DEFENSIN GENE EXPRESSION IN SOME PLANT SOURCES OF TAIF

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Plant defensins are promising future strategy as antimicrobial agents; however the number of characterized plant defensins is very low. We isolated and studied the expression of plant defensins in six plants from Taif region. Using RT-PCR and two pairs of common as well as three pairs of specific primers, the defensin gene expression was analyzed. Plants showed differences in defensin gene expression in floral buds and leaves, though floral buds represented higher gene expression. The amino acid sequence of the six isolated cDNA sequences showed high similarity with other defensin accessions from the nucleotide database, especially in the cysteine rich motif. Phylogenetic analysis revealed that the isolated sequences share the common features of plant defensins, especially the 8 conserved cysteines. The results of this study help to detect some valuable Saudi plant sources for the development of natural peptides as a replacement for chemical antibiotics.

Keywords: Plant, Defensin, gene expression, Antimicrobial peptides, Phylogenetic analysis.

INTRODUCTION

Defensins are subgroup of antimicrobial peptides widely present in eukaryotic cells. They are small cysteine-rich cationic proteins that are active against bacteria and fungi (YAMAGUCHI and OUCHI, 2012). They consist of 12-50 amino acids including six to 8 conserved cysteine residues. Most defensins function by binding to microbial cell membrane forming pore-like membrane openings which lead to the loss of essential ions and nutrients (ALBERTS et al., 2002; LEHRER and GANZ, 2002). According to the defensins knowledgbase database (http://defensins.bii.a-star.edu.sg/) there are 564 characterized defensins distributed as; 273 from vertebrates, 181 from vertebrates, 61 from plants, 6 defensins-likes, and 43 unclassified. Vertebrate defensins are distributed in three subfamilies; α- defensins, β- defensins, and θ-
defensins (TANG et al., 1999; TRABI et al., 2001). Currently, the 273 defensins from vertebrates are distributed as 189 β- defensins, 79 α- defensins, 5 θ- defensins (http://defensins.bii.a-star.edu.sg). The different forms of defensins are recognized depending on the location and connectivity of the cysteines (YANG et al., 2009).

Plant defensins are small size (45-54 aa), very diverse, and heat stable peptides that have conserved tertiary structure with α-helix and three antiparallel β-sheets (VALENTE et al., 2013; DE BEER and VIVIER et al., 2011). The tertiary structure of defensins is stabilized by disulphide bridges to LINk the α-helix to β-sheets to a structure known as the cysteine stabilizing motif. This motif is conserved in all plant defensins and other eukaryotes. Two other conserved motives have been identified in the plant defensin structure, namely the α-core and the γ-core (VALENTE et al., 2013; YANG et al., 2009). Plant defensins do not interact directly with plasma membrane like mammalian and insect defensin (STOTZ et al., 2009a; WANG et al., 2005; CARAVALHO et al., 2009). Despite the extreme primary sequence diversity among defensins, they share some general structural features including: (1) they lack a high amino acid signature that relate to the diverse array of activities that makes it difficult to predict their antimicrobial activity; (2) defensins have been co-evolved through a process in which they can accommodate mutations to avoid pathogen resistance; (3) They are amphipathic positively charged at physiological pH, that facilitates their interactions with the anionic head groups of microbial membrane lipids (VALENTE et al., 2013).

Plant defensins were first isolated from wheat and barley grains (COLILLA et al., 1990; MENDEZ et al., 1990) and they were called γ thionins since their size and cysteine content were found to be similar to the previously called thionins (CARRASCOL et al., 1981). Recently, they were isolated from roots, reproductive structures such as flowers and fruits (DE BEER and VIVIER, 2011; DE BEER and VIVIER, 2008). Plant defensins function as antimicrobial against a broad spectrum of plant pathogens that include bacteria (DE BEER and VIVIER, 2011) yeast (LIN et al., 2010; AERTS et al., 2009), oomycetes (DE BEER and VIVIER, 2011; PORTELES et al., 2010), and necrotrophic pathogens (LIN et al., 2010; PORTELES et al., 2010). They play an important role in the protection of germinating plant seeds, plant reproductive organs and fruits (VALENTE et al., 2013; STOTZ et al., 2009a; STOTZ et al., 2009b; KOVALCHUK et al., 2010). In addition, some members of plant defensins show important activities for medical applications, including protease inhibitor (DE BEER and VIVIER, 2011), anti-cancer agents (LIN et al., 2010), and HIV inhibitor (LIN et al., 2010). They also have insecticidal activity (DE BEER and VIVIER, 2011), activity against parasitic plants, and heavy metal tolerance.

Several studies reported that defensin genes are arranged in highly polymorphic multigene families (THOMMA et al., 2002; SCHUTTE et al., 2002; FEDOROVA et al., 2002; MERGAERT et al., 2003; GRAHAM et al., 2004). In the human genome, all known defensin genes are mapped in <1M bp region of chromosome 8p22-p23 (HARDER et al., 1997; LIU et al., 1997; LINZMERIER et al., 1999). Analysis of the sequenced plant genomes revealed that defensin genes are arranged in multigene families and are overrepresented in the genome of some plants species. Sixteen genes were found in the defensin gene family in Medicago truncatula (HANKS et al., 2005), whereas a family of 317 genes were detected in the defensins-like (DEFL) gene family in Arabidopsis thaliana (SILVERSTEIN et al., 2005).

Plant defensins gene expression is induced by pathogen attack, wounding, and some abiotic stresses, but generally they have constitutive expression pattern (PADOVAN et al., 2010; KOVALCHUK et al., 2010; BAHRAMNEJAD et al., 2009). A few plant defensins have tissue-specific
and developmentally regulated expression which was linked to specific biological functions other than plant defense. Two examples were demonstrated for the defensins from tomato and maize that play a role during pollination (STOTZ et al., 2009a; AMIEN et al., 2010). The expression of four defensin genes (Hc-AFP1-4) was studied in *Heliophila coronopifolia* (BEER and VIVIER, 2011). The expression of two peptides (Hc-AFP1 and 3) were more active compared to the other two (Hc-AFP1 and 3). However, peptides Hc-AFP1 and 3 were expressed in mature leaves, stems and flowers, whereas Hc-AFP2 and 4 were expressed in seed pods and seeds (BEER and VIVIER, 2011). LAY et al., (2003) tested the expression of NaD1 defensin in ornamental tobacco (*Nicotiana alata*) using In situ RNA blot analysis. Its 0.6 kb transcript was detected in anthers, ovaries, pistils, and petals. It was highly expressed in young flowers, expressed at low level in roots, whereas it was not detected in leaves. Similar expression pattern was observed in the case of FST (GU et al., 1992) and TPP3 (MILIGAN and GASSER, 1995) defensins. According to the finding of LAY et al., (2003), two types of defensins are produced in the flowers of Solanaceous plants. One type with C-terminal prodomain which is stored in the vacuoles and the other type that does not have the C-terminal prodomain is produced extracellularly (LAY et al., 2003).

The number of studied plant defensins (61) is extremely low compared to the characterized number of animal defensins (454) (http://defensins.bii.a-star.edu.sg), therefore there is a huge shortage in plant defensins studies. The isolation and characterization of a wide range of plant defensin peptides are crucial for the continuous development of economically and medically important products. Saudi Arabia has a very rich uncharacterized exotic plant flora that need to be characterized at the molecular level. In this study, we investigated the defensin gene expression of six plant species from the Saudi environment, Taif region. Results of this study will help detecting some useful Saudi plant sources for the novel use of natural peptides as an alternative for antibiotics for biocontrol.

**MATERIALS AND METHODS**

**Collection and preparation of plant samples**

Plant samples were collected from Taif region (Table 1). This included samples from floral buds because they are known to have high defensin gene expression and they are highly protected against pathogen attacks by microorganisms (LAY et al., 2003; BEER and VIVIER, 2011). Collected samples were frozen at -80°C or lypholysed (freeze-dried), ground into fine powder in a coffee grinder and stored at -80°C until used.

<table>
<thead>
<tr>
<th>No</th>
<th>Name</th>
<th>Scientific Name</th>
<th>Family</th>
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<tbody>
<tr>
<td>1</td>
<td>jelly bean tree</td>
<td><em>Parkensonia aculeate</em></td>
<td>Fabaceae</td>
</tr>
<tr>
<td>2</td>
<td>Taily Weed</td>
<td><em>Ochradenus baccatus</em></td>
<td>Resedaceae</td>
</tr>
<tr>
<td>3</td>
<td>Mexican tea</td>
<td><em>Chenopodium ambrosioides</em></td>
<td>Chenopodiaceae</td>
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<tr>
<td>4</td>
<td>Saltwort</td>
<td><em>Salsola imbricata</em></td>
<td>Amaranthaceae</td>
</tr>
<tr>
<td>5</td>
<td>Prickly Poppy</td>
<td><em>Argemone mexicana</em></td>
<td>Papaveraceae</td>
</tr>
<tr>
<td>6</td>
<td>Mole Plant</td>
<td><em>Euphorbia lathyris</em></td>
<td>Euphorbiaceae</td>
</tr>
</tbody>
</table>
Design of PCR primers

Full length mRNA for selected plant defensins from the Nucleotide database (http://www.ncbi.nlm.nih.gov/) were used to design DNA primers using software on the website of Macrogen company (http://dna.macrogen.com). Primers were ordered from the same company. Different primers that were designed for this study are summarized in Table (2).

Table 2. DNA primer sequence designed for cDNA synthesis and PCR amplification.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence 5'→3'</th>
<th>Accession #</th>
<th>PCR Product, bp</th>
<th>Organisms</th>
</tr>
</thead>
<tbody>
<tr>
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<td>F-atggctaagttgtcctatcat R-acatggaaagtaagaatatacctt</td>
<td>Common</td>
<td>250</td>
<td>plant defensin1</td>
</tr>
<tr>
<td>PD2</td>
<td>F-atggagaagaaactactagttgg R-acatcttttttgacgagcgc</td>
<td>Common</td>
<td>250</td>
<td>plant defensin2</td>
</tr>
<tr>
<td>TaD</td>
<td>F-atgcgtcactgtgtagctgtgc R-gcagtttcgctgagtagctctt</td>
<td>AB089942</td>
<td>250</td>
<td>Triticum aestivum</td>
</tr>
<tr>
<td>ZmD</td>
<td>F-atgcgcctgtcagcgtgagcgc R-agcagatctttgacgcagagcactt</td>
<td>JF797205</td>
<td>250</td>
<td>Zea mays</td>
</tr>
<tr>
<td>MtD</td>
<td>F-atgcgcttcgctttctgagcgc R-agcagatctttgacgcagagcactt</td>
<td>XM_003636567</td>
<td>250</td>
<td>Medicago truncatula</td>
</tr>
<tr>
<td>HvActin</td>
<td>F-tgcaagaaagagctttactctg R-gaagactcctctgtggagaa</td>
<td>AY145451.1</td>
<td>460</td>
<td>Hurdium valgure</td>
</tr>
</tbody>
</table>

RNA isolation

RNA was isolated from the collected plant samples. One ml of QIAzol was added to 10 mg of lyophilized powder and mixed (QIAGEN Inc., Valencia, CA). A volume of 0.3 ml chloroform was added to the homogenate. The mixture was then shaken for 30 s followed by centrifugation at 4°C and 13000 rpm for 20 min. The supernatant was transferred to a new tube. One fourth volume of 12 M lithium chloride was added and kept at -20°C overnight. Samples were centrifuged for 15 min at 4°C and 13000 rpm. RNA pellet was washed with 70% ethanol, briefly dried, and dissolved in DEPC water. The integrity of RNA was checked by agarose gel electrophoresis and its concentration and purity were determined at 260 nm and the OD260/280 ratio.

Synthesis of cDNA

A mixture of 2 µg of total RNA and 0.5 ng oligoDT primer in a total volume of 11 µl sterilized DEPC-water was incubated in the Multigene thermal Cycler (Labnet, USA) at 65°C for 10 min for denaturation. Then, 4 µl of 5X RT-buffer, 2 µl of 10 µM dNTPs and 100 U M-MuLV Reverse Transcriptase (SibEnzyme Ltd. AK, Novosibirsk, Russia) were added and the total volume was brought to 20 µl by DEPC water. The mixture was then re-incubated in the thermal Cycler at 37°C for 1h, then at 90°C for 10 min to inactivate the enzyme then, hold at 4°C. cDNA was kept at -20°C until used.

Semi-quantitative PCR

PCR was conducted in a final volume of 25 µl consisting of 1 µl cDNA, 1 µl of 10 picomol of each primer (forward and reverse), and 12.5 µl PCR master mix (Promega
Corporation, Madison, WI, USA). PCR was carried out using cycle sequence of denaturing at 94°C for 5 minute for one cycle, followed by 20 cycles which consisted of denaturation at 94°C for one minute, annealing at 55°C for 40 s, and extension at 72°C for one minute with additional cycle as a final extension at 72°C for 5 minutes. As a reference, expression of β-actin mRNA was tested using specific primers (Table 2). PCR products were electrophoresed on 2% agarose gel in TBE buffer at 100 volt for 50 minutes with ethidium bromide staining. PCR products were visualized under UV light and photographed. Densitometric analysis of band intensities was determined using NIH imageJ program (http://rsb.info.nih.gov/nih-imageJ).

**Sequencing of PCR products**

PCR fragments were sequenced at Macrogen campany (http://dna.macrogen.com, Korea) using the same primers used for PCR amplification. The expected protein sequence was obtained by translation of DNA sequences using the Expasy translation tool (http://web.expasy.org/translate). The obtained protein sequences were used for sequence alignment.

**Sequence alignment and phylogenetic analysis**

Various plant defensin sequences, 15 accessions, were retrieved from the Nucleotide database (http://www.ncbi.nlm.nih.gov/, Table 3). Protein sequences of these accessions were used for alignment with sequences obtained from the six defensin sequences isolated from plants used in this study. Clastual Omega for multiple alignments at the European Bioinformatic Institute web site (http://www.ebi.ac.uk/Tools/msa/clustalo) was used to align amino acid sequences. The aligned sequences were used to construct the phylogenetic tree at the same web site.

<table>
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<tr>
<th>No</th>
<th>Species</th>
<th>Accession #</th>
<th>Abbreviation</th>
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<tr>
<td>1</td>
<td>Arabidopsis thaliana</td>
<td>NM_128160</td>
<td>At-NM_128160</td>
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<tr>
<td>2</td>
<td>Brassica rapa</td>
<td>AB302324</td>
<td>Br-AB302324</td>
</tr>
<tr>
<td>3</td>
<td>Brassica juncea</td>
<td>DQ191751</td>
<td>Bj-DQ191751</td>
</tr>
<tr>
<td>4</td>
<td>Brassica oleracea</td>
<td>AJ311046</td>
<td>Bo-AJ311046</td>
</tr>
<tr>
<td>5</td>
<td>Capsicum annuum</td>
<td>EU560903</td>
<td>Ca-EU560903</td>
</tr>
<tr>
<td>6</td>
<td>Cicer arietinum</td>
<td>AY206395</td>
<td>Ca-AV206395</td>
</tr>
<tr>
<td>7</td>
<td>Heliophila coronopifolia</td>
<td>JN203138</td>
<td>Hc-JN203138</td>
</tr>
<tr>
<td>8</td>
<td>Lycopersicon esculentum</td>
<td>AJ133601</td>
<td>Le-AJ133601</td>
</tr>
<tr>
<td>9</td>
<td>Malus domestica</td>
<td>JQ342965</td>
<td>Md-JQ342965</td>
</tr>
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<td>10</td>
<td>Medicago truncatula</td>
<td>XM_003636567</td>
<td>Mt-XM_003636567</td>
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<tr>
<td>11</td>
<td>Nicotiana alata</td>
<td>HI471057</td>
<td>Na-HI471057</td>
</tr>
<tr>
<td>12</td>
<td>Psophocarpus tetragonolobus</td>
<td>JQ314214</td>
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<tr>
<td>13</td>
<td>Saccharum officinaru</td>
<td>EU531731</td>
<td>So-EU531731</td>
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<tr>
<td>14</td>
<td>Vicia faba</td>
<td>EU920043</td>
<td>Vf-EU920043</td>
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</table>
RESULTS AND DISCUSSION

Defensin gene expression was studied in six plants from Saudi environment; Taif region, using two pairs of common primes (PD, PD2) as well as three pairs of specific primers (TaD, ZmD, MtD). These primers were used to isolate defensin genes from the reproductive organs, floral buds. Floral buds are one of the most valuable plant organs because they are the bridge for genetic material to the next generation. They were collected from different plants from Taif region because they are the most exposed organs for pathogen attack and consequently are expected to have high expression of antimicrobial peptides genes (Table 1). Total RNA was isolated from the collected samples and used for cDNA synthesis which was used as a template for PCR amplification. Five primer pairs designed on common or specific defensin sequences from the genebank accessions were used for PCR amplification. Four primer pairs gave amplification products (Figure 1-4), whereas one primer pair did not give PCR product. PD1 primer amplified a 250 bp fragment in Parkensonia aculeate (Pa); Ochradenus baccatus (Ob); Chenopodium ambrosioides (Ca); Salsola imbricate (Si); Argemone Mexicana (Am). On the other hand, this fragment was not amplified in Euphorbia lathyris (El) (Figure 1a). The expression of B-actin was estimated as a constitutive expressed gene (Figure 1b) as a reference. The quantitative estimation of defensin genes was measured with Image J software (Figure 1c).

The quantitative analysis revealed major differences in gene expression level. Ca showed the highest expression, whereas Ob showed the lowest expression level indicating that not only different plants express defensin genes at different levels but also some plants like El lacked the expression of some defensin genes using the same primer pair.

Another primer, PD2, gave similar PCR fragment in five species; Pa, Ob, Ca, Am, El, whereas this fragment was not amplified in Si (Figure 2a). Interestingly, primer PD2 gave amplification product in El which gave negative results with primer PD1(Figure 1a, Figure 2a) which reveals that there might be a gene family of defensin in these plants members of which have tissue-specific expression pattern. B-actin gene expression was estimated as a reference constitutive expressed gene (Figure 2b). Regarding gene expression level, on the contrary of PD1 primer, El showed the highest level of expression and Ob gave the lowest expression (Figure 2c), while the amplified product was not detectable in Si plant.

When primer ZmD was used for PCR amplification, a little smaller fragment was amplified in all six studied species (Figure 3a). B-actin gene expression was estimated as a reference constitutive expressed gene (Figure 3b). Quantitative analysis of gene expression level showed that Am gave the highest expression, whereas Pa has the lowest level of expression (Figure 3c). Ob, Ca, and Si gave moderate expression levels.

MtD primer amplified similar DNA fragment in Pa, Ca, Si, Am, and El which was not amplified in Ob (Figure 4a). B-actin gene expression was estimated as a reference constitutive expressed gene (Figure 4b). Pa gave the highest level of expression, whereas Ca showed the lowest expression level and Ob showed no expression (Figure 4c). Pa, Ca, and Am are the three species which gave amplification products with all four primer pairs.
Figure 1. Gel electrophoresis of RT-PCR products of defensins using PD1 primer: **Pa**: *Parkinsonia aculeate*; **Ob**: *Ochradenus baccatus*; **Ca**: *Chenopodium ambrosioides*; **Si**: *Salsola imbricate*; **Am**: *Argemone mexicana*; **El**: *Euphorbia lathyris*.

Figure 2. Gel electrophoresis of RT-PCR products of defensins using PD2 primer: Abbreviations as in Figure 1.
Figure 3. Gel electrophoresis of RT-PCR products of defensins using ZmDPrimer: Abbreviations as in Figure 1.

Figure 4. Gel electrophoresis of RT-PCR products of defensins using MtDPrimer: Abbreviations as in Figure 1.
Plant leaves are the most abundant and most active fresh plant organ and floral buds are the most protected plant organ against pathogens. The expression level of defensin in floral buds was compared with their expression in leaves to monitor the relative expression in floral buds compared to leaves. ZmD primer gave amplification products with all six species under study; therefore it was used to estimate the expression of defensins in leaves and floral buds. This primer gave different level of expression in the floral buds in the six species. Am gave the highest expression and El produced the lowest expression (Figure 5a), but it is very interesting that the general expression pattern of defensins in floral buds is higher than its expression in leaves. This supports our assumption that floral buds represent one of the most exposed organs to microbial infections and are very valuable for the plant life; therefore they are more protected against pathogens. B-actin gene expression was estimated as a reference constitutive expressed gene (Figure 5b). Although the general gene expression in leaves was lower than floral buds, it showed variations among species (Figure 5a, 5c). El gave the lowest expression in leaves, whereas Ca gave the highest gene expression in leaves and the closest level to its floral buds expression (Figure 5c). This result indicates that there are differences in defensin gene expression among plants as well as among the different organs in the same plant species.

![Figure 5. Expression of defensins in leaf (L) and flower bud (F) tissues using ZmD primers: Abbreviations as in Figure 1.](image)

The six amplified DNA fragments resulted from this study were sequenced and were used to search the nucleotide database at the NCBI website (www.ncbi.nlm.nih.gov) for similar sequences. Sequences with high identity, 15, were selected and used in protein sequence alignment with the expected protein sequence of the six isolated defensin cDNAs from this study. Protein alignment revealed high similarity among the isolated defensin sequences and the sequences obtained from the database, especially the cysteine rich domain (Figure 6). All defensins used in this alignment have 8 cysteines including the six sequences isolated in this study. The 8 cysteins were conserved in all aligned sequences except *Vicia faba* (Accession #
EU920043) which has the first seven cysteine and lack the last one (Figure 6). Interestingly, the isolated six sequences are distributed among the sequences obtained from the database which indicates that the isolated sequences share the common features of plant defensins.

Figure 6. Partial protein sequence alignment of the six excisana genes isolated from plants under study and other 15 accessions from the genebank database. Pa: Parkensonia aculeata; Hc: Heliophila coronopifolia; Br: Brassica rapa; Bj: Brassica juncea; Bo: Brassica oleracea; At: Arabidopsis thaliana; Ca-AY: Cicer arietinum; Pt: Psophocarpus tetragonolobus; El: Euphorbia lathyris; Ob: Ochradenus baccatus; Vf: Vicia faba; Si: Salsola imbricata; Mt: Medicago truncatula; Md: Malus domestica; Ta: Triticum aestivum; Ca: Chenopodium ambrosioides; Am: Argemone excisana; So: Saccharum officinarum; Zm: Zea mays; Le: Lycopersicon esculentum; Na: Nicotiana alata. Conserved cysteines are highlighted and/or indicated by asterisks.

Amino acid sequences of the isolated defensins as well as the 15 similar accessions from the database were used to study the phylogenetic relationship among this group of plant defensin genes. The results showed that the six isolated sequences were distributed among the other sequences obtained from the genbank database. The phylogenetic tree started with three clades A, B, and C. Clade C contained Le and Na. Clade B was diverged into two sub-clades B1 which contained Ta and B2 which was diverged into B3 and B4 branches. Branch B3 contained Ca where branch B4 contained Am, So, and Zm (Figure 7). Clade A was diverged into two sub-clades A1 and A2 which carried Si, Mt, and Md. Sub-clade A1 was diverged into two sub-sub-clades A3 and A4 which carried Vf. Sub-sub-clade A3 was diverged into two main branches A5 and A6 which carried El and Ob; two isolated sequences in this study (Figure 6). The main branch A5 was diverged into two branches A7 and A8 which carried Ca-AY and Pt. Branch A7 was diverged into two sub-branches A10 and A9 which carried Pa. Sub-branch A10 was diverged into two termini A12 and A11 which carried Hc. Termini A12 was diverged into two
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sub-termini A13 which carried Br, Bj, and Bo, and A14 which carried At (Figure 7). The phylogenetic tree indicates that the two isolated sequences El and Ob were the closest two sequences of the isolated six defensins. Ca and Am were also close to each other on the phylogenetic tree, whereas Si was closer to Mt sequence. Br, Bo, and Bj located on the same termini of clade A (Figure 7).

Figure 7. Phylogenetic tree of amino acid sequence of defensin genes isolated from plants under study and other accessions from the genebank database. Abbreviations are as in Figure 6.

DISCUSSION

Defensin gene expression was studied in six plant species from Saudi environment; Taif region, using two pairs of common primes as well as three pairs of specific primers. In addition, these primers were used to isolate the defensin genes using RT-PCR and sequencing of cDNA. The obtained sequences were aligned with other similar defensin sequences from the Nucleotide database (www.nil.nih.gov) and used to establish the phylogenetic relationship among the isolated sequences and similar accessions from the database. Floral buds were used in this study because previous reports proved that they high level of defensin gene expression (LAY et al., 2003; SILVERSTEIN et al., 2005) and they are among plant tissues that are highly protected against pathogens. Out of five primer pairs, only four pairs gave positive amplification products including the common two pairs of primers, whereas one primer pair, TaD, did not amplify any fragments. One interesting result of this study is that the common two pairs of primers (PD1, PD2) could be used in the future to study the gene expression of defensins in other plant species.

Some primers did not amplify products in some plant species. Primer PD1 did not amplify product with El (Figure 1a), primer PD2 did not amplify product with Si (Figure 2a), primer ZnD is the only primer that gave amplification product with all six species (Figure 3a),
and primer MtD did not amplify product with Ob (Figure 4a). Absence of expression of some defensin fragments in some species could be explained by the differential presence of defensin genes in the defensin multigene family in different species or tissue specific expression.

Organization of defensin genes in multigene family was reported in previous studies (THOMMA et al., 2002; SCHUTTE et al., 2002; FEDOROVA et al., 2002; MEGAERT et al., 2003; GRAHAM et al., 2004). For example, the defensin gene family in Medicago truncatula was studied by HANKS et al. (2005) which resulted in the detection of sixteen genes that highly differed outside the eight cysteine residues. These genes were found to be expressed in different tissues including, leaves, flowers, seeds, and roots, and their expression was differentially induced by various biotic and abiotic stimuli (HANKS et al., 2005). In another study by SILVERSTEIN et al. (2005), a family of 317 defensin-like (DEFL) genes was detected in Arabidopsis thaliana. They were distributed into 16 clusters on five chromosomes. The expression of many of these DEFLs was tested and found to be expressed only in floral buds. In our study, some members of defensin gene family might not exist in some species; therefore they give negative results with certain primers (Figure 1a, 2a, 4a). Another explanation for the differential amplification of defensin genes could be due to that some primers did not find a perfect match in some members of the defensins gene family or members of this family might be expressed in a tissue-specific manner.

Our results indicated that there was a differential gene expression in the same tissue and between different tissues. This is clearly evident since there was a difference in the expression level in the floral buds (Figure 1-4) and between floral buds and leaves (Figure 5), although floral buds generally showed higher expression compared to leaves in all species (Figure 5). Similar defensin expression pattern was reported in several studies (BEER and VIVIER, 2011; LAY et al., 2003; HANKS et al., 2005; SILVERSTEIN et al., 2005). In one example, four plant defensin genes (Hc-AFP1-4) were isolated and studied from the native South African species Heliophila coronopifolia (BEER and VIVIER, 2011). The four defensins were divided into two groups depending on protein sequence homology and homology modelINg. Results of this study showed that recombinant peptides of Hc-AFP2 and 4 were more active compared to Hc-AFP1 and 3 which caused higher membrane permeability. However, the two groups of peptides showed differential expression in the native plant as Hc-AFP1 and 3 were expressed in mature leaves, stems and flowers, whereas Hc-AFP2 and 4 were expressed in seedpods and seeds (BEER and VIVIER, 2011). The expression of NaD1 defensin was estimated by In situ RNA blot analysis (Lay et al., 2003). A message of 0.6 kb of NaD1 transcript was detected in anthers, ovaries, pistils, and petals from ornamental tobacco (Nicotiana alata). At gene expression level, NaD1 transcript was not detected in leaves, whereas it was expressed at low level in roots, whereas it was highly expressed in younger flowers compared to mature ones. In addition, they tested the expression in different tissues of young flowers and found that the transcript was more abundant in epidermal cells of petals and sepals, whereas it was not detected in the tapetum tissue, the transmitting tissue of the vascular bundles of style and anthers (LAY et al., 2003). Other studies also showed high expression in young floral buds of FST (GU et al., 1992) and TPP3 (MILIGAN and GASSER, 1995). Similar to NaD1 both defensins (FST, TPP3) are abundant in young floral buds and their expression level decreases when floral buds grow older. In these three cases, NaD1, FST, TPP3 they were expressed in petals, pistils, and anthers, whereas they were not expressed in leaves. Tissue specific expression in our study agrees with previous results (GU et al., 1992; MILIGAN and GASSER, 1995; SILVERSTEIN et al., 2005; LAY et al., 2003) in that isolated
defensins in our study were expressed at high levels in floral buds, but, on the other hand, disagree with the previous results in that defensins in our study were expressed in leaves at lower levels. Solanaceous plants produce two types of defensins in their flower buds; one with C-terminal prodomain that is deposited in the vacuoles and the other type does not have the C-terminal prodomain which is produced outside the cell (LAY et al., 2003). Vacuole deposited defensins are produced in floral buds only. Based on this information and because our defensins are expressed in both floral buds and leaves, we would expect that defensins isolated in this study do not have the C-terminal prodomain.

In conclusion, two common primer pairs of plant defensins were designed and used to isolate, sequence, and study the defensins gene expression in 6 plants from Taif region. Also, the expression of defensins was compared in to main tissues, leaves and floral buds. The isolated and sequenced defensins have the common characteristics of plant defensins. These primers provide a tool to study defensins gene organization and expression in other plants as biodiversity indicator for the determination of valuable sources of defensins.

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