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Bioinformatics analysis of ubiquitin expression protein gene from *Heterodera latipons*

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ABSTRACT

Ubiquitin expression protein DNA clone (Hl-UBI) was isolated from *Heterodera latipons* collected from North Jordan. Its sequence of 285 nucleotides was also determined and deposited in the GeneBank. The 285-bp open reading frame coded for 76-amino acid protein having two domains; the ubiquitin domain and the C terminal extension. The first 59 amino acids were predicted with the ubiquitin domain with identity percentages of 78% to ubiquitin of *H. schachtii*, 77% to polyubiquitin of *Globodera pallida*, 74% to ubiquitin of *Globodera rostochiensis* and 72% to ubiquitin of *Heterodera glycines*. The other domain at the C-terminus, containing 17 amino acids, showed no homology to any known protein. Sequence analysis showed a calculated encoding amino acids molecular weight of 8.77 kDa, theoretical isoelectric point = 4.76, negatively charged residues = 12, positively charged residues = 9, extinction coefficient = 1490, estimated half-life = 30 h, instability index = 32.51 and grand average of hydropathicity = −0.537. The demonstrated subcellular localization analysis of cytoplasm, cell nucleus, mitochondrion, cell skeleton and plasma membrane of Hl-UBI protein occupied about 52.20, 21.70, 17.40, 4.30 and 4.30%, respectively. Sequence, homology and structural analysis confirmed that Hl-UBI gene was highly conserved during evolution and belonged to ubiquitin gene family.

1. Introduction

The Mediterranean cereal cyst nematode (MCCN), *Heterodera latipons*, is an obligate parasite that penetrates the root tissues and migrates toward the vascular cylinder to feed. The nematodes inject pharyngeal gland secretions into a few adjacent cells to dissolve the cell walls between these cells forming the syncytium, which is a very important structure for nematode feeding and development (Jones and Northcote, 1972; Davis et al., 2000). This process took place once the nematode becomes sedentary as a parasitism mechanism initiated by the effector proteins which incorporated into the host plant cells (Hussey, 1989). It is believed that these stylet-secreted proteins play a fundamental role in the root invasion and host defense suppression (Hussey, 1989; Davis et al., 2004, 2008).

The ubiquitin (UBI) proteins are existed in all eukaryotic cells, whose sequence is very well conserved. It is known to play crucial roles in a large variety of biological processes (Ciechanover, 1994; Ciechanover and Schwartz, 1994). The best-characterized function of ubiquitin is its role in protein degradation. The degradation process is done by serving ubiquitin as a covalent molecular signal in ubiquitin/proteasome system (Dreher and Callis, 2007). It is clear that the host UBI protein is involved in defense mechanisms of the plant. Also, the phytopathogens manipulate the host UBI proteins by some evolved mechanisms for their own benefit (Dielen et al., 2010; Shirsekar et al., 2010). There are many genes coding for ubiquitin in eukaryotes (Hershko et al., 2000; Jentsch and Pyrowolakis, 2000). These genes are coding for three types of UBI proteins: polyubiquitin, ubiquitin-like, and ubiquitin extension proteins. Within the ubiquitin extension proteins, a monomer is fused to the protein of the C-terminal extension. The *in vivo* cleavage of these genes in the initial translation products are done

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accurately and rapidly by hydrolases of the ubiquitin C-terminal (Wilkinson, 1997).

Genes encoding UBIs have been cloned from cereal cyst nematode, *Heterodera avenae* (Chen et al., 2017), soybean cyst nematode, *Heterodera glycines* (Gao et al., 2003), sugarbeet cyst nematode, *Heterodera schachtii* (Tytgat et al., 2004) and the Mediterranean cereal cyst nematode, *H. latipons* (Lafi et al., 2009). Interestingly, nematode encoded UBI carpoxyl extension proteins appear to represent a unique class, because there is no similarity between secretory proteins and their carpoxyl extension proteins with ribosomal carpoxyl extension proteins. Their specific gland expression indicates that nematode-produced UBI carpoxyl extension proteins are significant effectors (Gao et al., 2003; Tytgat et al., 2004); Nevertheless, their role in plant parasitism is yet to be elucidated.

Since we have cloned the DNA of ubiquitin gene of *H. latipons* in a previous work (Lafi et al., 2009) our objective in this study was to predict its characterization and structure using a bioinformatics analysis.

2. Materials and methods

2.1. Nematode culture

H. latipons populations were collected from the barley fields at Ar-Ramtha region in the North of Jordan. The nematode species were primarily identified based on qualitative and quantitative morphological characteristics of J_2s and cysts (Lafi et al., 2016).

2.2. Sequence analysis of Hl-UBI gene

Signal peptide for secretion analysis was performed using the SignalP 3.0 Server (Bendtsen et al., 2004) available on the Center for Biological Sequence Analysis website (http://www.cbs.dtu.dk/services/SignalP). Calculations of molecular weight, theoretical isoeletric point, negatively and positively charged residues, grand average of hydropathicity were calculated using ProtParm Tool (Gasteiger et al., 2005). The potential protein subcellular localization and transmembrane domains were estimated using PSORTII Prediction (Nakai and Horton, 1999) and TMHMM Server version 2.0 (Krogh et al., 2001), respectively.

2.3. Homology analysis of Hl-UBI protein

Sequence homology searches were done at the National Center for Biotechnology Informatics website with BLAST program (McCarter et al., 2000). Multiple sequence alignments were made using the ClustalW Tool presented in the BioEdit program (Hall, 1999). Alignment was performed between the translation products of *H. latipons* ubiquitin expression protein gene (accession no. FJ151169) with ubiquitin extension protein 2 of *H. schachtii* (AAP37976.1), polyubiquitin of *Globodera pallida* (CAL30085.1), ubiquitin extension protein of *H. schachtii* (AAP30081.1), ubiquitin extension proteins Ubi-1 of *Globodera rostochiensis* (AGI97006.1), ubiquitin extension proteins Ubi-2 of *G. rostochiensis* (AGI97007.1), ubiquitin extension protein of *H. glycines* (AAN32889.1) and ubiquitin extension protein of *H. glycines* (AAO33478.1).

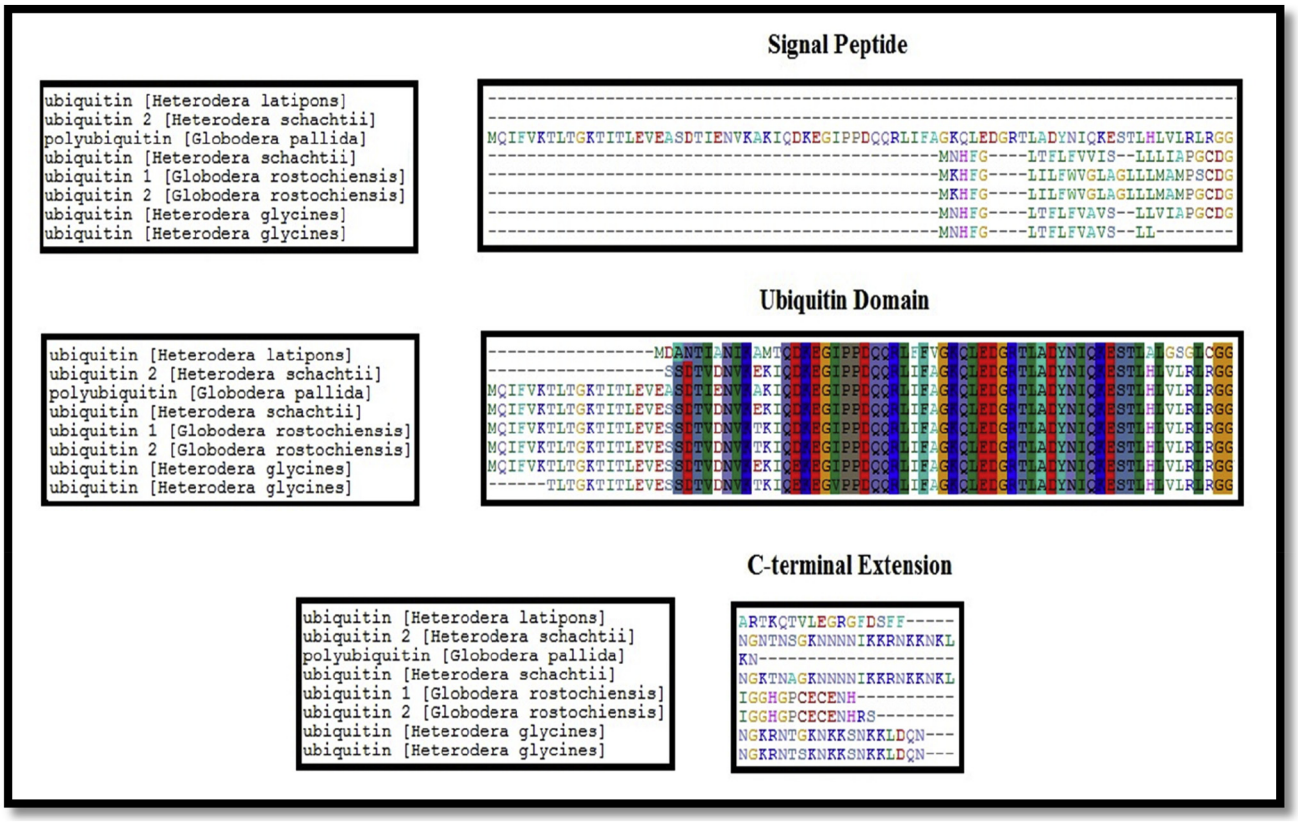

Fig. 1. Multiple alignment of the translation product of *Heterodera latipons* ubiquitin expression protein gene (accession no. FJ151169) with ubiquitin extension protein 2 of *Heterodera schachtii* (AAP37976.1), polyubiquitin of *Globodera pallida* (CAL30085.1), ubiquitin extension protein of *H. schachtii* (AAP30081.1), ubiquitin extension proteins Ubi-1 of *Globodera rostochiensis* (AGI97006.1), ubiquitin extension proteins Ubi-1 of *G. rostochiensis* (AGI97007.1), ubiquitin extension protein of *H. glycines* (AAN32889.1) and ubiquitin extension protein of *H. glycines* (AAO33478.1). The three domains, signal peptide for secretion, monoubiquitin, and C-terminal extension domain are indicated. Although the homology between the C-terminal domains of HlUBI and others is very low, the overall structure of both proteins is similar. Comparison between Hl-UBI and its homologues in other cyst nematodes reveals that several boxes of conserved amino acids are present in the signal peptide for secretion, the ubiquitin domain is almost identical, but a large variation is present in the C-terminal extensions between different nematode species. In black, identical residues; in gray, conserved substitutions. Identities and similarities are in color shades.

2.4. Structural analysis of Hl-UBI protein

Three-dimensional structural modeling of HlUB protein was built by the homology-based method using the SWISS-MODEL program (Biasini et al., 2014). The template used for modeling was the mouse ubiquitin.

3. Results

3.1. Sequence analysis of Hl-UBI gene

Based on the highly preserved sequences of the ubiquitins, the DNA designated Hl-UBI was isolated from *H. latipons*. Sequence analysis of Hl-UBI DNA (GenBank Accession No. FJ151169) have a calculated molecular weight of 8.77 kDa. The theoretical isoeletric point = 4.76, negatively charged residues = 12, positively charged residues = 9, extinction coefficient = 1490, estimated half-life = 30 h, instability index = 32.51 and grand average of hydropathicity = −0.537. No signal sequence and transmembrane domain were identified in the transcript using the SignalP 3.0 and TMHMM 2.0 servers. In comparison with the length of the nucleotide and amino acid sequences of cloned Hl-UBI, DNA was in good agreement with earlier reported sizes of other ubiquitins.

3.2. Homology analysis of Hl-UBI protein

The BioEdit sequence alignment of the ubiquitin proteins is shown in Fig. 1. The ubiquitin protein consists of three domains of the ubiquitin protein; signal peptide for secretion, ubiquitin domain, and the C-terminal extension. The open reading frame coded for a 76-amino acid protein having two domains; the ubiquitin domain and the C terminal extension. The first 59 amino acids were predicted with the ubiquitin domain, The identity percentages were 78% to ubiquitin extension protein 2 of *H. schachtii* (AAP37976.1), 77% to polyubiquitin of *G. pallida* (CAL30085.1), 74% to ubiquitin extension protein of *H. schachtii* (AAP30081.1), ubiquitin extension proteins Ubi-1 of *G. rostochiensis* (AGI97006.1) and ubiquitin extension proteins Ubi-2 of *G. rostochiensis* (AGI97007.1), 72% to ubiquitin extension protein of *H. glycines* (AAN32889.1) and 70% to ubiquitin extension protein of *H. glycines* (AAO33478.1). The other domain at the C-terminus, containing 17 amino acids, showed no homology to any known protein.

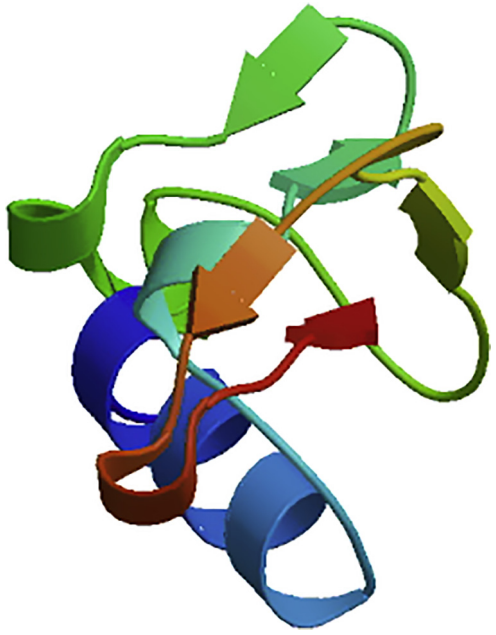

Fig. 2. Theoretical three-dimensional-structure modeling of the deduced Hl-UBI protein was based on the crystal structure of mouse ubiquitin as template using SWISS-MODEL.

3.3. Structural analysis of Hl-UBI protein

Phosphorylation sites of threonine occurred in Hl-UBI amino acid residue 13 and 65 by NetPhos 2.0 Server. Subcellular localization analysis demonstrated that Hl-UBI protein of cytoplasm, cell nucleus, mitochondrion, cell skeleton and plasma membrane occupied about 52.20, 21.70, 17.40, 4.30 and 4.30%, respectively. The three-dimensional structure of Hl-UBI protein was built by the homology-based modeling, based on the structure of mouse ubiquitin as template. The resolution based on the template was 1.70 Å. The sequence similarity between the Hl-UBI and mouse ubiquitin homologue was about 62.5%, indicating that the target sequence was well compatible with the template (Fig. 2). The overall folding pattern had 1 alpha helix (aquamarine blue), 2 310-helix (buff), and 6 beta folding (navy blue, yellow, green, yellow green, sorrel and red) (Fig. 2). Evaluation of atomic empirical mean force potential showed that five amino acid residues of Hl-UBI protein did not yield preferable result for closely related protein (Fig. 3).

4. Discussion

Ubiquitin is a small protein consisting of 76 amino acids and plays a key role in any stress response or protein degradation in eukaryotes (Masatoshi et al., 2000). In the ubiquitin extension proteins, an ubiquitin monomer and C-terminal extension protein are fused together. *In vivo*, Ubiquitin Proteins are cleaved rapidly by ubiquitin C-terminal hydrolases (Wilkinson, 1997). The analysis of DNA sequence of *H. Latipons* Hl-UBI gene in this study showed that the nucleotide sequence was >70% identity to that of those species in the order Tylenchida. In a previous bioinformatic analysis as well, Qiang et al., (2011) reported that the nucleotide sequence of the rice stem borer, *Chilo suppressalis*, was >72% similar to other known invertebrates and vertebrates. Furthermore, Zhang et al., (2008) showed that *Haritalodes derogate* ubiquitin shared >93% identity with other insects at amino acid level.

The Hl-UBI DNA of *H. latipons* in this work was found to have two domains; the ubiquitin domain, and C-terminal domain which had no homology with any other known protein. Unlike, previous studies reported three domains for the sugar beet cyst nematodes, *H. schachtii* and the potato cyst nematode, *G. rostochiensis*. The first domain seems to be a signal peptide for secretion and, as such, would be cleaved off before injection into the plant cell. As in

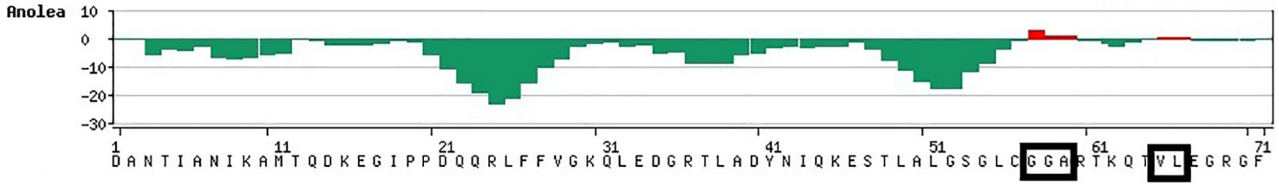

Fig. 3. Evaluation of atomic empirical mean force potential.

our study, the third, C-terminal domain also had no homology with any known protein (Tytgat et al., 2004; Chronis et al., 2013). The Hl-UBI differs significantly from others and is much shorter because of signal peptide domain. The result showed that Hl-UBI gene was highly conserved during evolution and belonged to ubiquitin gene family.

The overall structure of Hl-UBI and its homologues mostly resemble the ubiquitin extension proteins, and the C-terminal domain is also expected to be cleaved off. In the rice blast fungus, *Magnaporthe grisea*, the ubiquitin extension protein is highly expressed during the early infection stages (McCafferty and Talbot, 1998). Tytgat et al., (2004) assumed that the short C-terminal domain plays a regulatory role in the formation of feeding cells (syncytia) by *H. schachtii*, and this hypothesis is supported by the presence of the Hs-Ubi1 transcript, analyzed by RT-PCR, during all the female parasitic life stages to maintain the formation of feeding cells. However, this Ubi-1 was always absent in case of the adult males which leave the plant roots. Additionally, the very similar three-dimensional molecular modeling of the ubiquitins between the MCCN and mouse was also observed in the wild silk moth, *Bombyx mandarina* (Zhang et al., 2008). Therefore, it could be concluded that all ubiquitin genes in the various species might originated from the same ancestor's genes. Since all the ubiquitin genes of the organisms were so highly conserved, the researchers believed that the ubiquitin protein might not be used as a phylogenetic marker for evolutionary clock. However, some of the different relationships that appeared in this study could be due to the association with genetic differentiation to a certain extent of organisms exposed for a long time to the environmental stresses (Zhang et al., 2008). This conservative sequence suggested that the majority of amino acids of ubiquitin were essential in any mutation occurred over an evolutionary history done by the natural selection (Glickman and Ciechanover, 2002).

Yet, the actual function of the ubiquitin proteins is still unclear, although it could work as a chaperone. Several ubiquitin extension proteins have been illustrated with various lengths of C-terminal domains (Callis et al., 1990; Jones and Candido, 1993). Many reports had confirmed that ubiquitin gene expression plays a regulatory role in the feeding cell formation (Tytgat et al., 2004). Since the 1980s, ubiquitin studies have grown enormously and ubiquitin-dependent proteolysis degradation pathways have played major roles in a legion of biological processes (Varshavsky, 1997). It is well known that some abnormal protein degradation is an integral component of the cell physiology. So far, little is known for its physiological functions in nematode growth and development. Although previously Lafi et al. (2009) cloned a novel ubiquitin gene from *H. latipons*, the gene expression in various growth conditions, and the underlying mechanisms of this gene need to be further investigated to identify the precise biological properties in the various physiological processes. It could be concluded that our results, at least, provide several data for further studies on expressed characteristics and physiological functions of Hl-UBI gene. In addition, this work could provide valuable information for cyst nematodes identification and might be helpful for breeder and nematologist working to find resistant varieties against the cyst nematodes.

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