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Search for Candidate Gene for Enhancing Heat Tolerance in Plant

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ABSTRACT: High temperature can cause considerable pre and post- harvest damages, including scorching of leaves and twinges, sunburns on leaves, branches and stems, leaf senescence and abscission, shoot and root growth inhibition, fruit discoloration and damage, and reduced yield. A.thalianaplant represents a genetic model for experiments in genetic engineering. Seed plating theory micropropagation technique with stress parametersenhance the heat tolerance in A.thalianaplant. Genetic screening using PCR technique provides gene modification in A.thaliana plant. Kanamycin plating provides screening result for gene screening we use pCambia vector and EcoR1, Nco1 restriction endonuclease. Results of PCR show the heat tolerance capacity is enhance in A.thaliana. pCambia cloningrepresent the candidate gene for heat tolerance and screening gives the annotation of the DNA typing in A.thaliana.

Keywords: Micropropagation, kanamycin, ,Candidate gene.

I. INTRODUCTION

The world native vegetation requires expands plant production by 50% before 2030[1].Although includes global clime condition which is supposed to have negative effects on agricultural work rate through higher than optimal temperatures for plant creation[2]. One estimate temperature is assumed to rise by 2–3°C over the coming 35–40 years [3]. In this condition, genetic improvement of heat stress tolerance traits of plants which are essential for fungal pathogen.

Gaseous emissions due to the human activities are substantially adding to the existing concentrations of green house gases, particularly carbon dioxide, methane, chlorofluorocarbons and nitrous oxides.

Heat stress is the most destructive abiotic restricting the growth of plants. Heat stress can changes in various metabolic and physiological processes like oxidative damage of DNA, proteins and lipids also the production of reactive oxygen species (ROS)[4]. Various Numerous genese elaborate in heat tolerance. Candidate gene approach has also been used to recognize key genes which may control Abiotic stress (like Salinity, Drought & Cold) in plants species.[5-7]

Arabidopsis thaliana, are small flowering plant that is widely used as model plant in plant biology due to their short germination time (8-12 weeks) also it is the 1st plant to have its entire genome sequenced. Arabidopsis thaliana was reported as that fertile seed production through self pollination& limited requirements for growth facilities.[8]

This study intends to evaluate-DNA vectors for candidate genes probably associated with improved heat stress tolerance in A. thaliana. Additionally we looking at candidate gene for enhancing heat tolerance and heat sensitive in plant.A. thaliana has a small genome that already has been sequenced, our plant represents as a genetic model for experiments in genetic engeneering.

For the amplification of candidategene, two types of primers was designed that isouter primers and inner primers. The outer primers was designed by using PCR Cloning Kit User Manual[9]. Kanamycin plating provide screening result for gene screening we use pCambia vector and EcoR1, Nco1 restriction endonuclease. Results of PCR show the heat tolerance capacity is enhance in A.thaliana. pCambia cloning represent the candidate gene for heat tolerance and screening gives the annotation of the DNA typing in A.thaliana.

II. MATERIAL AND METHOD

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Plant material and growth conditions-
Study period- October 2019- November 2019

Our model plant (A. thaliana) is grown in an indoor chamber, the best average temperature to grow is 18 to 24°C (64 to 75 f) standard it's take 1 to 2 week to germinate.[10] We used leaves,twings, branches and stems also shoot and root for growth inhibition which was initially used to abiotic stress.

For this procedure we used some techniques like plant tissue culture, used of suitable promoter, suitable transformation gene and alsothe cloning process. Plant wereenergizein trays, before we start, the trays should be brushed/cleaned and seed free from old seeds also it should be free from fungus**.**

SOIL PREPRATION-

We used Miracle-Gro potting mix with suitable fertilizer to create Arabidopsis-familiar soil and mix it completely. Scoop dry soil into a autoclave bin,cover up the bins with aluminum foils and put a piece of autoclave tape. Autoclave on normal mode for 15-20 mindepending on how many bins are being autoclaved. Let it cool and the soil placed into tray. Once opened, autoclaved soil can be good for use for upto 2 weeks. Mix the soil in deionized water,cover with humidomes and let it soak for 3-6 h or overnight. The soil in trays is now ready for growing plants.

SEED STERILIZATION AND PLATING-

Put certain amount of seeds into 1.5ml eppendrof tubes. Add 1ml 70% of ethanol. Pour off water and add 5% ethanol, incubate for 5 min. Rinse with sterile water 4 times, add 0.1% Triton X-100 for 10 min. Moved sterile seeds to small fisher dishes with a wet whatman's filter paper on its bottom.

Before start, again the trays should be brushed/cleaned and seed free from old seeds also it should be free from fungus**.** Fill trays (15 x 9 x 8 cm) to the top with autoclaved soil add sterile water to the tray it usually takes usually 3-4 hr to let the soil soak thoroughly. And transfer the seeds into the soil(8/10 seeds per tray), after plating cover the tray with a humidome, taping it into the tray to keep the necessary humidity. Put the try in 4°C cold room for 3-5 days at dark, transfer a light(22 °C) 16h light/8 h dark to maintain photoperiod at 22 °C/ 18°C. Remove the humidome, and water with sterile water twice a week.

Process of cloning-

Plant tissue culture technique for Amplification of gene-

A.thaliana seeds were germinated in the laboratory. The amplification of candidate gene was carried out directly from these fresh leaves following the standard protocol Plant Direct PCR Kit (Finnzymes, 2009). Primary green leaf tissues were used as the tissue source for candidate gene. According to this protocol a small leaf sample was placed in 20 µL of dilution buffer.[11] The leaf was crushed with a 100 µL pipette tip by pressing it briefly against the tube wall. For the amplification of candidate gene, two types of primers were designed such as 1st amplification primers (outer primers) and 2nd amplification primers (inner primers). According to this protocol, the 15 bases of the 5′ end of the primer were homologous to 15 bases at one end of the vector pCambia and the 3′ end, the sequence was specific to the candidate (Clontech, 2010). gene.

2µL of cloning enhancer was mixed with 5 µL of inner fragments, heated at 37°C for 40 minutes in heat block followed by heating at 80°C for 40 minutes.[12] This mixture was stored at -20°C until further use. Preparation of linearized vector by restriction digestion was performed to achieve a successful In-Fusion reaction. The candidate gene was fused to completely digested pCambia.

After incubation, the fusion mixture was diluted with 40 µL TE buffer to prepare 50 µL fusion mixtures. From this mixture, 2.5 µL was transformed to 50 µL competent cell line DH5α (New England Biolab) by heat shock method and the cells were grown Followed by this, 100 µL, 250 µL 7 and 500µL protocol and transformed cells were spread on MB media plates containing 100 mg/L kanamycin and incubated at 37oC for overnight. Positive and negative control plates were set simultaneously. The positive control was performed with a circular vector of known concentration and the negative control was performed with known amount of linearized vector.

III. RESULT-

3.1- Amplification of Candidategene with outer and inner primers-

Forward primer's sequence- 5′-TCAGATTTCGGAAGAGAAGAAGG-3′

Reverse primer's sequence- 5′-CGTTGCTTTTGCTTTATGACTTT-3′

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Size of product – 2.05kb

Further properties of outer primers are describe in table given below

PRIMER	LENGTH
FORWARD	23bp
REVERSE	23bp

Table no.1: The properties of outer amplification primers for candidategene

Inner primer for candidate gene-

Forward primer's sequence- 5´-GAGTAAAGAAGAACCATGGGGAGAAGCATATTTTC-3´
Reverse primer's sequence- 5´-ATTCGGCCGGCCTCAGCCTCATTAGGCGCAAT-3´
Size of product – 1.5kb

Further properties of inner primers are describe in table given below -

PRIMERS	LENGTH
Forward	35bp
Reverse	32bp

Table no.2- The properties of inner amplification primers for candidategene

At the 5´- end, the highlighted part (15 bases) of the forward and reverse primers are homologous to one end of the vector pCambia and the 3´ end, the sequence of gene's was separate to the our candidategene. The melting temperature are shown in Table and the melting temperatures of gene sequences for forward and reverse primers are 64.1°C and 65.8°C respectively.

To get finest results of amplification, Bio-genic PCR kit used by us and whole Reaction of amplification was follow up with five different leaves and at the end product of PCR was examine by using gel electrophoresis. (figure no.- 1)

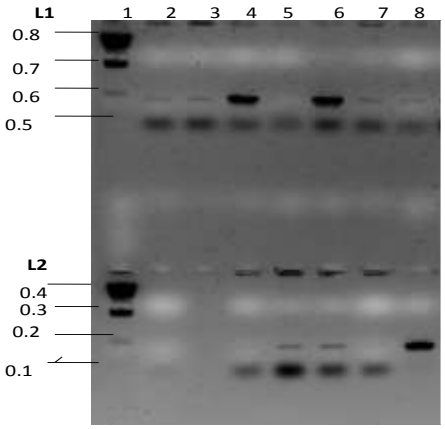

Figure1:Outer amplification primers are amplified with PCR products. Lane L1& L2isDNA ladder here Lane1, 2, 3, 4, 5,6,7 and lane 8 show band for candidate gene at about 0.1 & 0.5 kb position.

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In lane 1, it can be identified the one thin band is near 0.1kb .also in lane 2 & 5 band near 0.2kb and 0.5kb respectively. Visibility of this band could be due to the maximum amount of candidategene.

The purified candidategene product was amplified with using an annealing temperature of 85ºC. So the candidategene products was observed by using the gel electrophoresis(Figure- 2)

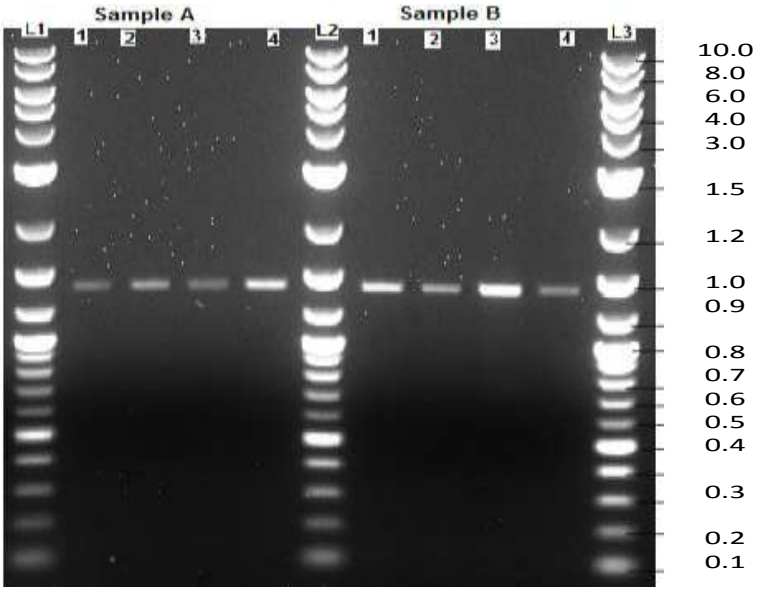

Figure2:Inner amplification primers are amplified with PCR products. Lane L1, L2 is & L3 are DNA ladder here Lane1, 2, 3, and lane 4 show band for candidate gene at about 2.0 kb position.

After amplification of candidategene it was digested by Ecoli and Nco1 restriction enzymes. The restriction digestions was carried out by using sample 2 and 4 (sample A and B) with both endonucleases and mixture of these enzymes. Result has been shown in Figure3 .

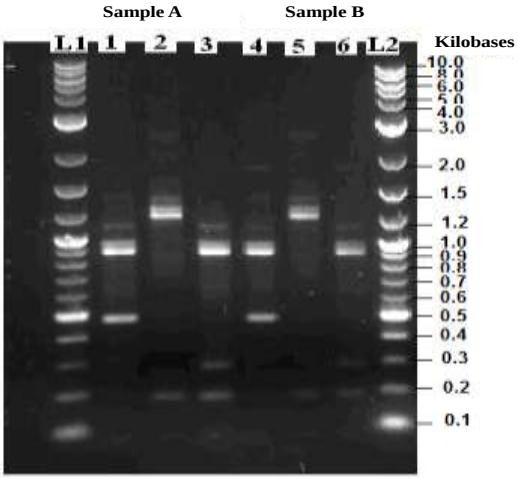

Figure3:Candidate gene are digested by restriction digestion .Again Lane L1 and L2 are two DNA ladder. Lane 1 and 4 digested with E.coliR1, lane 2 and 5 digested by Nco1, lane 3 , &6 digested by both E.coliR1 and Nco1 .

3.2. Digestion of vector pCambia-

T-DNA plasmid were digested by restriction enzymes BamHI and the product of digested gene were run on gel electrophoresis (shown in figure below). The vector map BamH1 was shown.

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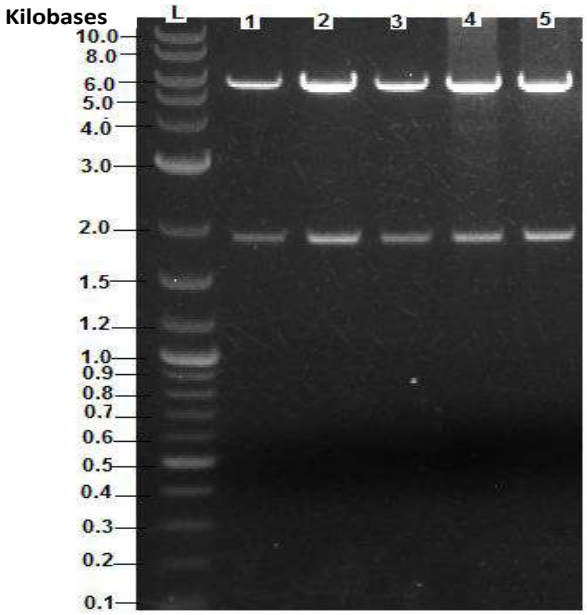

Figure 4: Two bands of vector T-DNA are shows in this gel image, after digestion with restriction enzyme BamH1. Lane L is the DNA ladder and here lane 1, 2, 3, 4, 5 shows the restriction digestion of pCambia.

3.3. Cloning and Transformation-

The modified cells was diluted by adding TE buffer. The diluted cells were spread on MS media containing kanamycin (100mg/L) and media plates were incubated for overnight at 37°C with negative control plates and positive control plates. CFUs no. was observed in this Table.

TYPES OF PLATES	NUMBER OF COLONIES
Positive control	Uncountable
Negative control	No growth
Transformed cells of 100µl suspensions	8
Transformed cells of 250µl suspensions	14
Transformed cells of 500µl suspensions	32

Table 3: Colony forming unit (CFU's) are showing on media plate with positive and negative control.

3.4. Screening-

Screening of this digestion was performed to confirm the restriction digestion. In this screening 8 colony were shown in this figure.

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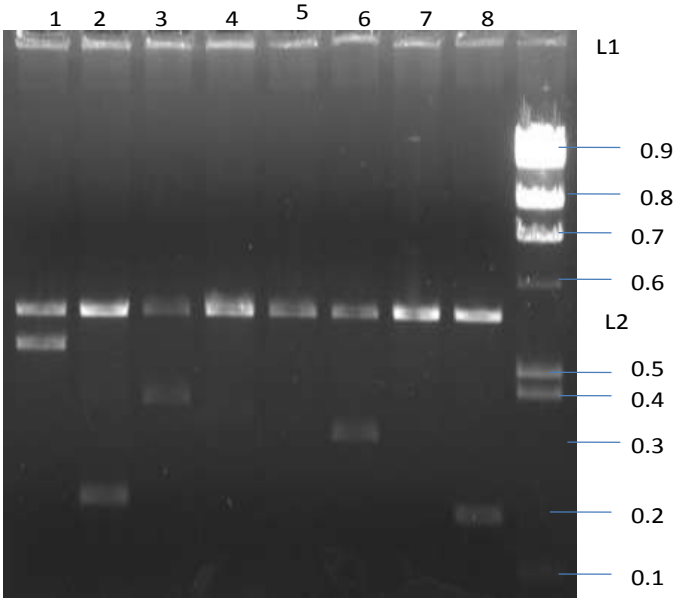

IV. DISCUSSION-

High temperature can cause considerable pre and post- harvest damages, including scorching of leaves and twinges, sunburns on leaves, branches and stems, leaf senescence and abscission, shoot and root growth inhibition, fruit discoloration and damage, and reduced yield[13]. A.thalianaplant represent a genetic model for experiments in genetic engineering. Seed plating theory micropropagation technique with stress parameter's enhance the heat tolerance in A.thalianaplant. Genetic screening using PCR technique provide gene modification in A.thaliana plant.[14] Kanamycin plating provide screening result for gene screening we use pCambia vector and EcoR1, Nco1 restriction endonuclease.

V. CONCLUSION-

Results of PCR shows the heat tolerance capacity is enhance in A.thaliana. pCambia clone the candidate gene for heat tolerance and screening gives the annotation of the DNA typing in A.thaliana.

REFERENCES-

[1]. Abedin J., Feldman J. and Meharg A. (2002) "Uptake Kinetics of Arsenic Species in Rice plants Plant Physiology, Vol. 128, (p. 1120-1128).

[2]. Chang-lan, Z. H. U., Ying-hui, X., Chun-ming, W., Ling, J., Hu-qu, Z., and Wan, J. M. (2005). Mapping QTL for heat-tolerance at grain filling stage in rice. Rice Sci. 12, 33–38

[3]. Eun Kyung Cho and Choo Bong Hong.(2004) . Molecular Cloning and Expression Pattern Analyses of Heat Shock Protein 70 Genes from Nicotiana tabacum. Journal of Plant Biology,**47(2)** : 149-159

[4]. Hatfield, J. L., and Prueger, J. H. (2015). Temperature extremes: effect on plant growth and development. Weather Clim. Extrem. 10, 4–10. doi: 10.1016/j.wace.2015.08.001

[5]. Jagadish, S. V. K., Septiningsih, E. M., Kohli, A., Thomson, M. J., Ye, C., Redoña, E., et al. (2012). Genetic advances in adapting rice to a rapidly changing climate. J. Agron. Crop Sci. 198, 360–373. doi: 10.1111/j.1439-037X.2012.00525.x

[6]. Knocz C. et al., (1992) "Methods in Arabidopsis research" World Scientific Publishing Co. Pte. Ltd. Printed in Singapore. Chapter 4 p. 100. Edited by Chua, N. And Schell, J. ISBN 981-02-904-5.

[7]. Pelleschi S, Guy S, Kim JY,Pointe C, Mahe A, Barthes L, et al. Ivr2, a candidate gene for a QTL of vacuolar invertase activity in maize leaves. Gene specific expression under water stress. Plant Mol Boil. 1999; 39;373-380. PMID: 10080702

[8]. Priyanka Das, Naveen Chandra Joshi. **(**2011).Minor modifications in obtainable Arabidopsis floral dip method enhances

transformation efficiency and production of homozygous transgenic lines harboring a single copy of transgene.Advances in Bioscience and Biotechnology.

[9]. Rotter D, Amundsen K, Bonos SA, Meyer WA, Warnke SE, Belanger FC. Molecular Genetics Linkage map for allotetraploid colonial bentgrass. Crop Sci. 2009; 49: 1609-1619.

[10]. Tomlinson, I. (2013). Doubling food production to feed the 9 billion: a critical perspective on a key discourse of food security in the UK. J. Rural Stud. 29, 81–90. doi: 10.1016/j.jrurstud.2011.09.001

[11]. Tondelli A, Francia E, Barabaschi D, Aprile A, Skinner JS, Stockinger EJ et al. Mapping regulatory genes are candidate for cold and drought stress tolerance in barley. Theor Appl Genet. 2005; 112:445-454. Dol;10.1007/s00122-0050144-PMID:16315028

[12]. Turgeon AJ. Turfgrass management. Prentice Hall, Englewood Cliffs, New Jersey; 1999.

[13]. Wahid A, Gelani, Ashraf M, Foolad MR. Heat tolerance in plants; An overview. Environ Exo Bot. 2007; 61:199-223.

[14]. Yu X, Bai G, Liu S, Luo N, Wang Y, Richmond DS, et al. Association of candidate genes with drought tolerance traits in divers perennial ryegrass accessions. J Exp Bot. 2013; 64:1537-1551. Doi:10.1093/jxb/ert018 PMID: 23386684.

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