African Journal of Biotechnology Vol. 11(29), pp. 7417-7423, 10 April, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.3494
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Cloning and characterization of functional keratin-associated protein 5-4 gene in maize

Lin Yang, Feng-Ling Fu, Long-Qun Deng, Shu-Feng Zhou, Tai-Ming Yong and Wan-Chen Li*

Maize Research Institute, Sichuan Agricultural University, Chengdu, Sichuan 611130, P.R. China.

Accepted 2 March, 2012

Keratin-associated proteins (KAPs) 5-4 which belongs to keratin-associated protein (KRTAP) type 5 family has two major groups: high/ultrahigh cysteine (HS) and high glycine-tyrosine (HGT). Based on bioinformatic prediction, we experimentally cloned a fragment containing an open reading frame of 1849 bp from maize, which encodes a protein of 408 amino acids. BLAST analysis indicated that KAP5-4 is homologous to the *qPE9-1* protein in rice. Conserved domains analysis predicted the presence of five domains. Real time reverse transcriptase polymerase chain reaction (RT-PCR) indicated that the expression of this gene is up-regulated in response to water-deficit stress in the root and leaf.

Key words: Maize, keratin-associated protein 5-4, water-deficit stress.

INTRODUCTION

In the hair cortex, hair keratins intermediate filaments are embedded in an interfilamentous matrix, which consists of hair keratin-associated proteins (KATAP, usually abbreviated as KAP) (Powell et al., 1991; Wu et al., 2008). KAP are considered to have originated independently, and to be essential for the formation of rigid hair shafts and resistant to them through their extensive disulfide bond cross-linking with the abundant cysteine residues of hair keratins or hydrophobic interactions with keratins (Rogers et al., 2006; Powell et al., 1991, 1995, Powell and Rogers, 1997). KAP were encoded by multigene families, which can be divided into two major groups: high/ ultrahigh cysteine (HS) and high glycine-tyrosine (HGT), and can be further grouped into 27 subfamilies, termed KAP1 to KAP27, based on phylogeny (Rogers et al., 2006, 2007). KAP 5-4 belongs to KAP type 5 family. Gene families in which duplications, rate variation and pseudogenization occur frequently are likely involved in functional innovation and adaptation (Hughes, 1999). Meanwhile, keratin-associated protein (KRTAP) participated in stability against stress (Hesse et al., 2004).

Maize is one of the most important foodstuff plants in the world; water-deficit stress poses serious threats to its production (Jiang et al., 2009; Lai et al., 2010; Zhuang et al., 2007). There were some researches on the gene expression analysis of maize, on how the reproductive organs respond to water-deficit stress conditions. Comparative analysis of a number of studies in drought-stressed maize (*Zea mays* L.) reporting quantitative trait loci (QTLs) for abscisic acid concentration, root characteristics, other morpho-physiological traits (MPTs) and grain yield (GY) reveals their complex genetic basis and the influence of the genetic background and the environment on QTL effects (Tuberosa et al., 2002). However, delineating a QTL to a single gene using genetic approaches is time-consuming and technically demanding (Fridman et al., 2000, 2004). As an alternative, microarray technology is a tool for analyzing genome-wide gene expression (Schena et al., 1995). Under water-deficit stress, placenta/pedicel and endosperm differed considerably in their transcriptional responses at nine days after pollination (DAP). Of the stress-response genes, 89% were upregulated in placenta/pedicel and 82% were downregulated in endosperm (Yu and Setter, 2003). Zhuang et al. (2007, 2008) used oligo microarray to examine 57452 transcripts expression at one and seven days after water-deficit

*Corresponding author. E-mail: aumdyms@sicau.edu.cn. Tel: 86-835-2882526. Fax: +86-835-2882154.

Abbreviations: KAP, Keratin-associated protein; **KRTAP,** keratin-associated protein; **HS,** high/ultrahigh cysteine; **HGT,** high glycine-tyrosine.

stress, respectively. Most of these transcripts had been previously reported to be associated with water stress. Zinselmeier et al. (2002) used oligonucleotide microarray containing 1,502 genes to examine genes expression at four days after silking and eight days after pollination in maize ear and kernel in response to water-deficit stress. This revealed 17 genes (10 upregulated, seven downregulated) that were affected by stress among these tissues and demonstrated that gene expression in the pedicel was more responsive than that in other tissues. However, the microarray technology measuring gene expression was Einsatz gewaltiger Mittel or was limited by the after points of process-time. Consequently, using the functional sequence of other species BLAST in the maize databases, understanding maize response to water-deficit stress requires a comprehensive evaluation of stress-induced changes in gene expression and is expected to advance our insight into crop improvement, and then, the successful use of *KAP5-4* genes encourages us to transform and overexpress endogenous *KAP5-4* genes in maize.

Here, we reported the cloning and characterization of a functional *KAP5-4* gene from maize, and its expression profile under drought stress. It is expected that the information obtained in such a study will help in the development of approaches to manipulate the genes to increase tolerance and improve yield of the maize crop.

MATERIALS AND METHODS

Plant materials, growth conditions, stress treatments and first strand synthesis

The seeds of maize inbred line 18-599 were sterilized and germinated in vermiculite. At the two leaf stage, the seedlings were transplanted into a plastic mesh grid for aquaculture and grown hydroponically at 28°C for a photoperiod of 12-h light/12-h dark (illumination of 20000 lux), with relative humidity of 60 to 80% and modest aeration. The nutrient solution was replaced every three days. At the three leaf stage, identical seedlings were subjected to drought stress treatments. For drought stress treatment, 16% polyethyleneglycols (PEG) was added to the nutrient solution. At 0 (control), 1, 2, 4, 6, 8, 12, 24, 48 and 72 h after stress treatment, leaves and roots were separately sampled from three seedlings, and frozen in liquid nitrogen immediately. RNA was isolated using the Trizol reagent (INVTROGEN, USA) according to the user manual and reverse transcribed to cDNA using PrimeScript RT reagent Kit (TakaRa China).

Database searches and data acquisition

The amino acid sequence of the *KAP5-4* was found in uniprot database (www.uniprot.org). The maize gene, *KAP5-4* was used to analyze the gene structure using maizesequence (www.maizesequence.org). Multiple sequence alignment was conducted among the deduced protein sequence and the deposited functional *KAP5-4* protein sequences in NCBI protein database. Phylogenetic analysis of these sequences was carried out by uniprot database (www.uniprot.org) and DNAman software. The family domains and functional domains of the deduced protein were analyzed using uniprot database (www.uniprot.rog) and maizesequence (www.maizesequence.org).

Open reading frame cloning

Primers were designed based on the predicted maize *KAP5-4* gene using primer 5.0. The 1289 bp fragments of *KAP5-4* gene including the predicted active sites was amplified using the forward primer (5' CATCAGCCAGCCACCACTC 3') and the reverse primer (5' CGAAGCCAACAGGCATCATAA 3'), respectively. PCR amplification was conducted using PrimeSTAR HS DNA Polymerase (TakaRa China) with proof reading activity. The temperature cycle was 2 min at 95°C for one cycles, 10 s at 98°C, 90 s at 68°C for 35 cycles, and 5 min at 68°C. The amplified product was purified using Universal DNA Purification Kit (TIANGEN China), dATP was added in the tail of sequences using the TaKaRa Taq^{TM} (TakaRa China), cloned into pMD19-T vector (TakaRa China), and sequenced by ShangHai Majorbio Bio-pharm Technology Co., Ltd (China) and SinoGeno Max Co., Ltd (China). The resulted sequence was identified at DNAman software and NCBI website (http://www.ncbi.nlm.nih.gov).

Real time RT-PCR

For real-time RT-PCR, a pair of primers (KAPs5-4F and KAPs5-4R) was designed to amplify a 103 bp fragment of the maize *KAP5-4* ORF. The GAPDH gene (GAPDHF and GAPDHR) was used as housekeeping control for template standardization (Table 1). The detection of amplification rates was performed using SsoFast EvaGreen Supermix (contained 2×reaction buffer with dNTPs, Sso7d-fusion polymerase, $MgCl_2$, EvaGreen dye and stabilizers; Bio-Rad USA). Real-time PCR analysis was performed on a Bio-Rad iCycler iQ5 Real-time PCR Systems. The annealing temperature (60 and 59°C) was used in the primers of KAPs5-4 and GAPDH. Real-time RT-PCR amplification was replicated at least three times.

RESULTS

Cloning of maize KAPs5-4 gene

By searching uniprot database (www.uniprot.org), a protein sequence deduced from maize encoding gene sequence B6UAE5 was found to be named *KAP5-4* protein. With the specific primers designed based on the identified encoding gene sequence, a fragment of 1224 bp was amplified from the cDNA sample of maize inbred line B73 (Figure 1). The sequence of the fragment was the ORF of *KAP5-4*. This ORF sequence was registered at 'maizesequence' (www.maizesequence.org) database with accession number GRMZM2G172320.

Deduced amino acid sequence, the domains and phylogenetic relationship of maize keratin-associated protein 5-4

The polypeptide encoded by maize *KAP5-4* gene is 408 amino acids long. The amplified sequence has the highest homology (54.839%) with the protein of the *qPE9-1* gene in rice. There were 328 identical positions and 82 similar positions (Figure 2). Multiple alignment

Table 1. The primers of real-time PCR.

Primer	Sequences from 5' to 3'
KAPs5-4F	CCCACTAATACCGATAACGAAG
KAPs5-4R	CAATGGCAGGAACAGCAGA
GAPDHF	CCATCACTGCCACACAGAAAC
GAPDHR	AGGAACACGGAAGGACATACCAG

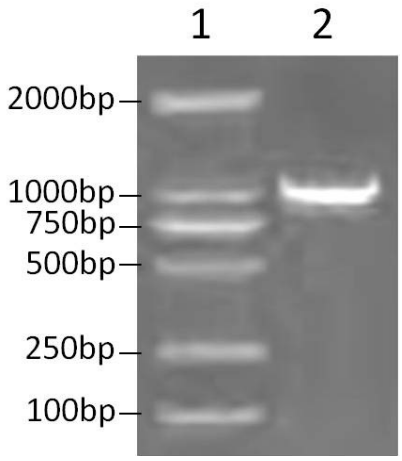

Figure 1. The fragment of the keratin-associated protein 5-4 protein in maize inbred line B73. 1, The band of the maker, 2, the band of the maize *KAP5-4* gene.

show that the variability of the amino acids sequences were 0.323, 0.275, 0.263, 0.260 and 0.384 in *Z. mays* (B6U8WT), *Z. mays* (B6UAE5), *Oryza sativa* subsp. Japonica (Q67UU9), *O. sativa* subsp. Indica (B8Y995) and *Homo sapiens* (Q6L8H1), respectively (Figure 3). These illustrated that the relation was close in the compared sequences. The domains of maize *KAP5-4* was forecasted by uniprot database (www.uniprot.rog) and maizesequence (www.maizesequence.org). There were five domains in the sequence of the *KAP5-4* (Table 2). These implied that maize *KAP5-4* has some functions which are associated with the domains.

Expression of maize keratin-associated 5-4 gene

In the root, the expression of gene *KAP5-4* gradually increased at 1 h and peaked at 24 h after water-deficit stress treatment, and then decreased, until a similar level at 72 h (Figure 4). In the leaf, the expression which was up-regulated gradually increased at 1 h and the first peaked at 4 h after water-deficit stress treatment, and then gradually decreased, until the second peak value was exhibited at 24 h after water-deficit stress treatment, until a lower level at 72 h (Figure 5). The expression in the root was higher than that in the leaf. These suggested that maize *KAP5-4* contributed to the response to water-deficit stress.

DISCUSSION

The length of fragment was same with the prediction online, and the amplified sequence was compatible with the searching result online. These suggested that the *KAP5-4* really exist in the genome of the maize. In the inbred B73, the 2.3-billion-base sequence (the largest genetic blueprint yet worked out for any plant species) includes more than 32,000 protein-coding genes spread across maize's 10 chromosomes (Palmer et al., 2003; Whitelaw et al., 2003; Schnable et al., 2009). The complex repetition and diversity of the maize genome make it a bigger challenge to explore the maize than other plants, and maize genomic survey sequence is available for use. This facilitates the discovery of functional genes.

In the five domains, the domain of the Von Willebrand Factor Type C (VWFC) emerged in the *qPE9-1* of the rice. The *qPE9-1* encoding keratin associated protein of 426 amino acid residues contained three VWFC domains (residues 99 to 153, 276 to 316 and 339 to 385), one transmembrane domain (residues 88 to 106), and one 4-disulfide-core domain (residues 153 to 166). An additional gene controlling grain size (*GS3*) has been identified in rice and also carries VWFC and trans-membrane domains (FAN et al., 2006), demonstrating the importance of these structures (Zhou et al., 2009; Huang et al., 2009). The domain of maize Prot_inh_BBI was same with the domain of the *Triticum aestivum wali* 6 protein, encoding a small protein that is related to the previously isolated *wali3* and *wali5* genes, whose molecular function were interrelated with water transport (Richards et al., 1994; Snowden and Gardner, 1993; Ji et al., 2010). Therefore, we forecasted that the maize *KAP5-4* gene had functional resemblance with the *wali6*, which is related to drought.

The results of the expression suggested that maize *KAP5-4* participated in the response to water-deficit stress. The originally upregulated expression of maize *KAP5-4* is likely to promote the content of analogous *wali* family genes, especially *wali6*, to induce stress signal transduction related genes (Snowden et al., 1993; Richards et al., 1994; Ji et al., 2010). The *wali6* was against aluminum stress in the root and induced expression (Richards et al., 1994; Snowden et al., 1993). These were why the expression of *KAP5-4* gene in the root was higher than that of leaf. The leaves and roots of maize seedlings had different expression profiles after PEG treatment and there was a lot of overlap between PEG- and drought-stress induced up-regulated transcripts (Zheng et al., 2004). In maize seedlings or tassels, the transcripts could be differentially expressed

```
ORYSI   1    MGEE-AVVMEAPRPKSPPRYPDLCGRRRMQLEVQILSREITFLKDELHFLEGAQPVSRSG   59
MAIZE   1    MGEEVAVVLEPPRPKSPPRYPDLCGRRRSQLELQMLNREIEFLKDELQLLEGVPPVSRS-   59
             **** ***:*.**************** ***:*:*.*** ******::***. *****

ORYSI   60   CIKEINEFVGTKHDPLIPTKRRRHRSCRLFRWIGSKLCICISCLCYCCKCSPKCKRPRCL   119
MAIZE   60   -CKEVIDFVGTKQDPLIPITKKTHRSCRLFWWIRSKLCICVPWFCCSCHCMPNCKRPCFL   118
               **: :*****:***** .:: ******* ** ******:. :* .*:* *:****  *

ORYSI   120  NCSCSSCCDEPCCKPNCSACCAGSCCSPDCCSCCKPNCSCCKTPSCCKPNCSCSCPSCSS   179
MAIZE   119  DCSCCSCPDLSCCYPSCKSCNK-PCFGPNSCSCCD--------ISCRKPDCPSCTSSCSS   169
             :***.** * .** *.*.:*   .* .*:.****.         ** **:*... .****

ORYSI   180  CCDTSCCKPSCTCFNIFSCFKSLYSCFKIPSCFKSQCNCSSPNCCTCTLPSCS--CKGCA   237
MAIZE   170  CCNRPCGNPDCS-----SCCTCNPSCCKPSCNSCCRPNCSSCCNLSCCKPSCSSCFSCCK   224
             **: .* :*.*:     ** ..  ** * ..   .: ****    :*  ****   . *

ORYSI   238  CPSCGCNGCGCPSCGCNGCGCPSCG--CNGCGLPSCG-CNGCGSCSCAQCKPDCGSCSTN   294
MAIZE   225  ANSSSCCKPKCSCFKALSCCKLQCSPNCCTCSLPSCSGCNPCGSC------KQCCSCPSD   278
             . *..*    *..  . .*   .*.  *  *.****. ** ****       :* **.::

ORYSI   295  CCSCKPSCNGCCGEQCCRCADCFSCSCPRCSSCFNIFKCSCAGCCSSLCKCPCTTQCFSC   354
MAIZE   279  CFDCKPNC-GCFGAQCCSCVRCCSCSCPRCSSCFGCFKY---FKCSNLFGCCSCKQCFKC   334
             * .***.* ** * *** *. * ***********. **      **.*  * . .***.*

ORYSI   355  QSSCCKRQPSCCKCQSSCCEGQ--PSCCEGHCCSLPKPSCPECSCGCVWSCKNCTEGCRC   412
MAIZE   335  QSSCCKGAPSCCKCQSSCCEGEDGSSSCWRSCCSVPKPDCPGCSCGCVWSCRKCTEGCRC   394
             ******  *************:  .*.*   ***:***.** *********::*******

ORYSI   413  PRCRNPCCLSGCLC  426
MAIZE   395  SGCRKPCCATGCLC  408
             . **:*** :****
```

Figure 2. The result of the comparison of the amino acid sequences of the keratin-associated protein in rice with the keratin-associated protein 5-4 in maize. ORYSI, Keratin associated protein (*O. sativa* subsp. Indica); MAIZE: keratin-associated protein 5-4 (*Z. mays*).

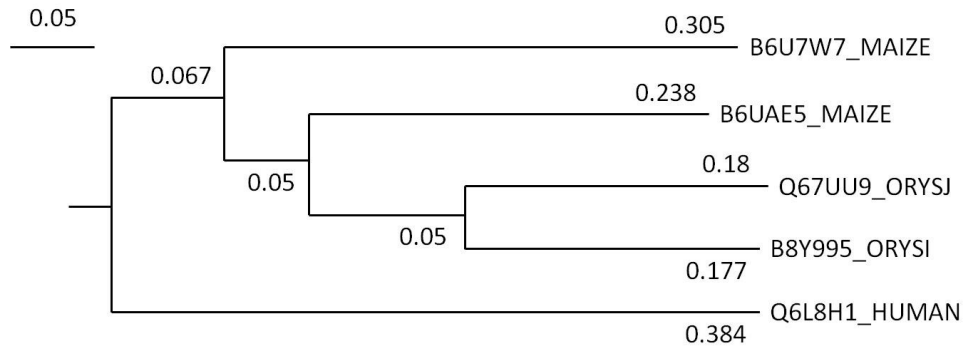

Figure 3. Neighbor-joining tree of the keratin-associated protein 5-4 sequences of other four species. B8Y995_ORYSI, Keratin associated protein (*O. sativa* subsp. Indica); Q67UU9_ORYSJ, DENSE PANICLE 1 (*O. sativa* subsp. Japonica); B6UAE5_MAIZE, keratin-associated protein 5-4 (*Z. mays*); B6U7W7_MAIZE, keratin-associated protein 5-4 (*Z. mays*); Q6L8H1_HUMAN: keratin-associated protein 5-4 (*H. sapiens*).

Table 2. The domains of the keratin-associated protein 5-4.

InterPro	Description	Beginning	Ending
IPR000020	Anaphylatoxin/fibulin	154	184
IPR018072	Conotoxin_a-typ_CS	170	180
IPR022353	Insulin_CS	299	313
IPR000877	Prot_inh_BBI	155	170
IPR001007	VWF_C	103	155

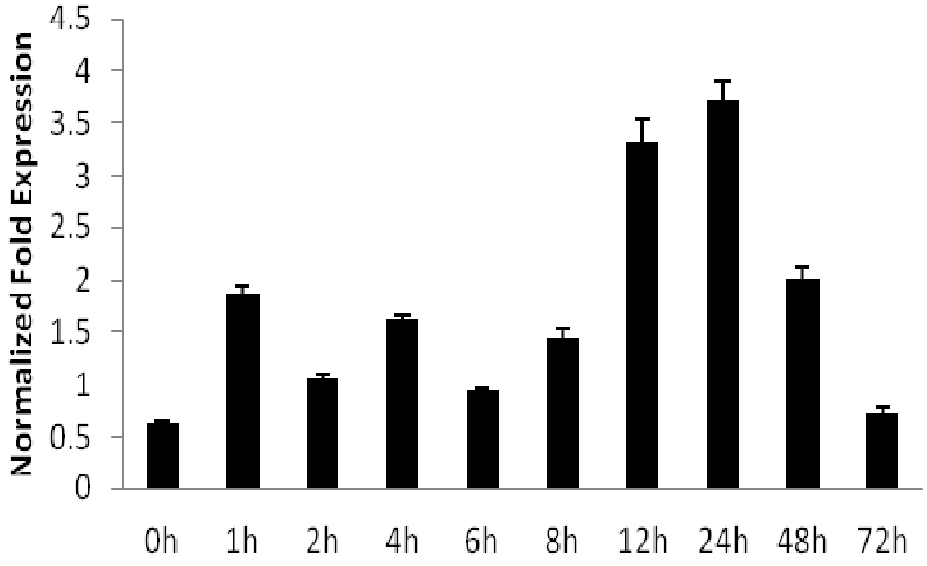

Figure 4. Normalized fold expression of the gene of keratin-associated protein 5-4 under water-deficit stress in root.

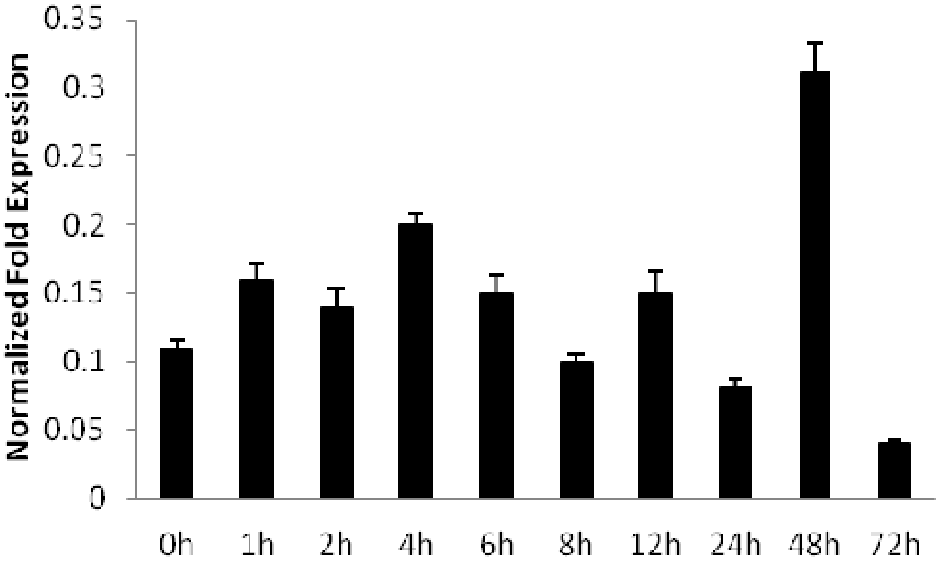

Figure 5. Normalized fold expression of the gene of keratin-associated protein 5-4 under water-deficit stress in leaf.

and upregulated under water- deficit stress for a long or short time (Jia et al., 2006; Zhuang et al., 2007, 2008). Therefore, we considered that the *KAP5-4* gene had response to drought stress, but the mechanism of signal transduction involving *KAP5-4* gene in maize remained to be explored. The maize *KAP5-4* gene has been identified, which have significantly increased expression and probably involved in water stress signaling pathway based on data analysis.

ACKNOWLEDGEMENTS

This work was supported by the Projects of Development Plan of the State Key Fundamental Research (973 Project) (2009CB118400) and the National Key Science and Technology Special Project (2008ZX08003-004 and 2009ZX08003-012B).

REFERENCES

Fridman E, Pleban T, Zamir D (2000). A recombination hotspot delimits a wild-species quantitative trait locus for tomato sugar content to 484 bp within an invertase gene. Proc. Natl. Acad. Sci. USA. 97(9): 4718-4723.

Fridman E, Carrari F, Liu YS, Fernie AR, Zamir D (2004). Zooming in on a quantitative trait for tomato yield using interspecific introgressions. Science. 305(5691): 1786-1789.

Fan CC, Xing YZ, Mao HL, Lu TT, Han B, Xu GC, Li XH, Zhang QF (2006). *GS3*, a major QTL for grain length and weight and minor QTL for grain width and thickness in rice, encodes a putative transmembrane protein. Theor. Appl. Genet. 112(6): 1164-1171.

Hesse M, Zimek A, Weber K, Magin TM (2004). Comprehensive analysis of keratin gene clusters in humans and rodents. Eur. J. Cell. Biol. 83(1): 19-26.

Huang XZ, Qian Q, Liu ZB, Sun HY, He SY, Luo D, Xia GM, Chu CC, Li JY , Fu XD (2009). Natural variation at the DEP1 locus enhances grain yield in rice. Nat. Genet. 41(4): 494-497.

Hughes AL (1999). Adaptive evolution of genes and genomes. Oxford University Press US.

Ji HF, Peng ZY, Bi YP, Shan L (2010). Recent Progress on the Genes Related to Aluminum Stress in Wheat. Chinese. Agric. Sci. Bull. 26(24): 10-19.

Jia J, Fu J, Zheng J, Zhou X, Huai J, Wang J, Wang M, Zhang Y, Chen X, Zhang J, Zhao J, Su Z, Lv Y, Wang G (2006). Annotation and expression profile analysis of 2073 full-length cDNAs from stress-induced maize (Zea mays L.) seedlings. Plant. J. 48(5): 710-727.

Jiang W, Fu FL, Zhang SZ, Wu L, Li WC (2009). Cloning and Characterization of Functional Trehalose-6-phosphate Synthase Gene in Maize. J. Plant. Biol. 53: 134-141.

Lai JS, Li RQ, Xu X, Jin WW, Xu ML, Zhao HN, Xiang ZK, Song WB, Ying K, Zhang M, Jiao YP, Ni PX, Zhang JG, Li D, Guo XS, Ye KX, Jiang M, Wang B, Zhang HS, Liang HQ, Zhang XQ, Wang SC, Chen SJ, Li JS, Fu Y, Springer NM, Yang HM, Wang J, Dai JR, Schnable PS, Wang J (2010). Genome-wide patterns of genetic variation among elite maize inbred lines. Nat genet 42(11): 1027-1030.

Palmer LE, Rabinowicz PD, O'Shaughnessy AL, Balija VS, Nascimento LU, Dike S, Bastide M, Martienssen RA, McCombie WR (2003). Maize Genome Sequencing by Methylation Filtration. Science. 302(5653): 2118-2120.

Powell BC, Nesci A, Rogers GE (1991). Regulation of keratin gene expression in hair follicle differentiation. Ann. NY. Acad. Sci. 642: 1-20.

Powell BC, Arthur J, Nesci A (1995). Characterization of a gene encoding a cysteine-rich keratin associated protein synthesized late in rabbit hair follicle differentiation. Differentiation. 58(3): 227-232.

Powell BC, Rogers GE (1997). The role of keratin proteins and their genes in the growth, structure and properties of hair. EXS. 78: 59-148.

Richards KD, Snowden KC, Cardner RC (1994). *Wali6* and *wali7* Genes Induced by Aluminum in Wheat *(Trificum aestivum* 1.) Roots. Plant. Physiol. 105(4): 1455-1456

Rogers MA, Langbein L, Praetzel-Wunder S, Winter H, Schweizer J (2006). Human hair keratin-associated proteins (KAPs). Int. Rev. Cytol. 251: 209-263.

Rogers MA, Winter H, Langbein L, Wollschlager A, Praetzel-WunderS, Jave-Suarez LF, Schweizer J (2007). Characterization of human KAP24.1, a cuticular hair keratin-associated protein with unusual amino-acid composition and repeat structure. J. Invest. Dermatol. 127(5): 1197-1204.

Schena M, Shalon D, Davis RW, Brown PO (1995). Quantitative monitoring of gene expression patterns with a complimentary DNA microarray. Science, 270(5235): 467-470.

Schnable PS, Ware D, Fulton RS, Stein JC, Wei FS, Pasternak S, Liang CZ, Zhang JW, Fulton L, Graves TA, Minx P, Reily DA, Laura Courtney L, Kruchowski SS, Tomlinson C, Strong C, Delehaunty K, Fronick C, Bill Courtney B, Rock SM, Belter E, Du FY, Kim K, Abbott RM, Cotton M, Levy A, Marchetto P, Ochoa K, Jackson SM, Gillam B, Chen WZ, Yan L, Higginbotham J, Cardenas M, Waligorski J, Applebaum E, Phelps L, Falcone J, Kanchi K, Thane T, Scimone A, Thane N, Henke J, Wang T, Ruppert J, Shah N, Rotter K, Hodges J, Ingenthron E, Cordes M, Kohlberg S, Sgro J, Delgado B, Mead K, Chinwalla A, Leonard S, Crouse K, Collura K, Kudrna D, Currie J, He R, Angelova A, Rajasekar S, Mueller T, Lomeli R, Scara G, Ko A, Delaney K, Wissotski M, Lopez G, Campos D, Braidotti M, Ashley E, Golser W, Kim H, Lee S, Lin J, Dujmic Z, Kim W, Talag J, Zuccolo A, Fan CZ, Sebastian A, Kramer M, Spiegel L, Nascimento L, Zutavern T, Miller B, Ambroise C, Muller S, Spooner W, Narechania A, Ren L, Wei S, Kumari S, Faga B, Levy MJ, McMahan L, Buren PV, Vaughn MM, Ying K, Yeh CT, Emrich SJ, Jia Y, Kalyanaraman A, Hsia AP, Barbazuk WB, Baucom RS, Brutnell TP, Carpita NC, Chaparro C, Chia JM, Deragon JM, Estill JC, Fu Y, Jeddeloh JA, Han YJ, Lee H, Li PH, Lisch DR, Liu SZ, Liu ZJ, Nagel DH, McCann MC, SanMiguel P, Myers AM, Nettleton D, Nguyen J, Penning BW, Ponnala L, Schneider KL, Schwartz DC, Sharma A, Soderlund C, Springer NM, Sun Q, Wang H, Waterman M, Westerman R, Wolfgruber TK, Yang LX, Yu Y, Zhang LF, Zhou SG, Zhu QH, Bennetzen JL, Dawe RK, Jiang JM, Jiang N, Presting GG, Wessler SR, Aluru S, Martienssen RA, Clifton SW, McCombie WR, Wing RA, Wilson RK (2009). The B73 maize genome: complexity, diversity, and dynamics. Science, 326(5956): 1112-1115.

Snowden KC, Gardner RC (1993). Five genes induced by aluminum in wheat (*Triticum aestivum L.*) roots. Plant. Physiol. 103(3): 855-861.

Whitelaw CA, Barbazuk WB, Pertea G, Chan AP, Cheung F, Lee Y, Zheng L, Heeringen S, Karamycheva S, Bennetzen JL, SanMiguel P, Lakey N, Bedell J, Yuan Y, Budiman MA, Resnick A, Van Aken S, Utterback T, Riedmuller S, Williams M, Feldblyum T, Schubert K, Beachy R, Fraser CM, Quackenbush J (2003). Enrichment of Gene-Coding sequences in Maize by genome filtration. Science, 302(5653): 2118-2120.

Tuberosa R, Salvi S, Sanguineti M, Landi P, Maccaferri M, Conti S (2002). Mapping QTLs regulating morphophysiological traits and yield: case studies, shortcomings and perspectives in droughtstressed maize. Ann. Bot. 89: 941-963.

Wu DD, Irwin DM, Zhang YP (2008). Molecular evolution of the keratin associated protein gene family in mammals, role in the evolution of mammalian hair. BMC Evol. Biol. 8: 241-256.

Yu LX, Setter TL (2003). Comparative transcriptional profiling of placenta and endosperm in developing maize kernels in response to water deficit. Plant. Physiol. 131(2): 568-582.

Zhuang YL, Ren GJ, Yue GD, Li ZX, Qu X, Hou GH, Zhu Y, Zhang JR (2007). Effects of water-deficit stress on the transcriptomes of developing immature ear and tassel in maize. Plant Cell. Pept. 26(12): 2137-2147.

Zhuang YL, Ren GJ, Zhu Y, Hou GH, Qu X, Li ZX, Yue GD, Zhuang JR (2008). Transcriptional profiles of immature ears and tassels in maize at early stage of water stress. Biol. Planta. 52(4): 754-758.

Zheng J, Zhao JF, Tao YZ, Wang JH, Liu YJ, Fu JJ, Jun Y, Gao P,

Zhang JP, Bai YF, Wang GY (2004). Isolation and analysis of water stress induced genes in maize seedlings by subtractive PCR and cDNA macroarray. Plant Mol. Biol. 55(6): 807-823.

Zhou Y, Zhu JY, Li ZY, Yi CD, Liu J, Zhang HG, Tang SZ, Gu MH, Liang GH (2009). Deletion in a quantitative trait gene *qPE9-1* associated with panicle erectness improves plant architecture during rice domestication. Genetics, 183(1): 315-324.

Zinselmeier C, Sun Y, Helentjaris T, Beatty M, Yang S, Smith H, Habben J (2002). The use of gene expression profiling to dissect the stress sensitivity of reproductive development in maize. Field Crop Res. 75: 111-121.