ISSN: 1680-5593

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Molecular Characterization and Expression Pattern of a Novel Cadmium/Zinc-Transporting *ATPase HMA1* Gene

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Abstract: Cadmium (Cd) of tobacco is a pollutant that is extremely toxic to the health of humans. Cadmium/zinc-transporting ATPase HMA1 gene has been characterized to function in the plant cadmium/zinc-transporting. The complete coding sequence of tobacco cadmium/zinc-transporting ATPase HMA1 gene was amplified by RT-PCR. The open reading frame of tobacco cadmium/zinc-transporting ATPase HMA1 gene was 2418 bp which encodes a protein of 805 amino acids. BLAST analysis revealed that tobacco cadmium/zinc-transporting ATPase HMA1 protein shares high homology with the cadmium/zinc-transporting ATPase HMA1 of potato (85%), Lycopersicon esculentum (84%), wine grape (71%), sweet orange (71%), soybean (69%) and thale cress (68%). Results also showed that tobacco cadmium/zinc-transporting ATPase HMA1 gene has a closer genetic relationship with the cadmium/zinc-transporting ATPase HMA1 gene of Lycopersicon esculentum. Prediction of transmembrane helices showed that tobacco cadmium/zinc-transporting ATPase HMA1 might be a transmembrane protein. The expression profile was studied and results indicated that tobacco cadmium/zinc-transporting ATPase HMA1 gene was moderately expressed in root, leaf and stem but hardly expressed in flower. These results established the primary foundation of utilizing tobacco cadmium/zinc-transporting ATPase HMA1 gene to descrease the cadmium content of tobacco and benefit the health of humans in the future.

Key words: Tobacco, gene, cadmium/zinc-transporting ATPase HMA1, expression pattern, established

INTRODUCTION

Cadmium (Cd) of tobacco is a pollutant that is extremely toxic to the health of humans. Cd has caused neurotoxicologic and behavioral changes in both humans and experimental animal studies (Liu et al., 2013; Counter et al., 2009). Cd exposure may be implicated in some neurological disorders including hyperactivity and increased aggressiveness in human (Liu et al., 2013; Maes et al., 2010). In the case of coronary risk with metal levels, Cd may be more important for females (Liu et al., 2013; Olsen et al., 2012). Cd was reported to damage bone microstructure and can negatively influence growth in newborns (Liu et al., 2013; Chen et al., 2011). Several studies have reported an inverse relationship between anthropometric measurements of the newborn and the placental or umbilical cord Cd level (Liu et al., 2013; Llanos and Ronco, 2009; Ronco et al., 2009). Cd exposure exerts inhibitory effects on testicular steroidogenesis (Liu et al., 2013; Pillai et al., 2012).

Cadmium/zinc-transporting ATPase HMA1 is a member of P (IB)-ATPase family that is localized to the chloroplast envelope and is involved in the plant transport of cadmium, zinc, copper and cobalt (Moreno *et al.*, 2008; Kim *et al.*, 2009; Higuchi *et al.*,

2009). It is essential for growth under high light conditions (Seigneurin-Berny et al., 2006). Cadmium/zinc-transporting ATPase HMA1 gene has been identified from many plants such as thale cress, tomato and potato. Until today, the tobacco cadmium/zinc-transporting ATPase HMA1 gene has not been reported yet. In present experiment, researchers will isolate the coding sequence of this tobacco gene, subsequently perform some necessary sequence analysis and tissue expression analysis for this gene. These will establish the primary foundation of utilizing tobacco cadmium/zinc-transporting ATPase HMA1 gene to descrease the cadmium content of tobacco and benefit the health of humans in the future.

MATERIALS AND METHODS

Samples collection, RNA extraction and first-strand cDNA synthesis: The tissues including leave, stem, root, flower from tobacco plants (Chinese local variety Yunyan 87) in the stage of anthesis were harvested and immediately frozen in liquid nitrogen and stored at -80°C. Total RNA extraction and first-strand cDNA synthesis for these tissue samples were performed as the methods describe by Liu (2009).

Isolation of the coding sequence: RT-PCR was performed to amplify the coding sequence of tobacco cadmium/zinc-transporting ATP ase HMA1 gene using the cDNA obtained from the pooled tissues above. The 20 µL reaction system was: 2.0 µL cDNA, 2.0 µL 2 mM mixed dNTPs, 2.0 μ L 10×Taq DNA polymerase buffer, 1.2 μ L 25 mM MgCl₂, 1.0 μL 10 mM forward primer, 1.0 μL 10 mM reverse primer, 2.0 units of Taq DNA polymerase (1U/1 μL) and 9.8 μL sterile water. The primers for tobacco cadmium/zinc-transporting ATPase HMA1 gene isolation were designed based on the tobacco EST sequences (GeneBank numbers FG145467 and AM812255) which are highly homologues with the coding sequence of cadmium/zinc-transporting ATPase HMA1 gene of Lycopersicon esculentum (Table 1). The PCR program initially started with a 94°C denaturation for 4 min followed by 35 cycles of 94°C/50 sec, 55°C (Table 1) 50 sec, 72°C/1 min then 72°C extension for 10 min, finally 4°C to terminate the reaction.

Quantitative Real Time PCR (qRT-PCR) for tissue expression profile analysis: qRT-PCR for evaluating the level of mRNA for cadmium/zinc-transporting ATPase HMA1 gene was performed by the ABI Prism 7300 Sequence Detection Systems (Applied Biosystems, Foster City, CA, USA). The 25 µL reaction volume of PCR reaction contained 1 µL SYBR Green real-time PCR Master Mix, 100 ng cDNA template and 200 nM each primer. Conditions for real-time PCR were: an initial denaturation at 95°C for 3 min, 40 cycles of 95°C for 15 sec, optimal annealing temperature for each specific primer for 15 sec (Table 2), 72°C for 20 sec. The gene relative expression levels were quantified relative to the expression of the reference gene, actin (GenBank Accession No. GQ339768) by employing the $2^{-\Delta \Delta C}$ value model (Livak and Schmittgen, 2001).

Sequence analysis: mRNA sequence prediction was conducted using GenScan Software (http://genes.mit.edu/GENSCAN.html). Protein conserved domain analysis was carried out by conserved domain architecture retrieval tool of BLAST at the National Center for Biotechnology Information (NCBI) server (http://www.ncbi.nlm.nih.gov/BLAST). Theoretical isoelectric point (pI) and Molecular weight (Mw) of the deduced

protein were computed using the Compute pI/Mw Tool (http://www.expasy.org/tools/pi_tool.html). Protein alignment analysis and phylogenetic tree analysis were performed with Clustalw Software (http://www.ebi.ac.uk/clustalw).

RESULTS AND DISCUSSION

Isolation result for tobacco cadmium/zinc-transporting *ATPase HMA1* **gene:** For tobacco cadmium/zinc-transporting *ATPase HMA1* gene, through RT-PCR with pooled tissue cDNAs, the resulting PCR products were 2418 bp (Fig. 1).

Sequence analysis: BLAST analysis of this cDNA sequence revealed that this gene was not homologous to any of the known tobacco gene and it was then deposited into the Genbank database (Accession No.: KJ159917).

The sequence prediction was carried out using the GenScan Software and results showed that the 2418 bp cDNA sequence represents one single gene which

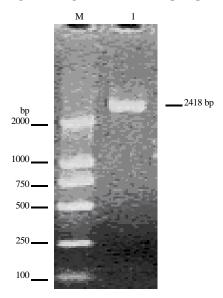


Fig. 1: PCR result for tobacco cadmium/zinc-transporting ATPase HMA1 gene; M: DL2000 DNA markers; 1: PCR product for tobacco cadmium/zinc-transporting ATPase HMA1 gene

Table 1: PCR primers for tobacco cadmium/zinc-transporting ATPase HMA1 gene isolation

Genes	Primer sequence	Ta (°C)	Length (bp)
Cadmium/zinc-transporting ATPase HMA1	Forward: 5'-ATGGAAGCTCTGCGTCTT-3'	55	2418
	Reverse: 5-TCACAAATGGGCTGCTTG-3'		

Table 2: qRT-PCR primers for tobacco cadmium/zinc-transporting ATPase HMA1, actin genes and annealing temperatures			
Genes	Primer sequence	Ta (°C)	Length (bp)
Cadmium/zinc-transporting ATPase HMA1	Forward: 5'-CTGCGGCTTTGTTTATTG-3'	53	418
	Reverse: 5'-GAGCCAACTTCCAGGTCA-3'		
Actin	Forward: 5'-CCATTCTTCGTTTGGACCTT-3'	56	257
	Reverse: 5'-TTCTGGGCAACGGAACCT-3'		

encodes a protein of 805 amino acids (Fig. 2). The theoretical isoelectric point (pI) and Molecular weight (Mw) of this deduced protein were also computed. The pI is 8.27. The molecular weight of this putative protein is 87498.28 Da.

Further, BLAST analysis of this protein revealed that it shares high homology with the

cadmium/zinc-transporting ATPase HMA1 of potato (Accession No: XP_006339845, 85%), Lycopersicon esculentum (Accession No: XP_004231883, 84%), wine grape (Accession No: XP_002278549, 71%), sweet orange (Accession No: XP_006466544, 71%), soybean (Accession No: XP_003550994, 69%) and thale cress (Accession No: NP 195444, 68%) (Fig. 3).

agçt cifgcgt citticaacttcat icgcagggaicaattitticaaictac aaari agaacg agaaitaaaggcicaaccgcaa ĊŢĊĸŢŦĠŶĊŦŢĊĊŢŦĊŢĠŢŢĠĠĠŢĊŦĠĸĠŶŦĠĸĊŢŦĠĸŢŦĠŢĠĠŦŢĸĸŢĠĸĸŢŦĸĹŦĠŦĠŶĸĸŢĸĸŢĸĸŢŦĸŢŦĊŊŦĠ octoctocacettit ocateactititatogggaacatati ogaacgtogetigetiettett ocaatettaatti octocatatatac oactacticactactocacegotica ҉าาที่อะรู้าลรู่าารู้าอรุ๊าธลู้อาทุ๊ธลรุ๊ลามู้อลรู้ารผู้ลอรู้ารรู้ลอรู้ารลู้ารลู้อยู้วิธรู้ลอรู้ารรู้อารู้ลารู้าลปุ้ลาทุ้ธรุ่ ŧĊagtaccagtegactgtagagttttcccgaggtagaacaacaattactattgagcatttgacaggagaagaatcagacctttaggaag gaggggacagtattcctggaggtgcacgaagctggagcagtattgttgatagtgaggggggaggaggaggtgtggagaggtcacaga tgTgtTatTatCtcTagCagTtgCgtTtcTtgGtcCatTccTttTcaAgtGgcCttTcaTtaCaCaCcGCagCttGcaGagGatCa ccGagCatTggGcCtTcaTggTgGCtgCatCacCttGtgCgtTagCtgTgGCacCatTggCttAtgCaaCtaCcaCtgCaGCttGc aÁnagesgeatatigeettaMaggesgasAasttettegeettegettegettegatgtettagtatitatetatitejaatjtejaatjaategetajaat ŦĠĸĠŢŦŢĠĸĸĠŦĠĸĸĠŦŦĠŦĠŖĸĸĔŦŦŶĸĊĠŦĸĠŦĸĠĸĸĠĊĸŶĸŦŢŦĸĠĸĸŶĸĸŶĸĸŶĸĸĠĊŦŖŦĸĠĸĊŖĸĠĸĸĠĸĠĸĠĸĠĸĠĸ CÖGTGGAĂAGCTTŤTCŤTGGTGTGTGGAATĂCAŢCACTC ACTTTŢCCÁCTCTGAAGĂTGĂATGAĞAGAGAGTTAĂAGĂGGCAGT.
ACGTCGTCTTŤTGGAGGTGATTŤTGTCCÁCGTGCTCTCTCTGAGTTAĂTAĂTGAAÄAGGTAACTCŤTTŢCCÁCTŤTGAGATAGCTCCŤT TGCAĞTTČTAGAGGTGTTĞTACAGACACTGCAGATCÁCGTAATTCĞTGŤTAŤGTĀCTĀCTĀCAŢGAGGCGAGTCCACGTAGAGTĀCCŤ. CŢGTGGGTATCAAGAAGTTĀCTĀCAGCCŢAAAGCÇAGAGGATAĀACŢTTŢTCĀTGTĀCAAGCAŢTTCAAGGGGTAACAGCGGCCŢ ATGGTAGGTGĀTGĞCAŤCAĀTGĀTGĀTGCCCÇAGCACTTGCTGCAGCTACCGTTGGTATTTGTGCTGGGGAACGAGTTAGTGCGGGGCT ĠŢŦĠĊĠĠŖŦĠŢĸĊŢĠŦŢĠĊŢĸĊŖĸĠĸĸŶŦĸŢŦŦŶĊĠĠŦĠĴĊĊĠĸŢŦŦĞŦĠŢŦĠĊĠĸĸĸŦŶŦĊŢĊĠĸŖĸĸŶĸĸŶĸĸŶĸŦŶĊŦŶĠĸŶĊ ĸĄŢĠŖĠĠĸĊŢŦĠĊŦŦŢĠŦĊŦĸĠĊĸŢŦĊŢŦŦŢĠĠĊĊŦĊŦĊŢŦĸĹĸŦĹĸĠĬĸŦŢĠĠĠĸĠŢĊĊŢĊĊĜĸĊŢĠŦĠĠŦŢĠĸĊĠĠŢĊĊ aoğt oğcağoc†to†to†to†ttöto†cakttöto†to\$toöttoTaaktoötoöaa&atootoatoakoakookooko ĸŢŦĠŖĊĸĸĸŦŢĠĊĸŦŦĠŦĊŦĸĸŢŦĸŢĠŦŢĠŦŢĠĸŖĠĊĸŦĠĠŖĸĊĊŢĸĊŖŦĸġĊĸĊĊĸŢŦĊĸĸĠĊĸĠĊĊĸĸŦŦŢĠŦĸĸ

Fig. 2: The complete coding sequence of tobacco cadmium/zinc-transporting ATPase HMA1 gene and its encoding amino acids; *indicates the stop codon

Sweet orange	METTLLHSTIHVASFPSLTRSIRLKRVNSLKPTLSFTHPV
Thale cress	MEPATLTRSSSLTRFPYRRGLSTLRLARVNSFSILPPKTL
Soybean	MEAIPYSIPSTKLHSSLHIYTGVTRIRSLPLRPPPIS
Wine grape	METLPFRGVPSLSCARRRVNNSFEPFHHRTLFFYNLSLPKSLFPPLNFPL
Potato	MEALRLSTSFAGINCSIYKSTRRLKVNRNLLLSSLKPK
Lycopersicon esculentum	MEALRLSTSFSGINCSIYKSTRRLKVNRNLLFSSLKPI
Common tobacco	mealrlstsfaginfsiyktrrrikvnrnvllsslksk
Sweet orange	IRFNFKPLNYRPVNCLSHPHINHOHHHHHHHHHHHHHNDCS-
Thale cress	LROKPLRISASLNLPPRSIRLRAVEDHHHDHHHDDEODHHNHHHHHHOHG
Soybean	IKPLYSPNFLILHRHRLRCVAESSNNHHHHDHDHHHGHHHHHHHHHHHSHG
Wine grape	LPRHRALOFVAKAEDSHHHHHHPHNDHHHDDHHHHHGHGHGHHHHHHGSG
Potato	SSVPIRSSAOFRGRIHCSACSCSSHSHHHHHHDHSHDHHNHHHHHH
Lycopersicon esculentum	SSVPIRSSAOFRGRIHCSACSCSSHSHOHHHHHHHHHHHHHHHHHHHH
Common tobacco	PSISIRSSTOFRGRIHCAACNCGHSHHHHHHHDHEHGHDHHHH
	* * .* :
Sweet orange	OLSGPOKAVIKFAKATRWLDLANFLREHLOLCCCAAALFLAAA
Thale cress	CCSVELKAESKPOKMLFGFAKAIGWVRLANYLREHLHLCCSAAAMFLAAA
Soybean	ANLTGPOKAVIAFAKATRWMDLADILREHLHLCCFSTALFVAAA
Wine grape	STLSRTQESFLRIAKAIRWADLADFLRENLHLCCCSTGLFLAAA
Potato	GPDEGDGKLTKFQEVFLKFANAIRWTQLANYLRENLELCCCSAVLFIAAA
Lycopersicon esculentum	GHDEGDGKLTKFQEVFLKFANAIRWTQLANYLRENLELCCCSAVLFIAAA
Common tobacco	GHDEGDGKLTKSQELFLKFARAIRWTHLANILREHLELCCCSAALFIAAA
Sweet orange	ACPYLLPKPAIKPLQNAFLAVAFPLVGVSASLDALTDIAGGKVNIHVLMA
Thale cress	VCPYLAPEPYIKSLQNAFMIVGFPLVGVSASLDALMDIAGGKVNIHVLMA
Soybean	ICPHTLPKPLVKPLQNSLIFVAFPLVGVSASLDALIEISSGKVNIHVLMA
Wine grape	ACPYLIPKPAVKPLQNAFIFVAFPLVGVSASLDALIDITGGKVNIHVLMA
Potato	VCPYFLPKPAVLPLQRIFALIAFPLVGVSASLDALVDITGGKINIHVLMA
Lycopersicon esculentum	VCPYFLPGPAVLPLQRIFALIAFPLVGVSASLDALVDITGGKINIHVLMA
Common tobacco	ACPYFLPQPAVLPLQRVFTLIAFPLVGVSASLDALMDITGGKINIHVLMA
	: ** : .. : :.******** :*:.**:*****

Fig. 3: Continue

J. Anim. Vet. Adv., 13 (3): 159-164, 2014

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Soybean
Wine grape
Potato
  Lycopersicon esculentum
Common tobacco
  Sweet orange
Thale cress
Soybean
Wine grape
Potato
                                                                                                                      Lycopersicon esculentum
Common tobacco
Sweet orange
Thale oress
Soybean
Wine grape
Potato
Lycopersicon esculentum
Common tobacco
                                                                                                                       GTATITIEHLTGEVKPLEAKVGDRIPGGARNLDGRMILKATKTWNESTLN
                                                                                                                       GTATITIEHLTGEVKPLEAKVSURIFSGAKNLUVKLIKAIKIMLO2L
GSATITIEHLTGEVKPLEAKAGDRIPGGARNLDGRIIVKATKAMDSILM
GSATITIEHLTGEVKPLEAKVGDRIPGGSRNLDGRIIVEVMTWKESTLS
GRSTITIEHLTGEVKPVERTVGERIPGGARNLDGMLIVKAKKTWKESTLS
GRSTITIEHLTGEVKPLDKKEGDNIPGGARNLDGMLIVKAKKTWKESNLS
GRSTITIEHLTGEVKPLDKKEGDNIPGGARNLDGMLIVKAKKTWKESNLS
                                                                                                                       RIVQLTEEAQLNKPKLQRWLDEFGEQYSKVVVVLSLAIALIGPFLFKWSF
KIVQLTEEAHSNKPKLQRWLDEFGENYSKVVVVLSLAIAFLGPFLFKWPF
RIVQLTEEAQSNKPKLERWLDEFGERYSQVVVVLSIAIAVIGPFLFKWPF
RIVQLTEEAQLNKPKLQRWLDEFGDHYSKVVVVLSIAVAFIGELLFKWPF
Sweet orange
Thale cress
Soybean
Wine grape
Potato
                                                                                                                       RIVQLIEERQLSKPRLQRWLDKFGEQYSKAVVLLSLAVAFLGFFFKWFF
RIVQLIEERQLSKPRLQRWLDKFGEQYSKAVVLLSLAVAFLGFFFFKWFF
RIVQLIEERQLSKPRLQRWLDKFGEQYSKAVVLLSLAVAFLGFFFFKWFF
RIVQLIEERQLSKPKLQRWLDKFGEQYSKAVVLLSLAVAFLGFFFKWFF
  Lycopersicon esculentum
Common tobacco
                                                                                                                       IGTSVCRGSVYRALGLMVAASPCALAVAPLAYATAISSCARKGILLKGGQ
LSTAACRGSVYRALGLMVAASPCALAVAPLAYATAISSCARKGILLKGAQ
VSTSACRGSIYRALGLMVAASPCALAVAPLAYAIAISSCARKGILLKGGH
ISTSVCRGSVYRALGLMVAASPCALAVAPLAYATAISACARKGILLKGGH
FSTTACRGSIYRALGLMVAASPCALAVAPLAYATAISACAKRGILLKGGQ
FSTTACRGSIYRALGLMVAASPCALAVAPLAYATAISACAKRGILLKGGQ
ISTAACRGSVYRALGLMVAASPCALAVAPLAYATAISACAKRGILLKGGG
ISTAACRGSVYRALGLMVAASPCALAVAPLAYATAISACAKRGILLKGGE
Sweet orange
Thale cress
Soybean
Wine grape
Potato
   Lycopersicon esculentum
Common tobacco
                                                                                                                         VLDALASCHTIAFDKTGTLTTGGLMFKAIEPIYGHWIRSKKTHDISCCIF
                                                                                                                      VLDALASCHIJAFDKTGILITGGIMFKAIEPIYGHWIRSKKHDISCCIP
VLDALASCHIJAFDKTGILITGGLTKKAIEPIYGHQF-GTNSSVITCCIP
VLDALASCHIJAFDKTGILITGGLTKAIEPIYGHVWNNESNVPSCCIP
VLDALASCHIJAFDKTGILITGGETKAIEPIYGHGVAYRSKFVSCCIP
VLDALASCHSJAFDKTGILITGEFMCKAIEPIHGHAKSVGKG-FASCCNP
VLDALASCHSJAFDKTGILITGEFMCKAIEPIHGHAKSVGKG-FASCCNP
VLDALASCHSJAFDKTGILITGEFMCKAIEPIHGHAKSVGKG-FASCCNP
VLDALASCHSJAFDKTGILITGEFMCKAIEPIHGHGKRIAS-----CCVP
Soybean
Wine grape
Potato
Lycopersicon esculentum
Common tobacco
                                                                                                                      NCEKEALAVAAAMEKGTTHPIGRAVVDHSIGKDLPSVSIDRFEYFPGRGL
NCEKEALAVAAAMEKGTTHPIGRAVVDHSVGKDLPSIEVESFEYFPGRGL
TCEKEALAVASAMEKGTTHPIGRAVVDHSGEKDLPSVSVESFEYFPGRGL
SCEIEALAVAAAMERGTTHPIGRAVVDHCVGKDLPPVAVENFESLPGRGL
SCEKEALAVAAAMERGTTHPIGRAVVDHSAGKDLPSISVESFENLPGRGI
SCEKEALAVAAAMERGTTHPIGRAVVDHSTGKDLPSISVESFENLPGRGI
SCEKEALAVAAAMERGTTHPIGRAVVDHSTGKDLPSISVESFENLPGRGI
...
  Lycopersicon esculentum
Common tobacco
                                                                                                                       TATVNG IESGTEGGKELKASLGSVDFITSLCKSEDESRKIKEAVNASSYG
TATVNGVKTVAEESRLRKASLGSIEFITSLFKSEDESKQIKDAVNASSYG
 Sweet orange
Thale cress
Thale cress
Soybean
Wine grape
Potato
Lycopersicon esculentum
Common tobacco
                                                                                                                      TATVNIGVKTVABESRLRKASLGSIEFITSLFKSEDESKQIKDAVNASSYG
TATVNSIESGTGGAKLLKASLGSIDFITSLCQSEDESKIKEANNISSYG
SATLISIESGIGGGELLKASIGSLEYILSLCKSEDELKKIKEAMSTSSYG
IATLSSFEPRLGGGKPWKAFLGSVEYITSLCDSEDESRVEEAVNISSHG
IATLSSFEPRLGGGKPWKAFLGSVEYITSLCDSEDESRVEEAVSTSSHG
FATISSFEPRLGGGKPWKAFLGSVEYITSLFBEDESRRVEAVSTSSHG
FATISSFEPRLGGGKPWKAFLGSVEYITSLFBEDESRRVKEAVSTSSHG
**:.... **:*:::* ** ...** .:::::::::.**
                                                                                                                      RGFVHAALSVN-EKVTLIHLEDRPRPGVSDVIAELKDHARLRVMMLTGDH
KDFVHAALSVD-QKVTLIHLEDQPRPGVSGVIAELKSWARLRVMMLTGDH
SEVVHAALSVN-QKVTLIHLEDRPRPGVVDVIQELQDEAKRVMMLTGDH
SDFVHAALSVN-KKVTLIHEDERRPGVLDVILALQDQAKLRVMMLTGDH
VDFVHAALSVNNQKVTLFHFEDKPRPGVLDVVOTLQNQAKLRVIMLTGDH
VDFVFAALSVNNQKVTLFHFEDKPRPGVLDVIQTLQNQAKLRVIMLTGDH
GDFVHAALSVNNQKVTLFHFEDKPRPGVLDVIQTLQNQAKLRVIMLTGDH
GDFVHAALSVNNQKVTLFHFEIASTCSSRRCTDIADQLTRVMYCDMRRVH
  Sweet orange
Thale cress
Soybean
Wine grape
Potato
  Lycopersicon esculentum
Common tobacco
                                                                                                                      ESSAQRVANAVGINEVYCSLKPEDKLNHVKSTSRDMGGGLIMVGEGINDA
DSSAMRVANAVGITEVYCNLKPEDKLNHVKNIAREAGGGLIMVGEGINDA
DSSAMRVANAVGITEVYCNLKPEDKLSHVKDISRDMGGLIMVGEGINDA
ESSARRVANSOVGINEFHCNLKPEDKLSHVKDISRDMGGLIMVGDGINDA
ESSAMRVANAVGIKEVYCSLKPEDKLSHVTSISRDGGGLIMVGDGINDA
EASARRVANTVGIKEVNCSLKPEDKLSHVTSISRDTGG-LIMVGDGINDA
KASAKRVAKTVGIKEVNCSLKPEDKLSHVTSISRDTGG-LIMVGDGINDA
VELLKLWVSRS----YYSLKPEDKLSHVTSISRDTGG-LIMVGDGINDA
 Sweet orange
Thale cress
  Wine grape
Potato
  Potato
Lycopersicon esculentum
Common tobacco
                                                                                                                       PALAAATVGIVLAQRASATAIAVADVLLLRNNISGVPFCVAKSRQTTSL
                                                                                                                       PALAAATVGIVLAQRASAIAIAVADVLLLRNNISGVPTCVARSRQITSLV
PALAAATVGIVLAQRASATAIAVADVLLLRESISAVPTCIAKSRQITSLV
PALAAATVGIVLAHRASATAIAVADVLLRESISAVPTCIAKSRQITSLV
PALAAATVGIVLAERASAATAVADVLLLDDNISGVPTCVAKSRQITSLV
PALAAATVGIVLAERASAAATAVADVLLLQDNISGVPTCVAKSRQITSLI
PALAAATVGIVLAERASAAATAVADVLLLQDNISGVPTCVAKSRQITSLI
PALAAATVGIVLAERASAAATAVADVLLLQDNISGVPTCVAKSRQITSLI
PALAAATVGIVLAERASAAATAVADVLLLQDNISGVPTCVAKSRQITSLI
  Soybean
Wine grape
Potato
  Lycopersicon esculentum
Common tobacco
                                                                                                                      KQNVALALSCIILASLPSVLGFLPLWLTVLLHEGGTLVVCLNSVRALNDP

KQNVALALTSIFLAALPSVLGFVPLWLTVLLHEGGTLLVCLNSVRGINDP

KQNVALALTSILMASLPSVLGFLPLWLTVLLHEGGTLLVCLNSVRALNEP

KQNVALALSCILLASLPSVLGFLPLWLTVLLHEGGTLLVCLNSVRALNEP

KQNVVLALCSIILASLTSVMGFLPLWLTVLLHEGGTLLVCLNSVRALNEP

KQNVVLALCSIILASLTSVMGFLPLWLTVLLHEGGTLLVCLNSVRALNEP

KQNVVLALCSIILASLTSVLGVLPLWLTVLLHEGGTLLVCLNSVRALNEP

KQNVALALSSILLASLTSVLGVLPLWLTVLLHEGGTLLVCLNSVRALNEP
  Sweet orange
Thale cress
  Soybean
Wine grape
Potato
  Lycopersicon esculentum
Common tobacco
                                                                                                                       SWSWRQDIGHLINGFKSKHSVLQKKDARSNIMPAASL
SWSWKQDIVHLINKLRSQEPISSSSNSLSSAH----
SWSWKHDIFHLISEIKSRLLSLKINIITGSNSIITINL
TWSWKQDLVPVVDKFKSTIMFLRRHTITSSSTRAAPL
TWSWREDISQIIDRMRSLVMFLRHGTLBSTIQAAHL—
TWSWREDISQIIDRLRSLIMFLRHGTLBSTIQAAHL—
TWSWREDVSQMIDKLHSLIMFLRHGTLPSTIQAAHL—
TWSWREDVSQMIDKLHSLIMFLRHGTLPSTIQAAHL—
****:
  Common tobacco
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Fig. 3: The alignment of cadmium/zinc-transporting ATPase HMA1 proteins

From the results obtained above, it can be concluded that this protein is the tobacco cadmium/zinc-transporting ATPase HMA1 and the new gene is the tobacco

cadmium/zinc-transporting ATPase HMA1 gene. Based on the results of the alignment of different species of cadmium/zinc-transporting ATPase HMA1 proteins, a

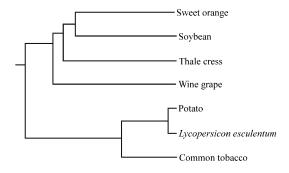


Fig. 4: The phylogenetic tree for seven kinds of cadmium/zinc-transporting ATPase HMA1 genes

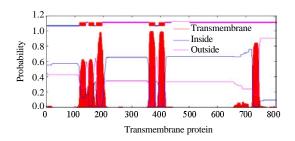


Fig. 5: The transmembrane protein prediction of tobacco cadmium/zinc-transporting ATPase HMA1

phylogenetic tree was constructed using the ClustalW Software as shown in Fig. 4. Phylogenetic analysis revealed that the tobacco cadmium/zinc-transporting ATP ase HMA1 gene has a closer genetic relationship with that of Lycopersicon esculentum.

The prediction of transmembrane helices in protein using the TMHMM Server v. 2.0 (http://www.cbs.dtu.dk/services/TMHMM/) showed that tobacco cadmium/zinc-transporting ATPase HMA1 might be a transmembrane protein (Fig. 5).

Tissue expression profile: Tissue expression profile analysis was carried out and results revealed that the tobacco cadmium/zinc-transporting *ATPase HMA1* gene was moderately expressed in root, leaf and stem but hardly expressed in flower (Fig. 6).

Modern comparative genomics research has revealed that virtually all (99%) of the protein-coding genes of humans share high homology with the that of mouse for human and mouse both belong to mammalian (Hardison, 2003; Liu, 2009). This extensive conservation in protein-coding regions implied that this conservation of protein-coding sequences may be expected in tobacco and other plants of solanaceae. From the sequence analysis of cadmium/zinc-transporting ATPase HMA1 genes, it can be seen that the coding sequences of cadmium/zinc-transporting ATPase HMA1 genes were highly conserved in three solanaceae plants-tobacco, potato and Lycopersicon esculentum.

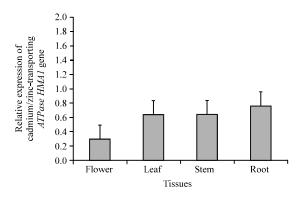


Fig. 6: Expression analysis of cadmium/zinc-transporting ATPase HMA1 gene mRNA in various tobacco tissues

The phylogenetic tree analysis revealed that the tobacco cadmium/zinc-transporting ATPase HMA1 gene has a closer genetic relationship with that of Lycopersicon esculentum. This implied that researchers can use lycopersicon esculentum as model organism to study the tobacco cadmium/zinc-transporting ATPase HMA1 gene or use tobacco as model organism to study the Lycopersicon esculentum cadmium/zinc-transporting ATPase HMA1 gene.

From the tissue distribution analysis in the experiment it can be seen that cadmium/zinc-transporting ATP ase HMA1 gene was moderately expressed in root, leaf and stem and hardly expressed in flower. For cadmium/zinc-transporting ATP ase HMA1 functions in the transport of cadmium, zinc, copper and cobalt (Moreno et al., 2008; Kim et al., 2009; Higuchi et al., 2009), the suitable explanation for this is that the transport process of cadmium, zinc, copper and cobalt is mainly existed in root, leaf and stem in the stage of anthesis. These merit further study.

CONCLUSION

Researchers first isolated the tobacco cadmium/zinc-transporting ATP ase HMA1 gene. These will establish the primary foundation of utilizing tobacco cadmium/zinc-transporting ATP ase HMA1 gene to descrease the cadmium content of tobacco and benefit the health of humans in the future.

ACKNOWLEDGEMENTS

This research was supported by grants from the Science and Technology Development Plan of Yunnan Provincial Tobacco Monopoly Administration (Corporation) (2013YN05).

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