Abstract – A partial mRNA sequence was identified from *Celosia cristata* purified leaf mRNA population at the early vegetative stage. Based on its deduced amino acid sequence analysis, it was predicted to encode a homologue of Herpes UL31 protein superfamily in test plant. The bacterial origin of the isolated fragment was eliminated on the basis of some experimental evidences. Since the cloned mRNA fragment was significantly closer to the viral protein rather than to their eukaryotic sequences, thereby it may be a great value for evolutionary biologists, as it may certify a horizontal transfer of genetic information between viruses and eukaryotic taxa. The present report may lead to further investigations with regard to the structures and biological importance of viral UL31 gene in plant system. A possible correlation between the product of the isolated clone and anti-viral mechanism was proposed.

**Keywords:** antiviral, *Celosia*, expression, UL31 protein

Identification of a partial mRNA containing UL31 viral protein domain from *Celosia cristata* expressed sequences

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**Introduction**

*Celosia cristata* (commonly known as cockscomb) belongs to the class Magnoliopsida, order Caryophyllales and family Amaranthaceae. It is commonly planted as ornamental or eatable plant in Africa, South America, India and some parts of Asia (National Research Council, 2006). It has potent medicament value. It is used for the treatment of hematemesis, abnormal uterine bleeding, hematochezia, hemorrhoidal bleeding, leukorrhea, chronic dysentery with persistent diarrhoea, redness of the eye and dizziness (Kirtikar & Basu, 1935; reviewed by Surse et al. 2014). Several biological activities like Antioxidant, Antiviral, anti-aging; Anthelmintic actions are attributed for this plant (Pyo et al. 2008; Woo et al. 2011; Rubini et al. 2012). Its propagation is successfully followed to ensure the raising of plant in the nurseries and fields, too.

*C. cristata* mainly contains flavonoids. Its leaves contain two glycoproteins CCP-25 and CCP-27 having antiviral and ribosome-inactivating activities (Balasubrahmanyam et al. 2000; Gholizadeh et al. 2004). These antiviral proteins present strong antioxidant activity through the increase in activities of redox enzymes such as peroxidase, catalase and polyphenyl oxidase (Gholizadeh et al. 2004). Amaranthine type of betacyanins has been isolated from this plant. Its seeds have been reported to contain 4-hydroxy phenethylalcohol, kaempferol, quercetin, β-sitostanol, 2-hydroxy octadecenoic acid, stigmasterol and various saponins (Xiang et al. 2010).

The vitality of number of activities is predicted to be present in *C. cristata* plant. Nowadays, attempts are made to propagate the research and pharmacotherapeutic potentials of this plant. Its aesthetic and medicinal uses are definitely going to be evaluated in detail. In this regards, the present research work reports the isolation and identification of a cDNA fragment containing viral protein domain from the expressed sequences of *C. cristata* plant that may help us to understand more about its antiviral potentials in the future.

The correspondence sequence was submitted to EMBL databases under accession number FM955595.1.
Materials and Methods

**Materials.** Plasmid vector pGEM-T easy was from Promega (Cat. no. A1360). DH5α E. coli strain was used for bacterial transformation. Trizol reagent was from CinnaGen (Cat. no. RN7713C; RNXTM). mRNA purification kit was provided by QIAGEN (Cat. No. 70022). AcessQuick™ RT-PCR System was purchased from Promega (Cat. no. A1701). DNA extraction kit (Cat. no. K0513) was purchased from Fermentas. All of the other chemicals used in this research work were of molecular biology grades. The seeds of *C. cristata* plant taken from lab stock.

**Total RNA isolation and mRNA purification.** Total RNA of test material was isolated using Trizol reagent. Briefly, about 0.2 g of test material was fine powdered and 2 ml of Trizol reagent was added to homogenize it. After addition of 200 μl of chloroform and incubation on ice for 5 min, the mixture was centrifuged at 13000g for 15 min. The upper phase was transferred to another tube and RNA was precipitated with an equal volume of isopropanol and washed in 75% ethanol. Poly (A+) RNA was purified from total RNA using oligo dT-columns according to the provided protocol of kit. The integrities of the total RNA and purified mRNA were also analyzed on non-denaturing gel. The quantity of the RNA in the starting materials for the next experiment was measured spectrophotometrically (Ausubel et al. 1991).

**RT-PCR based amplification.** Primer set used for the amplification was designed based on already reported plants antiviral proteins sequences by online Primer3 software (Gholizadeh et al. 2005). In order to analyze the expressed sequence, the RT-PCR reaction was performed. For this, about 0.5 g of mRNA mixed with 25 μl Master Mix and 1 μl of primers and adjusted to a final volume of 50 μl. The mixture was incubated at 95°C for 3 minutes in 25 cycles. The primer sequences and the details of PCR steps were presented in Table 1.

**Sequencing and analysis.** The amplified product cloned in pGEM-T easy vector (Ausubel et al. 1991) and proceeded for the sequencing in Microsynth DNA sequencing center at Switzerland. The nucleotide and deduced amino acid sequences of the isolated mRNA was analyzed by computing at BLAST (Basic Local Alignment Search Tool) and CDART (Conserved Domain Architectural Tool) at http://www.ncbi.nlm.nih.gov/BLAST and http://.expasy.org/tools/.

**Results and Discussion**

A partial mRNA was amplified from *C. cristata* leaf mRNA population prepared at its early vegetative stage. The integrities of total RNA, cDNA and amplicon were presented in Fig. 1. Nucleotide and amino acid sequence analysis data revealed that the amplified fragment spanning 551bp in length and might be a partial ORF encoding a homologue of Herpes viral UL31 protein member in test plant (Fig. 2).

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**Table 1. Primer set & PCR amplification steps**

<table>
<thead>
<tr>
<th>Primer sequences</th>
<th>PCR amplification steps</th>
</tr>
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<tbody>
<tr>
<td>Forward: 5'TATGGCGCAAACAAACAAATAA3'</td>
<td>Denaturation: 93°C / 1min</td>
</tr>
<tr>
<td>Reverse: 5'GTGTCGTTGCATTGATCAGG 3'</td>
<td>Annealing: 57°C / 1.5min</td>
</tr>
<tr>
<td></td>
<td>Extension: 72°C / 2min</td>
</tr>
<tr>
<td></td>
<td>Final Extension: 72°C / 10min</td>
</tr>
</tbody>
</table>

Primer sequences were designed by Primer3 software at: http://www.primer3plus.com/
Homology search data showed that this sequence is about 40% identical to the UL31 protein of U(L)31 and U(L)34 protein of herpes simplex virus type 1 (Fig. 3). Despite the primary structures, comparison of the secondary structures of *Celosia* UL31 homologue and herpes simplex virus protein does more support the homology between these two sequences (Fig. 3). The low primary sequence similarity score between viral and plant sequences may indicate that the isolated mRNA has not been derived from the viral contaminants. Besides this, the possibility for the viral origin of the isolated clone is strongly eliminated based on the following experimental evidences: (a) *Celosia* poly A+ RNA was purified from total RNA extract and used as initial

**Fig. 1.** Agarose gel analysis of the expression. The integrities of total RNA (left), ladder DNA marker and cDNA population (middle), and the end product of RT-PCR as amplicon were analyzed on agarose gel.

**Fig. 2.** Sequence analysis of the isolated mRNA. The nucleotide (a) and deduced amino acid (b) sequences of the cloned partial mRNA, and the predicted Herpes UL31 protein superfamily conserved domain are presented.

Homology search data showed that this sequence is about 40% identical to the UL31 protein of U(L)31 and U(L)34 protein of herpes simplex virus type 1 (Fig. 3). Despite the primary structures, comparison of the secondary structures of *Celosia* UL31 homologue and herpes simplex virus protein does more support the homology between these two sequences (Fig. 3). The low primary sequence similarity score between viral and plant sequences may indicate that the isolated mRNA has not been derived from the viral contaminants. Besides this, the possibility for the viral origin of the isolated clone is strongly eliminated based on the following experimental evidences: (a) *Celosia* poly A+ RNA was purified from total RNA extract and used as initial
material, (b) Oligo dT primer was used for cDNA synthesis from mRNA sample and PCR reaction was performed on cDNA population.

UL31 protein of herpes simplex virus type 1 has been known to form a complex that accumulates at the nuclear rim and is necessary for the envelopment of viral nucleocapsids (Reynolds et al. 2001). UL31 of herpes simplex virus type 1 has been also recently reported to be efficiently required for the expression of viral gene products upon infection of the host cells (Roberts & Baines 2011).

Celosia expressed mRNA sequence was significantly closer to viral protein rather than to their eukaryotic sequences. This similarity may be an important value for evolutionary biologists, as it can certify a horizontal transfer of genetic information between viral and eukaryotic taxa through co-existing? We think that such a viral homologue may play an important biological role in plant system, which needs to be investigated. We here propose a possible correlation between the product of the isolated clone and antiviral mechanism through the “homology-dependent RNA degradation” strategy. A serious attempt is recommended to be carried out for the identification of the biological importance of such a viral-type sequence in the expressed genome of plant system in the future.

**Fig. 3. Sequence comparison.** The primary and the secondary structures of the isolated clone and the viral UL31 protein were compared by CLASTAW and PSIPRED, to form a complex that accumulates at the nuclear rim and is necessary for the envelopment of viral nucleocapsids (Reynolds et al. 2001). UL31 of herpes simplex virus type 1 has been also recently reported to be efficiently required for the expression of viral gene products upon infection of the host cells (Roberts & Baines 2011). Celosia expressed mRNA sequence was significantly closer to viral protein rather than to their eukaryotic sequences. This similarity may be an important value for evolutionary biologists, as it can certify a horizontal transfer of genetic information between viral and eukaryotic taxa through co-existing? We think that such a viral homologue may play an important biological role in plant system, which needs to be investigated. We here propose a possible correlation between the product of the isolated clone and antiviral mechanism thought the “homology-dependent RNA degradation” strategy. A serious attempt is recommended to be carried out for the identification of the biological importance of such a viral-type sequence in the expressed genome of plant system in the future.

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References


