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Original Research Article

CLONING AND EXPRESSION OF UNIVERSAL STRESS PROTEIN 2 (USP2) GENE IN *ESCHERICHIA COLI*

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Abstract: *Different types of abiotic stresses inhibit the normal growth of plants by changing their physical biochemical, morphological, and molecular traits. It links to the polygenic traits, which is controlled with the help of different genes, due to this polygenetic the manipulation of foreign genetic makeup is very difficult. Drought stress is the very major type of threat to reduce the yield of cash crops in Pakistan and as well as in all over the world. Gene manipulation is the solution to face this problem by producing genetically modified crop plants that have the ability to survive in drought conditions. Universal stress protein gene has been already identified in bacteria which showed its response under stressed conditions, by manipulation of universal stress protein gene. It was found from our study that the bacterial cells transformed with the USP2 gene isolated from cotton induced abiotic stress tolerance under heat, osmotic, and salt stress. It was suggested from our findings that the USP2 gene could be used to produce abiotic stress tolerance transgenic crop plants to enhance crop plant yield and quality.*

Keywords: drought, heat, salt, universal stress protein gene, gene manipulation, polygenic traits, tress tolerance

Introduction

There are many abiotic stresses which inhibt the normal growth of plant. These unfavorable conditions stop the growth of crops, some abiotic factors are drought tolerance, temperature flactuation, higher concentration of salt, and cold stress (Jaleel *et al*., 2009). Loss of water at normal or higher temperature is known as drought cause abnormalities in biological and chemical functions and also change the morphology of cells, due to loss of water plant loses their turger pressure. the composition of cell membrane is also changed and its interections between all other components. Plants shows their response against drought stress at moleculer and cellular level by activating the set of metabolic activities (Fahramand *et al*., 2014). At moleculer level, set of different genes are involved to start the defence machanism of plant to servive under stres conditions, but the study of genetic material of plant at proteomic and transcriptomic level shows that regulation of stress related genes are at cellular level. It also shows that the expression of gens are vary plant to plant (Ribas *et al*., 2006).

The activation of the metabolic reaction in stress condiotion depends upon the two types of pathway known as ABA-dependent or ABA-independent pathway,the end product of these pathways played an important role in the activation of stress related pathway (Majid *et al*., 2017; Qamar *et al*., 2017; Shinozaki and Yamaguchi-Shinozaki, 2007). Abiotic stresses depends upon the biological processes of plant. The Plants contain bundle of protein contaning universal stress protein domains. The study of plant taxa shows 200 different protein containing USP domains (Isokpehi *et al*., 2011; Sivamani *et al*., 2000). In cotton two genes are isolated which encode universal stress protein GUSP1 and GUSP2 (Fahramand *et al*., 2014; Mahajan and Tuteja, 2005; Wang *et al*., 2003). The number of copies of genes increased during drought stress. The role of universal stress protein first studied in tomato shows that two genes has been in the activtion of USP protein. One gene produced light harvesting chlorophyll 'a' and 'b' binding protein (LHCB) and second produced osmo protective compound which is proline (Fahramand *et al*., 2014). Some proteins are also discovered which played an important role to prevent plant cells from cell to lose water (dehydration) (Van Loon and Van Lanen, 2013; Van Loon *et al*., 2016). Universal stress proetin is present in cytoplasm also known as cytoplasmic protein presenrt in bacteria. By chinging the concentration of cellular components with the help of different types of abiotic stresses increased the expression of universal stress protein (Jaleel *et al*., 2009). This group of universal stress protein is also present in different types of bacteria,

archaea, fungi, and plants. Six universal stress proteins (USPa, USPb, USPc, USPd, USPe, USPf) present in E. *coli* (Robinson, 2016) are further divide into two groups on the basis of their sequence (Miki and McHugh, 2004; Tranel and Wright, 2002; Zhu *et al*., 2000). Universal stress protein plays an important role in response to heat shocks, cold shock and metabolic control (Adugna *et al*., 2006; Deng *et al*., 2011). Universal stress protein contain USP specfic domain that's why it named as universal stress protein(USP). A universal protein contain more then one USP domains due to which it performs variable functions in different organisms. With the help of 2-D-gel electrophoresis first universal stress protein was discovered named as C-13.5 name based on its migration during experiment. In further different studies role of universal stress protein is identified. Universal stress protein play different role in cell to prevent heat cold and from drought (Hanin *et al*., 2001; Lloyd *et al*., 2005). In drought stress universal stress protein shows intraction with annexin protein but the detailed mechnism is still unknown. Under high salt conditions E.*coli* activates ion transporter channel and start transport of salt ion outside from bacterial cell. The activation of this transpoter channel occur only when it gives induction with a comple of KdpD and KdpE, when phosphorylation occured this complex is formed (Gelvin, 2003; Hood *et al*., 1986). The universal stress protein also having ATP binding motifs which is present on its N terminal. But th C terminal region takes different form depends upon the protein which intract. But in same cases C terminal also binds with ATP molecule due to this binding USP capables to bind with 2 ATP molecule. Some Universal stress proteins have good percentage of glycine aminoacid which allows universal stress protein intract with other proteins (Li *et al*., 2005). Our research was based on universal stress protein 2 (USP2) which helps plant under stress condition to maintain itself, universal stress protein2 provids resistance against drought, heat, osmotic and salt stress. Universal stress protein2 was islolated from *Gossypium arboreum* and transformed in *E.coli* for its expressional studies.

Material and methods

Place of study/research

All the reseach work of cloning was done in moleculer lab of CRiMM department of the Unversity of Lahore. Luria bertan (L.B) media was prepared by using diffent ingridents like Yeast extract 5g/l, NaCl 10g/l, Tryptone 10g/l and Agar used to solidify the media was 0.5% and autoclaved it on 121^0C temperature, 15 Pascal pressure for 20 minute to avoid any type of contamination, after autoclaving supplemented with different antibiotics like kanamycin 50mg/ml and ampicillin 100mg/ml for bacterial growth. This media was used to grow bacteria contained all the nutrients required for bacterial growth.

Sterilization of working area

The surface of laminar flow cabinet was sterilized by using 70% ETOH use as a disinfectant after media preparation and autoclaving of the media placed in laminar flow cabinet and other things which was used during inoculation of bacterial culture process like Pipette, spreader, colony picker, petri plates, paraffin, scissor, pipette tips, and others. After placing the material and disinfection of the cabinet turn on UV light for 20 minute to kill microbes present on the surface of things.

Revival of stock of bacterial culture

Liquid broth

After media preparation inoculated the bacterial culture for its maximum growth and for plasmid isolation, PCR reaction etc. take 10 ml of freshly prepared L.B media in 250ml flask, add 1ml of stock culture of each (puc, pet, top10, bl21) culture, supplemented with 10 µl antibiotic which is specific according to bacterial strain and was placed on shaker incubator overnight at 37^0C and 250 rpm.

Solidifying media

After autoclaving of media, it was placed in laminar flow cabinet to cool down and to avoid contamination. When media is bear able to touch add required antibody and pour it in plastic petri plate, uncovered the petri plate until media is solidify. Take 5µl of stock culture and spread it on petri plate with the help of spreader, after this wrap petri plates with paraffin and placed petri plates in incubator temperature was 37^oC overnight for bacterial growth.

Strains of bacteria and plasmids

Different strains of bacteria and plasmids are used which are listed below.

Isolation of plasmid: After revival of culture the next step was isolation of plasmid by using plasmid extraction kit method.

Gel electrophoresis

Gel electrophoresis is another technique which was used to identify isolated plasmid quantity and it is also used for PCR product conformation, run with 1Kb ladder to conform the size of the product. First make 50X TAE (tris acetate EDTA) stock concentration then diluted with distilled water and convert 50X TAE into 1X TAE (working concentration). Take 20ml of stock of 50X TAE and dissolved it into 80ml of distilled water to make 1X TAE. Dissolved above given quantity of agarose (used as solidify agents) into required ml of TAE and heat till agarose was dissolved properly after this wait till the gel was bear able to touch and add ethedium

bromide and mix them well and pour into gel caster where the comb was already set before pouring gel. When gel was solidify remove comb and pour sample into gel well with dye (bromo phenol blue) and also run 1Kb ladder, after loading run gel at 100 voltage for 30 min from negative pole to positive pole to identify required segment gel was observed under UV transilluminator.

PCR (polymerase chain reaction)

Polymerase chain reaction was the next step after plasmid isolation and conformation of plasmid by using gene specific primer to amplify specific segment at specific temperatures.

Restriction of plasmid and gene

After conformation of plasmid and amplification of specific gene segment the next step was restriction, restrict plasmid and gene with two different enzymes to avoid frame shifting and for normal functioning protein. The restriction is done with enzyme Nde1 and BamH1 which produced sticky ends, or overhangs have same complementary sequences. After preparing restriction mixture incubate PCR tubes at 37^{o}C for 2 hours after this perform gel electrophoresis to observed restricted fragments on gel.

PCR purification method

After restriction of plasmid and gene cut the segment of plasmid and gene present on gel with the help of cutter under UV transluminator and put in eppendroff, after cutting purify the nucleic acids by using quegene kit method, in this kit method different buffers are used to purify contents.

Ligation

After nucleic acid purification the next step is ligation of the plasmid with gene with the help of enzyme T4 DNA ligase. Ligation was done after preforming nanodrop (which is used to detect nucleic acid concentration in elution buffer). The nanodrop value for PUC (which contain USP2 gene) is 6.1ng/ µl, and nanodrop value of pet vector is 12ng/ µl. the ligation is done with 1:2. After preparing reaction mixture incubated overnight at 22^{o}C, after overnight incubation speared whole reaction sample on kanamycin contain L.B agar plate and incubated overnight

Competent cells formation

After ligation next step to prepared competent cells of bacterial strains top 10, for preparing competent cells or the transformed cells was speared on kanamycin contain L.B agar plate and incubated overnight, after incubation first growing colony pick and cultured into L.B media, isolate plasmid and perform PCR to conform USP2 gene ligation and transformation.

Results

Isolation of plasmids (PUC57 and PET 30b)

This gel electrophoresis shows the isolated plasmid from stock solutions of bacteria. Plasmids were isolated by using plasmid extraction kit method and stored at -20. The size of Puc plasmid was 3220b.p and size of Pet 30b was 5400bp (Figure 1).

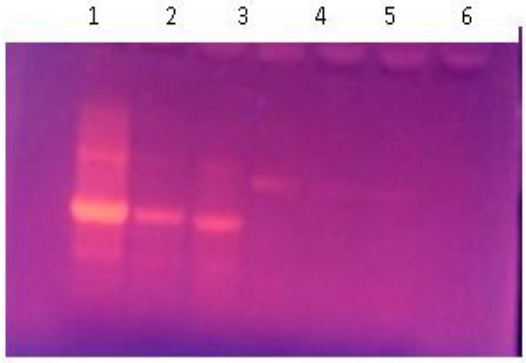

Fig 1: lane 1,2,3 shows isolated puc plasmids from bacteria and lane 4,5,6 shows pet plasmids.

Conformation of universal stress protein through PCR

After isolation of plasmids the PUC plasmid was amplified through PCR by using gene specific primer and run along with 1Kb ladder to confirm the size of gene which was about 500 base pair long (Figure 2).

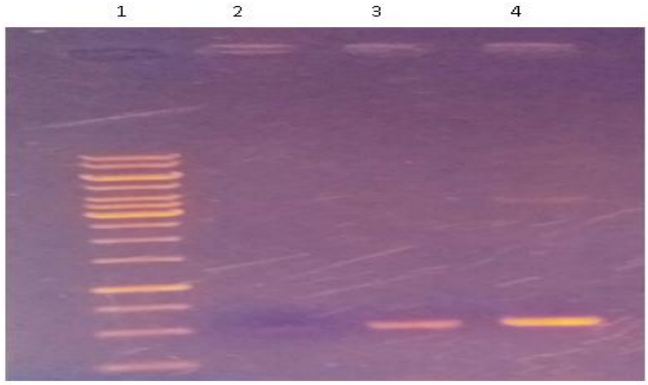

Fig 2: Conformation of usp2 gene present in puc plasmid through pcr amplification by using gene specific primer with 1kb ladder for conformation of gene size.

Restriction of plasmid and gene

After conformation of the gene in puc plasmid the next step was restriction of Pet plasmid and universal stress protein with Ndel and BamHl which produce sticky ends to avoid frame shifting (Figure 3).

Ligation and transformation

After restriction the next step was ligation of plasmid with gene with the help of T4 DNA ligase at 22^{o}C overnight. Transform ligated cells into freshly prepared competent cells, and spread it on ampicillin containing nutrient agar plate (Figure 4).

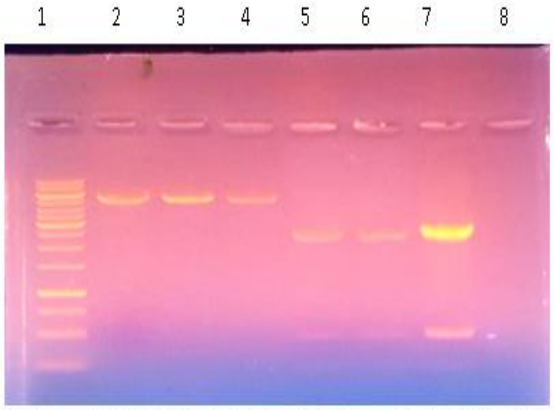

Fig 3: lane 1,2,3 shows pet 30b and lane 4,5,6 shows puc 57 plasmids restrict with Nde1 an BamH1 enzymes at 37°C for two hours and run with 1kb ladder to identify the size of restricte fragment

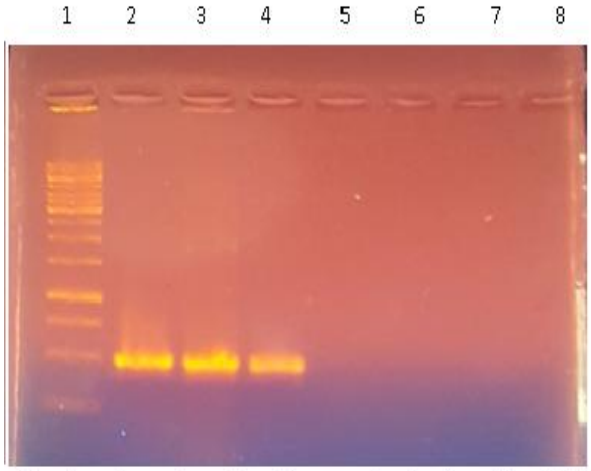

Fig 4: conformation of usp2 gene after ligation with T4 DNA ligase and then amplified through PCR gene specific primer.

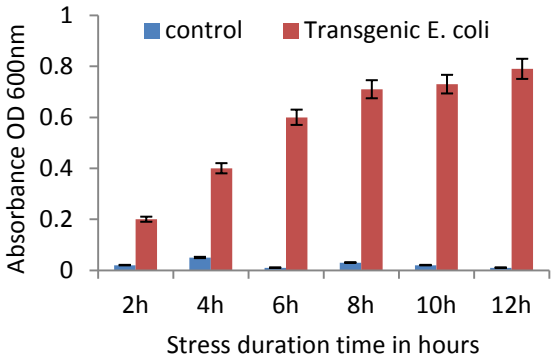

Figure 6. Transgenic and control/no-trangsgenic *E.coil* for heat stress

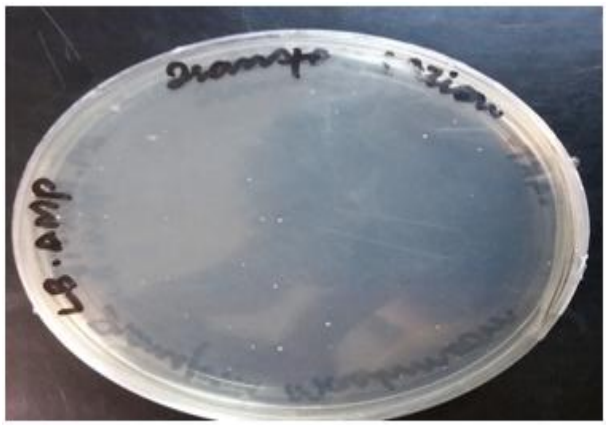

Fig 5: Shows transformation of usp2 gene into Pet 30b after ligation with T4 DNA ligase enzyme at 22°C overnight

Conformation of the clone of universal stress protein through PCR

After transformation colonies grown on ampicillin containing agar plate were recultured into broth of nutrient media at 37 °C overnight. Then plasmid was isolated and amplified through PCR to confirm clone of gene (Figure 4 and 5). The results from figure 6, revealed that there was a gradual increase in the expression of USP2 gene for heat stress in *E. coli* with passage of time interval of 2 hours to 12 hours. The non-transgenic cells were died in the culture while increasing the duration of heat stress. It was also found from figure 7 that there was also gradual increase in the osmotic adjustment in the transgenic bacterial cells as compared with non-transgenic bacterial cells. Similarly, the tolerance of transgenic bacterial cells was enhanced with increasing time interval as compared with the non-transgenic bacterial cells.

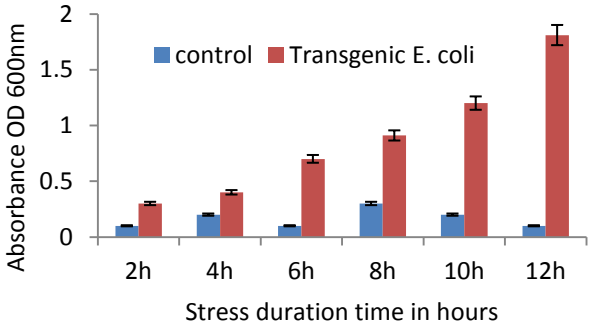

Figure 7. Transgenic and control/no-trangsgenic *E.coil* for osmotic stress

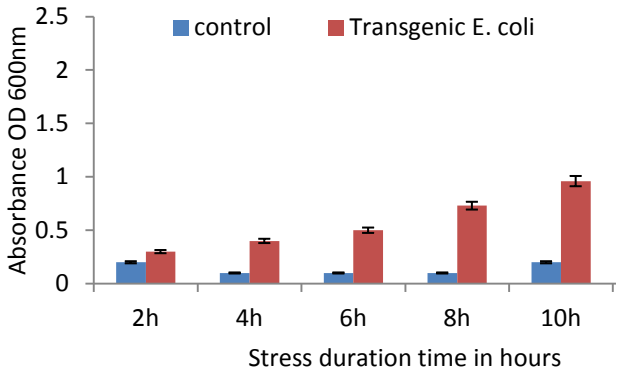

Figure 8. Transgenic and control/no-trangsgenic *E.coil* for salt stress

Discussions

The transformation technique is used to protect crop plants from abiotic and biotc stress conditions which help in minimizing the damage through stress conditions in crop plants (Howe and Jander, 2008; Lawrence and Novak, 2006). It Provides defense mechanism against stress. Transformation of USP2 gene was done with the tag of ubiquitin promoter (Jabeen *et al*., 2008; Sultan *et al*., 2009) along with report gene like GUS gene. In our study the report universal stress protein was successfully transformed into vector expression strain of bacteria by using pet vector which was restricted with Nde1 and BamH1. The restriction which is produced sticky ends and ligation with T4DNA ligase. The ligated universal stress protein gene is very close to vector promoter region to obtained maximum expression and number of copies. Universal stress protein helps to provide resistance in plant against drought tolerance. Similar results have been reported in various studied (Ibl and Stoger, 2012; Reyes *et al*., 2010). To achieve the maximum transformation rate efficiency was carried out by changing the media components concentration by providing kanamycin 250mg/l (Grover, 2012; Li and Greene, 2010). But in this research, the maximum growth of bacteria is obtained by using optimized concentration of bacterial culture media with 50mg/ml kanamycin. It has been found that cloning of gene is usually a major step to transform gene in crop plants with desireable charecteristices. Universal stress protein helps to maintain water level in plant cells specially in cotton to provide drought resistance. Universal stress protein is a regulatory protein and its activity is increased by introducing its intractions with other morphological, moleculer and biochemical traits (Jin *et al*., 2010; Yang *et al*., 2014). The transformed bacterial cells which showed tolerance under heat stress, osmotic stress and salt stress conditions indicated that the cells with USP2 gene shows tolerance against changing environmental conditions. The abiotic stress under such conditions of transgenic bacterial cells may indcue the change and ability to withstand against stressful environmental conditions (Engel *et al*., 2014; Pouly *et al*., 2013; Rossier *et al*., 2013). The use of USP2 gene for crop plants may be helpful to produce abiotic stress tolerance in crop plants to increase crop plant yield. It was suggested from our study that the USP2 gene may be used to produce transgenic crop plants against abiotic stress conditions.

Conflict of intrest

The authors showed absence of conflict of interest for manuscript publication.

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