Detection and Amplification of the E8 Promoter in Tomato Plant and Sequencing of the E8 Gene

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Abstract

Tomato (Lycopersicon esculentum Mill) is edible, often red berry-type fruit and a rich source of Fe, Vitamin A, B and C. It is widely cultivated all over the world. E8 gene is related to ethylene biosynthesis in plants. Ethylene is responsible for growth and ripening of fruits. The present study was carried out to detect and amplify the E8 Promoter in the leaves and fruits of Tomato, Spring onion, Capsicum and Lettuce. The CTAB Protocol of Doyle and Doyle (1990) were used for DNA extraction from leaves and fruits and quality was checked on gel electrophoresis. PCR program for E8 was optimized and PCR products were sequenced. The sequencing results were aligned and Blast. The result indicated that in Tomato fruit the E8 gene is detected and amplified as compare to leaves and other plants. This E8 primer will be utilized for different fruits as well.

Key words: E8 promoter; Tomato; PCR; Sequencing

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INTRODUCTION

The scientific name of the tomato is *Lycopersicon esculentum* Mill. The tomato is used in sandwich, burger, ketchup and food salad, The common name of the tomato is Tomato, Tamati, Tamatiso, uTamatisi, the family belongs to tomato is family Solanaceae, kingdom plantae, Angiosperms, Eudicots, Asterids amd order Solanales and genus Solanum, specie *S*. lycopersicum (Gao et al., 2010). The Spanish explorers introduced tomato into Spain and it was later taken to Morocco, Turkey and Italy. In Italy and France, it was termed love apple. It was widely believed that the tomato was poisonous and its use as a food crop was only accepted in the 18th century. Tomato is now one of the most popular and widely grown vegetables around the world (Zulu, 2001). The first known British tomato grower was Patrick Bellow of Castle town who successfully reared plants from seeds in 1554. Tomatoes can make people healthier and decrease the risk of conditions such as cancer, osteoporosis and cardiovascular disease. People who ate tomatoes regularly have a reduced risk of contracting cancer diseases such as lung, prostate, stomach, cervical, breast, oral, colorectal, esophageal, pancreatic, and many other types of cancer. Some studies show that tomatoes and garlic should be taken together at the same time to have its cancer preventive effects. Tomato is an excellent fruit or vegetable for rapid skin cell replacement. Tomato juice can be used for healing sunburn because of its unique vitamin C. Tomato have vitamin A & E , tomato juice is good to yourself from fatigue and sleepiness (Bhowmik et al., 2012).

Ethylene (C_2H_4) is an important gaseous plant hormone involved in almost all phases of growth and development of plants there are certain compounds, which are known to release ethylene. Application of these ethylene releasing compounds could be much easier than the gaseous form of ethylene (Siddiq et al., 2009).

Ethylene is a gaseous plant hormone involved in specific developmental processes, as well as in response to many external stresses. Ethylene biosynthesis is increased in response to stimuli such as wounding, pathogen attack, and drought during normal development, ethylene promotes a number of events, including senescence, seed germination, abscission, and fruit ripening.

In climacteric fruits such as tomatoes (Lycopersicon *esculentum),* bananas, and avocados, the initiation of ripening is associated with a burst in ethylene biosynthesis, accompanied by a large increase in the respiration rate Ethylene biosynthesis during

fruit ripening is autocatalytic, such that a small amount of ethylene stimulates a massive increase in ethylene production (Michelle et al., 1996).

MATERIALS AND METHODS

Plant sample for DNA Isolation

For DNA isolation Seeds of tomato, Spring onion, Capsicum and Lettuce were germinated in Petri dishes. When seedling emerges then transferred to disposable glass in soil and DNA extracted from young leaves and when fruit mature.

Extraction Method

The CTAB-based extraction method of Doyle and Doyle (1990) was adapted with some modifications; the method was optimized for DNA extraction by varying the concentration of NaCL, Tris-HCL, β Mercapthaethonal etc.

Standardized Extraction Method

Pre heat CTAB buffer (pH 8) containing 1M Tris-HCL, 5M NaCl, 0.5M EDTA and 20g CTAB in water bath at 65˚C for ten to thirty minutes, grind 100-200 gm, young plant tissue to find powder in the presence of liquid nitrogen by using pre-chilled pestle and mortar. Transfer the content in 1.5ml micro centrifuge tube and add, 1ml CTAB buffer solution, followed by 20-30µl β-marcapthethanol, gently shake and incubate at 65˚C for 25 minutes. During incubation, shake the sample gently after 10 minutes. After incubation add 300µl Sodium acetate and kept the sample on ice for 20 minutes. Centrifuge at 12000rpm for 10 minutes at 4˚C. Take the supernatant in a fresh tube and add 1volume Tri-Chloromethane: Iso-pentanol (24:1) to remove protein. Repeat the process of centrifugation and Tri-Chloromethane: Iso-pentanol (24:1). Carefully transfer the supernatant into fresh tube and add 1 volume Iso-Propanol to facilitate sedimentation of DNA and place the sample at -20˚C for 30 minutes. Centrifuge briefly and discard the supernatant and wash the pellet with 75% Alcohol. Vacuum dry the pellet for 10 minutes then ads 100-200µl distil water, to elute the DNA and add 1µl RNAase and incubate at 37˚C for 30 minutes.

DNA Quality Confirmation

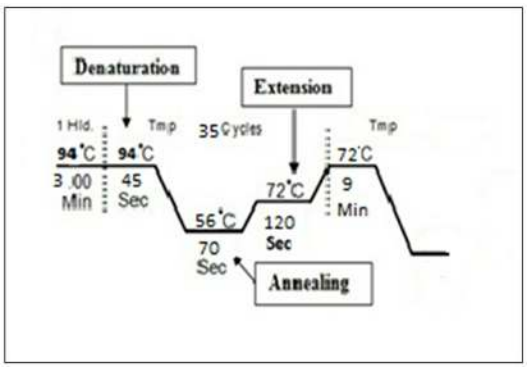
DNA quality was confirmed on 1% gel electrophoresis.

PCR Master Mix	10µl
E8 F-Primer	0.5 µl
E8 R-Primer	0.5 µl
PCR Water	6µl
Template	1 µl
Total Reaction	18 µl

PRIMERS

NAME	Sequence	Num of Nucleotides
E8F	5`GACCTTCTTTTGCACTGTGAATGATT`3	26
E8R	5`CTAGAAGGAATTTCACGAAATCGGC`3	25

PCR PROGRAM



RESULTS AND DISCUSSION

Table1 shows four genomes of four different crops tomato, capsicum, green onion and lettuce were detected at their fruiting and we check out the quality of DNA by the help of gel electrophoresis and the third one green onion had not the good quality of DNA because they contain the un-wanted macro molecules like proteins etc.

Similar sort of results were reported by Deikman and Fischer (1988).

Figure 1: Total isolated DNA from tomato, capsicum, onion and lettuce. Lane-1 shows reflects vibrant band of tomato DNA, Lane-II shows capsicum DNA, Lane-III shows green onion DNA and Lane-IV shows lettuce DNA.

PCR Product

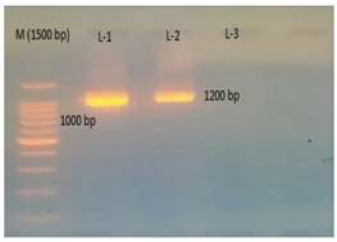


Figure 2: Shows the final PCR product marker is 1500 Base pair. Lane-I and Lane-II have good quality of PCR product and Lane-III have no result and the product is 1200 Base pair.

Figure 2 shows the final PCR results the DNA are bind to the primer and the primer is E8 promoter and we get the objective that the primer are bind to the to the tomato gene that are responsible for the ethylene production in this picture we have a marker that specify the location of the DNA at the genome the marker are 1500 bp that are shown in the picture and we have the 3 Lanes which is two are bind with good quality and the final product is 1200 bp.

Sequence of the E8 promoter

>150721-14_G05_E-8_E-8F.ab1 1219

GGCCGGGATCTTACTAGGCGATTTCGTGAATTC
CTTCTAGGCCGATTTCGTGAAATTGCCTTCTAGG
CCGATTTCGTGAAATTCCTTCTAGGCCGATTTCG
GGAAATTCCTTCTAGGCCGATTTCGTGAAATTCC
TTCTAGGCCGATTTCGTGAAATTCCTTCTAGGCC
GATTTCGTGAAATTCCTTCTAGGCCGATTTCGTG
AAATTCCTTCTAGGCCGATTTCGTGAAATTCCTT
CTAGGCCGATTTCGTGAAATTCCTTCTAGGCCG
ATTTCGTGAAATTCCTTCTAGGCCGATTTCGTGA
AATTCCTTCTAGGCCGATTTCGGGAAATTCCTTC
TAGGCCGATTTCGGGAAATTCCTTCTAGACCGA
TTTCGGGAAATTCCTTCTAGACCGATTTCGCGAA
GTTCCTTCTAGGCCGATTTCGTGAAATCCCTTCA
AGGCCGATTTCGTGAAATTCCTTCTAGGCCGAT
TTCGTGAAAGTCCTTCTAGGACGATTTCGCGGG
AGGCTTCTAGAGAAGTTTTGAGACAACTGTTATA
GGTAGCCTTCGAGAAGATCATTCTTTGACGACT
ACGTGTCTTTTATTCTATAAATATACTAAGATCTT
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AAGGTTTCTAGAAGATGCACACCTAAATTATATT
TGATTTTACTCACTTCACTGAACTTGTGAATTCC
TTCATCATATACACCTACTCCTATTATGACTACA
AGTTGGCAAAGAATGATATGAATGCTACTTAGAT
AATCATAGTCACCTAGATCATTAATTTATCAAAG
ATAATATCAAACTTCTCCCTAAAATTTGAGCAAA
CTTCTCACTAAACTTGTGGACTAACCCGAAATCT
CAGAAATTATATTTATACTGGAAAGTCAGATAAA
TGTCTGCCAAGATTTCTATGGTGGGATAAACAAA
TTATATGGATTACATAGTGGAATATTAGGAAAT
GTACAGGCTTTATCATGGATATATTCTTAAATCA
AATCTTATTAAAGTATTTGATAAGGGCGTTCGG
AAAATTCCTTTAGGCGATCGGAAATCCTCTTAA
GGGAATTCCTGAAGGCCGTTTTGGAATTCTGA
AAGCCTTTAGGGAATCCTCAAAGCCGTAGGGG
ATTCCCAAAGCCCTTAGCGGATTCCCAATGCC
GATTCGGATTCCTAAATCCGGTGCGGAATACTT
TCGCAAGTAG

Blast the E8 sequence

Lycopersicon esculentum ethylene-responsive fruit ripening gene E8 promter and 5'UTR, partial sequence

Sequence ID: gb|AF515784.1| Length: 2191 Number of Matches: 1

Range 1: 1074 to 1624 GenBank Graphics

Score	Expect	Identities	Gaps	Strand
492 bits(266)	7e-135	465/551(84%)	54/551(9%)	Plus/Minus

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Query  564   TTGACGACTACGTGTCTTTTATT-CTATAAATATACTAAGATCTTCTATGTAAAATGATT  622
Sbjct  1624  TTGACAACTACATTTCTTTTATTTTTATAAATTTACTAATATCTTCTATGCAAAATTATT  1565

Query  623   CGGTGACTTTCTATCCTTTAAGGTTTCTAGAAGATGCACACCTAAATTATATTTGATTTT  682
Sbjct  1564  CGGTGCCTTTCTAAACTTTAAGGTTTTTATTTGATGTACACCTAAATTATATTTTATTTT  1505

Query  683   ACTCACTTCACTGAACTTGTGAATTCCTTCATCATATACACCTACTCCTATTATGACTAC  742
Sbjct  1504  AATCACTTCACTGAACTTGTTTATTCCTTCATCATATACACCTACTCCTATTATGACTAC  1445

Query  743   AAGTTGGCAAA-G-AATGATATGAATG-CTACTTAGAT-AATCATAGTCACCTAGATCA-  797
Sbjct  1444  AAGTTGGCAAAAGTAATGATATGAATTTCTACTTAAATAAATAATAGTCACCTAGATAAA  1385

Query  798   TTAATTTATCAAA-GATAA-TATCAAAC-TTCTC-CCTAAAATTTGAGCAAA-CTTCTCA  852
Sbjct  1384  TTAATTTAACAAAAGATAAATATCAAACCTTCTCACCTAAAATTTGAGCAAAACTTCTCA  1325

Query  853   CT-AAACTTGTGGACTAA-CCCGAAA-TCT-CAGAAA-TTA-TATTTA-TACTGGAAA-G  904
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Query  905   TCAGAT-AAATGTCTGC-CAAGA-TTTCTATGGT-GGGA-TAAA-CAAATTA-TAT-GGA  956
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Query  1006  -CTTAAA-TC-AAA-T-C-TTATT-AAA-GT-ATTT--GA-TAAGGGC-G-TT-CGGAAA  1058
Sbjct  1144  TCTTAAAATTTAAAATTCATTATTTAAAAGTTATTTTCGAATAAGGGCCGATTTCGTGAA  1085

Query  1051  ATTCCTT-TAG  1060
Sbjct  1084  ATTCCTTCTAG  1074
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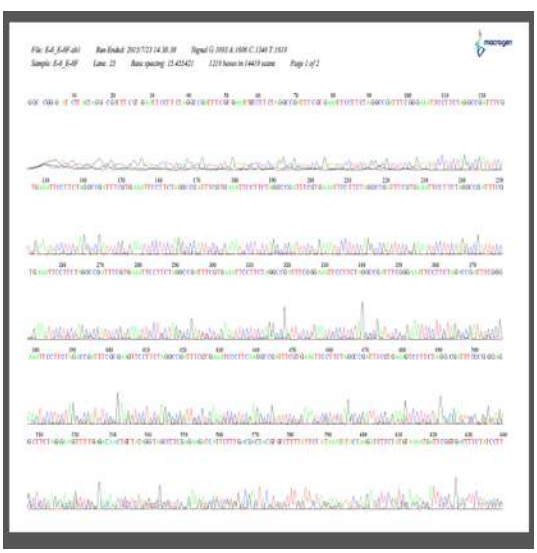
CHROMATOGRAM



CONCLUSION

The E8 gene which is responsible for the Ethylene production in many of crops especially in Tomato for fruit ripening that is successfully achieved in PCR result, that are bind to the primer and we detect and identified this gene, and later we sequenced the E8 promoter.

REFERENCES

- Zulu K. (2001). Production of the tomato Natal Department of Agriculture and Environmental Affairs. *Vegetable production guideline.*
- Gao G, Bergefurd B and Precheur B. (2010). Growing Tomatoes in the Home Garden. *Agriculture and Natural Resources.* HYG-1624-10. 1-12.
- Bhowmik D, Kumar KPS, Paswan S and Srivastav S. (2012). Tomato A Natural Medicine and Its Health Benefits. *Journal of Pharmacognosy and Phytochemistry*. 1(1):33-43.
- Michelle L, Kneissl and Deikman J. (1996). The Tomato *E8* Gene influences Ethylene Biosynthesis in Fruit but Not in Flowers. *Plant Physiol.* 112:537-547.
- Siddiq S, Yaseen M, Mehdi AR, Khalid A and Kashif S. (2009). Growth and yield response of tomato (*lycopersicon esculentum* mill) to soil applied calcium carbide and methionine. *Pak. J. Bot.* 41(5): 2455-2464.
- Doyle JJ and Doyle JL. (1990). Isolation of plant DNA from fresh tissue. *Focus*. 12:13-15.
- Deikman J and Fischer RL. (1988). Interaction of a DNA binding factor with the 5'-flanking region of an ethylene-responsive fruit ripening gene from tomato. *EMBO J.* 7(11):3315–3320.