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Mobile genetic element excision footprints of *Drosophila melanogaster* found in heat shock protein coding regions of Solanaceous crops

M. K. CHANDRA PRAKASH[1], REENA ROSY THOMAS[1] and SUKHADA MOHANDAS[2]

[1]Section of Economics & Statistics, [2]Division of Biotechnology, Indian Institute of Horticultural Research, Hessaraghatta Lake Post, Bangalore 560 089, India
Email : mk_chandraprakash@yahoo.com

ABSTRACT: Mobile genetic elements are discrete sequences in the genome that are able to transport themselves to other locations within genome, which may have direct consequences on gene expression. More than 96 per cent of the transposable elements, occurring in heat shock promoters are P transposable element. In natural populations of *Drosophila melanogaster*, the promoters of heat-shock genes are especially susceptible to the insertion of transposable elements. These mobile elements often leave small rearrangements called transposon footprints at sites where they excise. It was proved that transposable elements insertion and subsequent excision resulted in the production of 8 bp direct repeats. The transposable elements often insert into the genes' regulatory regions is due to their high expression level. In this context, Heat Shock Protein sequences, mainly HSP90 and HSP83 in Solanaceae crops were analysed for presence of transposable element excision footprints using *in silico* methods. It was found that out of the 17 hsp sequences, 14 hsp coding sequences were having 8 bp transposable element excision footprints consistently at the same location. These footprints left in individual sequences are surprisingly not random; excision footprints predominate consistently in each sequence. This suggests that the excision event and footprint formation involves DNA repair of hsp sequences flanking the transposable element. Identifying these footprints are useful for discovering genes that encodes for heat shock proteins in Solanaceae crops.

Keywords : Heat Shock Protein, Solanaceous crops, transposable elements

INTRODUCTION

The major component of all genomes is mobile genetic elements (MGE) and represent between 3 to 50% of the content of the genome depending on the species (Capy *et al*., 1997). Mobile genetic elements includes Transposable Elements (TEs) also known as transposons and can be regarded as parasite DNA that does neutral and deleterious effects. Transposons play an important role in the evolution of gene function and may be involved in the restructuring of genomes due to their ability to restructure or rearrange chromosomes (Agrawal *et al.*, 2001; Witte *et al.*, 2001). The genome size differences observed among crop plants are largely due to unequal accumulation of repetitive DNA sequences mainly transposable elements (Casa *et al*., 2004). These movable elements (TEs) are semi-parasitic DNA sequences that can replicate and spread through the host's genome. These elements can insert within genes or regulatory sequences, which can disrupt gene function or expression. Some types move by conservative process, which involves the excisions of the sequences from its original position followed by its reinsertion elsewhere.

P-transposable element hybrid dysgenesis:

The P transposable element, a DNA transposon in *Drosophila melanogaster* arises from transposition. This event called hybrid dysgenesis occurs when females from laboratory strains of *D. melanogaster* are crossed with males from wild populations having P elements (fig-1). The off spring resulting from such crosses are

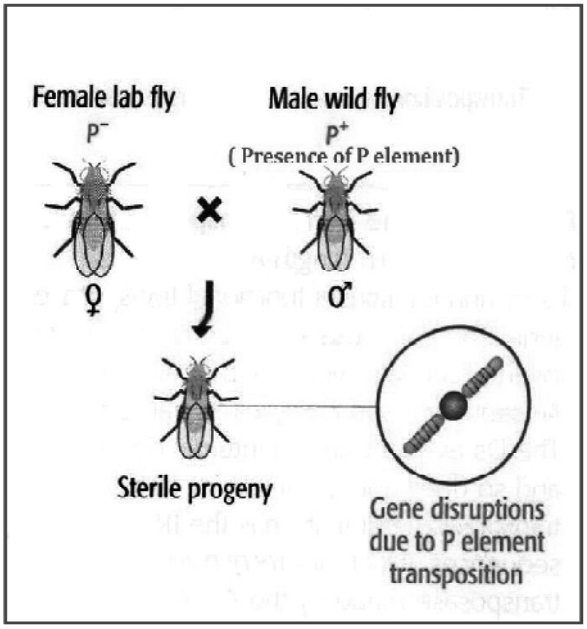

Fig. 1. Cross between a male P⁺ (contains a repressor that prevents P element transposition) and a female P- (lacking the repressor) resulting in sterile progeny in *D. melanogaster*

sterile and have chromosomal abnormalities along with a variety of other genetic malfunctions. A specific class of such regulatory mutations are known to affect the expression of heat shock genes (Walser, 2006). These mutations are insertions of a P transposable element into the regulatory region of heat shock genes. They affect gene expression, thermotolerance (Lerman, 2005); Chen *et al.*, 2007a), development and longevity (Chen *et al.*, 2007b). It was proved that transposable elements insertion and subsequent excision results in the production of 8 bp footprint. This footprint sequence flanking the P element is a direct repeat of the heat shock consensus elements created by the P element insertion. The likely reason why P elements often insert into these genes' regulatory regions is their high expression levels.

MATERIALS AND METHODS

Transposable element excision foot prints in HSP sequences : The TE insertion and excision events happened in most of the Heat Shock Protein (HSP) sequences (Chen, 2012). The Transposable elements binds in heat shock protein regions and is later excised. The promoters of heat-shock genes are especially susceptible to the insertion of transposable elements (TE). The rate of TE was reported to be 14.7 times higher than the average rate of TE insertions in promoter regions of non-heat shock genes (Walser, 2006). On the event of excision of Transposable element in the proximal promoter of HSP 83 gene, a 8 bp sequence footprint was left in these region. The 8-bp sequence flanking the TE is a direct repeat of the heat shock consensus elements (Chen and Wagner, 2012) created by the Transposable element insertion and subsequent excision. As the DNA in the promoters (i.e., regulatory regions) of heat-shock genes is unusually accessible, these genes might harbor many transposable elements. More than 96 percent of the transposable elements occurring in heat shock promoters are P-transposable element insertions (Walser,

Table 1. Summary of HSP90/83 sequences of Solanaceae crops

Sl. No.	Crop	length	GI reference
1	*Nicotiana tabacum* hsp90 mRNA for heat shock protein 90,	2,297 bp	AB264546.1 GI:110083390
2	*Solanum tuberosum* clone 054G03 Hsp90-2-like mRNA,	2,188 bp	DQ252488.1 GI:81074297
3	*Nicotiana benthamiana* molecular chaperone Hsp90-1 mRNA,	2,100 bp	AY368904.1 GI:38154481
4	*Lycopersicon esculentum* molecular chaperoneHsp90-1mRNA,	2,100 bp	AY368906.1 GI:38154488
5	*Nicotiana tabacum* heat shock protein 90 (OINtHsp90) mRNA,	2,306 bp	AY519499.1 GI:46093889
6	*Nicotiana benthamiana* molecular chaperone Hsp90-2 mRNA,	2,100 bp	AY368905.1 GI:38154484
7	*Lycopersicon esculentum* molecular chaperone Hsp90-2mRNA,	2,100 bp	AY368907.1 GI:38154492
8	*Capsicum Chinese* mRNA for putative Hsp90-2,	2,100 bp	AB372259.1 GI:171854656
9	*Nicotiana benthamiana* molecular chaperone Hsp90-3 mRNA,	2,100 bp	GQ845021.1 GI:260100691
10	*Nicotiana tabacum* cultivar Bright Yellow 2 molecular chaperone Hsp90 mRNA,	2,100 bp	HQ834904.1 GI:322517782
11	*Nicotiana tabacum* NtHsp90 mRNA for Heat shock protein 90,	2,100 bp	AB689674.1 GI:392465168
12	*Nicotiana attenuata* heat shock protein 90-1 mRNA,	2,100 bp	GU265722.1 GI:315307965
13	*Nicotiana attenuata* heat shock protein 90-2 mRNA,	2,100 bp	GU265723.1 GI:315307967
14	PREDICTED*: Solanum lycopersicum* heat shock protein 83-like (LOC101264183), mRNA	2,642 bp	XM_004234170.1 GI:460376867
15	PREDICTED: *Solanum lycopersicum* heat shock protein 83-like, transcript variant 1 (LOC101260143), mRNA	2,595 bp	XM_004240669.1 GI:460390178
16	*Solanum lycopersicum* molecular chaperone Hsp90-1, mRNA	2,100 bp	NM_001247507.1 GI:350535173
17	*Solanum lycopersicum* molecular chaperone Hsp90-2 (HSC80), mRNA	2,100 bp	NM_001247510.1 GI:350535223

2006; Chen *et al.*, 2007a). P transposable element insertions are widespread in the Drosophila genome, and they are especially abundant in heat-shock genes (Walser, 2006; Chen *et al.*, 2007a). If these footprint sequences or its complementary left by the transposable element excision are located in the DNA sequences, it would enable us to locate the heat shock protein coding regions in Solanaceous crops.

RESULTS AND DISCUSSION

The sequences associated with heat stress mainly HSP90 and HSP83 in Solanaceae crops were analysed using *in silico* methods from the public domain datasets. A computer program has been used to locate the TE Excision footprint locus in each HSP90/83 sequences. A total of 17 published gene coding sequences of HSP 90/ 83 from Solanaceae crops comprising of *Solanum lycopersicum, Solanum tuberosum, Lycopersicon esculentum, Capsicum chinense, Nicotiana benthamiana, Nicotiana tabacum,* and *Nicotiana attenuate* were analysed (Table 1) for transposable element excision footprints. Of these 14 hsp sequences were found to be having element excision footprints which were of 8 bp

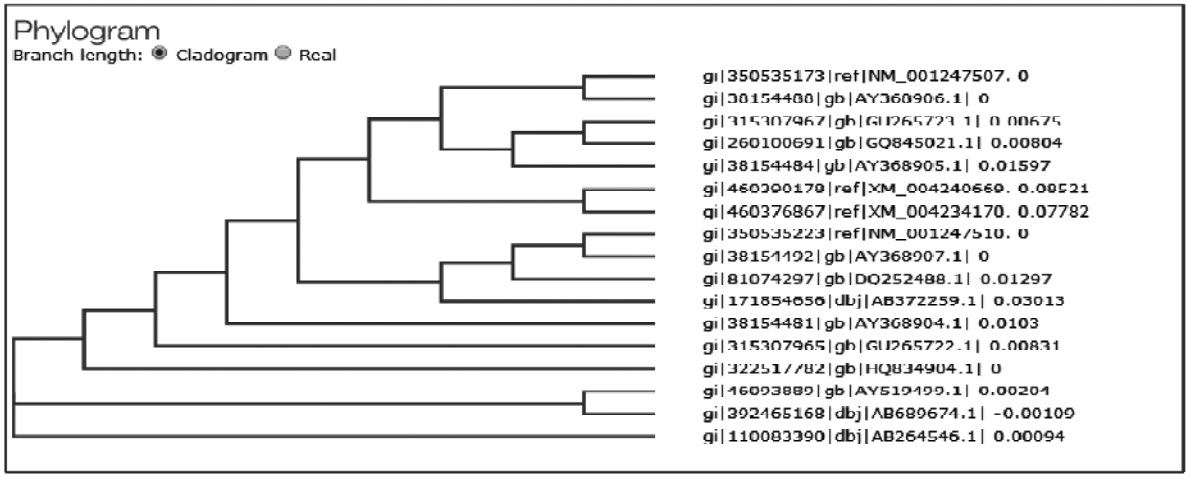

Fig. 2. Multiple sequence alignment showing an evolutionary conservedness and relationship by which they share a common ancestor

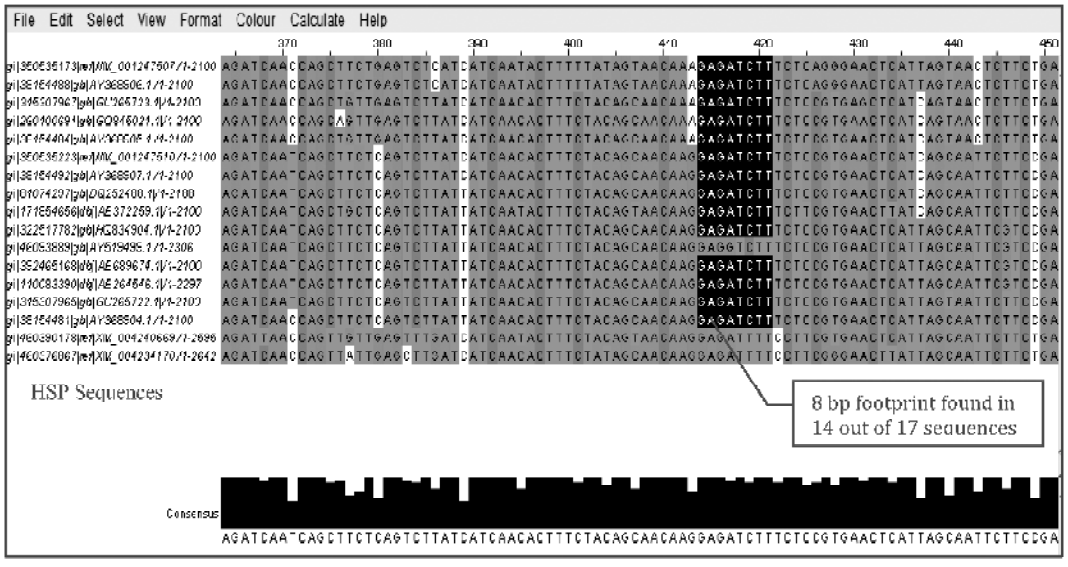

Fig. 3. MSA showing alignment of 17 hsp sequences having 8 bp TE excision footprints

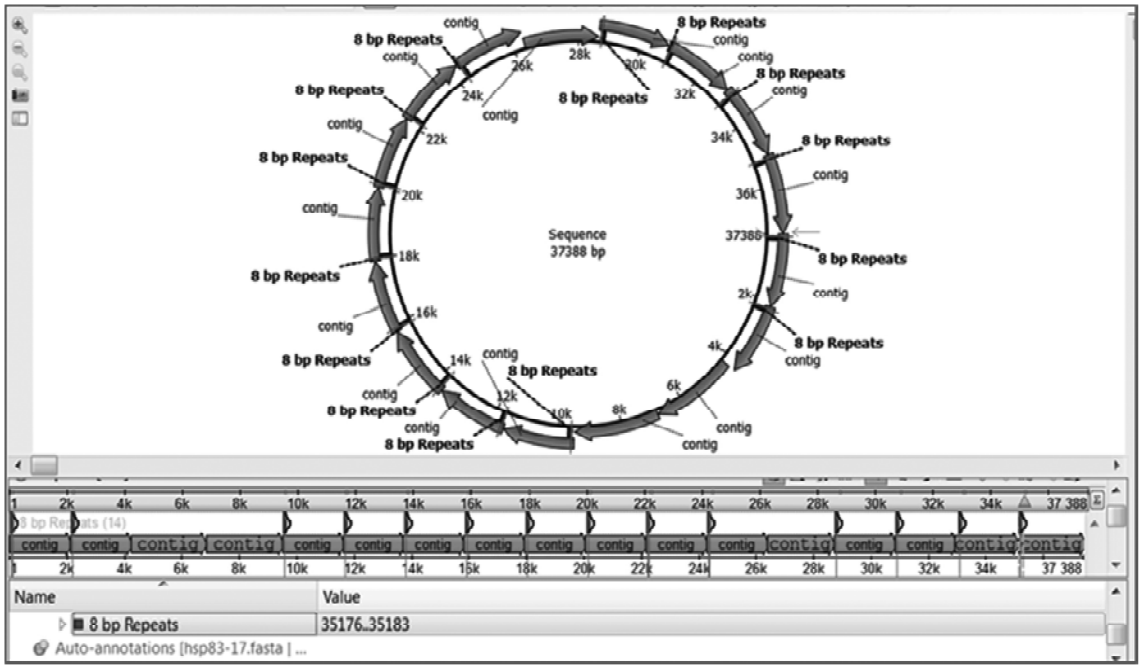

Fig 4. UGENE software showing presence of footprints in 14 out of 17 HSP90/83 sequences as circular view

in length. Also it was found that these footprints left in individual sequences are not random.

All the above sequences were subjected to Multiple Sequence Alignment (MSA) which is a sequence alignment tool for three or more biological sequences, generally protein, DNA, or RNA. The query sequences are assumed to have an evolutionary conserved and relationship by which they share a common ancestor (Fig.2). From the MSA result, the sequence homology is inferred to assess the sequences' that shared evolutionary origins.

From the alignment for hsp sequences across the Solanaceous crops, it was observed that the transposable element excision footprints having 8 bp is predominant consistently in the same position (Fig. 3). These sequences encodes for heat stress tolerance and are also molecularly evolutionarily conserved across Solanaceae crops (ChandraPrakash, 2013).

Locus of TE Footprints in Heat Shock protein sequences:

All the 17 HSP90/83 sequences were plotted as circular view using UGENE software. It was observed that the presence of foot prints at regular intervals indicates that the TE insertion happened in the same region of each sequence. The arrow shown in clockwise direction indicates individual sequences of Solanaceae crops. The 8 bp footprints shown in circular ring as radial lines. Approximately 2000 bp sequence of each HSP 90/83 is plotted as horizontal bar scaled as 1k to 34k for the 17 sequences. The small triangular mark indicates the footprints for the 14 sequences suggesting the presence of footprint in same locus.

CONCLUSION

Transposable Elements (TE) also known as transposons can be regarded as parasite DNA that does neutral and deleterious effects. The P transposable element, a DNA transposon in *Drosophila melanogaster* arises from transposition. *P* element insertions are widespread in the *Drosophila* genome and they are abundant in heat-shock genes. It was proved that transposable elements insertion and subsequent excision results in the production of 8 bp footprint. This footprint sequence flanking the P element is a direct repeat of the heat shock consensus elements created by the P element insertion. The transposable elements often insert into the genes' regulatory regions due to their high expression level. Mostly it binds in heat shock protein regions and is later excised. It was found that out of the published 17 hsp sequences, 14 hsp coding sequences were having TE excision footprints. The transposon insertion and subsequent excision leaving footprint formation involves DNA repair of HSP sequences flanking the element. Formation of these footprints left in individual sequences are surprisingly not random, they are found consistently

at the same location. If these TE excision footprints are identified, this would enable to locate the heat stress tolerant regions, which would eventually discover potential candidate genes that encodes for heat shock proteins in Solanaceous crops.

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