pattern, prototype and validation" pp, 184-189, IEEE 2010

[4] Antonin Chazalet, "Service Level Checking in the Cloud Computing Context" pp, 297-304, IEEE 2010

[5] Mohammed Alhamad, Tharam Dillon, Elizabeth Chang, "Conceptual SLA Framework for Cloud Computing", pp, 606-610, IETE 2010.

[6] Rodziah Atan, Nor Shahida Mohd Jamail, Service Level Agreement Checking in Cloud Computing in Terms of Verification and Validation Concepts, ISBN : 978-0-9853483-3-5 SDIWC, pp. 727-737, 2013

[7] Sarwan Singh, Manish Arora , "Monitoring and Controlling Multi Level SLA in Cloud Environment using Agent" IJARCSSE, volume 3 issue 7, pp. 1006-1010, July 2013

[8] Manish Arora, M. Syamala Devi, "Distributed algorithm for multi-agent environment" , IJITKM, volume 4 no.2, pp. 519-525, 2011

CHARACTERIZATION OF ANTI RADIATION AND WATER REPELLENT FABRICS FOR MOBILE POUCH

Mr. S. Chandra Kumar[1], Dr.J.Srinivasan[2], Ms.V.Bhanu Rekha[3]

[1,2,3] *Department of Fashion Technology*

Kumaraguru College of Technology Coimbatore 49. Tamilnadu (India)

I. INTRODUCTION

The effect of mobile phone radiation on human health is the subject of recent interest and study, as a result of the enormous increase in mobile phone usage throughout the world. Mobile phones use electromagnetic radiation in the microwave range. Other digital wireless systems, such as data communication networks produce similar radiation. Tissues nearest to where the phone is held can absorb this energy. This may lead to brain tumour, brain cancer, heart attack, headache, hair loss etc.

The rate at which energy is absorbed by the human body is measured by the Specific Absorption Rate (SAR), and its maximum levels for modern handsets have been set by government regulating agencies in many countries. In India, a SAR limit is 2 W/kg. In the case of a person using a cell phone, most of the heating effect will occur at the surface of the head, causing its temperature to increase by a fraction of a degree.

In this case, the level of temperature increase is an order of magnitude less than that obtained during the exposure of the head to direct sunlight. The brain's blood circulation is capable of disposing of excess heat by increasing local blood flow. However, the cornea of the eye does not have this temperature regulation mechanism and exposure of 2–3 hours duration has been reported to produce cataracts in rabbits' eyes at SAR values from 100-140W/kg, which produced temperatures of 41°C.

Radiation can be controlled by two methods, absorption and reflection. In this study, aluminium, bamboo and bamboo charcoal fabrics are used as anti radiation materials. Aluminium works in the principle of reflection, whereas bamboo and bamboo charcoal work in the principle of absorption.

II.MATERIALS AND METHODS

2.1. Anti radiation materials selected for the mobile pouch

Aluminium and bamboo were chosen as anti radiation materials based on the two principles of anti radiation namely reflection and absorption. Aluminium works under the principle of reflection, whereas bamboo charcoal works under the principle of absorption.

Aluminium finds its applications in various avenues in technical textiles. They are used in Anti-static protective clothing in the petrochemical industry, pilot suits, fire workers suit, etc. and as sound absorbers in all kinds of silencers and are especially used as shielding fabrics for utility workers in high field areas.

Bamboo-charcoal has a porous structure. Bamboo-charcoal yarn has a cross-section filled with various micro-gaps and micro-holes, similar to that found in charcoal gas filters and hence compared to other conventional fabric; it has better moisture absorption and ventilation.

In this study, mobile pouch is produced consisting of three layers:

- An outer water repellent cotton knitted fabric layer
- Middle layer with an anti radiation material (A layer of aluminium foil sandwiched between 2 layers of aluminium or bamboo charcoal knitted fabric)
- An anti scratch inner velvet fabric layer to protect the mobile's screen from scratches

Fig 1: A- Aluminium yarn, B – Bamboo fiber, C – Bamboo charcoal yarn

2.2. Preparing the outer water repellent layer

Cotton knitted material was sourced and finished with fluorocarbons to render water repellent finish. Fluorochemical repellents are unique in that they confer both oil and water repellency to fabrics. The ability of fluorochemicals to repel oils is related to their low surface energy. The finish is given on a padding mangle machine. Once the chemical bath is set, process is started. Initially the fabric is fed through roller1 above the chemical bath to roller2 inside the chemical bath, which is dipped in chemicals. Thus padding of chemicals on fabric is done. Then the fabric is passed between roller 3 & 4 which is above the chemical bath, this squeezes the fabric before sending it to the drying chamber to remove excess of water before curing it through curing chamber in a higher temperature of about 160^{o}C.

2.3. Preparing the Middle Layer By Knitting Single Jersey Fabric

Middle layer consists of an anti radiation material comprising a layer of aluminium foil sandwiched between 2 layers of aluminium or bamboo charcoal knitted fabric.

Aluminium yarns or bamboo charcoal yarns are knitted in tubular knitting machine as single jersey fabric. The count of aluminium yarn used was 30s Ne. The count of bamboo charcoal yarn used is 60s Ne. Initially the yarn is fed through a guide and then to the needle. The outcome is obtained as single jersey fabric.

Fig 2: A – Bamboo charcoal knitted jersey, B – Aluminium knitted jersey, C – Aluminium foil

2.4. Preparing the Inner Anti Scratch Layer with Velvet Fabric

Velvet fabric is sourced and used as the inner layer because it can protect the mobile from scratches.

2.5. Thickness Tests for the Various Fabrics Used For the Mobile Pouch: Standard- ASTM D 5729 (07.02)

Thickness tester has been used to test the thickness of the prepared fabrics. Clean the circular pressure foot and the anvil (base plate). Set the gauge dial to zero. Keep the sample below the presser foot without wrinkles. The presser foot is lowered on to the sample slowly at a uniform rate. The thickness of the substrate is noted from the dial till the movement of the pointer has stopped. Repeat the process taking 5 readings at different points of the sample and find out the average thickness of the fabric.

2.6. Water Repellent Durability Test

Water repellency is tested by placing a drop of water on the fabric and observing whether the drop resides on top of the fabric or whether it penetrates. Lower the surface tension of the liquid, the better the fabric's resistance to oily stains.

2.7. Oil Repellent Test

Oil repellency is tested by placing a drop of oil on the fabric and observing whether the drop resides on top of the fabric or whether it penetrates. Lower the surface tension of the liquid, the better the fabric's resistance to oily stains.

2.8. Radiation Tests

Radiation test is carried on with an analogue type multimeter. In this analogue multimeter, AC is kept 0 V and DC at 2.5 V. A copper wire, which has good conductivity, is connected on the two knobs. Now a mobile phone, whose radiation has to be tested, is kept on the copper wire. When a call or message is received by the phone, the radiation is shown by pointer in volts. The radiation tests were taken for Aluminium fabrics and bamboo charcoal fabrics. The radiation control was taken for varying layers of each fabric to find a better radiation control.

With trials taken for each fabric the number of layers which showed a better radiation control were selected with 2 layers for aluminium fabric and 5 layers for bamboo charcoal fabric. As the aluminium fabric gave a better control of radiation from the mobile phones, aluminium knitted fabric was selected to prepare the middle layer. For the middle layer, aluminium foil was sandwiched between two layers of aluminium knitted fabrics.

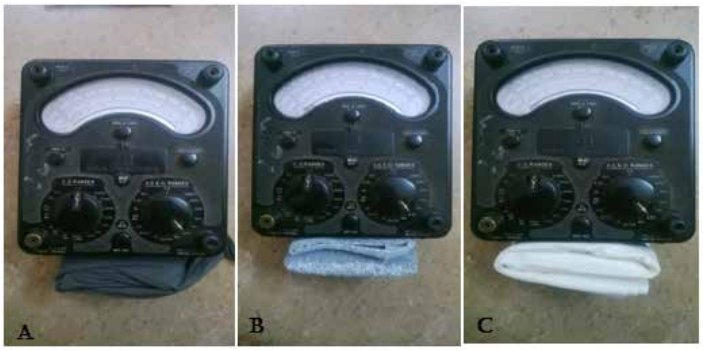


Fig 3: Testing radiation control A -Aluminium fabric, B - Bamboo charcoal fabric and C – Bamboo fabric

III.RESULTS AND DISCUSSIONS

3.1. Oil / Water Repellency Tests

The oil and water were dropped on the cotton finished fabric and drops were not absorbed by the fabric. They rolled down the fabric, displaying the effect of the fluorocarbon finish given to the cotton fabric

Fig 4: Testing for water and oil repellency on cotton fabric

3.2. The Product

The dimensions of the product were 100 cms in length and 26 cms in width. Accordingly the outer, middle and inner layers were cut and assembled using an single needle lock stitch sewing machine,

Fig 5: Stitched and Assembled Product

3.3. Results for Radiation Tests

The following table shows the values of radiations measured with a multi meter. The values are recorded for 60 different types of mobiles and are tested for Aluminium knitted fabric, bamboo charcoal and finally the middle sandwich layer

Table 1: Radiation control level for aluminium, bamboo charcoal fabric and sandwich middle layer

S. NO.	MOBILE PHONE	Radiation in Volts without anti radiation material	Radiation in Volts with 2 layers aluminum fabric	Radiation in Volts with 5 layers charcoal fabric	Radiation in Volts with 2 layers aluminum fabric and 1 layer aluminum foil
CATEGORY - 1					
1	C1– model-A	0.3	0.05	0.2	0.01
2	C1– model-B	0.25	0.01	0.1	0
3	C1 – model-C	0.32	0.05	0.25	0.01
4	C1 – model-D	0.29	0.02	0.16	0
5	C1– model-E	0.36	0.05	0.2	0.02
6	C1 – model-F	0.3	0.05	0.2	0.01
7	C1– model-G	0.25	0.01	0.1	0
8	C1– model-H	0.3	0.05	0.01	0.01
9	C1– model-I	0.4	0.1	0.2	0.05
10	C1– model-J	0.1	0.05	0	0
11	C1– model-K	0.1	0.05	0	0
12	C1– model-L	0.1	0.09	0	0
13	C1– model-M	0.2	0.06	0	0
14	C1– model-N	0.1	0.03	0	0
15	C1– model-O	0.1	0.05	0	0

16	C1– model-P	0.4	0.12	0.2	0.06
17	C1– model-Q	0.3	0.05	0.16	0.01
CATEGORY – 2					
1	C2– model-A	0.2	0.01	0.1	0
2	C2– model-B	0.05	0.03	0.01	0
3	C2– model-C	0.1	0.05	0	0
4	C2– model-D	0.25	0.05	00	0
5	C2– model-E	0.2	0.09	0.1	0
6	C2– model-F	0.1	0	0	0
7	C2– model-G	0.1	0	0	0
8	C2– model-H	0.2	0.1	0.05	0
9	C2– model-I	0.19	0.05	0.01	0
10	C2– model-J	0.16	0.06	0	0
11	C2– model-K	0.1	0	0	0
12	C2– model-L	0.5	0.1	0.2	0.1
13	C2– model-M	0.26	0.1	0	0
14	C2– model-N	0.19	0	0	0
CATEGORY – 3					
1	C3– model-A	0.5	0.1	0.3	0.1
2	C3– model-B	0.3	0.09	0.2	0.03
3	C3– model-C	0.2	0	0	0
4	C3– model-D	0.5	0.12	0.3	0.12
5	C3– model-E	0.5	0.06	0.26	0.01
6	C3– model-F	0.5	0.2	0.22	0.1
7	C3– model-G	0.6	0.2	0.4	0.16
8	C3– model-H	0.3	0	0.1	0
9	C3– model-I	0.4	0.1	0.24	0.03
10	C3– model-J	0.2	0	0.1	0
11	C3– model-K	0.1	0	0	0
CATEGORY – 4					
1	C4– model-A	0.29	0.1	0	0
2	C4– model-B	0.3	0.1	0.1	0
3	C4– model-C	0.3	0.1	0.1	0.1
4	C4– model-D	0.27	0.09	0.05	0
5	C4– model-E	0.09	0	0	0
6	C4– model-F	0.06	0	0	0
7	C4– model-G	0.6	0.22	0.3	0.2
8	C4– model-H	0.6	0.21	0.42	0.21

CATEGORY – 5					
1	C5– model-A	0.26	0	0.1	0
2	C5– model-B	0.24	0	0	0
3	C5– model-C	0.3	0.1	0.12	0.1
4	C5– model-D	0.1	0	0	0
5	C5– model-E	0.34	0	0.1	0
CATEGORY – 6					
1	C6– model-A	0.2	0	0.05	0
2	C6– model-B	0.25	0	0.05	0
3	C6– model-C	0.3	0.12	0.15	0.12
4	C6– model-D	0.2	0	0.09	0
5	C6– model-E	0.26	0.02	0.01	0.02

The table above shows that category – 3 and category – 4 brands have recorded the maximum radiations of about 0.6 V when tested without any radiation protection. 2 layers of aluminium have given better protection against radiation rather than the protection given by 5 layers of bamboo charcoal fabric. So Aluminium fabric has been selected for the middle anti radiation layer of the mobile pouch. The radiation control results of the final sandwiched layers (aluminium foil sandwiched between 2 layers of aluminium knitted fabric) show very good protection from the radiation.

Table 2: Thickness of the Fabric

S. No	**Fabric**	**No. of layers used**	**Thickness in mm**
1	Cotton fabric	**1**	**0.6**
2	Aluminum fabric	**2 X 0.75**	**1.5**
3	Aluminum foil	**1**	**0.34**
4	Velvet fabric	**1**	**0.96**
Total thickness of the pouch around the mobile			**3.4**

The thickness of the final product with aluminium knitted fabric is 3.4 mm. This is the thickness of the layer which gives protection from the radiation released from the mobile.

Costing for the finished mobile pouch

Table 3: Costing of the Product

S.no	Items	Cost in Rs.	Cost for one pouch in Rupees.
1	Aluminium yarn	175 (1 cone)	6.3
2	Knitting charges for aluminium fabric	142 per meter	26.56
3	Aluminium foil	1	1
4	Cotton	90 per meter	4.3
5	water repellent finish	750 per meter	35.70
6	Velvet	40 per meter	5
7	Stitching charge	-	10
8	Logo		2
TOTAL			**90.86**

The cost of the mobile pouch is Rs. 90.86. This could be sold for Rs. 100/- with profit added to it. When mass produced the cost could be slashed down to Rs. 75/-

IV. CONCLUSIONS

1) From the radiation test it is found that, bamboo fabric has no radiation control, 2 layers of aluminium fabric can control 82% of radiation and 5 layers of bamboo charcoal fabric can control 75% of radiation.
2) Aluminium foil when used in small amount helps to prevent from radiation, but when used in large amount it blocks the signal.
3) 2 layers of aluminium fabric and one later of very thin aluminium foil can control radiation upto 98%
4) Radiation from phone can be prevented from penetrating our body, when mobile phone is carried in an anti radiation pouch.

REFERENCES

[1] Electromagnetic Fields and the Risk of Cancer. Report of an Advisory Group on Non-ionising Radiation. Doc NRPB,3(1),1-138(1992).
[2] Health Effects Related to the use of Virtual Display Units. Report of an Advisory Group on non-ionising radiation.Doc NRPB,5(2),1-75(1994)
[3] ELF Electromagnetic Fields and the Risk of Cancer. Report of an Advisory Group on Non-ionising Radiation.Doc NRPB,12 (1), 1-179(2001).

[4] Possible Health Effects from Terrestrial Trunked Radio (TETRA). Report of an Advisory Group on Non-ionising Radiation.Doc NRPB, 12(2), 1-80(2001).

[5] Binhi, Vladimir N: Repiev, A & Edelev, (2002), Magnetobiology: underlying physical problems.San Diego: Academic Press, Pp.1-16.

[6] Wikipedia. Mobile phone [online]. Available at: http://en.wikipedia.org/wiki/mobile_phone

[7] SaeidSanei, J.A. Chambers, Centre of Digital Signal Processing, Cardiff University, UK

[8] Harland JD, Liburdy RP (1997). "Environmental magnetic fields inhibit the antiproliferative action of tamoxifen and melatonin in a human breast cancer cell line". Bioelectromagnetics 18, Pp:555-62.

[9] Aalto S,Haarala C, Bruck A, Sipila H, Hamalainen H, Rinne JO (July 2006). "Mobile phone affects cerebral blood flow in humans". Journal of Cerebral Blood Flow and Metabolism 26(7).Pp:885-90.

[10] http://sarshield.com/radiation-chart/

[11] http://www.lessemf.com/faq-shie.html

[12] http://www.positivehealth.com/article/environmental/emf-protective-devices

[13] http://www.cellphonetechnicians.com/923-2/

[14] http://en.m.wikipedia.org/wiki/Electromagnetic_shielding

[15] www.youtube.com/multimeterasemfdetector

[16] http://www.indiamart.com/antiradiationmaterials/mobilecase/

[17] www.fibre2fashion.com

[18] http://www.fibre2fashion.com/industry-article/3/213/metallic-fibres11.asp.

PLANAR GRAPH IN DATA ENCRYPTION

M. Yamuna[1] and A.Elakkiya[2]

[1,2] *School of Advanced Sciences, VIT University, Vellore, (India)*

ABSTRACT

Transfer of data and its safety is an important issue in this information world. Planar graphs are not in wide use in data encryption. In this paper we propose a method of encryption using planar graphs. We also have developed a program for the proposed algorithm using MATLAB.

Keywords: Decryption, Encryption, Graph.

I INTRODUCTION

Before the modern era, cryptography was concerned solely with message confidentiality (i.e., encryption) - conversion of messages from a comprehensible form into an incomprehensible one and back again at the other end, rendering it unreadable by interceptors or eavesdroppers without secret knowledge (namely the key needed for decryption of that message). Encryption was used to ensure secrecy in communications, such as those of spies, military leaders, and diplomats. Yadhu Ravinath et.al have proposed a selective encryption mechanism using message specific key and spanning tree concept of graph theory. The mechanism provides protection of privacy in communication as it avoids the formation of self-loops and parallel edges and key is exchanged only among the authenticated neighbours only [1]. Esam Suliman Mustafa Ahmed et.al proved the effect of encryption delay on TCP based application is discussed. Increasing the encryption delay and then comparing the effect of that delay on TCP protocol through different scenarios is the methodology of the study, using OPNET [2]. Hussein Th.Khamees et.al used the stream cipher which is the best way with the algorithm Geffe generator with a specific length to Encryption the information from the plain text in the first compute [3]. Graph theory has contributed to the development of various encryption techniques. In this paper we propose a method using graph duals.

II PRELIMINARY NOTE

In this section we provide the basic results of graph theory which are requested for proposed encryption scheme.

2.1 Graph

In the most common sense of the term, a graph is an ordered pair G = (V, E) compromising a set V of vertices or nodes together with a set E of edges or links, which are 2 – elements subset of V (that is an edge is related with two vertices, and the relation is represented as an unordered pair of the vertices with respect to the particular edge)[4].

2.2 Weighted Graph

A weighted graph is a graph in which each branch is given a numerical weight. A weighted graph is therefore a special type of labeled graph in which the labels are numbers (which are usually taken to be positive) [5]. The graph in Fig. 1 is an example of a weighted graph [6].

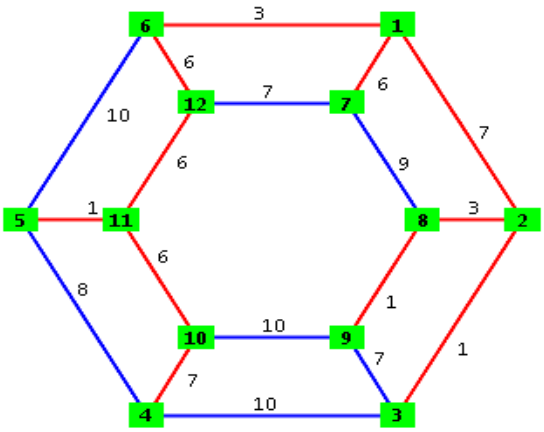


Fig. 1

2.3 Simple Graph

A simple graph, also called a strict graph, is an unweighted, undirected graph containing no graph loops or multiple edges [7]. In Fig. 2 G1 [8] represents a simple graph and G2 a multiple graph [9].

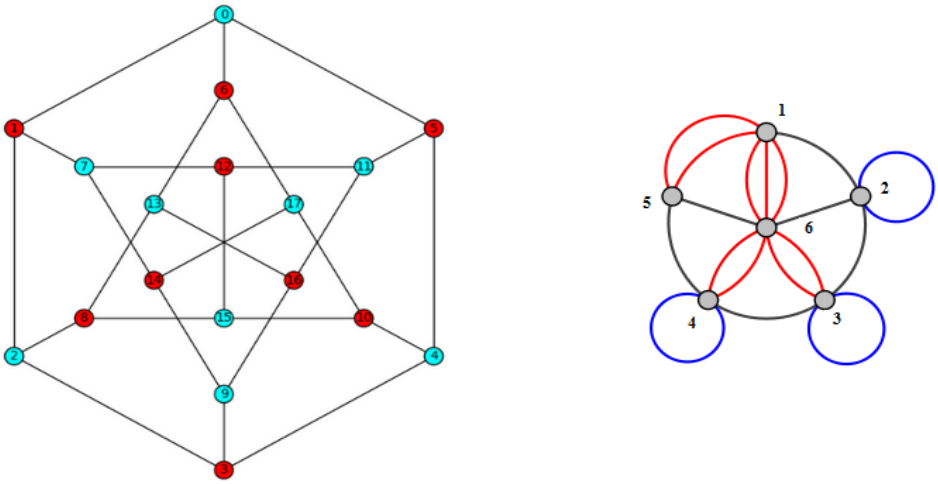


Fig. 2

2.4 Planar Graph

In graph theory, a planar graph is a graph that can be embedded in the plane, i.e., it can be drawn on the plane in such a way that its edges intersect only at their endpoints. In other words, it can be drawn in such a way that no edges cross each other. Such a drawing is called a plane graph or planar embedding of the graph [10]. The graph in Fig. 1 is an example of a weighted graph [11]

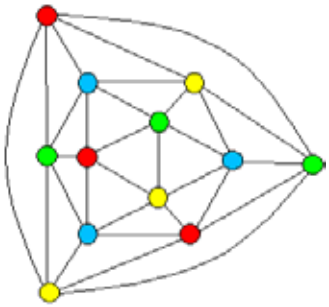


Fig. 3

2.5 Dual Graph

The dual graph of a plane graph G is a graph that has a vertex corresponding to each face of G, and an edge joining two neighboring faces for each edge in G. In Fig. the red graph is the dual graph of the blue graph [12].

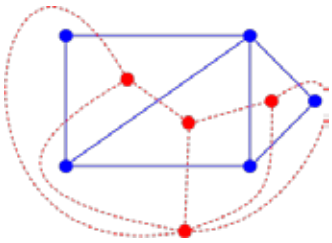


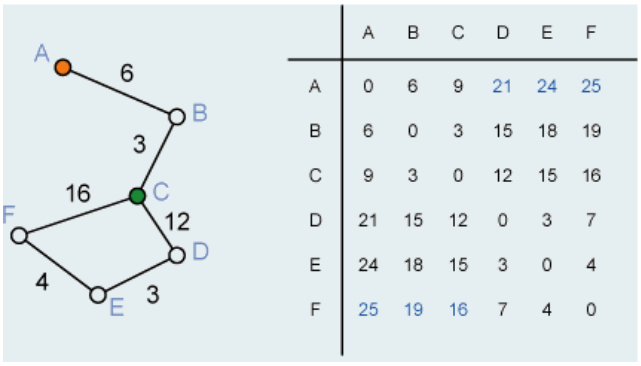
Fig. 4

2.6 Degree

In graph theory, the degree of a vertex of a graph is the number of edges incident to the vertex, with loops counted twice. The degree of a vertex v is denoted deg (v). The maximum degree of a graph *G*, denoted by Δ(*G*), and the minimum degree of a graph, denoted by δ(*G*), are the maximum and minimum degree of its vertices In Fig.2 the degree of all the vertices in G1 has degree 3 and the degree of vertices 1, 2, 3, 4, 5 in G2 are 6, 5, 6, 6, 4, 9 respectively [13].

Distance Matrix

In mathematics, computer science and graph theory, a distance matrix is a matrix (two-dimensional array) containing the distances, taken pairwise, of a set of points. This matrix will have a size of n x n where n is the number of points, nodes or vertices (often in a graph)[14]. Snapshot – 1 provides a weighted graph and its related distance matrix [15].



	A	B	C	D	E	F
A	0	6	9	21	24	25
B	6	0	3	15	18	19
C	9	3	0	12	15	16
D	21	15	12	0	3	7
E	24	18	15	3	0	4
F	25	19	16	7	4	0

Snapshot – 1

III PROPOSED METHOD

In this paper we aim to encrypt any message S using a graph G and its dual G^*. If there is a vertex of degree two in a planar graph G, then there will more than one edge between a pair of vertices in G*. So while we decrypt, more than one edge between a vertex pair creates a dilemma in picking the right edge. If G is a simple planar graph with degree of each vertex atleast 3, then G* is always simple. So, we use only graphs where G, G^* are simple and degree of every vertex in G is atleast 3. The basic idea is that we pick a random edge sequence in graph G. We then pick the corresponding edge sequence in the dual G* and assign weights to the edges and use the graph for encryption.

3. 1 Encryption Chart

We can use any normal encryption chart. Table – 1 provides a sample chart.

A	B	C	. . .	Z	Blank space
1	2	3		26	27

Table – 1

We can include any number of characters depending on the need of the message to be encrypted.

3. 2 Encryption Algorithm

Let | S | = K. Let us label the edges in G as $e_1, e_2, \ldots$ and the corresponding edges in G^* as $e_1^*, e_2^*, \ldots$. Let **S = SECRET** be the message to be encrypted.

Step 1 Choose a planar graph G with atleast K edges and construct G^*.

Since | S | = 6 we choose a graph with atleast 6 edges as seen in Fig. 5. The dual graph G* is also seen in Fig. 5.

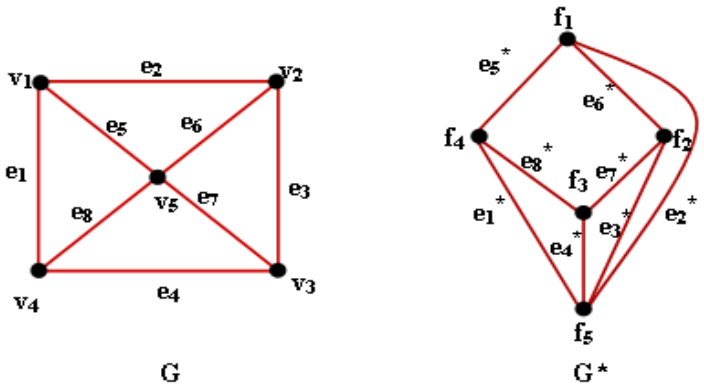


Fig. 5

Step 2 Randomly pick K edges from G say $e_1, e_2, \ldots e_k$.

We randomly choose the edges $e_7, e_6, e_4, e_5, e_8, e_3$ from Fig. 5.

Step 3 Convert each character in S into numerical values using Table –1 to generate a sequence $w_1, w_2, \ldots w_k$.

In our example the sequence $w_1, w_2, \ldots, w_6$ is 19 5 3 18 5 20 (from Table – 1).

Step 4 Assign the values $w_1, w_2, \ldots w_k$ as weights of the $e_1^*, e_2^*, \ldots e_k^*$.

Assigning the weights 19 5 3 18 5 20 to the edges e_7*, e_6*, e_4*, e_5*, e_8*, e_3*, the graph is as seen in Fig.6

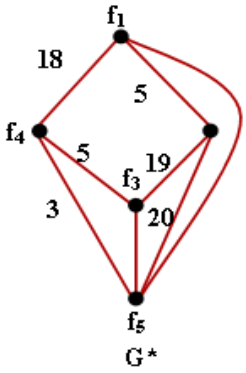


Fig. 6

Step 5 Assign arbitrary weights to the remaining edges in G^* and to edges in G.

We assign weights 2, 9 to the remaining edges e_1, e_2 in G and weights 24, 27 to the edges e_1*, e_2* in G*. The resulting graph is as seen in Fig. 7.

Step 6 Send G, G^* to the receiver.

Finally we send the graph in Fig. 7 to the receiver (the lines with message is highlighted in blue).

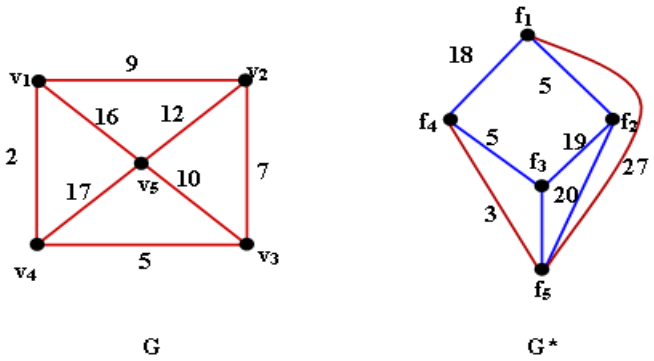


Fig. 7

For decrypting the message we reverse the procedure.

Suppose the received message is as seen in Fig. 8.

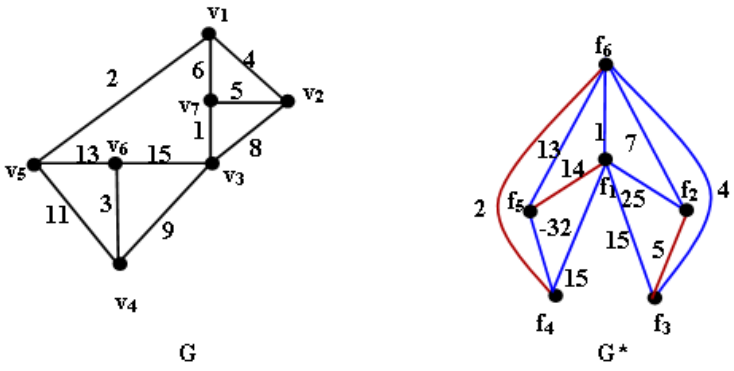
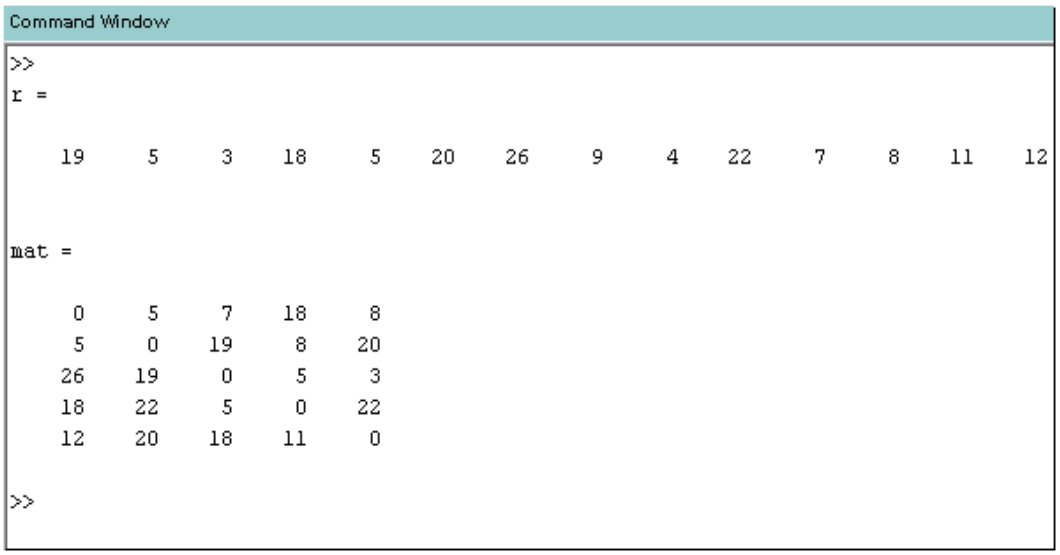


Fig. 8

Suppose the edge sequence given to us is (v_1, v_2), (v_7, v_3), (v_6, v_3), (v_5, v_6), (v_6, v_4) (v_3, v_2), (v_1, v_5), (v_1, v_7) (Note than instead of edges labeling the edges are provided using vertex labels). We pick the corresponding edge sequence from G*. The edge sequence is (f_2, f_6), (f_1, f_3), (f_1, f_4), (f_1, f_5), (f_4, f_5), (f_3, f_6), (f_1, f_6), (f_1, f_2). The corresponding weight sequence is 7 15 15 4 27 4 1 25. The message is decrypted as **GOOD DAY** from Table – 1.

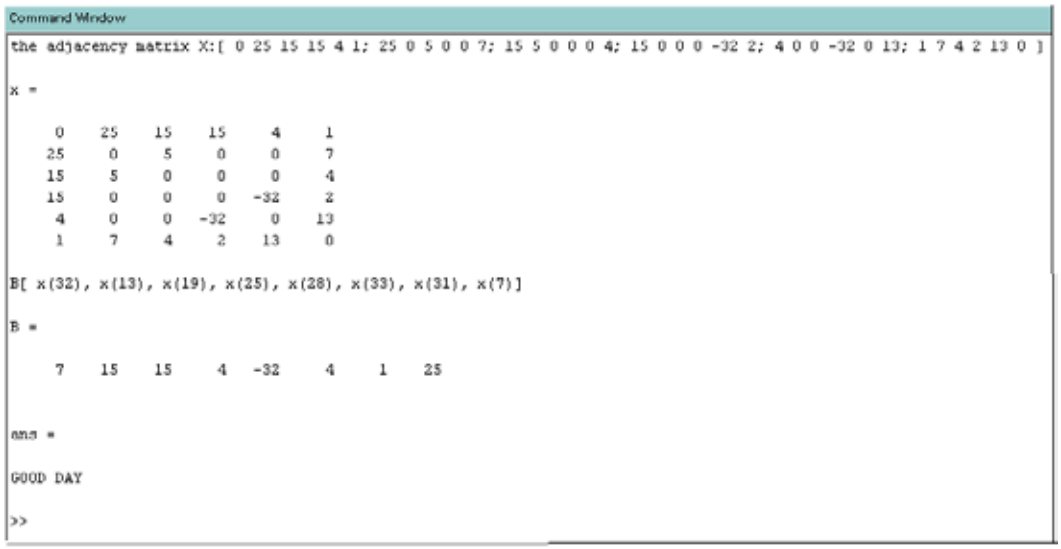
IV IMPLEMENTATION OF THE ALGORITHM

We have created a MATLAB code for encryption and decryption of the message. A graph can be represented by its adjacency matrix. In the MATLAB code we finally encrypt the graph as an a distance matrix to the receiver. Snapshot – 2 provides the output screen for the message SECRET encrypted in the example. We have used ASCII conversions for the message to be converted to a string and then into a distance matrix.



Snapshot – 2

It can be noted that the first six characters in the output string matches with the conversion using Table – 1 as in Sec 3. 2. The distance matrix can be send to the receiver instead of the graphs.



Snapshot – 3

Snapshot – 3 provides the output screen for the decryption example discussed in Sec 3. 2. It can be verified that the output matches with the example discussed. We have used the regular ASCII conversions for converting numbers to strings and strings to numbers. The program provides fast output for graphs with 10 vertices. It is expected that output would be generated fast with advanced models.

V CONCLUSION

- The edge sequence can be picked in any random order. If G is a graph with e – edges and the message to be encrypted is of length k, then the number of possible combinations for choosing edges = eC_k.
- For each of these combinations we can choose the edge sequence from G*. So the number of ways of encrypting the message is eC_k.
- Unless the edge sequence is known the message cannot be decrypted, which means that one has to try eC_k combinations to decrypt a message.

Also numerous graphs are available in public domain, that it is difficult to find the encrypted graph and a fake one. So the proposed method is safe for encryption of any message.

REFERENCES

[1] Yadhu Ravinath, Vipul Mangla, Arkajit Battacharya, Peeyush Ohri, Graph Theory Application in Selective Encryption Mechanism for Wireless Ad Hoc Network, International Journal of Emerging Trends and Technoloy in Computer Science, Vol 2, Issue 2, March - April 2013

[2] Essan suliman Mustafa Ahmed Dr.Amin Babiker A/Nabi Mustafa, The Effect of Encryption Algorithms Delay on TCP Traffic over Data Networks, IOSR Journal of Computer Engineering, Volume 17, Isuue 1, Ver . II (Jan – Feb 2015), pp 85-91.

[3] Hussien Th Khamees, Jalal A. Kahlf and A.Al-sajee, Encryption and Decryption of Data by using Geffe Algorithm, International Journal of Modern Engineering Research, Vol 2, Issue 3, May –June 2012 pp-1354 – 1359.

[4] http://en.wikipedia.org/wiki/Graph_theory

[5] http://mathworld.wolfram.com/WeightedGraph.html

[6] http://www.maplesoft.com/support/help/Maple/view.aspx?path=examples/GraphTheory

[7] http://mathworld.wolfram.com/SimpleGraph.html

[8] http://www.sagemath.org/doc/reference/graphs/sage/graphs/graph.html

[9] http://en.wikipedia.org/wiki/Glossary_of_graph_theory

[10] http://en.wikipedia.org/wiki/Planar_graph

[11] www.mathpages.com/home/kamth266/kamth266.htm

[12] http://en.wikipedia.org/wiki/Dual_graph

[13] http://en.wikipedia.org/wiki/Degree_%28graph_theory%29

[14] http://en.wikipedia.org/wiki/Distance_matrix

[15] http://www.gitta.info/Accessibiliti/en/html/StructPropNetw_learningObject4.html