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DNA CLONING AND MOLECULAR CHARACTERIZATION OF *H2A* AND *H4* GENES IN PEPPER

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ABSTRACT

The complete coding sequences (CDS) of "Yunnan Purple Pepper No.1" (C*apsicum annuum* L.) *H2A* and *H4* genes were amplified using the reverse transcriptase polymerase chain reaction based on the conserved sequence information of some Solanaceae plants and known highly homologous pepper ESTs. The nucleotide sequences analysis of these two genes revealed that pepper *H2A* gene encodes a protein of 142 amino acids that has high homology with the *H2A-like* protein of 5 species: matrimony vine, grape, alfalfa, arabidopsis and maize. Sequence analysis of the second gene revealed that the pepper *H4* encodes a protein of 103 amino acids that has high homology with the proteins of 4 species: tomato, tobacco, eggplant and petunia. The tissue expression analysis indicated that the pepper *H2A* and *H4* genes were moderately expressed in the seven tissues. Our experiment established the primary foundation for further research on these two pepper genes.

Key words: *Capsicum annuum* L.; *H2A*; *H4*; Gene expression profile, Bioinformatics analysis.

INTRODUCTION

Histones are a kind of highly alkaline proteins found in eukaryotic cell nuclei (Wang *et al.*, 2004). They package and order the DNA into structural units called nucleosomes. Histone are the most important protein components of chromatin, acting as spools around which DNA winds, and play a important role in gene regulation (Redon *et al*., 2002). The unwound DNA in chromosomes would be very long without histones. For example, the length of DNA in cell is about 1.8 meters, but it has about 90 micrometers (0.09 mm) of chromatin when DNA wind on the histones (Redon *et al.*, 2002; Bhasin *et al.*, 2006). Histones were discovered in 1884 (Wurtele *et al.*, 2010). But until the early 1990s, histones were regarded to be inert packing material for eukaryotic nuclear DNA. During the 1980s, eukaryotic histones were demonstrated to repress gene transcription (Krogan *et al.*, 2002; Billon and Côté, 2011; Song *et al.*, 2011; Eriksson *et al.*, 2012; Wurtele *et al.*, 2012). It is now known that histones play both positive and negative roles in gene expression, forming the basis of the histone code. There are five major families of histone proteins called H1 (H5), H2A, H2B H3, and H4. Histones H2A, H2B, H3 and H4 are known as the core histones, while histones H1 and H5 are known as the linker histones (Alva *et al.*, 2007; Ward *et al.*, 2009). In the core histones of H2A, H2B, H3 and H4, two of each histones dimers form one octameric nucleosome core (Luger *et al*., 1997). 147 base pairs of DNA wrap around this core particle 1.65 times in a left-handed super-helical turn (Bhasin *et al*., 2006). H1, a linker histone, binds the nucleosome at the entry and exit sites of the DNA, so as to lock the DNA into place and to allow the formation of higher order structure. The 10 nm fiber or beads on a string conformation is the most basic such formation (de Napoles *et al.*, 2004). Histones are highly conserved through evolution (Kayne *et al.*, 1988; Wurtele *et al.*, 2010). The histone H2A, H2B, H3 and H4 are relatively similar in structure. They all have a 'helix-turn-helix-turn-helix' motif which allows the easy dimerisation. They also share the feature of long ''tails' on one end of the amino acid structure which should be the location of post-translational modification (Krogan *et al.*, 2002; Billon and Côté, 2011; Song *et al.*, 2011; Eriksson *et al.*, 2012). Histones make some types of interactions with DNA: such as alpha-helices in H2B, H3, and H4 with a net positive charge interact with negatively charged phosphate groups on DNA; Nonpolar interactions between the histone and deoxyribose sugars on DNA; Hydrogen bonds between the amide group on the main chain of histone proteins and the DNA backbone; Non-specific minor groove of the H3 and H2B N-terminal tails insertions into two minor grooves of each on the DNA molecule. Histone modifications act in diverse biological processes such as DNA repair, gene regulation, spermatogenesis (meiosis) and chromosome condensation (mitosis) (Strahl and Allis, 2000; Song *et al*., 2011). Histones undergo posttranslational modifications that alter their interaction with DNA and nuclear proteins. Long tails of the H3 and H4 histones protruding from the nucleosome can be covalently modified at several places (Jenuwein and Allis, 2001). Such modifications include methylation, phosphorylation,

SUMOylation, ADP-ribosylation, acetylation, ubiquitination and citrullination (Krogan *et al.*, 2002; Billon and Côté, 2011; Song *et al.*, 2011; Eriksson *et al.*, 2012; Wurtele *et al.*, 2012).

In our study, two novel pepper histone genes, *H2A* and *H4*, were isolated from pepper (*Capsicum annuum* L.). Their expression patterns are presented. It provides primary information for further understanding the biochemical functions of *H2A* and *H4* in pepper.

MATERIALS AND METHODS

Sample collection: All plants were obtained from the college of horticulture and landscape, Yunnan agricultural university. Pepper tissues (root, stem, leaf, flower, pericarp, placenta and seed) were instantly frozen in liquid nitrogen and stored at -80°C before use.

RNA extraction and first-strand cDNA synthesis: Total RNA and first-strand cDNA synthesis for samples were carried out according to the methods described by Deng *et al.*, (2012).

PCR amplification: PCR was performed to isolate the pepper genes from pooled cDNAs from different tissues. Reactions were performed as described previously (Deng *et al.*, 2012; Huo *et al.*, 2012). The PCR program of the *H2A* and *H4* genes consisted of 94°C for 3 min, followed by 34 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 2 min, and a final extension at 72°C for 10 min and 4°C hold.

The mRNA and amino acid sequences for *H2A* and *H4* from various plant species archived at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov) were used to locate conserved regions by multiple sequence alignment with CLUSTALW 1.8. The following primers were designed: H2A-F: 5'- AAA GCA ATC TTC AAA TCT AG -3', H2A -R: 5'- ATC CTT ACC CAA AAC AC -3'; H4-F: 5'-CCC CAA ATC CAT AAA GAG-3', H4-R: 5'-CAC AAC TCC AGC CTT CTC-3'

RT-PCR expression profile: The reverse transcriptase (RT)-PCR primers for pepper *H2A* and *H4* used for expression profile analysis were the same as those used for PCR. *Actin*, a housekeeping gene as a positive control was selected and the primers were 5'-TGC AGG AAT CCA CGA GAC TAC-3' and 5'-TAC CAC CAC TGA GCA CAA TGT T-3'. PCR was optimized to ensure sufficient product intensity within the linear phase of amplification.

Bioinformatics analysis: Software from NCBI and ExPASy was used for analysis sequence of pepper *H2A* and *H4* genes; GenScan software was used for prediction the cDNA sequences. Software from ExPASy was used for Putative protein theoretical molecular weight (Mw) and isoelectric point (pI) prediction; SignalP 3.0 server was used for signal peptide prediction; Software from PSort server was used for subcellular localization prediction; TMHMM-2.0 server was used for transmembrane topology prediction; Conserved domains and similar proteins was performed using the software from NCBI; Software from Clustal and from mega-software with standard parameters was used for the alignment of the nucleotide sequences and deduced amino acid sequences; Software from NPS was used for secondary structures of deduced amino acid sequences prediction; Software from ExPASy was used for the 3D structure prediction based on existing 3D structures by the amino acids homology modeling.

RESULTS

RT-PCR of pepper *H2A* and *H4:* RT-PCR with pooled tissue cDNAs for pepper *H2A* and *H4* genes yielded products of 520 bp and 540 bp. Sequence prediction showed that the 520 bp and 540 bp cDNAs represent 2 genes that encode 142 and 103 amino acid proteins, respectively.

Physical and chemical characteristics of pepper H2A and H4: The complete coding sequences (CDS) and the encoded amino acids of pepper H2A and H4 are presented in Figures 1 and 2.

Putative protein Mw, pI, amino acid composition, atomic composition, extinction coefficients, estimated half-life, instability index, aliphatic index and GRAVY were computed. pI of the putative proteins of *H2A* and *H4* was 10.3 and 11.8, Mw was 14685 and 11425 Da, repectively. Total number of negatively charged residues (Asp + Glu) of the *H2A* and *H4* genes were 10 and 7. Total number of positively charged residues (Arg + Lys) of *H2A* and *H4* genes were 22 and 25. Formulas were $C_{644}H_{1067}N_{199}O_{191}S_1$ and $C_{500}H_{848}N_{166}O_{136}S_2$, total number of atoms was 2102 and 1652, extinction coefficient (assuming all pairs of Cys residues form cystines) was 4470 and 5960, instability index was 38.16 indicating stable and 47.22 indicating unstable, aliphatic index was 88.73 and 85.15, and GRAVY was -0.283 and -0.550, respectively. Extinction coefficient (assuming all Cys residues are reduced) and estimated half-life (mammalian reticulocytes, in vitro) of the two pepper genes were 30h.

Prediction and analysis of structures and conserved domains of pepper H2A and H4: The putative H2A and H4 proteins were also analyzed. The H2A conserved domains were identified as H2A superfamily. H4 conserved domains has a H4 specific hits.

The results of secondary structure prediction showed that deduced pepper H2A protein contained 35.21% alpha helices, 7.04% extended strands, 8.45% beta turns and 49.3% random coils. Deduced pepper H4

protein contained 44.66% alpha helices, 14.56% extended strands, 11.65% beta turns and 29.13% random coils.

The pepper H2A and H4 proteins did not contain putative signal peptides (Bendtsen *et al*., 2004) and were not potential membrane proteins (Moller *et al*., 2001). Pepper H2A was probably located with 84.4% likelihood in the chloroplast stroma and H4 was probably located with 95.1% likelihood in the nucleus (Nakai *et al*., 1999).

Analysis of sequence identity and evolutionary relationships of pepper H2A and H4: The homology of the pepper H2A protein was determined and analyzed. The results showed that the pepper H2A protein had high homology with this protein of five other plant species (Figure 3). H4 shared homology with H4 protein in the species shown in Figure 4.

Phylogenetic trees were constructed on the basis of the alignments as shown in Figure 5 and 6. Pepper H2A and H4 were most closely related to H2A and H4 of matrimony vine and tomato.

Tissue expression: In order to examine the differential distributions of *H2A* and *H4* genes in tissues of pepper, the relative mRNA expression levels of *the two pepper genes* were evaluated by RT-PCR. The results revealed that the pepper *H2A* and *H4* genes were moderately expressed in the seven tissues (figures 7 and 8).

Figure 1 Complete CDS and amino acid sequence of H2A in pepper; * indicates the stop codon

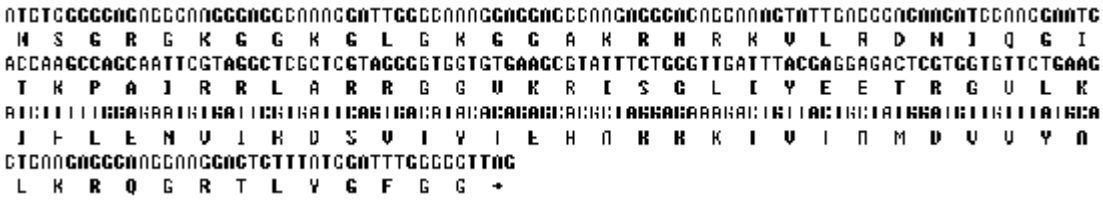

Figure 2 Complete CDS and amino acid sequence of H4 in pepper; * indicates the stop codon

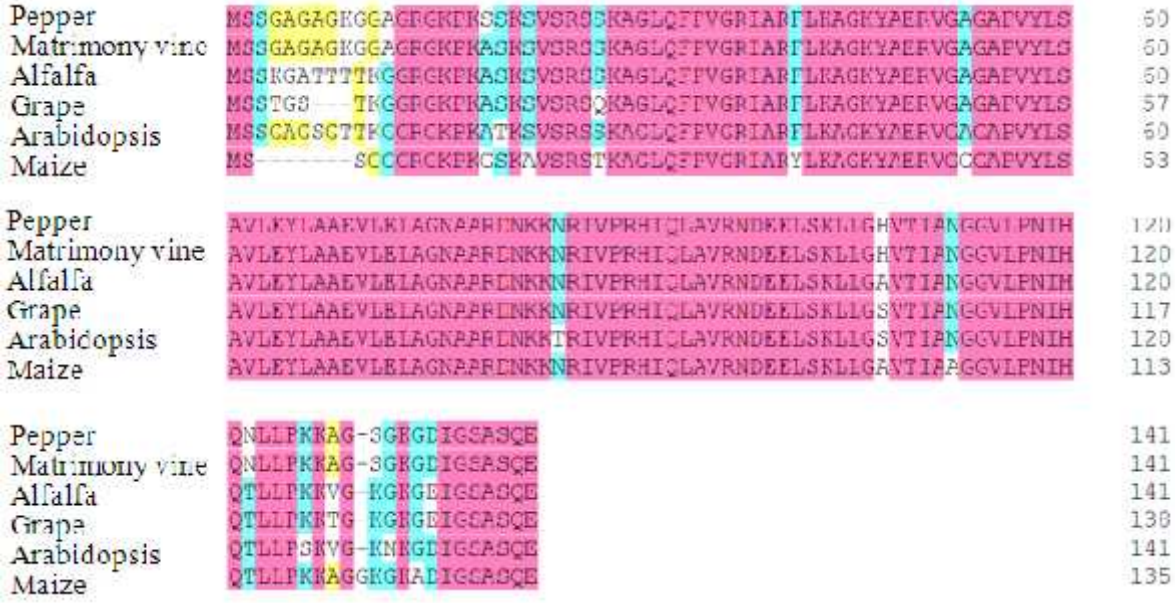

Figure 3. Multiple amino acid sequence alignment of H2A

Figure 4. Multiple amino acid sequence alignment of H4

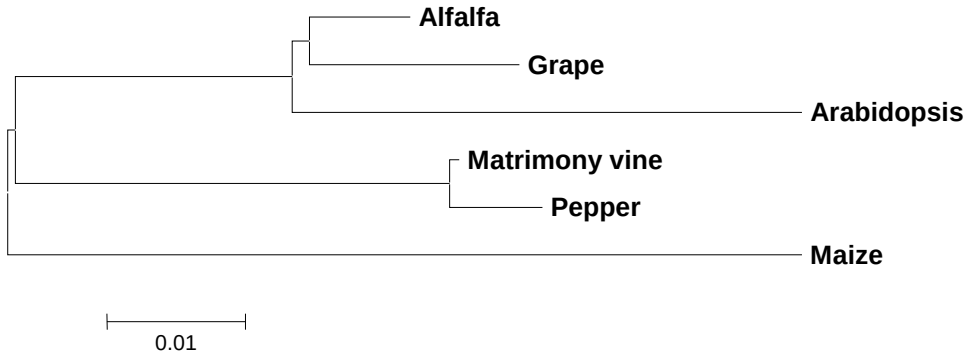

Figure 5. Phylogenetic tree of H2A gene using software ClustalX and Mega 4.0.

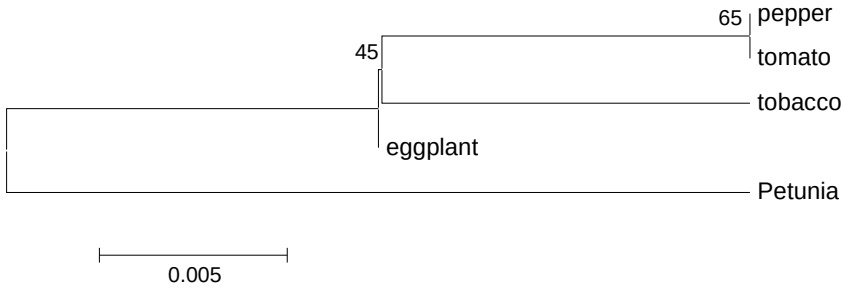

Figure 6. Phylogenetic tree of H4 gene using software ClustalX and Mega 4.0.

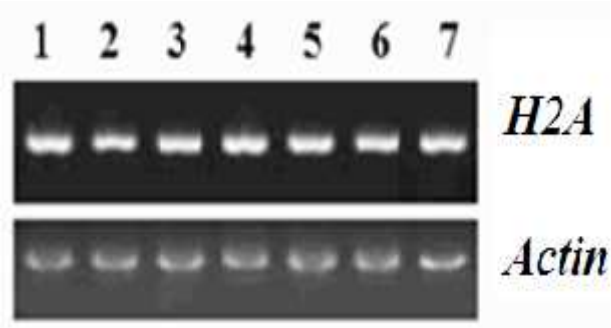

Figure 7. Tissue expression of pepper H2A; Actin served as the internal control

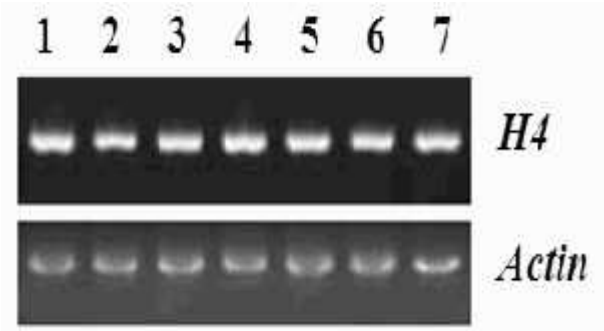

Figure 8. Tissue expression of pepper H4; Actin served as the internal control

DISCUSSION

Comparative genomics determines the relationship of genome structure and function of different species (Hardison, 2003). Several researchers have learned that H2A and H4 proteins from different species are highly conserved (Kayne *et al.*, 1988; Redon *et al.*, 2002). Therefore, we can use matrimony vine or tomato as a model organism to study the pepper H2A and H4 or use pepper as a model organism to study the tomato, potato or tobacco H2A and H4 genes.

In this study, pepper H2A and H4 genes were isolated and characterized. Many genes encoding H2A and H4 from several plant species have been isolated and characterized at the genetic and chemical levels (Alva *et al.*, 2007; Redon *et al.*, 2002). However, cloning of the H2A and H4genes from pepper has not been previously reported. This work verifies for the first time the presence of H2A and H4 in pepper.

The isolated H2A cDNA was 429 bp long encoding 142 amino acids. Comparison of amino acid sequence showed high homology to matrimony vine, alfalfa, grape, Arabidopsis and maize. The isolated H4 cDNA was 312 bp long encoded 103 amino acids. Sequence analysis revealed that the pepper H4 has high homology with the proteins of 3 species-tobacco, tobacco and petunia. From the alignment analyses for pepper H2A and H4 proteins, we also found that pepper H2A and H4 proteins did not show complete identity with matrimony vine, tomato or other plants. This implied that pepper H2A and H4 has some different functions compared to matrimony vine, tomato and other plants.

Our results show that the pepper *H2A* and *H4* genes were expressed in the seven tissues. The analysis of expression level showed that two pepper genes were moderately expressed in the all seven tissues. The results demonstrated that the pepper *H2A* and *H4* genes were house-keeping genes.

Conclusions: In summary, we first isolated the pepper *H2A* and *H4* genes and performed necessary functional analysis and tissue expression profile analysis. The results will be extremely important in elucidating the molecular mechanism of gene expression.

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REFERENCES

Alva, V., M. Ammelburg and A. N. Lupas (2007). On the origin of the histone fold. BMC Struct. Biol. 7: 17.

Bendtsen, J. D., H. Nielsen, G. von Heijne, and S. Brunak (2004). Improved prediction of signal peptides: SignalP 3.0. J. Mol. Biol. 340: 783-795.

Bhasin, M., E. L. Reinherz and P. A. Reche (2006). Recognition and classification of histones using support vector machine. J. Comput. Biol. 13 (1): 102–12.

Billon, P. and J. Côté (2011). Precise deposition of histone H2A.Z in chromatin for genome expression and maintenance. Biochim. Biophys. Acta. 1819 (3–4): 290–302.

Deng, M.-H., J.-F. Wen, J.-L. Huo, H.-S. Zhu, X.-Z. Dai, Z.-Q. Zhang, H .Zhou and X.-X. Zou (2012). Relationship of metabolism of reactive oxygen species with cytoplasmic male sterility in pepper (*Capsicum annuum* L.) Scientia Horticulturae. 134: 232-236.

de Napoles, M., J. E. Mermoud, R. Wakao, Y. A. Tang, M. Endoh, R. Appanah T. B. Nesterova, J. Silva, A. P. Otte, M. Vidal, H. Koseki and N. Brockdorff (2004). Polycomb group proteins Ring1A/B link ubiquitylation of histone H2A to heritable gene silencing and X inactivation. Dev. Cell. 7 (5): 663–76.

Eriksson, P. R., D. Ganguli, V. Nagarajavel and D. J. Clark (2012). Regulation of histone gene expression in budding yeast. Genetics. 191(1):7-20.

Hardison, R. C. (2003). Comparative genomics. PloS. Biol. 1: 58.

Huo, J., P. Wang, H. Wei and Y. Zeng (2012). Molecular cloning, sequence characterization and tissue transcription profile of a novel gene PQBP1 from Chinese Banna Mini-pig Inbred Line (BMI). J. Anim. Vet. Adv. 11(10):1674-1680.

Jenuwein, T. and C. D. Allis (2001). Translating the histone code. *Science* 293 (5532): 1074–1080.

Kayne, P. S., U. J. Kim, M. Han, J. R. Mullen, F. Yoshizaki and M. Grunstein(1988). Extremely conserved histone H4 N terminus is dispensable for growth but essential for repressing the silent mating loci in yeast. Cell. 55 (1): 27–39.

Krogan, N. J., J. Dover, S. Khorrami, J. F. Greenblatt, J. Schneider, M. Johnston and A. Shilatifard (2002). COMPASS, a histone H3 (Lysine 4) methyltransferase required for telomeric silencing of gene expression. J. Biol. Chem. 277 (13): 10753–5.

Luger, K., A. W. Mäder, R. K. Richmond, D. F. Sargent and T. J. Richmond (1997). Crystal structure of

the nucleosome core particle at 2.8 A resolution. *Nature* 389 (6648): 251–60.

Moller, S., M. D. Croning and R. Apweiler (2001). Evaluation of methods for the prediction of membrane spanning regions. Bioinformatics. 17: 646-653.

Nakai, K. and P. Horton (1999). PSORT: a program for detecting the sorting signals of proteins and predicting their subcellular localization. Trends Biochem. Sci. 24: 34-35.

Redon, C., D. Pilch, E. Rogakou, O. Sedelnikova, K. Newrockand and W. Bonner (2002). Histone H2A variants H2AX and H2AZ. Curr. Opin. Genet. Dev. 12 (2): 162–9.

Song, N., J. Liu, S. An, T. Nishino, Y. Hishikawa and T. Koji (2011). Immunohistochemical analysis of histone H3 modifications in germ cells during mouse spermatogenesis. Acta Histochemica et Cytochemica 44 (4): 183–190.

Strahl, B. D. and C. D. Allis (2000). The language of covalent histone modifications. *Nature* 403 (6765): 41–45.

Wang, H., L. Wang, H. Erdjument-Bromage, M. Vidal, P. Tempst, R. S. Jones and Y. Zhang (2004). Role of histone H2A ubiquitination in Polycomb silencing. Nature. 431 (7010): 873–8.

Ward, R., A. Bowman, H. El-Mkami, T. Owen-Hughes and D. G. Norman (2009). Long distance PELDOR measurements on the histone core particle. J. Am. Chem. Soc. 131 (4): 1348–1349.

Wurtele, H., G. S. Kaiser, J. Bacal, E. St-Hilaire, E. H. Lee, S. Tsao, J. Dorn, P. Maddox, M. Lisby, P. Pasero and A. Verreault (2012). Histone H3 lysine 56 acetylation and the response to DNA replication fork damage. Mol. Cell. Biol. 32 (1): 154-172.

Wurtele, H., S. Tsao, G. Lépine, A. Mullick, J. Tremblay, P. Drogaris, E. H. Lee, P. Thibault, A. Verreault and M. Raymond (2010). Modulation of histone H3 lysine 56 acetylation as an antifungal therapeutic strategy. Nat. Med. 16 (7): 774-780.