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Short Communication

PCR Amplification of Boiling Soluble Encoding Genes from *Lantana camara*

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Abstract

Lantana camara is one of the most invasive species worldwide which can tolerate wide variety of soil types, has high reproductive potential, easy dispersal of seeds and extreme adaptability in hot and dry environments. However the molecular basis of its invasiveness is still not well known. In the present study effort has been made to find out homologous boiling soluble protein encoding genes. PCR amplification using conserved specific primers resulted in discrete bands of various lengths. Clustal-W analysis revealed the presence of conserved amino acid residues in the derived ORF sequences. Based on the findings we propose the molecular basis of invasiveness of Lantana camara.

Key words: Boling soluble proteins, Lantana, PCR

Introduction

Alien species were introduced intentionally by our forefathers to gratify social or pleasure purposes. With an abound increase in rate and the magnitude of transportation and trade there has been a bountiful inclusion of such species which are becoming a threat to the indigenous population. *Lantana camara* is such an example, being included in the list of "100 of the World's Worst Invasive Alien Species". It is an invasive weed spread wide and across America, many parts of Africa, Australia and India. *Lantana camara* survives in the range of warmer areas of the world, can tolerate wide variety of soil types, has high reproductive potential, easy dispersal of seeds and extreme adaptability in hot and dry environments [1]. Allelopathy, presence of a variety of phytochemicals and a potential to invade any habitat are a few of many allied characters that advocate invasiveness. These characters provide them a selective advantage over the originals, enabling them to colonize quickly at the cost of natives.

As a foreign plant inhabits a novel environment and establishes itself, the molecular-biochemical mechanisms intrinsic to its physiology help cope with stress tolerance and thus its survival [2]. A combination of biotic and abiotic stresses generates responses in plants both at cellular and molecular levels like accumulation of regulatory proteins or isozymes which may be involved in stress tolerance [2]. A medley of selective genes with diverse roles in stress culmination are induced or repressed by these processes [3,4]. Such examples are the production of Late embryogenesis abundant proteins (LEA) and heat shock proteins which are expressed both constitutively and under stress. These stress-induced proteins (HSPs, dehydrins, LEAs) are highly hydrophilic and possess a specific characteristic termed as "boiling stability"

[5] as they remain stable even after boiling. Molecular chaperones that succour in folding of cellular proteins making the cellular perturbation trivial are referred as heat shock proteins. LEA proteins were first identified in cotton as a set of proteins that are highly accumulated in the embryos at the late stage of seed development [6]. Three major categories of LEA proteins have been found owing to the homology in their amino acid sequence domains (7, 6). Correlation studies between gene expression of the stress related proteins and biotic or environmental stresses, speculated a protective and essential role for the survival of plant cells under various extreme conditions [8]. Earlier research also indicated that hydrophilins represent less than 0.2% of the total protein of a given genome [9]. An evolutionary role, in the detrimental environment conditions, of these stress proteins has been suggested as the conserved lysine rich regions were discovered through bioinformatic analyses of hydrophilins from several kingdoms including plant, bacteria and fungi [5]. The ability to tolerate and thrive in, many a times impossible conditions, can be owed to these stress proteins. There may lie a possibility that invasive alien plants may contain such kind of boiling stable proteins which helps them grow under any abiotic conditions like high temperature, drought, or high salt. Ecology, habitat and invasiveness of these plants have been an area of attention lately, but the molecular mechanisms which maybe a fundamental criteria in their preseverance in different habitats is still not documented. Although some recent studies have documented that cellular concentration of HSPs can be directly related to wide range temperature tolerances [10,11,12]. However, the role of boiling stable proteins (BSPs) is still not well documented. Therefore, the aim of the present study is to provide knowledge of the fascinating molecular adaptations of IAS plant *Lantana camara* in relation to boiling stable proteins (BSPs) in general.

Materials and methods

The leaves (5g) of *Lantana camara* (pink variety) were collected and washed with double distilled water. After drying the leaves, total DNA was extracted by using the CTAB protocol [13]. The DNA was spooled out with a capillary after centrifugation and then washed with 70% ethanol. It was vacuum dried and dissolved in minimum volume of TE buffer. PCR was used to amplify DNA of *Lantana camara* using some specifically designed conserved primer sets (as given in Table 1) from different boiling soluble genes using Primer 3 and Primer Blast software. 10 ul of DNA was used as a template for PCR amplification with a pair of gene specific primers in a final reaction mixture of 20ul containing PCR buffer(1X), dNTP mixture (0.1mM), 1.5mM $MgCl_2$, 25 picomoles of forward and reverse primer and 0.5 units of DNA polymerase. The cycling conditions consisted of an initial denaturing step at 95^{o}C for 5min, followed by 40 cycles at 95^{o}C for 45sec (Denaturation), 52^{o}C for 45sec (Annealing) and 72^{o}C for 1min(Extension) and the final elongation step at 72^{o}C for 10 mins. The PCR amplified DNA was purified using gel extraction kit (Banglore Genei kit) and eluted in 20-30ul of TE. The genes were sequenced through customer care service of Chromous Biotech and then subsequently used for clustal W (https://www.ebi.ac.uk/Tools/msa/clustalw2/). Suitable ORF were derived by using ORF finder (https://www.ncbi.nlm.nih.gov/orffinder/

Results and discussion

The gel electrophoresis of the generated PCR products (14) resulted in discrete bands of distinct base pair lengths (Fig 1) obtained by using different primers from boiling stable proteins encoding genes. Prominent bands of suitable length were excised from the gel (A1, A2, A3 and A5) as shown in Fig. 1 and used for nucleotide sequencing. The procured sequences were legible, except A3 being too short, which were thereby used for finding ORF (Fig 2). The protein sequence of

the selected ORF was used for multiple sequence alignment using by Clustal W against the putative and known sequences corresponding to the proteins involved in stress tolerance like LEA2, LEA3, WZY3-1 among different plants like *Triticum aestivum*, *Hordeum vulgare* etc as shown in Figure 3. A fair content of sequence similarity could be seen within the alignment in the sequences (Fig. 3). The sequences used for alignment have been identified as homologus, boiling stable protein encoding genes and have been proved to play a functional role in dehydration tolerance in plants [13]. The invasive species have been said to "eurythermal", the ability to maintain physiological function over a wide range of temperatures, a conducive property as the temperature rises which is considered to be enhanced in invasive, than native species [14]. The expression of heatshock proteins (Hsps) has also been analyzed as a potential subcellular mechanism underlying temperature tolerances and hence stability which may enhance invasiveness [14] .based on our results we hypothesize that boiling stable proteins can probably be the reason for the establishment and manifestation of these species in a foreign habitat. Further investigations into the same may hold an important and substantial role in understanding the relation between boiling stable proteins and stress tolerance at molecular level in invasive alien species. Detailed analysis of the regulation of gene expression of the genes involved should in the future elucidate the mechanisms governing environmental stress tolerance in invasive plant species.

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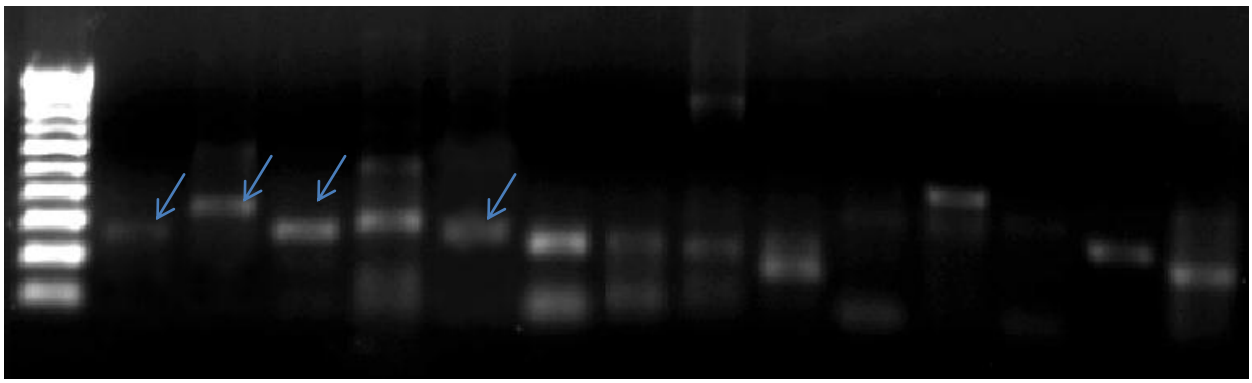

Fig 1: Agarose gel electrophoresis of the PCR products generated after DNA amplification with specific primers. The blue arrows show the bands selected and further used for sequencing i.e A1, A2, A3 and A5. L= Ladder, A1-A14 are the PCR products named arbitrary.

Table 1: Primers used to amplify *Lantana camara* boiling stable proteins related genes with their accession numbers and the source of origin.

	PCR products	PRIMERS (F/R)	PROTEIN	Acc no of gene
1	A1	CCGCACAGTACACCAAGGAA ACTTACACCAAATGGGCGGA	LEA 2 PROTEIN	AY148491.1| Triticum aestivum LEA2 protein
2	A2	CCAAGCAGAAGACGTCGGAG GGGCGGAAATCACACAAGAG	LEA 2 PROTEIN	AY148491.1| Triticum aestivum LEA2 protein
4	A3	CAAGCAGAAGACGTCGGAGA CCCTGGTGATCTTTCTCGGT	LEA 2 PROTEIN	AY148491.1| Triticum aestivum LEA2 protein
6	A4	AGGCCAAGGACAAGACTGC ACGGACTCCTTGGTGTACTG	LEA 3 PROTEIN	AY148492.1| Triticum aestivum LEA3 protein
8	A5	GAAGACAGAGATGGCCAAGCA GGAAAGCAAATCAAGCGCGAA	LEA 3 PROTEIN	AY148492.1| Triticum aestivum LEA3 protein
9	A6	GGTCTCAAGGGAAGGAAGCT CCTTTCTTCTCCTCCTCGGG	DEHYDRIN LIKE PROTEIN	AY574032.1| Triticum aestivum dehydrin-like gene
13	A7	CAACACCTACGGACAGCAAG CGGCACCTCAAACTTTCACA	dehydrin WZY2 mRNA	EU395844.2| Triticum aestivum dehydrin WZY2
15	A8	TTGCAGCCAAGTGAGCAAGA CTGCCGTATGACCTTGCTGT	dehydrin WZY2 mRNA	EU395844.2| Triticum aestivum dehydrin WZY2
16	A9	CACAAGTTGCAGCCAAGTGA ATGACCTTGCTGTCCGTAGG	dehydrin WZY2 mRNA	EU395844.2| Triticum aestivum dehydrin WZY2
17	A10	GAGAAGCACCACAAGCACAA CCTTGTGGTCCTGCTTCTTC	Chinese Spring abscisic stress-ripening protein mRNA	HQ287799.1| Triticum aestivum
18	A11	GGGGAGTACGAGCGGATC CCACCTCCTCCTCGATCTTG	Chinese Spring abscisic stress-ripening protein mRNA	HQ287799.1| Triticum aestivum
19	A12	GAATGGATCCCGGAAAGCAC GGGAATGAACCAAGCCACAG	Triticum aestivum AP2-containing protein (Dreb1)	AF303376.1| Triticum aestivum AP2-containing protein (Dreb1)
21	A13	ACTTGGTCGTTGGAGGAGAG GCTGCGTCTGTCTCTTGGAT	Triticum aestivum cold acclimation protein WCS120	M93342.2|WHTCOAC Triticum aestivum cold acclimation protein WCS120 (WCS120)
23	A14	AGCTCACTTGGTCGTTGGAG GCTGCGTCTGTCTCTTGGAT	Triticum aestivum cold acclimation protein WCS120	M93342.2|WHTCOAC Triticum aestivum cold acclimation protein WCS120 (WCS120)

1. Product name A1

a. >lcl|ORF1 CDS Strand +2

ATGATCTGCCACCCGCAGTCGAGCGCAGCCCCTCCCATGTCCCACTGCAA

CGGCTGGACCTTCACGTGGAGCGTGGCGTCTCGCGCCTCTACAACCGCGC

GCATCCGCACAACGGCCCGCCTAAAAAAATCTCCACGGATCGCCGCCCAC

TTGGCCCCCCCTTGGGTTGCGCTCCCATTATGA

Protein sequence: >lcl|ORF1 Strand +2

MICHPQSSAAPPMSHCNGWTFTWSVASRASTTARIRTTARLKKSPRIAAHLAPPWVALPL

2. Product name A2

a. >lcl|ORF3 CDS Strand +2

ATGTCCCATTACCCTCTCGTTTGCCCAGGCTATATTTGGGTGAAAAAATC

GATCGCGCTCCTTCTGGGACTTTGTTCACTCAAAGAAACCCTATCCCCTT

TTTGCTCATGGATTACTCCATGCTTGATAGCTATGACGGATCCATTCCGT

TGCCTGAGGGAGTCAAGTTCCCAGATGCATTGTCCCTTGCCTTGTCTGAC

CCTTCCATATATGGCACCCCTTTTCTTGGCCTAA

Protein sequence: >lcl|ORF3

MSHYPLVCPGYIWVKKSIALLLGLCSLKETLSPFCSWITPCLIAMTDPFR

CLRESSSQMHCPLPCLTLPYMAPLFLAX

3. Product name A5

>lcl|ORF2 CDS Strand +3

ATGGCGACCCTCGGCGCGGCCTCGGGTTGTGGCATGATCACACTGGCGCT

GGGGCCAGCGATCCCGACGACGGGGGGAGCAGTGGGGAGATGA

Protein sequence: >lcl|ORF2 MATLGAASGCGMITLALGPAIPTTGGAVGR

Figure 2: Protein sequences generated from ORF finder of the selected PCR products A1, A2 and A5 as named initially and were further used for alignment.

1. **Product Name A1**

Protein sequence: >lcl|ORF1 Strand +2

MICHPQSSAAPPMSHCNGWTFTWSVASRASTTARIRTTARLKKSPRIAAHLAPPWVALPL

```
this               PQSSAAPPMSHCNGWTFTWSVASRASTTARIRTTARLKKSPRIAAHLAPPWVALPL----
BAF79928.1         AAQYAQERSSDAAQYTKESAVAGKDMTGSVLQQAGETVVGAVVGAKDA---VANTLGMGG
ANB44749.1         AAQYAQERSSDAAQYTKESAVAGKDKTGSVLQQAGETVVGAVVGAKDA---VANTLGMGG
APJ36638.1         AAQYAQERSSDAAQYTKESAVAGKDKTGSVLQQAGETVVGAVVGAKDA---VANTLGMGG
AY148491.1         TAQYAQERSSDAAQYTKESAVAGKDKTSGVLQQASETVVNAVVGAKDA---VANTLGMGG
AAN74638.1         TAQYAQERSSDAAQYTKESAVAGKDKTSGVLQQASETVVNAVVGAKDA---VANTLGMGG
XP_020173192.1     TAQYAQERSSDAAQYTKESAVAGKDKTSGVLQQASETVVNAVVGAKDA---VANTLGMGG
AHZ35570.1         TAQYAQERSSDAAQYTKESAVAGKDKTSGVLQQASETEVNAVVGAKDA---VANTLGMGG
XP_020200916.1     TAQYAQERSSDAAQYTKESAIAGKDKTGSVLQQAGETVVNAVVGAKDA---VANTLGMGG
AHZ35571.1         TAQYAQDRSSDAAQYTKESAVAGKDKTGSVLQQAGETVVSAVVGAKDA---VANTLGMGG
BAC80266.1         TAQYAQERSSDAAQYTKESAVAGKDKTGSVLQQAGETVVSAVVGAKDA---VANTLGMGG
                   . . *    * .  :*   ::*..  * . :. :.    .. :.*: *   ** .*
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2. Product name A2

Protein sequence: >lcl|ORF3 Strand +2

MSHYPLVCPGYIWVKKSIALLLGLCSLKETLSPFCSWITPCLIAMTDPFR

CLRESSSQMHCPLPCLTLPYMAPLFLAX

```
this               ---------------------------SLKETLSPFCSWITPCLIAMTDPF-RCLRESSSQ
AY148492.1         EKTEMAKQKAAETTE-----------AAKQKASETAQYTKES-VAGKDKTGSVLQQAGET
AAN74639.1         EKTEMAKQKAAETTE-----------AAKQKASETAQYTKES-VAGKDKTGSVLQQAGET
ALD18912.1         EKTEMAKQKAAETTE-----------AAKQKASETAQYTKESAVAGKDKTGSVLQQAGET
AAN74637.1         EKTEAAKQKAAETTEAARQKAAEATEAAKQKASETAQYTKESAVAGKDKTGSVLQQAGET
ALD18913.1         EKTEAAKQKAAETTEAARQKAAEATEAAKQKASETAQYTKESAVAGKDKTGSVLQQAGET
XP_020173182.1     EKTEAAKQKAAETTEAARQKAAEATEAAKQKASETAQYTKESAVAGKDKTGSVLQQAGET
ACH89913.1         EKTEAAKQKAAETTEAAKQKAAEATEAAKQKASDTAQYTKESAVAGKDKTGSVLQQAGET
AKC92683.1         EKTEAAKQKAAETTEAAKQKAAEATEAAKQKASDTAQYTKESAVAGKDKTGSVLQQAGET
ACH89911.1         EKTEAAKQKAAETTEAAKQKAAEATEAAKQKASDTAQYTKESAVAGKDKTGSVLQQAGET
APJ36638.1         EKTEAAKQKAAETAEAAKQKASEAAQYAQERSSDAAQYTKESAVAGKDKTGSVLQQAGET
AIZ11400.1         EKTEAAKQKAAETAEAAKQKASETAQYAQERSSDAAQYTKESAVAGKDKTGSVLQQAGET
                                            ::  *  ..: . . :* .*     *.::..
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3. Product name A5

Protein sequence: >lcl|ORF2 Strand +3 MATLGAASGCGMITLALGPAIPTTGGAVGR

```
this               ----------MATLGAASGCGM-------------------ITLALGPAIPTTGG-----
ACZ60124.1         EEKHHKHKEHLGEMGAVAAGAFALYEKHEAKKDPDHAHKHKIEEEIAAAVAVGSGGYAFH
ACZ60133.1         EEKHHKRKEHLGEMGAVAAGAFALYEKHEAKKDPDHAHKHKIEEEIAAAVAVGSGGYAFH
ACZ60122.1         EEKHHEHKEHLGEMGAVAAGAFALYEKHEAKKDPDHAHKHKIEEEIAAAVAVGSGGYAFH
ACZ60132.1         EEKHHKHKEHLGEMGAVAAGAFALYEKHEAKKDPDHAHKHKIEEEIAAAVAVGSGGYAFH
ACZ60120.1         EEKHHKHKEHLGEMGAVAAGAFALYEKHEAKKDPDHAHKHKIEEEIAAAVAVGSGGYAFH
XP_003577811.1     EEKHHKHKEHLGEMGAVAAGAFALYEKHEAKKDPENAHRHKIAEEVGAAAAVGAGGFVFH
EMS61201.1         EEKHHKHKEHLGEMGAAAAGAFALYEKHEAKKDPEHAHKHKIEEEVAAAAAVGAGGFVFH
XP_020170879.1     EEKHHKHKEHLGEMGAAAAGAFALYEKHEAKKDPEHAHKHKFEEEVAAAAAVGAGGFVFH
HQ287799.1         EEKHHKHKEHLGEMGAAAAGAFALYEKHEAKKDPEHAHKHKIEEEVAAAAAVGAGGFVFH
ADQ85915.1         EEKHHKHKEHLGEMGAAAAGAFALYEKHEAKKDPEHAHKHKIEEEVAAAAAVGAGGFVFH
                             :. :**.:. .:                  :   :..* .. .*
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Fig 3: Alignment of A1, A2 and A5 PCR products against the putative sequences of boiling stable proteins taken from different plants like *Triticum aestivum*, *Hordeum vulgare* using Clustalw. 'this' here represents the sequence of our pcr products in each alignment. The following are the accession numbers of the sequences used for alignment .Here"*"-asterisk denotes that residue at that position is exactly same.":"-colon indicates residues at that position are very similar. "."-dot indicates residues are more or less similar

Table 2: The accession numbers, type and the source of the sequences of the proteins used in the alignment in Clustalw

S.no.	Accession Number	Protein Name	Source
1	AY148491.1	LEA2 protein	*Triticum aestivum*
2	AAN74638.1	LEA2 protein	*Triticum aestivum*
3	XP_020173192.1	ABA-inducible protein PHV A1-like	*Aegilops tauschii subsp. tauschii*
4	AHZ35570.1	LEA protein	*Triticum aestivum*
5	BAC80266.1	ABA inducible protein	*Triticum aestivum*
6	BAF79928.1	Group3 late embryogenesis abundant protein	*Triticum aestivum*
7	ANB44749.1	LEA-3 protein	*Triticum aestivum*
8	APJ36638.1	WZY3-1	*Triticum aestivum*
9	XP_020200916.1	ABA-inducible protein PHV A1-like	*Aegilops tauschii subsp. tauschii*
10	AHZ35571.1	LEA protein	*Triticum aestivum*
11	AY148492.1	LEA3 protein (LEA3)	*Triticum aestivum*
12	AAN74639.1	LEA3 protein	*Triticum aestivum*
13	ALD18912.1	LEA3	*Triticum turgidum subsp. durum*
14	ACH89913.1	Late embryogenesis abundant protein	*Hordeum vulgare subsp. vulgare*
15	ALD18913.1	LEA3	*Triticum turgidum subsp. durum*
16	AAN74637.1	LEA1 protein	*Triticum aestivum*
17	ACH89911.1	Late embryogenesis abundant protein	*Hordeum vulgare subsp. vulgare*
18	AKC92683.1	Late embryogenesis abundant protein	*Hordeum vulgare*
19	APJ36638.1	WZY3-1	*Triticum aestivum*
20	AIZ11400.1	Late embryogenesis abundant protein 3	*Eremopyrum triticeum*
21	XP_020173182.1	Late embryogenesis abundant protein, group 3	*Aegilops tauschii subsp. tauschii*
22	HQ287799.1	cultivar Chinese Spring abscisic stress-ripening protein mRNA,	*Triticum aestivum*
23	ADQ85915.1	Abscisic stress-ripening protein	*Triticum aestivum*
24	XP_020170879.1	Abscisic stress-ripening protein 2-like	*Aegilops tauschii subsp. tauschii*
25	EMS61201.1	Abscisic stress-ripening protein 2	*Triticum urartu*
26	XP_003577811.1	PREDICTED: Abscisic stress-ripening protein 3-like	*Brachypodium distachyon*
27	ACZ60124.1	Abscisic stress ripening	*Musa ABB Group*
28	ACZ60122. 1	Abscisic stress ripening	*Musa ABB Group*
29	ACZ60132.1	Abscisic stress ripening	*Musa AAB Group*
30	ACZ60133.1	Abscisic stress ripening	*Musa balbisiana*
31	ACZ60120.1	Abscisic stress ripening	*Musa itinerans var. itinerans*