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Non-specific lipid transfer proteins in maize

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Abstract

Background: In plant, non-specific lipid transfer proteins (nsLTPs) are small, basic proteins that have been reported to be involved in numerous biological processes such as transfer of phospholipids, reproductive development, pathogen defence and abiotic stress response. To date, only a tiny fraction of plant nsLTPs have been functionally identified, and even fewer have been identified in maize [*Zea mays* (Zm)].

Results: In this study, we carried out a genome-wide analysis of nsLTP gene family in maize and identified 63 nsLTP genes, which can be divided into five types (1, 2, C, D and G). Similar intron/exon structural patterns were observed in the same type, strongly supporting their close evolutionary relationship. Gene duplication analysis indicated that both tandem and segmental duplication contribute to the diversification of this gene family. Additionally, the three-dimensional structures of representative nsLTPs were studied with homology modeling to understand their molecular functions. Gene ontology analysis was performed to obtain clues about biological function of the maize nsLTPs (ZmLTPs). The analyses of putative upstream regulatory elements showed both shared and distinct transcriptional regulation motifs of *ZmLTPs*, further indicating that ZmLTPs may play roles in diverse biological processes. The dynamic expression patterns of *ZmLTPs* family across the different developmental stages showed that several of them exhibit tissue-specific expression, indicative of their important roles in maize life cycle. Furthermore, we focused on the roles of maize nsLTPs in biotic and abiotic stress responses. Our analyses demonstrated that some *ZmLTPs* exhibited a delayed expression pattern after the infection of *Ustilago maydis* and differentially expressed under drought, salt and cold stresses, and these may be a great help for further studies to improve the stress resistance and tolerance in maize breeding.

Conclusions: Our results provide new insights into the phylogenetic relationships and characteristic functions of maize nsLTPs and will be useful in studies aimed at revealing the global regulatory network in maize development and stress responses, thereby contributing to the maize molecular breeding with enhanced quality traits.

Background

As its name implies, plant lipid transfer protein (LTPs) were termed because of their functions that transfer phospholipids and fatty acids between membranes *in vitro* [1]. They were also named non-specific LTPs (nsLTPs) due to the character of non-specific binding to different lipids. Plant nsLTPs are small, basic proteins, usually about 6.5 ~ 10.5 kDa in size, characterized by an eight cysteine motif (8CM) backbone with the general form C-Xn-C-Xn-CC-CXC-Xn-C-Xn-C [2]. Almost all nsLTPs carry an N-terminal signal peptide in their nascent polypeptides. Thus, these proteins are likely secreted to the cell exterior for functioning. Many nsLTPs also possess a sequence for the post-translational addition of a glycosylphosphatidylinositol

(GPI)-anchor, which attaches the protein to the exterior side of the plasma membrane [3]. The conserved features of plant nsLTPs include four defined disulfide bonds formed by eight Cys residues. Furthermore, the crystal structures of plant nLTPs were comprised of four or five alpha helices (α -helices), with a central hydrophobic cavity where the lipid binding takes place [4].

Based on the molecular weight (Mw) of the mature proteins, plant nsLTPs can be classified into two main types, nsLTP1 (9 kDa) and nsLTP2 (7 kDa) [5]. According to the sequence similarity, Boutrot et al. classified 49 out of the 52 rice *nsLTPs* and 45 out of the 49 Arabidopsis *nsLTPs* into nine types (I, II, III, IV, V, VI, VII, VIII, and Y) [2]. Recently, *nsLTPs* have been categorized into four major and several minor types by sequence similarity, intron position and spacing between the cysteine residues, as well as a potential glycosylphosphatidylinositol

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modification site within their encoded proteins [6]. *In vitro* studies showed that plant nsLTPs have the ability to facilitate the inter-membrane exchange and transfer of various amphiphilic molecules including phospholipids, glycolipids, steroids, acyl-CoAs and fatty acids. Several structures of plant nsLTPs have been resolved by X-ray and NMR spectroscopic techniques [4,7,8]. The typical fold of nsLTP1 is characterized by four to five α -helices connected by four disulfide bridges, partly wrapped by a long C-terminal segment. The overall structure delimits a large central hydrophobic cavity, where the alkyl moiety of lipids is inserted into. The backbone folds of nsLTP1 and nsLTP2 show structural similarities, however, drastic changes in their central hydrophobic cavity. Compared with these two earliest nsLTP types, the three-dimensional model of Arabidopsis DIR1 (AtDIR1) follows the general nsLTPs fold with five α -helices stabilized by four disulfide bonds around a central tunnel-shaped cavity. It was reported that most of the putative functions of nsLTPs are related to their ability to bind lipids in their hydrophobic cavity [9-11].

In the ensuing years, some members of nsLTPs have been functionally identified in plant species, including the involvement of cuticular waxes and cutin syntheses [12]. A recent study demonstrated that LTPg1, a Type G nsLTP from Arabidopsis, contribute either directly or indirectly to cuticular lipid accumulation [13]. In addition, nsLTPs are expressed in diverse organs and cells, including callus, germinating and maturing seeds, leaves, roots, stems, ovaries, anthers, and pollens [6,14]. The localization of nsLTP transcripts in anthers has been well reported in Arabidopsis and rice, and abundant Type III nsLTPs (also termed Type C nsLTPs) were expressed specifically in the anther tapetum, with levels peaking at the developmental stage of maximal pollen-wall exine synthesis [15]. A lipid transfer protein, OsC6, is widely distributed in anther tissues such as the tapetal cytoplasm, required for postmeiotic anther development in rice [16]. With respect to plant defense, nsLTPs are also recognized to be pathogenesis-related proteins and constitute the PR-14 family [17]. Some of them were demonstrated to share structural similarities with oomycetous elicitors and act as competitors with elicitors for specific receptors on membrane [18,19]. In addition, nsLTPs participate in long-distance signaling during pathogen defense [9]. Studies on purified nsLTPs confirmed their roles in abiotic stress tolerance [20]. Transcript levels of *nsLTPs* increased in response to drought, salt and cold stresses in many cases [21]. Stabilization of membranes, cuticle deposition and/or changes in cell wall organization have been claimed as their putative roles in the responses to these stress factors. In tobacco, nsLTP genes up-regulated during drought-induced cuticular wax deposition [22]. Overexpression of the pepper CALTP1 gene in Arabidopsis

increased its tolerance to NaCl and drought stresses at various vegetative growth stages [23]. Arabidopsis *LTP3* act as a target of MYB96 to be involved in plant tolerance to freezing and drought stresses [24].

In previous studies, only a small portion of *nsLTPs* from maize have been well characterized. These include, *MZm3-3*, which is expressed specifically in the tapetum during male gametogenesis [25]; *Zm-LTP* (*ZmLTP1.2*; GRMZM2G010868), which binds to calmodulin (CaM) in a Ca^{2+} -independent manner [26]; *ZmLTP3* (*ZmLTP1.1*; GRMZM2G126397), which is induced by mannitol, salt and SA treatments [27]; *BETL-9* (*ZmLTPd6*; GRMZM2G087413), which is transcribed in the outer surface of the developing endosperm [28]. As the genome of inbred line B73 has been sequenced completely, it is the time to initiate functional annotation and to perform more comprehensive analyses of maize nsLTP gene family. To date, genome-wide overview of the maize nsLTP gene family was yet to be reported. Therefore, as the first step to elucidate the functions of ZmLTPs, a genome-wide study for this gene family is necessary. In this study, we identified 63 genes encoding 77 putative nsLTPs in the maize genome that can be classified into five types. Detailed analyses including primary sequence, phylogenetic relationships, gene structure, chromosome location, gene duplication and divergence, three-dimensional structure, gene ontology, promoter and expression profilings were performed. Based on microarray, RNA-sequencing and real-time quantitative PCR (qRT-PCR), we analyzed the expression patterns of ZmLTP genes in different tissues and developmental stages, and took a further step towards understanding plant responses to biotic and abiotic stresses. Our analysis provides novel insights on ZmLTP gene family to support further functional research on nsLTP gene family in plants, particularly the proteins that may have important functions in response to biotic and abiotic stresses.

Results and discussion

Identification of nsLTP members in maize and sorghum

In order to identify the complete and non-redundant nsLTPs in maize, an accurate scan of the maize proteome was performed. Initially, 130 potential ZmLTPs were identified in the maize genome following the removal of those sequences with an incomplete 8CM domain. Then, each of the deduced protein sequences was manually assessed through the analysis of the cysteine residue patterns. Subsequently, 40 proline-rich or hybrid proline-rich proteins, which were characterized by a high proportion of proline, histidine and glycine residues in the sequence comprised between the signal peptide and the 8CM, were removed (Additional file 1: Table S1). Next, two α -amylase/trypsin inhibitors, three prolamin storage proteins and three 2S albumin storage proteins were also discarded. Additionally, five transcript forms

lacking the GPI-anchor signal peptide (GRMZM2G174680_P02, GRMZM2G083725_P02, GRMZM2G006047_P01, GRMZM2G005991_P02, GRMZM2G116167_P02) were not taken into consideration. As a result, 77 proteins were confirmed as maize nsLTPs which were encoded by 63 nsLTP genes (Additional file 2: Table S2). Additionally, the same approach was used to identify nsLTPs in sorghum, which resulted in the identification of 58 nsLTP genes (Additional file 3: Table S3 and Additional file 4: Table S4).

Sequence analysis and classification of putative maize nsLTPs

Previously, Edstam et al. have proposed that the plant nsLTPs can be divided into four major and several minor types according to sequence similarity, intron position and spacing between the cysteine residues [6]. In one of the major types, Type G, the transcripts encode a C-terminal signal sequence in addition to the N-terminal one, leading to a post-translational modification where a GPI-anchor is added to the protein. The classification of the identified type G ZmLTPs was based on the presence of a GPI modification site, as well. In the second round of classification, the remaining sequences were grouped according to the identity matrix calculated from the multiple sequence alignments. When compared with the classification proposed by Edstam et al., we found that 73 out of the 77 ZmLTPs could be classified into five types (1, 2, C, D and G). Among the nsLTPs that were present in four flowering plants (maize, sorghum, rice and Arabidopsis), the variation in the number of nsLTPs across types and species was detected (Additional file 5: Table S5). In type G, 26, 24, 27, and 29 nsLTPs were found in maize, sorghum, rice and Arabidopsis, respectively. Whereas, only 2 nsLTP genes were distributed to Type E in Arabidopsis. Noticeably, a similar number of nsLTP genes were found in Type 1 and Type 2. It is thus conceivable that this difference may be due to gene duplication and loss. The main characteristic of plant nsLTPs is the presence of eight cysteine residues in a highly conserved region (C-Xn-C-Xn-CC-Xn-CXC-Xn-C-Xn-C). In order to establish a specific 8CM consensus for each obtained nsLTP type, we conducted a multiple sequence alignment using the 8CMs from the 77 ZmLTPs (Additional file 6: Figure S1). The amino acid sequence alignment of the 8CMs of ZmLTPs reveals a variable number of inter-cysteine amino acid residues (Table 1).

In this study, all the characteristics of the 77 ZmLTPs were summarized in Additional file 2: Table S2. 96% of the nsLTP precursors were initially synthesized with a signal peptide of 16-46 amino acids. The putative subcellular targeting of the 77 ZmLTP pre-protein sequences was analyzed. As expected, most of the proteins are predicted to be secreted except for ZmLTPg21, which have

been predicted to be cytoplasm protein (Additional file 2: Table S2). At the pre-protein level, the ZmLTPd1 and ZmLTPd4 deduced proteins are identical, as are the ZmLTPd2 and ZmLTPd10 deduced proteins. After cleavage of their signal peptide, the ZmLTPg20.1 and ZmLTPg20.2 mature proteins are identical. Therefore, before potential post-translational modifications, the 63 ZmLTP genes encode 74 different mature proteins. 29 ZmLTPs were found to have one or more phosphorylation sites and most of the phosphorylated sites were located on serine residues at the C-terminal (Additional file 7: Table S6). To clearly understand the sequence characteristics of ZmLTPs, we further analyzed the pI (isoelectric point) values, Mw values, and CXC motifs of all available ZmLTPs. As shown in Additional file 2: Table S2, maize nsLTPs are small and their molecular masses usually range from 6,854 Da to 11,107 Da except for Type G. Type 1 and Type D nsLTPs were mostly 9 kDa proteins and Type 2 and Type C nsLTPs were 7 kDa proteins. The Mw value of Type G nsLTPs was much higher than that of other types due to the presence of supernumerary amino acid residues located at the C-terminal of the deduced mature proteins. Judging from the pI value, Type 1, 2, C and D nsLTPs are mostly alkaline proteins. The majority of Type G nsLTPs are weakly alkaline or acidic (Additional file 2: Table S2). The average molecular mass and the theoretical pI are 8,963 Da and 9.27 pI, respectively. As for the CXC motif, most residues at the X position in Type 1 nsLTPs are hydrophilic, while in Type 2, C, D and G, the X position is usually occupied by a hydrophobic residue (Additional file 6: Figure S1). These conserved hydrophobic or hydrophilic residues may play significant roles in the biological functions of ZmLTPs [29].

Phylogenetic analysis of the maize, sorghum, rice and Arabidopsis nsLTPs

To analyze the phylogenetic relationship of the nsLTPs among maize, sorghum, rice and Arabidopsis, 274 nsLTPs from these four species were analyzed (Additional file 8: Figure S2). We performed a multiple sequence alignment of the 8CM domain sequences from maize, sorghum, rice and Arabidopsis and then generated a phylogenetic tree by the neighbor-joining method. Previously, Edstam et al. has divided the plant nsLTPs into ten types [6]. On the basis of the comparison between the previous dataset and ours, the six groups classified here were in agreement with the Type 1, 2, C, D, E and G of nsLTPs. As shown in Additional file 8: Figure S2, members in Type 1 and 2 formed specific clades, indicating that the genes in these major nsLTP types share a common ancestor. The sequences from the minor Type C and E also formed separated clades. Since relatively high bootstrap values were observed in the internal branches of Type 1 and Type C, it clearly showed the derivation of statistically

Table 1 Some characteristics for the different types of non-specific lipid transfer proteins found in maize

Type	Number of members	GPI-anchored	Spacing pattern										
1	8	No	C	X _{9,10}	C	X _{14,15,17}	CC	X _{18,19}	CXC	X ₂₁₋₂₄	C	X ₁₃	C
2	9	No	C	X ₇	C	X _{12,13}	CC	X _{8,9}	CXC	X ₂₃	C	X ₆	C
C	2	No	C	X ₉	C	X _{14,19}	CC	X ₉	CXC	X ₁₂	C	X ₆	C
D	16	No	C	X _{9,10,13,14}	C	X _{11,12,14,16-18}	CC	X _{9,11,12}	CXC	X _{11,21-24,26}	C	X ₆₋₁₀	C
G	26	Yes	C	X _{5,6,9,10,12}	C	X _{8,13-18,21}	CC	X _{12-14,18}	CXC	X _{21,22,24-27,29,41}	C	X _{8-10,13,20}	C

Character "X" represents any amino acid, and the Arabic numeral following "X" stands for the numbers of amino acid esidues.

reliable pairs of possible homologous proteins sharing similar origin from a common ancestor. It was also worth noting that Type E nsLTPs seem to be monophyletic, and this may mean that the monocotyledon plants discarded these genes during the evolutionary divergence between monocots and dicots.

Intron-exon structure of the maize nsLTP gene family

As a kind of evolutionary relic, the intron-exon arrangement carries the imprint of the evolution of a gene family. Investigation of ZmLTP gene structures revealed low diverse distribution of intronic regions amid the exonic sequences, and 31 ZmLTP genes (36 Alternative splicing forms) were predicted to be interrupted by 1-2 introns positioned 4 to 67 bp downstream of the codon encoding the eighth cysteine in the 8CM (Additional file 9: Figure S3). Alternative splicing forms of the same gene usually had similar intron-exon structures, indicating that the distinct proteins from a single transcript may share similar functions. Additionally, it is interesting to find a similar exon/intron pattern in each group. For instance, the nsLTP genes in Type 1, 2 and C lacked an intron, while all members in Type G contained 1-3 introns. Except for *ZmLTPd6*, *ZmLTPd13*, *ZmLTPd14* and *ZmLTPd15*, no intron was detected in the coding regions of Type D genes. Our comparative analysis with the gene structures of *AtLTPs* and *OsLTPs* indicated that the exon/intron structures of *ZmLTPs* are similar to those of *Arabidopsis* and rice (Additional file 10: Figure S4).

Chromosomal localization and gene duplication of ZmLTP genes

In silico mapping of the gene loci showed that 63 *ZmLTPs* were unevenly assigned to maize ten chromosomes (Figure 1A). Chromosome 1 contained the maximum number of *ZmLTPs* (11), while the minimum number (2) were presented on Chromosome 9. However, several chromosomes lacked *ZmLTPs* in some regions, such as the long arm of chromosome 5 as well as the short arms of Chromosome 3, 6 and 9. The exact position (in bp) of each *nsLTP* on maize chromosome is given in Additional file 2: Table S2.

Gene duplication is generally believed to be a major driving force in evolutionary innovation, giving rise to genomic complexity. Maize originated from an ancient allotetraploid and has undergone several rounds of whole-genome duplication events during its gene evolution [30,31]. Segmental and tandem duplications are known to be the main causes leading to gene family expansions. In this study, a total of seven tandem repeats (*ZmLTPg6/ZmLTPg7/ZmLTPg8*; *ZmLTP2.7/ZmLTP2.8*; *ZmLTPg20/ZmLTPg21*; *ZmLTPd10/ZmLTPd11*; *ZmLTPd12/ZmLTPd13*; *ZmLTP1.6/ZmLTP1.7* and *ZmLTPd15/ZmLTPd16*) were identified in the maize genome. One significant cluster of three Type G genes was found on Chromosome 1. What's more, six direct repeat tandems were identified on chromosome 6, 7, 8 and 10. Genes in the same cluster were closely related to one another. For instance, *ZmLTPd15* and *ZmLTPd16* protein sequences shared 95% similarity and the two genes were physically located next to each other in the same chromosomal region. In addition, five sister pairs (*ZmLTPg6/ZmLTPg16*, *ZmLTPg11/ZmLTPx2*, *ZmLTPg13/ZmLTPg21*, *ZmLTPd7/ZmLTPd13* and *ZmLTP2.7/ZmLTP2.9*) appeared to be generated from segmental duplication events due to their positions on the same duplicated gene blocks (Figure 1A and Additional file 11: Table S7). Furthermore, we analyzed the evolution of nsLTP genes among maize, sorghum and rice (Figure 1B and Additional file 11: Table S7). 22 out of 63 maize nsLTP genes had collinear genes in rice, while 35 had syntenic members in sorghum. According to this analysis, two paired nsLTP genes (*ZmLTPg21/OsLTPg17* and *ZmLTPg13/OsLTPg17*) were located in genomic regions with synteny between the maize and rice genomes. Seven paired nsLTP genes (*ZmLTPg16/SbLTPx1*, *ZmLTPg11/SbLTPg18*, *ZmLTPx2/SbLTPg18*, *ZmLTP2.9/SbLTP2.5*, *ZmLTPg21/SbLTPg10*, *ZmLTPg13/SbLTPg10* and *ZmLTPg13/SbLTPg4*) were located in genomic regions with synteny between the maize and sorghum genomes, indicating that these genes may be derived from a common ancestor. Interestingly, one of the sister pairs (*ZmLTPg6/ZmLTPg16*) had a unique, syntenic *nsLTPs* in sorghum, which indicated that they might be generated from segmental duplication after the divergence of maize and sorghum.

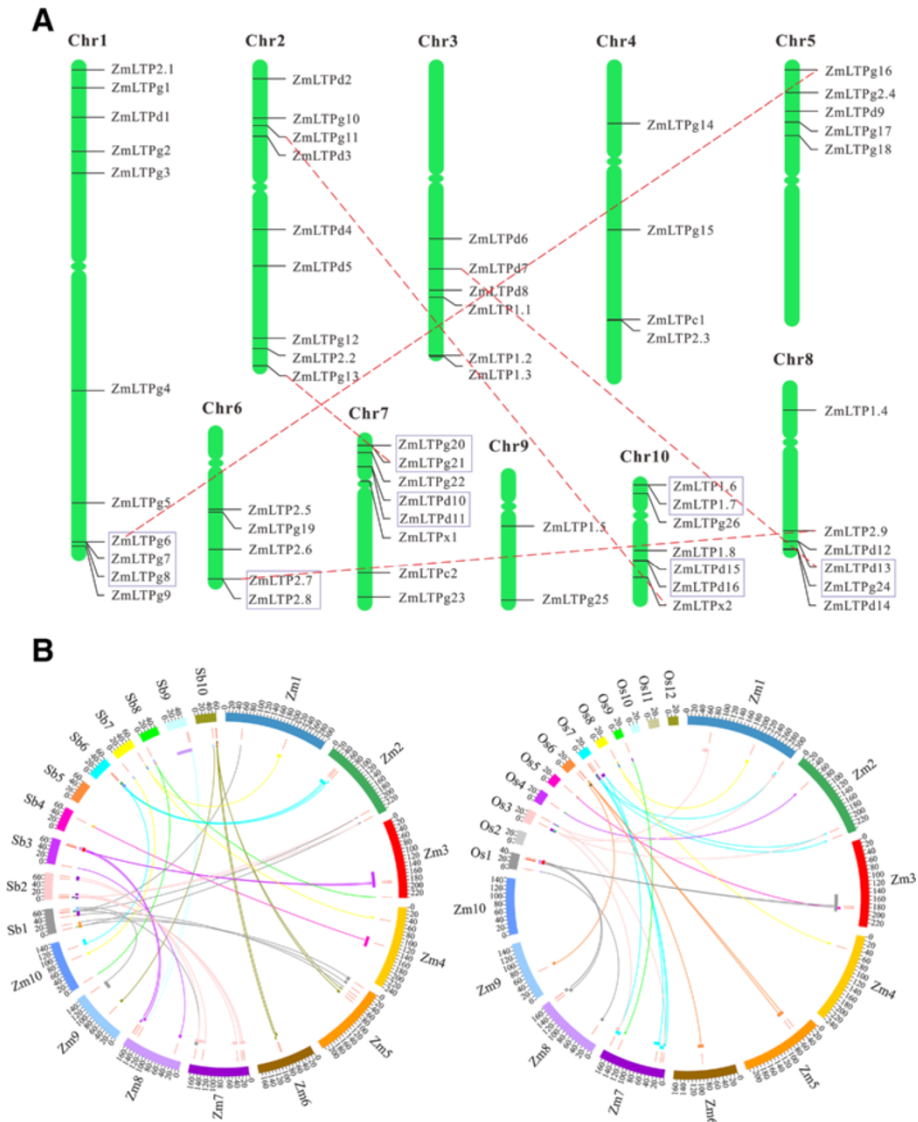


Figure 1 Genome distribution and synteny analysis of nsLTP genes from maize and its relatives (rice and sorghum). (A) Distribution of 63 ZmLTP genes on ten maize chromosomes. Chromosomal distances are given in Mb. Chromosome numbers are indicated at the top of each bar. The gene names on the right side of each chromosome correspond to the approximate locations of each nsLTP gene. The tandem duplicated gene clusters are marked in boxes, and segmental duplication genes are connected by dashed lines in red. (B) Synteny analysis of nsLTP genes from maize and its relatives (rice and sorghum). Positions of putative nsLTP genes are shown using red line. Boxes represent the syntenic blocks. Colors are assigned to the syntenic regions according to the colors of the corresponding chromosomes. Innermost colored lines show synteny between nsLTP genes.

Divergence rate of the maize nsLTP genes

The ratio (K_a/K_s) of nonsynonymous substitution rate (K_a) versus synonymous substitution rate (K_s) is widely used as an indicator of selective pressure [32]. As shown in Table 2, the K_a/K_s ratios of 5 duplicated gene pairs were less than 1, indicating that they seemed to be under purifying selection; however, the K_a/K_s ratios of 9 duplicated gene pairs were more than 1, suggesting that they subjected to positive selection. Positive selection was thought to be one of the major forces for the emergency of new motifs/functions in protein after gene duplication,

and the divergence of the duplicated genes was driven by positive selection [33]. Based on the substitution rates analysis, we found that 64.29% of the duplicated gene pairs had strong positive selection pressure, implying that positive selection contributed to further gene diversification in the *ZmLTP* family. In addition, the divergent times between the duplicated gene pairs were analyzed (Table 2). The duplication events for the 5 segmental duplications were estimated to have occurred approximately between 9.43 and 34.06 million years ago (Mya), while the duplication events for the 9 tandem duplications occurred

Table 2 Ka/Ks analysis and estimate of the absolute dates for the duplication events between the duplicated ZmLTP genes

Duplicated pair	Ka	Ks	Ka/Ks	Date (Mya)	Duplicate type	Purifying selection	Group
<i>ZmLTPg6/ZmLTPg16</i>	0.091	0.123	0.7423	9.43	Segmental	Yes	Type G
<i>ZmLTPg11/ZmLTPx2</i>	0.127	0.236	0.5382	18.12	Segmental	Yes	Type G
<i>ZmLTPg13/ZmLTPg21</i>	0.334	0.379	0.8816	29.18	Segmental	Yes	Type G
<i>ZmLTPd10/ZmLTPd11</i>	0.359	0.519	0.6920	39.91	Tandem	Yes	Type D
<i>ZmLTPd15/ZmLTPd16</i>	0.022	0.119	0.1828	9.13	Tandem	Yes	Type D
<i>ZmLTP2.7/ZmLTP2.9</i>	0.475	0.443	1.0720	34.06	Segmental	No	Type 2
<i>ZmLTPd7/ZmLTPd13</i>	0.306	0.237	1.2910	18.24	Segmental	No	Type D
<i>ZmLTP1.6/ZmLTP1.7</i>	0.401	0.368	1.0911	28.28	Tandem	No	Type 1
<i>ZmLTP2.7/ZmLTP2.8</i>	0.339	0.314	1.0806	24.15	Tandem	No	Type 2
<i>ZmLTPd12/ZmLTPd13</i>	0.688	0.401	1.7169	30.81	Tandem	No	Type D
<i>ZmLTPg6/ZmLTPg7</i>	0.681	0.483	1.4094	37.17	Tandem	No	Type G
<i>ZmLTPg6/ZmLTPg8</i>	0.624	0.469	1.3300	36.06	Tandem	No	Type G
<i>ZmLTPg7/ZmLTPg8</i>	0.607	0.484	1.2533	37.24	Tandem	No	Type G
<i>ZmLTPg20/ZmLTPg21</i>	0.738	0.656	1.1245	50.46	Tandem	No	Type G

approximately between 9.13 and 50.46 Mya. Fossil data along with the phylogenetic studies estimated that the different grass families diverged from a common ancestor 50 to 70 Mya [34]. The divergence times on the nodes of the tree were estimated with the non-parametric rate smoothing method of Sanderson, assuming that maize and rice diverged 50 Mya [35]. Duplication events for one tandem duplicated gene pair (*ZmLTPg20/ZmLTPg21*) occurred around 50.46 Mya, after origin of grasses and before divergence of rice and maize (within last 70 to 50 million years). The ancestor of maize and sorghum diverged about 12 Mya and subsequently a whole genome triplication (WGT) event occurred in the maize approximately 5 Mya [34]. Duplication events for 10 gene pairs (*ZmLTPg11/ZmLTPx2*, *ZmLTPg13/ZmLTPg21*, *ZmLTPd10/ZmLTPd11*, *ZmLTP2.7/ZmLTP2.9*, *ZmLTPd7/ZmLTPd13*, *ZmLTP1.6/ZmLTP1.7*, *ZmLTP2.7/ZmLTP2.8*, *ZmLTPd12/ZmLTPd13*, *ZmLTPg6/ZmLTPg7* and *ZmLTPg6/ZmLTPg8*) occurred within last 39.91 to 18.12 million years, after divergence of rice and maize, but before maize and sorghum were separated from each other. Duplication events for the other two gene pairs (*ZmLTPg6/ZmLTPg16* and *ZmLTPd15/ZmLTPd16*) occurred around last 9 million years, after maize and sorghum were separated, and before a whole genome duplication (WGD) event occurred in the maize. Therefore, evolutionary origin of the maize nsLTP genes might undertake three evolutionary stages.

The features of the three-dimensional structures of major nsLTPs

In order to better understand the non-specific binding between the hydrophobic ligands and ZmLTPs, ZmLTP1.2,

ZmLTP2.1 and ZmLTPd5 were selected as representative sequences of Type 1, 2, and D for structural modeling (Additional file 2: Table S2). The crystal structures of maize ZmLTP1.6 (PDB ID: 1FK7) [7], wheat nsLTP2 (PDB ID: 1TUK) [8], AtDIR1 (PDB ID: 2RKN) [4] were selected as templates for structural modeling based on searches against the PDB using the Basic Local Alignment Search Tool (BLAST) at NCBI (<http://blast.ncbi.nlm.nih.gov/>) with the target Type 1, 2 and D protein sequences as baits.

Based on the ZmLTP1.6, nsLTP2 and AtDIR1 structures, the amino acids 29-121 of ZmLTP1.2, 32-98 of ZmLTP2.1, and 37-115 of ZmLTPd5 could be modeled with the sequence identities of 88.17%, 62.69%, and 51.95%, respectively. Our structural analysis showed that ZmLTP1.2 and ZmLTP1.6 have typical features of plant nsLTPs, including two conserved pentapeptides, T-T/A-A-D-R (positions 46-50) and P-Y-T-I-S (positions 87-91). It has been reported that these two consensus pentapeptides (T/S-X-X-D-R/K and P-Y-X-I-S) were important in catalysis or binding [5]. ZmLTPd5 and AtDIR1 contain high proline content (positions 25-32) that is not highlighted in nsLTP2 and ZmLTP2.1. It is noteworthy that proline-rich regions are involved in protein-protein interactions [36]. As aforementioned, X is a hydrophilic residue in the CXC motif of Type 1 nsLTPs. However, a hydrophobic residue was found at the X position in Type 2 and Type D nsLTPs. As illustrated in Figure 2A, in the CXC motif, asparagines between the two cysteines in ZmLTP1.2 and ZmLTP1.6 are replaced by a hydrophobic amino acid, phenylalanine, in ZmLTP2.1 and nsLTP2, whereas in ZmLTPd5 and AtDIR1, the X position are occupied by a hydrophobic residue, leucine.

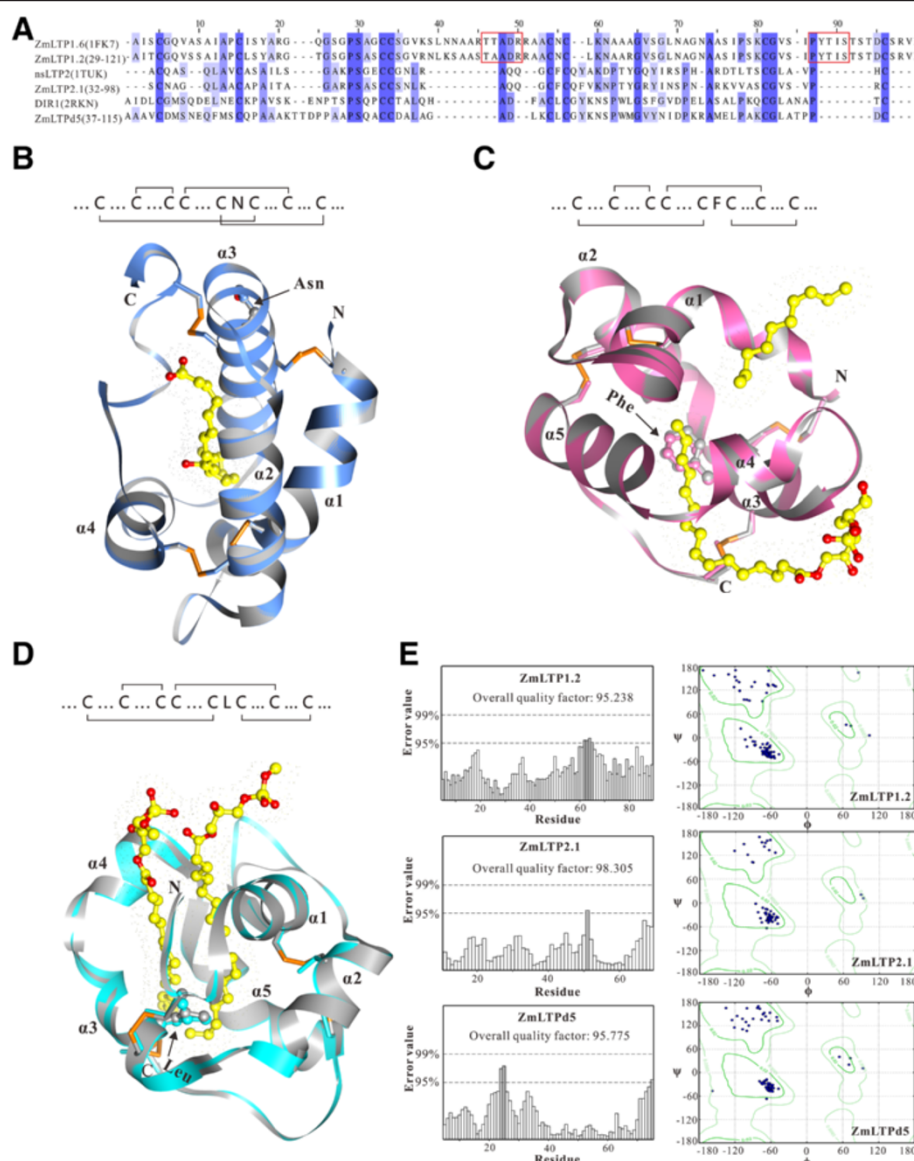


Figure 2 The structure features of maize nsLTPs. (A) Sequence alignment of maize ZmLTP1.6 (1FK7; amino acids (aa) 1-93), wheat nsLTP2 (1TUK; aa 1-67), AtDIR1 (2RKNN; aa 1-77), ZmLTP1.2 (aa 29-121), ZmLTP2.1 (aa 32-98) and ZmLTPd5 (aa 37-115). Identical residues are highlighted by blue background. Consensus residues Thr-X-X-Asp-Arg and Pro-Tyr-X-Ile-Ser are marked in boxes. **(B)** Schematic representation of the cysteine pairing pattern and superposition of the backbone trace of ZmLTP1.2 (blue) and ZmLTP1.6 (grey) complexed with ricinoleic acid (shown as ball-and-stick; carbons in yellow and oxygen in red). The four disulfide bonds are shown in orange. **(C)** Schematic representation of the cysteine pairing pattern and superposition of the backbone trace of ZmLTP2.1 (pink) and nsLTP2 (grey) complexed with LPG (L- α -palmitoyl glycerol) lipid ligands (shown as ball-and-stick; carbons in yellow and oxygen in red). The four disulfide bonds are shown in orange. **(D)** Schematic representation of the cysteine pairing pattern and superposition of the backbone trace of ZmLTPd5 (cyan) and the crystal structure of AtDIR1 (grey) in complex with two lysophosphatidyl choline molecules (shown as ball-and-stick; carbons in yellow and oxygen in red). The four disulfide bonds are shown in orange. **(E)** ERRAT result depicting overall quality factor and Ramachandran plot of ZmLTP1.2, ZmLTP2.1 and ZmLTPd5.

The hydrophobic residues in the CXC motif of ZmLTP2.1, nsLTP2, ZmLTPd5 and AtDIR1 are buried inside the molecule, whereas the hydrophilic residue of ZmLTP1.2 and ZmLTP1.6 are at the surface (Figure 2B, C and D). In the template protein ZmLTP1.6, the Cys residues 1-6, 2-3, 4-7, and 5-8 are paired,

whereas the Cys residues 1-5, 2-3, 4-7, and 6-8 are paired in the template protein AtDIR1, similarly to the Type 2 nsLTPs. These observations suggested that the central residue of the CXC motif may govern the cysteine pairing and influence the overall fold of the protein. The crystal structure homology models

were validated in terms of stereochemical quality by Ramachandran plot and the ERRAT prediction (Figure 2E).

From the superposition structure, we find that ZmLTP1.2 and ZmLTP1.6 share a common structural fold stabilized by four disulfide bonds, and the four prominent α -helices are packed against a flexible C-terminal arm formed by a series of turns, which forms a cap over the hydrophobic cavity (Figure 2B). The three-dimensional structure of ZmLTP2.1 showed that two adjacent hydrophobic cavities seem able to expand the hydrophobic cavity to accommodate the alkyl ligand (Figure 2C). The structure of ZmLTPd5 is in accordance with AtDIR in the aspects of the order and orientation. Their α -helix topology is well conserved and the four cysteine bridges superimpose well, showing high similarity in the three-dimensional structure (Figure 2D). Interestingly, the Arabidopsis mutant defective (*dir1-1*) was compromised in the production or transmission of an essential mobile signal to promote long-distance signaling [9]. Whether other Type D nsLTPs have the functions related to systemic resistance signalling remain to be further investigated.

Gene ontology analysis of the maize nsLTPs

In order to comprehend the unique aspects of maize nsLTPs, Gene Ontology (GO) enrichment analysis of the functional significance was performed. Out of 77 ZmLTP proteins, annotation could not be performed for 24 proteins. Besides, 53 ZmLTPs were defined in 22 significant GO terms (Additional file 12: Table S8). The analysis showed that 53 ZmLTPs were separated into two main categories (biological process and molecular function), which included 18 and 4 significant GO terms, respectively (Figure 3A). For the enriched biological processes, the common categories are “response to stimulus” followed by “response to abiotic stimulus” and “response to stress”, “multicellular organismal process”, “biological regulation” and “establishment of localization”. Noteworthy, about 47 ZmLTPs were shown to participate in “lipid transport” (GO: 0006869), which is concordance with the molecular role of nsLTP in transporting hydrophobic molecules *in vitro*, suggesting that ZmLTPs play an important role in carrying membrane components to the growing site of elongating cells. The enriched GO terms also include the parent term “response to abiotic stimulus” with enriched children term “response to cold”, and 22.64% (12 of 53) ZmLTPs were exhibited to participate in “response to stress stimulus” (particularly in cold). This highlights the putative association of nsLTP proteins in stress tolerance behavior of maize. In case of molecular functions, about 88.68% (47 of 53) ZmLTPs were shown to

participate in “lipid binding” (GO: 0008289). One of the interesting observations was that some significantly enriched terms are involved in “water binding” and “ice binding”. To summarize, the GO analysis indicated that ZmLTPs may be involved in diverse biological processes.

Key *cis*-elements within the promoter regions of ZmLTP genes

In-silico analysis of 1 kb upstream region (from translation start site) of 63 maize nsLTP genes revealed the presence of various regulatory elements, which are associated with development, abiotic or biotic stress signaling, and hormone signaling (Additional file 13: Table S9). To avoid the biases in analysis, only *cis*-elements identified in at least ten different genes were considered. As shown in Figure 3B, except for TATA-box and CAAT-box, G-box is the most frequently found type of *cis*-elements, and the regulatory element involving light responsiveness seems to be enriched in ZmLTPs. Nearly 80% of ZmLTPs contained Skn-1 motifs which might account for endosperm expression. Stress-responsive *cis*-regulatory elements identified in this study included fungal elicitor responsive element (Box-W1), defense and stress responsive element (TC-rich repeats), heat stress responsive element (HSE), low temperature responsive element (LTR), anaerobic-response element (ARE and GC-motif), MYB binding site (MBS) involving in drought-inducibility and phytohormone-responsive elements, like auxin responsive element (TGA-element and AuxRR-core), methyl jasmonate responsive element (CGTCA-motif and TGACG-motif), salicylic acid responsive element (TCA-element), abscisic acid responsive element (ABRE) and gibberellic acid-responsive element (GARE). With a few exceptions, ZmLTPs contained circadian elements, which may be responsible for its distinct diurnal expression pattern. Other regulatory elements, such as O₂-site (involved in zein metabolism regulation), DRE (involved in responses to dehydration, low temperatures and salt), CAT-box (related to meristem expression) and CCGTCC-box (related to meristem specific activation) were also presented. Especially, the promoters of 39 ZmLTPs contained MBS elements ranging from 1 to 5 copies (Additional file 13: Table S9), indicating the important role of MYB transcription factors in regulating ZmLTPs. Some recent reports have shown that nsLTP can act as targets of MYB to regulate plant tolerance to freezing and drought stresses [24,37], suggested that the ZmLTP members participate in some abiotic stress signaling.

The diverse roles of nsLTPs in maize development

As for multigene family, the analysis of gene expression patterns often provides useful clues to decide genes function. To investigate the temporal and spatial expression

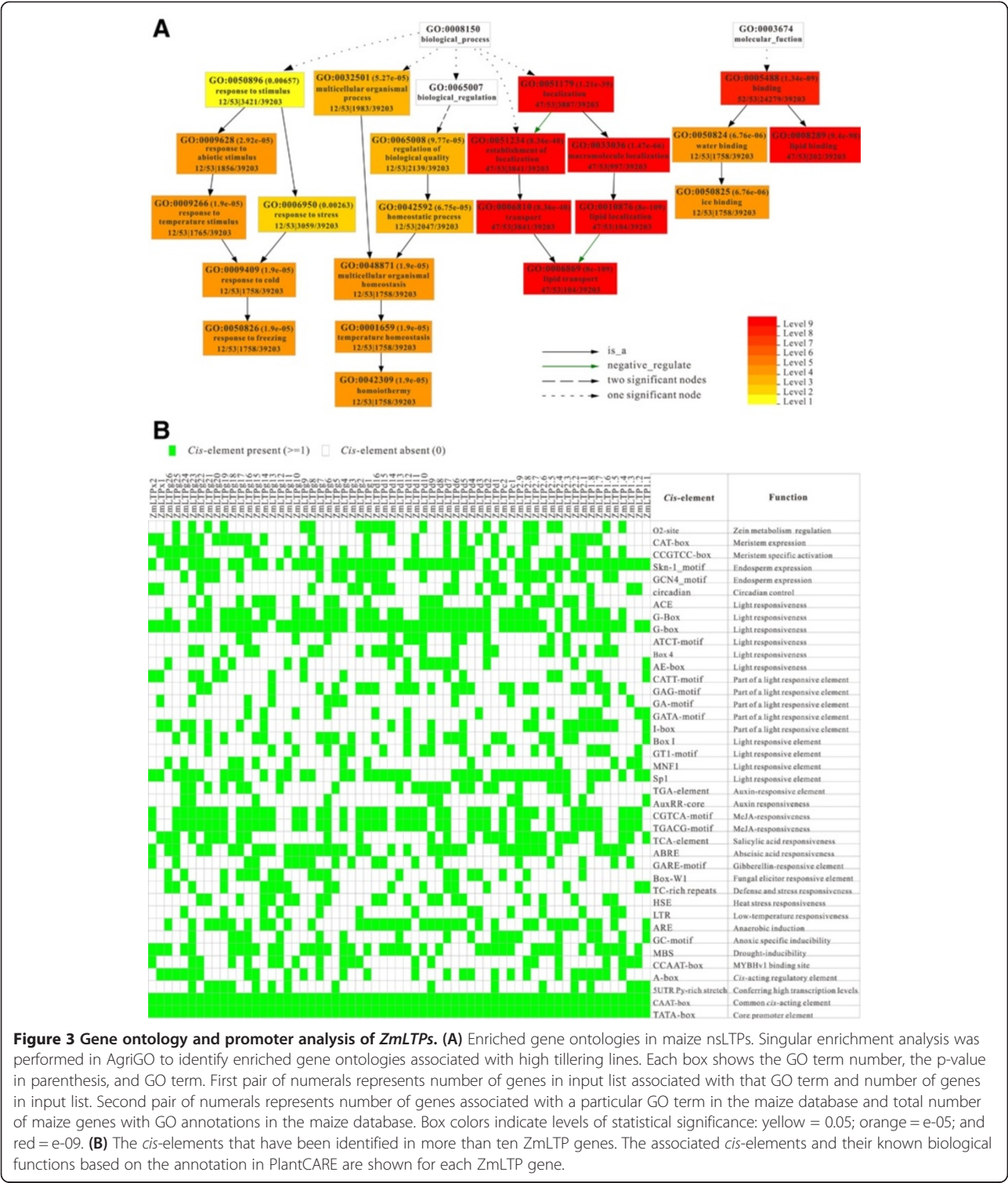
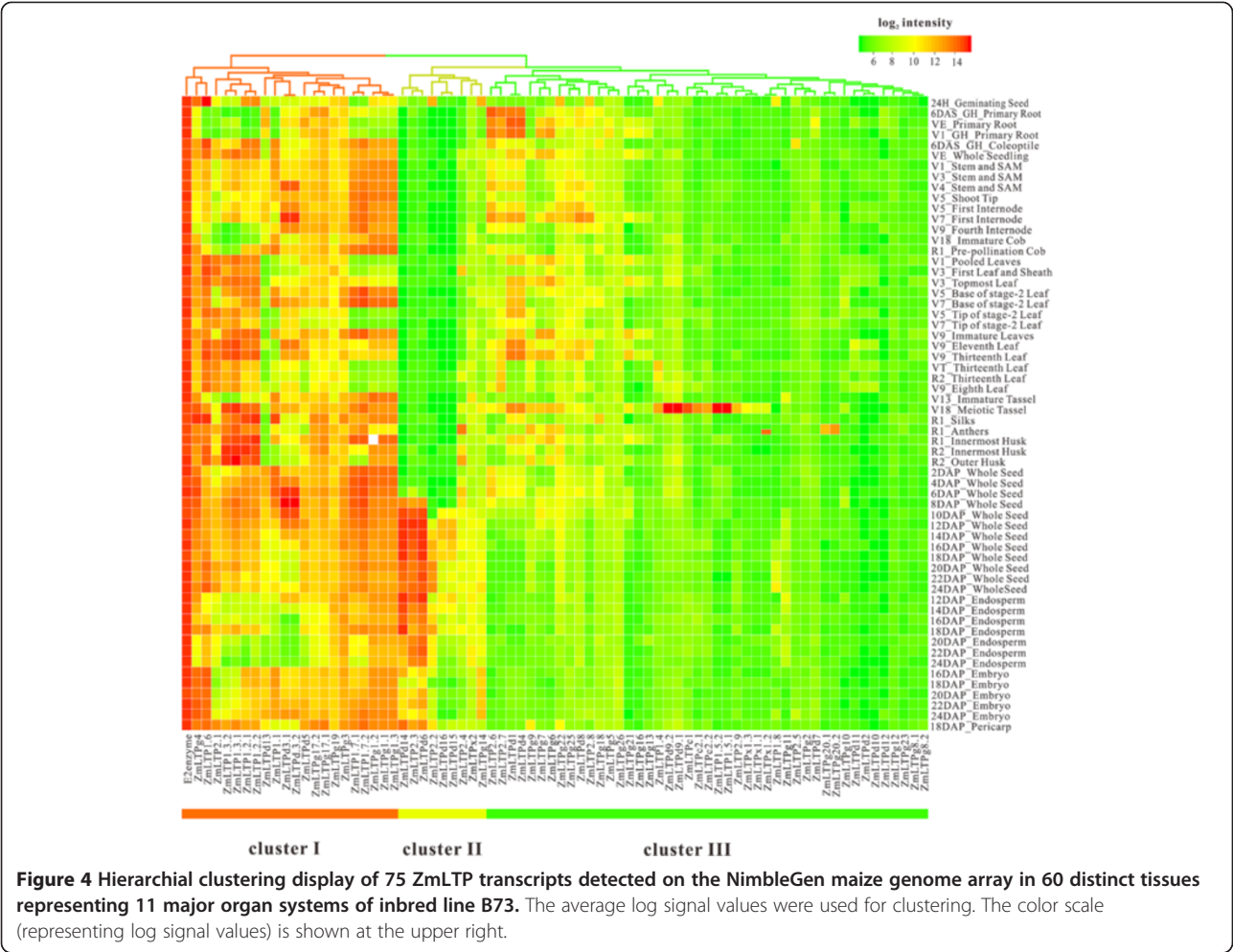


Figure 3 Gene ontology and promoter analysis of *ZmLTPs*. (A) Enriched gene ontologies in maize nsLTPs. Singular enrichment analysis was performed in AgriGO to identify enriched gene ontologies associated with high tillering lines. Each box shows the GO term number, the p-value in parenthesis, and GO term. First pair of numerals represents number of genes in input list associated with that GO term and number of genes in input list. Second pair of numerals represents number of genes associated with a particular GO term in the maize database and total number of maize genes with GO annotations in the maize database. Box colors indicate levels of statistical significance: yellow = 0.05; orange = e-05; and red = e-09. **(B)** The *cis*-elements that have been identified in more than ten *ZmLTP* genes. The associated *cis*-elements and their known biological functions based on the annotation in PlantCARE are shown for each *ZmLTP* gene.

patterns of the nsLTP genes in the maize life cycle, hierarchical clustering was performed to visualize a global transcription profile of the *ZmLTP* genes across the 11 organs during diverse developmental stages. As depicted in Figure 4, the heatmap can be apparently divided into three

clusters. Cluster I contains 21 members (excluding the E2 enzyme as the reference). Cluster II has 9 members, and 45 members belong to Cluster III. Genes in Cluster I obviously have relatively high expression levels, with the mean log-signal values of each gene ranging from 9.0 to 14.9



(Additional file 14: Table S10). Conversely, Cluster III contains those genes with relatively low expression levels and the average log-signal value range is between 5.2 and 9.2. In Cluster II, genes are expressed at a moderate level with the mean log-signal values ranging from 6.1 to 8.5. To elucidate putative differentially expressed genes in specific organs or stages, the coefficient of variation (CV value; $CV = S/X_{mean}$, where S represents the standard deviation and X_{mean} describes the mean expression level of a gene across all the tissues) of each gene in the three clusters was calculated (Additional file 14: Table S10). A house keeping gene in maize, which encodes a ubiquitin-conjugating enzyme (E2), was used as internal reference in our expression analysis [38]. The results showed a huge variation among all the genes with the CV values ranging from 1.01% to 52.97%. Cluster I has the least expression variability from 1.01% to 26.80%, indicating a most stable expression pattern relative to other ZmLTP genes. *ZmLTP1.1*, the most changeable genes in cluster I, whose homolog in rice, *Photoperiod-sensitive dwarf 1* (*Psd1*, *Os01g60740*), was previous demonstrated to

encode a lipid transfer protein that may participate in regulation of plant cell division and elongation [39]. One gene (*ZmLTP1.2*), which can bind to calmodulin in a Ca^{2+} -independent manner [26], displayed high expression levels at nearly all of the maize organs and/or stages of development analyzed except for root, immature cob and endosperm. A total of 12 nsLTP protein-encoding genes were found to be highly expressed in anthers. 7 *ZmLTPs* were found to be highly expressed in leaf at different developmental stages, whereas, 5 *ZmLTPs* specially accumulated at V5_Base of stage-2 Leaf, V7_Base of stage-2 Leaf, V9_Immature Leaves, V9_Eleventh Leaf and V9_Thirteenth Leaf. Cluster II showed the highest expression variability, ranging from 11.95% to 52.97%. There are 8 genes with CV values more than 15% in Cluster II, among which 3 genes (*ZmLTPd14*, *ZmLTP2.3* and *ZmLTPd6*) have CV values more than 45% and display transcript accumulation at late seed developmental stages (10 to 24 DAP) and endosperm. It has been reported that *BETL9* (*ZmLTPd6*) has its expression restricted to the endosperm transfer cells, which share their position as

entrance gates to the developing seed for nutrients coming from the maternal tissues [28]. There is an increasing evidence that this protein might be involved in the defense of the developing seed against mother plant-borne pathogens [40,41]. Genes in Cluster III showed an apparent fluctuation, with the CV values ranging from 4.82% to 31.83%. In this context, Cluster III can be further divided into two subclusters, with genes in the first subcluster having higher signal intensity values than those in the second subcluster. In the first subcluster, four *ZmLTPs* (*ZmLTP2.6*, *ZmLTP2.7*, *ZmLTPd1* and *ZmLTPd4*) displayed high expression levels during root development and this finding suggested that *ZmLTPs* may participate in maize root development. The organ-specific expression dynamics revealed the distinct expression patterns of *ZmLTP* genes throughout the entire life cycle in maize. For example, two *ZmLTP* genes, *ZmLTPg20* and *ZmLTP2.5*, were specifically expressed in the anthers and coleoptile, respectively. There are 6 genes (*ZmLTP1.4*, *ZmLTP1.5*, *ZmLTP2.9*, *ZmLTPc1*, *ZmLTPc2* and *ZmLTPd9*), with CV values among 15.54% to 23.92% in the second subcluster, distinctly increasing their expression levels in meiotic tassel. Among them, *ZmLTPc2* is highly homologous to *MZm3-3*, which is expressed specifically in the tapetum during male gametogenesis of maize [25]. Furthermore, four nsLTP genes (*Os09g35700*, *Os01g49650*, *Os01g12020* and *Os08g43290*) were reported to be expressed in rice anther, which are putatively related to exine synthesis in sporophytic anthers of progressive developmental stages [42]. Interestingly, these four rice nsLTPs were the homologs of *ZmLTPc2*, *ZmLTP2.9*, *ZmLTP1.4* and *ZmLTPc1*, respectively. Synthesis of lipidic components in anthers, including the pollen exine, is essential for plant male reproductive development. Therefore, this finding further suggested that nsLTPs play significance roles in male reproductive development.

Pathogen responses of *ZmLTPs* to the fungal infection

Plant nsLTPs are capable of inhibiting bacterial and fungal pathogens and are, therefore, thought to play an important role in plant defense [18]. To discover the *ZmLTP* genes involved in maize pathogen response, microarray data collected after the treatments of 12, 24 hours, 2, 4, 4.5, 8 days of *Ustilago maydis* (*U. maydis*) infection were analyzed. *U. maydis* is a ubiquitous pathogen of maize which depends on living tissue for proliferation and development. A total of 41 probe sets found on the maize 18 k Gene-Chip could be assigned to 36 different *ZmLTP* genes (Additional file 15: Table S11). The log₂ ratio values (Treated/Control) were illustrated by a heatmap, showing the fold change in expression of each *ZmLTP*. As shown in Additional file 16: Figure S5, most of the *ZmLTPs* exhibited a delayed expression pattern after the infection of *U. Maydis*. 23 *ZmLTPs* distinctly increased

their expression levels and accumulated mostly on the 4 and 8 days post infection, especially of *ZmLTPg1*, *ZmLTP1.1* and *ZmLTP1.7*, suggesting that these genes might participate in the pathogen response. Interestingly, some *ZmLTP* genes show up-regulated expression levels over time, especially of *ZmLTP2.8*, demonstrating a similar response pattern to this kind of fungus infection. Besides, the expression levels of *ZmLTPd2* and *ZmLTP2.1* show a rapid increase after infection, suggesting their high sensitivity to *U. maydis* infection. One nsLTP from Arabidopsis (*AtDIR1*) has been suggested to be involved in long-distance signaling during pathogen defense [9]. Therefore, it is reasonable to find a transcript accumulation of the *ZmLTPd5*, a *AtDIR1* homologue from maize, after the infection of *U. maydis*. In addition to the genes up-regulated after *U. maydis* infection, there are also some *ZmLTP* genes show a decline of expression levels as time goes by, such as *ZmLTPg23* and *ZmLTPd6*. These genes might function in other biological processes or respond to other kinds of pathogen attacks. Taken together, these results presented here indicated that many genes in this family might participate in the pathogen response.

Transcriptional responses of maize nsLTP genes against abiotic stresses

Apart from their inducibilities upon pathogen infection, nsLTP genes are also responsive to abiotic stresses like drought, cold and salt [21,43]. To investigate the possible role of *ZmLTPs* under drought stress, the microarray data of two inbred lines, drought-tolerant line Han21 and drought-sensitive line Ye478, under different drought treatments were used [44]. Following whole-chip data processing, 57.14% (36 of 63) *ZmLTP* genes, about 41 probes (some genes have two or more probe sets) in the microarray, were extracted for analysis (Additional file 15: Table S11 and Figure 5A). Using fold change >2 and *P* value <0.05, *ZmLTP* genes with changeable expression profiles in at least one conditions were regarded as differentially expressed genes (Figure 5B). The result showed that 14 and 13 *ZmLTPs* were differentially regulated by drought and/or re-watering treatment(s) in Han21 and Ye478 maize inbred lines, respectively. Interestingly, some *ZmLTPs* appeared to be up-regulated in Han21 under drought stress, suggesting that they may have a role in drought stress response in maize. For example, the transcript levels of *ZmLTP2.4*, *ZmLTP2.7* and *ZmLTP2.8*, were significantly up-regulated under moderate and severe drought stresses (Figure 5A). Furthermore, *ZmLTP2.6* and *ZmLTPd8* showed high-abundance transcript levels under moderate drought condition compared to untreated samples. To verify the expression patterns of these predicted genes under drought stress, 8 differentially expressed genes were selected for qRT-PCR analysis and the primers used in this assay are listed in Additional file 17: Table

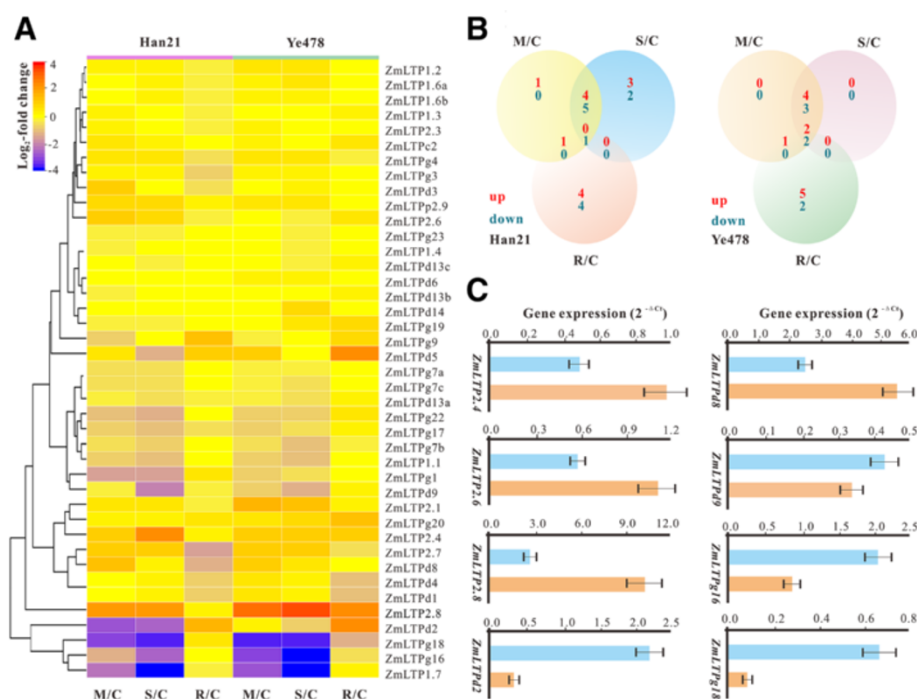


Figure 5 Differential gene expression of *ZmLTPs* under drought stress. (A) Expression profiles of 36 *ZmLTPs* under moderate drought stress (M/C), severe drought stress (S/C) and re-watering (R/C) as compared to control seedlings in Han21 and Ye478, respectively. Log2-based fold changes were used to create the heatmap. **(B)** Venn diagrams for the number of differentially expressed *ZmLTP* genes under moderate drought stress (M/C), severe drought stress (S/C) and re-watering (R/C) as compared to control seedlings in Han 21 and Ye478, respectively. A gene is considered differentially expressed if it is up- or down-regulated at least two-fold with a P value <0.05 under any of the given conditions. **(C)** Quantitative RT-PCR analysis for selected *ZmLTP* genes in drought treatments. The maize UBQ1 gene was used as endogenous control to normalize data. The values represented are the mean of two biological replicates, each with three technical replicates. Error bars represent standard deviations determined with two biological replicates.

S12. As shown in Figure 5C, *ZmLTP2.4*, *ZmLTP2.6*, *ZmLTP 2.8* and *ZmLTPd8* were obviously up-regulated in water deficient, whereas *ZmLTPd2*, *ZmLTPd9*, *ZmLTPg16* and *ZmLTPg18* were down-regulated under drought stress. Taken together, qRT-PCR-based expression profiling for these selected genes confirmed the outcome of microarray analysis.

In addition to DNA microarray analysis, RNA-seq analysis was performed to investigate the functions of *ZmLTPs* involved in the differential drought sensitivity of maize reproductive (ovary) and vegetative tissues (leaf) under both drought and well-watered conditions [45]. Nearly 66.67% (42) of *ZmLTPs* were reliably detected by RNA-seq data (Additional file 18: Table S13). Out of 42 expressed genes, 3 genes (*ZmLTP2.8*, *ZmLTPg14* and *ZmLTP2.2*) were distinctly upregulated and 17 genes were down-regulated in the drought-stressed ovary tissue, while 10 (*ZmLTPd3*, *ZmLTP2.8*, *ZmLTPd4*, *ZmLTPd1*, *ZmLTP2.6*, *ZmLTPg2*, *ZmLTP1.1*, *ZmLTPg3*, *ZmLTPg1* and *ZmLTPd5*) were significantly up-regulated and 2 (*ZmLTP2.9* and *ZmLTPg24*) were down-regulated in the drought-stressed leaf meristem, suggesting that these *ZmLTP* genes may exhibit tissue-specific expression under drought stress.

Many nsLTPs in plants have been reported to be closely related to drought stress. Plants have a cuticle on the surface of their leaves that provides a protective barrier against the environmental adversities, such as drought stress. In *Arabidopsis*, *AtLTPg4*, which have close phylogenetic relationship with *ZmLTPg14* in maize, play a role in wax and/or cutin monomer transport [46]. In our study, the expression level of *ZmLTPg14* was increased in drought-stressed ovary tissue, implying a possible role in drought resistance. *ZmLTP1.2*, which was reported as the target of calmodulin, was significantly down-regulated in drought-stressed ovary tissue but up-regulated in drought-stressed leaf meristem. As expected, a stresses-response gene, *ZmLTP1.1*, also increases its expression level in the drought-stressed leaf meristem, whereas it was down-regulated in the drought-stressed ovary tissue. In any case, the analysis of the expression profiles based on both microarray and RNA-seq methods proved that *ZmLTPs* may play important roles in maize tolerance to drought stress. While further work is required for understanding how *ZmLTPs* are mechanistically linked to drought stress responses in maize.

Apart from understanding the effect of drought stress on the transcript levels of various maize nsLTP genes, it is also important to conceive about the expression level of these genes under the salt stress. Root is the first organ directly exposed to salt stress. To better understand the roles of ZmLTP genes in salt stress responses, RNA-seq analysis was performed to explore the expression profiles of ZmLTPs in three root types of maize [primary root (PR), seminal root (SR) and crown root (CR)] under salt stress. Nearly 60.32% (38 of 63) of ZmLTPs were detected by RNA-seq. As shown in Additional file 19: Figure S6, 3 ZmLTP genes (*ZmLTP2.4*, *ZmLTPd3* and *ZmLTPg17*) and 4 genes (*ZmLTP2.8*, *ZmLTPd3*, *ZmLTPd11* and *ZmLTPg17*) were found to be at least two-fold up-regulated in the PR and SR under salt stress, respectively. Under salt stress, 11 ZmLTP genes (*ZmLTP1.1*, *ZmLTP1.5*, *ZmLTP2.4*, *ZmLTP2.8*, *ZmLTPd3*, *ZmLTPd9*, *ZmLTPd11*, *ZmLTPg2*, *ZmLTPg4*, *ZmLTPg5* and *ZmLTPg10*) significantly increased their expression levels in CR. It can be concluded that the gene expression profiles of ZmLTPs are more variable in the CR than those in PR and SR under salt stress. One ZmLTP gene, *ZmLTPd3*, was highly induced by salt stress in PR, SR and CR, suggesting that it may play an important role in salt stress response in maize. In addition, *ZmLTP1.1* was specifically expressed in CR, which is accordance with previous report that *ZmLTP1.1* may participate in salt resistance [27].

It is well-known that plants are constantly exposed to a variety of environmental stresses. Among them, the low temperature constitutes a key factor that can influence plant growth, development and crop productivity. The accumulation of soluble sugar and proline are important to protect plants against cold stress [47]. In previous research, the expression of rice cold-inducible nsLTP gene in transgenic *Phalaenopsis amabilis* improved its adaptive responses to cold stress. The examination of transgenic plants showed the increased accumulation of several compatible solutes such as total soluble sugars and proline [48]. To further dissect the biological function of ZmLTPs in cold tolerance, we investigate the possible roles of ZmLTPs under cold stress condition in two inbred maize lines, chilling-sensitive ETH-DL3 and chilling-tolerant ETH-DH7 [49]. Following whole-chip data processing, 54% (34 of 63) ZmLTPs have corresponding probe sets in the microarray, and a total of 36 probe sets on the maize 58 k NSF Array (some genes have two probe sets) were extracted for analysis (Additional file 20: Table S14). Three ZmLTPs (*ZmLTPd12*, *ZmLTPg9* and *ZmLTP1.5*) were defined as differentially expressed genes in response to cold stress (Additional file 21: Figure S7). Surprisingly, all of the three differentially expressed genes were up-regulated under severe cold stress. Among them, *ZmLTPd12* and *ZmLTPg9* were differentially expressed under the severe cold stress conditions in ETH-DH7, and *ZmLTP1.5* and

ZmLTPd12 was up-regulated under severe cold stress conditions in ETH-DL3.

Our results revealed effects of drought, salt and cold stress on transcript abundance of several members of maize nsLTP gene family, indicative of their important roles in abiotic stresses. Moreover, the promoters for most of ZmLTPs were found to contain one or more important *cis*-regulatory elements such as ABRE, MBS, TC-rich repeat and C-repeat/DRE (Additional file 13: Table S9), which might be involved in regulation of expression of ZmLTPs under these stress conditions. Thus, expression profiling against abiotic stresses provided novel insights into specific and/or overlapping expression patterns of nsLTP genes. These analyses will contribute to the studies for unraveling the molecular mechanisms of signal transduction, stress response, and developmental processes.

Conclusions

We have identified 63 nsLTP genes in maize genome, and the members in this family can be divided into five types (1, 2, C, D and G). Comprehensive study of ZmLTPs provided some important features of this gene family such as gene structure, duplicated event, and three-dimensional structure. Additionally, gene ontology analysis was performed to obtain clues about biological function of the ZmLTPs. The analyses of putative upstream regulatory elements further indicated that ZmLTPs may be involved in diverse biological processes and provided new insights into the shared and different transcriptional regulation machineries of ZmLTPs. The expression profiles of ZmLTPs across the different developmental stages showed that some members of this family exhibit tissue-specific expression, indicative of their important roles in performing diverse developmental and physiological functions during the maize life cycle. Further, we focused on the roles of maize nsLTPs in stress responses. The result indicated that some ZmLTPs exhibited a delayed expression pattern after the infection of *Ustilago maydis* and differentially expressed under drought, salt and cold stress, which may be used in further studies for improving stress tolerance in maize. In a word, our results are significant contributions to maize molecular breeding with enhanced quality traits.

Methods

Retrieval and identification of nsLTPs in maize and sorghum genomes

Our search strategy used successive iterations of hidden Markov model (HMM) and BLAST searches to identify small, secreted nsLTPs in maize. The complete genome sequence of maize was downloaded from the maize sequence database (release version 5b; <http://www.maizesequence.org/index.html>). Starting with protein sequences annotated with the Pfam domain PF00234 (plant lipid

transfer/seed storage/trypsin-alpha amylase inhibitor), we scanned maize proteome sequences using the hmmsearch from the HMMER 3.0 package [50] with default parameters. To increase the probability of detecting putative nsLTPs in maize, BLASTP searches were further performed against maize proteome sequences using all known nsLTP amino acid sequences from Arabidopsis and rice as baits. The results were merged, and redundant sequences were deleted. Then, the deduced protein sequences of candidate nsLTPs were manually examined to harbor the 8CM and proteins lacking the essential cysteine residues were removed. Additionally, the proteins encoding for the putative proline-rich proteins were excluded from further analysis. The remaining candidate proteins were submitted to the InterProScan (<http://www.ebi.ac.uk/InterProScan>) to verify the presence of nsLTP domains. The protein sequences of the 2S-albumins (At2S1 to At2S4) [51], and alpha amylase inhibitor (RATI) [52] were BLAST-searched against the rest of candidate nsLTP proteins to exclude possible cereal storage proteins. In addition, sorghum genome sequences were downloaded from the Joint Genome Institute plant genomics database (release version 1.4; <http://www.Phytozome.net>) and the same approach was used to identify nsLTPs in sorghum.

Primary sequence analysis

All identified nsLTPs were analyzed for the presence of potential signal peptide cleavage sites using SignalP v4.0 program [53]. The subcellular localization of nsLTP was predicted by LocTREE 3 (<https://roslab.org/services/loctree3/>). Following signal peptide removal, molecular weight and isoelectric point were computed for mature proteins with ExPasy's pI/Mw tool (http://web.expasy.org/compute_pi/). The eight Cys residues of the 8CM domain were excluded during pI calculation, since Cys residues in a disulfide bond have no charge. Three prediction tools, PredGPI [54], big-PI Plant Predictor [55] and GPI-SOM [56] were used to determine the presence of GPI-anchor sites. Phosphorylation sites of the nsLTPs were predicted by P3DB (<http://www.p3db.org/>). A sequence identity matrix of the mature nsLTP sequences was computed using BioEdit v7.0.9.0 (<http://www.mbio.ncsu.edu/bioedit/bioedit.html>), which enable us to determine the gene subfamily assignment. The nomenclature was based on the guidelines proposed by Edstam et al. [6].

Sequence alignment and phylogenetic reconstruction

The nsLTP amino acid sequences of Arabidopsis (*A. thaliana*) and rice (*O. sativa*) were downloaded from the TAIR (<http://www.Arabidopsis.org/>) and TIGR (<http://rice.plantbiology.msu.edu/>) databases, respectively. Multiple alignments of the 8CM part of the mature proteins

were carried out using the ClustalW implemented in MEGA 5 software. Manually refined alignments were used as input in ProtTest v2.4 [57], which was set to all candidate models, a BIONJ tree, and the slow optimization strategy. The best model (WAG + I + G) was selected by the Akaike information criterion implemented in ProtTest. Then, the amino acid sequences of 8CM of maize, rice and Arabidopsis, totally 274 sequences were loaded into PhyML v3.0 software [58]. The confidence level of each node was estimated by the bootstrap procedure using 100 resampling repetitions of the data [59].

Gene structure and conserved intron splicing site analysis

To explore the diverse exon-intron organization of maize nsLTP genes, we compared the predicted coding sequence (CDS) of maize nsLTP genes with their corresponding genomic sequences using GSDS software (<http://gsds.cbi.pku.edu.cn>). For a better visualization and comparison, the 5' UTR sequences were removed beforehand. The positions of the 8CM were retrieved with PAL2NAL (<http://www.bork.embl.de/>) by converting a multiple sequence alignment of 8CM sequences and the corresponding gene sequences into a codon alignment, and then marked in red [60].

Genome distribution and gene duplication analysis

The genes were plotted separately onto the ten maize chromosomes according to their ascending order of physical position (bp), from the short arm telomere to the long arm telomere. As a gene family may be expanded through tandem and segmental duplication events, we intended to identify the mechanisms involved in the expansion of ZmLTP gene family. ZmLTP genes separated by no more than 10 intervening genes were considered as tandem duplication [61]. The putative orthologs from rice, sorghum and Arabidopsis were assigned to corresponding maize nsLTP proteins according to searches from the TIGR database (http://rice.plantbiology.msu.edu/annotation_pseudo_apk.shtml). Syntenic information from all examined genes was collected from the Plant Genome Duplication Database (PGDD, <http://chibba.agtec.uga.edu/duplication>). The ideograms were created using Circos software [62].

Estimation of synonymous and non-synonymous substitution rates

Synonymous and nonsynonymous substitution rates of duplicated genes were calculated by DnaSP v5.0 [63]. The ratio of nonsynonymous to synonymous nucleotide substitution rates between paralogs was analyzed to detect the mode of selection. Ka/Ks ratio >1, <1 and =1 represent positive selection, purifying selection and neutral selection, respectively. The dates of the duplication events

were calculated by the equation $T = Ks/2\lambda \times 10^{-6}$ Mya, ($\lambda = 6.5 \times 10^{-9}$) [64,65].

Homology modeling of maize nsLTPs

All the maize nsLTP proteins were searched against the Protein Data Bank (PDB) by BLASTP with the default parameters to identify the best template. The data was fed in SWISS-MODEL [66] for predicting the protein structure by homology modeling. To visualize and edit the PDB models, interactive molecular graphics program Chimera v1.8 was used [67]. Ramachandran plots for the evaluation of the model were calculated using Chimera, and the amino acid environment was evaluated using ERRAT at the UCLA-DOE Structure Analysis and Verification Server (<http://nihserver.mbi.ucla.edu/ERRAT/>). A sequence alignment of various nsLTPs was made using the ClustalW implemented in MEGA 5 software, and the alignment was manually inspected and adjusted via Jalview (<http://www.jalview.org/>).

Gene ontology analysis

The GO enrichment analysis was performed using the web-based tool AgriGO (<http://bioinfo.cau.edu.cn/agriGO/index.php>). The singular enrichment analysis (SEA) method was used to identify enriched GO terms, and the significantly enriched functional clusters were selected with an adjusted q-value less than 0.05. The program provides the output defining three categories of GO classification, namely, biological processes, cellular components and molecular functions.

Promoter analysis of maize nsLTP genes

To investigate *cis*-elements in promoter sequences of maize nsLTP genes, the upstream sequences (~1000 bp) of each identified *ZmLTPs* were retrieved from B73 maize genome sequence using a Perl script. The PlantCARE website (<http://bioinformatics.psb.ugent.be/webtools/plantcare/html/>) was used to identify *cis*-elements in the promoters [68]. To avoid biases in analysis, only *cis*-elements identified in at least ten different genes were considered further.

Microarray-based gene expression analysis

To gain an insight into the family-wide expression profile, microarray-based data analyses of both development and stress responses were carried out. In order to achieve the expression profilings of *ZmLTP* genes among different organs and development stages, transcriptome data of the genome-wide gene expression atlas of maize inbred line B73 were obtained from publicly available database PLEXdb with the accession number ZM37. Gene expression data of drought stress and pathogen infection were downloaded from GEO database with accession numbers GSE16567 and GSE10023, respectively. The microarray data of cold stress were downloaded from ArrayExpress

database with accession number E-MTAB-1315. To identify probe sets for *ZmLTP* genes, probe match against the maize GeneChip assemblies were performed on the Affymetrix Netaffx website (<http://www.affymetrix.com/estore/>) and PLEXdb. The data were normalized using a robust multi-chip average (RMA) algorithm [69]. Limma package was applied to data processing, and heatmaps representing log2-transformed probe intensities were generated with the gplots package [70]. A hierarchical clustering algorithm was applied to determine similar patterns in expression profiles. Using fold change >2 and *P* value <0.05, *ZmLTP* genes having changed expression profiles in at least one of the three conditions examined were regarded as differentially expressed genes under drought stress, whereas the response must be at least 2.8-fold in cold stress.

Differential gene expression profiles based on RNA-seq

Previous maize drought transcriptome performed by RNA-seq analysis was used to investigate the expression patterns of drought-responsive genes in drought-treated and well-watered fertilized reproductive (ovaries) and vegetative tissue (basal leaf meristem) [45]. The ovaries and the basal leaf meristem each have four libraries corresponding to well-watered and drought-stressed tissue, with one biological replicate respectively. The transcript abundance of each gene was estimated by fragments per kilobase of exon per million fragments mapped (FPKM). To achieve the expression patterns of *ZmLTP* genes in three root types (PR, SR and CR) under salt stress, the RNA-seq data were downloaded from the ArrayExpress database with accession number E-GEOD-53995. The transcript abundance of each gene was estimated by reads per kilobase per million mapped reads (RPKM).

Real-time PCR analysis

Maize seeds were incubated at 30°C for germination and sown in plastic pots containing sandy loam soil of 0.1 m³ each. Pots of each plant were divided into 3 groups with 2 replicates. Plant growth was carried out in a controlled condition of 14 h photoperiod, 28 ± 2°C day/night temperature and 55% air humidity. At 34 days from the date of sowing (at this time, all plants had 3–4 fully developed leaves), one set of pots was kept as control (well-watered) and two other sets were used for drought by withholding the water for 6 days. At the end of this period, the volumetric soil water content (measured at the 5 cm depth from the top of soil level) was approximately 12.5% for the drought-stressed plants, compared with approximately 30% for the control plants. The leaves of the seedlings were harvested, immediately frozen in liquid nitrogen and stored at -80°C. Then, total RNA was extracted from leaves after subjecting to drought stress using Trizol reagent, followed by DNase I treatment to remove any genomic DNA contamination.

About 2 µg of total RNA was reverse-transcribed using Takara RNA PCR Kit to generate cDNA. QRT-PCR was performed on iCycler iQ5 Multicolor real-time PCR detection system (Bio-Rad) by using Power SYBR Green PCR Master Mix (Applied Biosystems). Two biological replicates of each sample and three technical replicates of each biological replicate were used. The thermal cycling conditions were as follows: 50°C for 2 min, 95°C for 10 min, 40 cycles of 95°C for 15 s and 60°C for 1 min. Quantification of gene expression was performed by $2^{-\Delta C_t}$ method. Maizeubiquilin-1 (UBQ1) gene was used as internal control for normalization.

Availability of supporting data

All the supporting data are included as additional files.

Additional files

Additional file 1: Table S1. Maize genes encoding proteins with a Pfam domain PF00234 which belong to hybrid proline-rich proteins, alpha-amylase/trypsin inhibitors, prolamin storage proteins and 2S albumin storage proteins.

Additional file 2: Table S2. The occurrence of nsLTPs in maize, and some of their features.

Additional file 3: Table S3. Designations used for nsLTP sequences in this report, and their corresponding database accession numbers.

Additional file 4: Table S4. Sorghum genes encoding proteins with a Pfam domain PF00234 which belong to hybrid proline-rich proteins, alpha-amylase/trypsin inhibitors, prolamin storage proteins and 2S albumin storage proteins.

Additional file 5: Table S5. A summary of nsLTP genes in maize, sorghum, rice and Arabidopsis.

Additional file 6: Figure S1. Multiple sequence alignment of ZmLTPs. Amino acid sequences of the 8CMs were aligned using ClustalW implemented in MEGA5 software suite program and manually refined. The conserved cysteine residues are blue boxed.

Additional file 7: Table S6. Predicted phosphorylation sites in ZmLTPs.

Additional file 8: Figure S2. Unrooted phylogenetic tree between maize, sorghum, rice and Arabidopsis nsLTP gene families. Different species are denoted with different colors (maize in black, sorghum in green, rice in blue and Arabidopsis in red).

Additional file 9: Figure S3. The map of intron-exon arrangement of maize nsLTP genes. Introns and exons are drawn to scale with the full encoding regions of their respective. Exons are depicted as green boxes, introns as connecting thin lines and 8CM as red boxes. Non-translated regions, when supported by full-length cDNA sequences, are shown in blue boxes.

Additional file 10: Figure S4. The map of intron-exon arrangement of Arabidopsis and rice nsLTP genes. Introns and exons are drawn to scale with the full encoding regions of their respective. Exons are depicted as green boxes, introns as connecting thin lines and 8CM as red boxes. Non-translated regions, when supported by full-length cDNA sequences, are shown in blue boxes.

Additional file 11: Table S7. Syntenic sites harboring nsLTP genes in maize, sorghum and rice.

Additional file 12: Table S8. Genes list corresponding to the GO enrichment analysis by the AgriGO program.

Additional file 13: Table S9. Analysis of *cis*-elements in the 1 kb sequence upstream of the translation initiation codon in ZmLTP genes.

Additional file 14: Table S10. Average log2 signal intensity values and CV values of 75 detected ZmLTP transcripts in sixty distinct tissues representing eleven major organ systems of inbred line B73 ordered by three clusters.

Additional file 15: Table S11. The probe sets of ZmLTP genes on the maize 18 k GeneChip in this study.

Additional file 16: Figure S5. Normalized signal intensities of 41 probe sets representing 36 ZmLTP genes displayed for the microarray experiment, which was treated with *Ustilago maydis*. Log2 ratio values (shown by a green-red gradient) represents the fold change of the gene expression in the fungal infections.

Additional file 17: Table S12. Primers used for real time PCR analysis.

Additional file 18: Table S13. List of FPKM values of 42 ZmLTP genes in maize reproductive (ovary) and vegetative tissue (leaf) under both drought and well-watered conditions.

Additional file 19: Figure S6. Expression profiles of 38 ZmLTP genes in three root types [primary root (PR), seminal roots (SR) and crown roots (CR)] under salt stress.

Additional file 20: Table S14. The probe sets of ZmLTP genes on the maize 58 k NSF array in this study.

Additional file 21: Figure S7. Microarray-based expression profiles of 36 probe sets representing 34 ZmLTP genes under cold stress condition in two inbred maize lines, chilling-sensitive ETH-DL3 and chilling-tolerant ETH-DH7.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

KW conceptualized, designed the project. XZ and KW carried out computational analysis, microarray and RNA-seq expression profiling. KW and XZ performed the experiments. XZ and KW wrote the manuscript. Both authors read and approved the final manuscript.

Acknowledgments

We are grateful to the providers who submitted the microarray and RNA-seq data to the public expression databases which can be applied freely. We sincerely thank Dr. Yanmei Wang in Tsinghua University for revising our manuscript. We also thank Yanhui Chen, Yina Lin, Si Pan, and Xiaoyao Liu in our laboratory for useful discussions.

Received: 3 July 2014 Accepted: 9 October 2014

Published online: 28 October 2014

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doi:10.1186/s12870-014-0281-8

Cite this article as: Wei and Zhong: Non-specific lipid transfer proteins in maize. *BMC Plant Biology* 2014 **14**:281.

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Table S1. Maize genes encoding proteins with a Pfam domain PF00234 which belong to hybrid proline-rich proteins, alpha-amylase/trypsin inhibitors, prolamin storage proteins and 2S albumin storage proteins.

hybrid proline-rich proteins			
GRMZM2G379898	GRMZM2G351505	GRMZM2G037255	GRMZM2G094639
GRMZM2G059964	GRMZM2G372102	GRMZM2G372074	GRMZM2G345700
GRMZM2G162276	AC155352.2_FG010	GRMZM5G873271	GRMZM2G406313
GRMZM2G410338	GRMZM2G398807	GRMZM2G104945	GRMZM2G058208
GRMZM2G136367	GRMZM2G304378	GRMZM2G040689	AC234161.1_FG001
AC234161.1_FG002	GRMZM2G407189	GRMZM2G429000	GRMZM2G391272
GRMZM2G391286	GRMZM2G091534	GRMZM2G031354	GRMZM2G106324
GRMZM2G027221	GRMZM2G027167	GRMZM2G477697	GRMZM2G477685
alpha-amylase/trypsin inhibitors			
GRMZM2G304548	GRMZM2G404688		
prolamin storage proteins			
GRMZM2G060429	GRMZM2G138689	GRMZM2G138727	
2S albumin storage proteins			
GRMZM2G012148	GRMZM5G822180	GRMZM2G410134	

Table S2. The occurrence of non-specific lipid transfer proteins in maize, and some of their features.

¹ECM, eight-cysteine motif.

²AA, number of amino acids.

³Mw, molecular weight in Dalton.

⁴pI, isoelectric point (cysteine residues were not taken into account in the pI caculation).

⁵Subcellular target for the protein. S = secretory pathway, C = cytoplasm.

Name	Gene ID	Protein ID	Chr.	W/C	Locus	No. of introns in ORF	ECM ¹	Signal peptide		Mature protein		Subcellular localization ⁵	Sorghum Ortholog	Rice Ortholog	Arabidopsis Ortholog
								AA ²	AA	Mw ³	pI ⁴				
Type 1															
ZmLTP1.1	GRMZM2G126397	GRMZM2G126397_P01	3	W	184434264..184435351	0	CX{9}CX{14}CCX{19}CXCX{24}CX{13}C	28	95	9856.47	10.62	S	SbLTP1.2	OsLTP1.2, OsLTP1.4	AtLTP1.6, AtLTP1.10
ZmLTP1.2.1	GRMZM2G010868	GRMZM2G010868_P01	3	C	230606444..230608235	0	CX{10}CX{14}CCX{19}CXCX{22}CX{13}C	28	93	9087.23	11.4	S	SbLTP1.5, SbLTP1.6, SbLTP1.7	OsLTP1.21, OsLTP1.22, OsLTP1.14, OsLTP1.17, OsLTP1.18	AtLTP1.1, AtLTP1.4, AtLTP1.5, AtLTP1.8
ZmLTP1.2.2		GRMZM2G010868_P02	3	C	230607424..230608235	0	CX{10}CX{14}CCX{19}CXCX{22}CX{13}C	28	91	8873.99	11.4	S	SbLTP1.5, SbLTP1.6, SbLTP1.7	OsLTP1.21, OsLTP1.22, OsLTP1.14, OsLTP1.17, OsLTP1.18	AtLTP1.1, AtLTP1.4, AtLTP1.5, AtLTP1.8
ZmLTP1.3.1	GRMZM2G107839	GRMZM2G107839_P01	3	W	231823510..231824603	0	CX{9}CX{14}CCX{19}CXCX{22}CX{13}C	30	127	12983.2	10.11	S	SbLTP1.4	OsLTP1.12, OsLTP1.19, OsLTP1.20	AtLTP1.7, AtLTP1.11, AtLTP1.12
ZmLTP1.3.2		GRMZM2G107839_P02	3	W	231823868..231825026	0	CX{9}CX{14}CCX{19}CXCX{22}CX{13}C	30	93	9228.69	11.1	S	SbLTP1.4	OsLTP1.12, OsLTP1.19, OsLTP1.20	AtLTP1.7, AtLTP1.11, AtLTP1.12
ZmLTP1.4	GRMZM2G096234	GRMZM2G096234_P01	8	C	25946151..25947246	0	CX{9}CX{15}CCX{19}CXCX{23}CX{13}C	26	100	9890.46	4.53	S	SbLTP1.1	OsLTP1.1	-
ZmLTP1.5.1	GRMZM2G025026	GRMZM2G025026_P01	9	W	61863515..61865024	0	CX{9}CX{17}CCX{19}CXCX{21}CX{13}C	24	95	10163.43	4.52	S	SbLTP1.9	-	-
ZmLTP1.5.2		GRMZM2G025026_P02	9	W	61863624..61864642	0	CX{9}CX{17}CCX{19}CXCX{21}CX{13}C	24	93	9921.16	4.61	S	SbLTP1.9	-	-
ZmLTP1.6	GRMZM2G101958	GRMZM2G101958_P01	10	C	4165442..4166593	0	CX{9}CX{14}CCX{18}CXCX{22}CX{13}C	28	93	9026.15	11	S	SbLTP1.5, SbLTP1.6, SbLTP1.7	OsLTP1.21, OsLTP1.22, OsLTP1.14, OsLTP1.17, OsLTP1.18	AtLTP1.1, AtLTP1.4, AtLTP1.5, AtLTP1.8
ZmLTP1.7.1	GRMZM5G898755	GRMZM5G898755_P02	10	C	4243325..4244555	0	CX{9}CX{14}CCX{19}CXCX{23}CX{13}C	35	105	10164.64	9.86	S	SbLTP1.8	-	-
ZmLTP1.7.2		GRMZM5G898755_P01	10	C	4243574..4244555	0	CX{9}CX{14}CCX{19}CXCX{23}CX{13}C	35	94	9131.41	10.42	S	SbLTP1.8	-	-
ZmLTP1.8	GRMZM2G439268	GRMZM2G439268_P01	10	C	84608056..84608542	0	CX{9}CX{14}CCX{19}CXCX{22}CX{13}C	30	93	9799.24	5.21	S	SbLTP1.3	OsLTP1.7	-
Type 2															
ZmLTP2.1	GRMZM2G137329	GRMZM2G137329_P01	1	C	3000161..3000884	0	CX{7}CX{13}CCX{8}CXCX{23}CX{6}C	25	73	7399.45	11.05	S	SbLTP2.1, SbLTP2.3	OsLTP2.3, OsLTP2.7, OsLTP2.8, OsLTP2.9, OsLTP2.10, OsLTP2.11, OsLTP2.12	AtLTP2.5, AtLTP2.11, AtLTP2.12
ZmLTP2.2	GRMZM2G081464	GRMZM2G081464_P01	2	C	220468734..220469483	0	CX{7}CX{13}CCX{8}CXCX{23}CX{6}C	28	71	7474.71	11.38	S	SbLTP2.7	OsLTP2.6, OsLTP2.13	-
ZmLTP2.3	GRMZM2G406552	GRMZM2G406552_P01	4	C	195759880..195760460	0	CX{7}CX{13}CCX{8}CXCX{23}CX{6}C	19	78	7988.29	9.7	S	-	-	-
ZmLTP2.4	GRMZM2G387360	GRMZM2G387360_P01	5	W	23838144..23838818	0	CX{7}CX{13}CCX{8}CXCX{23}CX{6}C	28	68	7042.98	10.26	S	SbLTP2.1, SbLTP2.3	OsLTP2.3, OsLTP2.7, OsLTP2.8, OsLTP2.9, OsLTP2.10, OsLTP2.11, OsLTP2.12	AtLTP2.5, AtLTP2.11, AtLTP2.12
ZmLTP2.5	GRMZM2G039383	GRMZM2G039383_P01	6	C	86281901..86282589	0	CX{7}CX{12}CCX{8}CXCX{23}CX{6}C	20	74	7654.79	10.9	S	SbLTP2.7	OsLTP2.6, OsLTP2.13	-
ZmLTP2.6	GRMZM2G403007	GRMZM2G403007_P01	6	C	129702914..129703989	0	CX{7}CX{13}CCX{8}CXCX{23}CX{6}C	32	69	7214.41	10.28	S	SbLTP2.5	OsLTP2.4	AtLTP2.4, AtLTP2.9
ZmLTP2.7	GRMZM2G004909	GRMZM2G004909_P01	6	W	162646836..162647576	0	CX{7}CX{13}CCX{8}CXCX{23}CX{6}C	32	69	7114.24	10.16	S	SbLTP2.5	OsLTP2.4	AtLTP2.4, AtLTP2.9
ZmLTP2.8	GRMZM2G320373	GRMZM2G320373_P01	6	W	162662690..162663517	0	CX{7}CX{13}CCX{9}CXCX{23}CX{6}C	28	70	7370.69	11.61	S	SbLTP2.6	OsLTP2.5	-
ZmLTP2.9	GRMZM2G393150	GRMZM2G393150_P01	8	C	152130321..152131030	0	CX{7}CX{13}CCX{9}CXCX{23}CX{6}C	26	74	7797.14	10.36	S	SbLTP2.4	OsLTP2.2	-
Type C															
ZmLTPc1	AC225127.3_FG003	AC225127.3_FGP003	4	C	195060826..195061640	0	CX{9}CX{14}CCX{9}CXCX{12}CX{6}C	23	80	7855.96	8.76	S	SbLTPc2	OsLTPc1	AtLTPc2
ZmLTPc2.1	GRMZM2G073377	GRMZM2G073377_P01	7	W	141644107..141645231	0	CX{9}CX{19}CCX{9}CXCX{12}CX{6}C	41	68	6854.02	9.98	S	SbLTPc1	OsLTPc2	-
ZmLTPc2.2		GRMZM2G073377_P04	7	W	141644109..141644635	0	CX{9}CX{19}CCX{9}CXCX{12}CX{6}C	41	78	7922.28	10.02	S	SbLTPc1	OsLTPc2	-
Type D															
ZmLTPd1	GRMZM2G031102	GRMZM2G031102_P01	1	C	31896751..31897513	0	CX{14}CX{14}CCX{11}CXCX{24}CX{10}C	20	91	9301.72	9.4	S	SbLTPd9, SbLTPd11, SbLTPd12	OsLTPd5, OsLTPd6, OsLTPd8	AtLTPd7,AtLTPd8
ZmLTPd2	GRMZM2G071771	GRMZM2G071771_P01	2	W	10823770..10824501	0	CX{9}CX{16}CCX{9}CXCX{26}CX{7}C	26	77	8046.4	10.4	S	SbLTPd5	OsLTPd4	-
ZmLTPd3.1	GRMZM2G136364	GRMZM2G136364_P01	2	C	55687675..55688855	0	CX{14}CX{14}CCX{12}CXCX{24}CX{10}C	16	103	10110.55	9.51	S	SbLTPd9, SbLTPd11, SbLTPd12	OsLTPd5, OsLTPd6, OsLTPd8	AtLTPd7,AtLTPd8
ZmLTPd3.2		GRMZM2G136364_P02	2	C	55687675..55688855	0	CX{14}CX{14}CCX{12}CXCX{24}CX{10}C	16	103	10215.73	8.99	S	SbLTPd9, SbLTPd11, SbLTPd12	OsLTPd5, OsLTPd6, OsLTPd8	AtLTPd7,AtLTPd8
ZmLTPd4	GRMZM2G099867	GRMZM2G099867_P01	2	W	127959950..127960704	0	CX{14}CX{14}CCX{11}CXCX{24}CX{10}C	20	91	9301.72	9.4	S	SbLTPd9, SbLTPd11, SbLTPd12	OsLTPd5, OsLTPd6, OsLTPd8	AtLTPd7,AtLTPd8
ZmLTPd5	GRMZM2G164440	GRMZM2G164440_P01	2	C	156327934..156329194	0	CX{10}CX{17}CCX{9}CXCX{24}CX{7}C	-	-	-	-	-	SbLTPd4	OsLTPd3	AtLTPd1,AtLTPd2
ZmLTPd6	GRMZM2G087413	GRMZM2G087413_P01	3	W	138623892..138624577	1	CX{10}CX{17}CCX{9}CXCX{22}CX{9}C	20	87	9889.67	9.27	S	SbLTPd7, SbLTPd8, SbLTPd13	OsLTPd9, OsLTPd10, OsLTPd13	AtLTPd10
ZmLTPd7	GRMZM2G396418	GRMZM2G396418_P01	3	C	162372093..162372586	0	CX{10}CX{18}CCX{9}CXCX{22}CX{8}C	28	78	8282.39	9.69	S	-	-	-
ZmLTPd8	GRMZM2G065557	GRMZM2G065557_P01	3	W	179414637..179416401	0	CX{14}CX{14}CCX{11}CXCX{24}CX{10}C	27	91	9360.23	11.37	S	SbLTPd9, SbLTPd11, SbLTPd12	OsLTPd5, OsLTPd6, OsLTPd8	AtLTPd7,AtLTPd8
ZmLTPd9.1	GRMZM2G094632	GRMZM2G094632_P02	5	W	39411646..39412216	0	CX{10}CX{16}CCX{11}CXCX{23}CX{7}C	24	101	11106.72	5.7	S	SbLTPd1	OsLTPd11	-
ZmLTPd9.2		GRMZM2G094632_P01	5	W	39411646..39412902	0	CX{10}CX{16}CCX{11}CXCX{23}CX{7}C	24	92	9939.33	6.82	S	SbLTPd1	OsLTPd11	-
ZmLTPd10	AC233926.1_FG002	AC233926.1_FGP002	7	W	30954067..30954378	0	CX{9}CX{16}CCX{9}CXCX{26}CX{7}C	26	77	8046.4	10.4	S	SbLTPd5	OsLTPd4	-
ZmLTPd11	GRMZM2G000221	GRMZM2G000221_P01	7	W	31086409..31087079	0	CX{9}CX{16}CCX{9}CXCX{26}CX{7}C	27	78	8153.49	6.77	S	SbLTPd6	-	-
ZmLTPd12	GRMZM2G471051	GRMZM2G471051_P01	8	C	162752046..162752849	0	CX{9}CX{16}CCX{9}CXCX{21}C{8}C	29	78	8162.44	7.98	S	-	OsLTPd2	-
ZmLTPd13	GRMZM2G170969	GRMZM2G170969_P01	8	C	162756043..162756672	0	CX{10}CX{11}CCX{9}CXCX{22}CX{6}C	29	71	7437.4	9.19	S	-	-	-
ZmLTPd14	GRMZM2G091054	GRMZM2G091054_P01	8	C	171565757..171566487	1	CX{10}CX{17}CCX{9}CXCX{22}CX{9}C	24	87	9554.06	6.78	S	SbLTPd7, SbLTPd8, SbLTPd13	OsLTPd9, OsLTPd10, OsLTPd13	AtLTPd10
ZmLTPd15	GRMZM2G155555	GRMZM2G155555_P01	10	C	96605920..96606680	1	CX{10}CX{15}CCX{9}CXCX{22}CX{9}C	27	93	10346.2	9.84	S	-	OsLTPd14	-
ZmLTPd16	GRMZM2G063375	GRMZM2G063375_P01	10	C	96617537..96618949	1	CX{10}CX{15}CCX{9}CXCX{22}CX{9}C	27	93	10302.08	9.46	S	-	OsLTPd14	-
Type G															
ZmLTPg1.1		GRMZM5G850455_P01	1	W	14103040..14104304	0	CX{9}CX{14}CCX{12}CXCX{26}CX{9}C	23	188	19357.29	9.13	S	SbLTPg8, SbLTPg12	OsLTPg2, OsLTPg19	AtLTPg6, AtLTPg13, AtLTPg14
ZmLTPg1.2	GRMZM5G850455	GRMZM5G850455_P02	1	W	14103040..14104361	2	CX{9}CX{14}CCX{12}CXCX{26}CX{9}C	23	169	16940.38	8.21	S	SbLTPg8, SbLTPg12	OsLTPg2, OsLTPg19	AtLTPg6, AtLTPg13, AtLTPg14
ZmLTPg1.3		GRMZM5G850455_P03	1	W	14103040..14104361	1	CX{9}CX{14}CCX{12}CXCX{26}CX{9}C	23	224	22784.06	8.96	S	SbLTPg8, SbLTPg12	OsLTPg2, OsLTPg19	AtLTPg6, AtLTPg13, AtLTPg14
ZmLTPg2	GRMZM2G174680	GRMZM2G174680_P01	1	C	52625970..52627201	1	CX{9}CX{16}CCX{13}CXCX{21}CX{9}C	29	160	15631.12	6.38	S	SbLTPg6	OsLTPg4	-
ZmLTPg3	GRMZM2G078876	GRMZM2G078876_P01	1	C	66023613..66026784	2	CX{9}CX{16}CCX{14}CXCX{25}CX{9}C	25	156	15026.19	8.71	S	SbLTPg5	OsLTPg5, AtLTPg10	-
ZmLTPg4	GRMZM2G083725	GRMZM2G083725_P01	1	C	194657095..194659202	2	CX{9}CX{14}CCX{12}CXCX{29}CX{9}C	23	158	15977.04	6.97	S	-	-	-

ZmLTPg5	GRMZM2G097137	GRMZM2G097137_P01	1	W	263198227..263201072	1	CX{9}CX{15}CCX{12}CXCX{27}CX{9}C	28	133	12510.03	8.38	S	SbLTPg3	-	-
ZmLTPg6	GRMZM2G006047	GRMZM2G006047_P02	1	C	287110810..287111891	2	CX{9}CX{14}CCX{12}CXCX{26}CX{9}C	32	150	14309.15	9.87	S	SbLTPx1, SbLTPg9,	OsLTPg7, OsLTPg14, OsLTPg16, OsLTPg18	AtLTPg15, AtLTPg19, AtLTPG26
ZmLTPg7	GRMZM2G005991	GRMZM2G005991_P01	1	C	287146113..287147400	3	CX{10}CX{17}CCX{12}CXCX{24}CX{8}C	27	174	17391.17	8.63	S	SbLTPg4	OsLTPg8	AtLTPg16, AtLTPg20
ZmLTPg8.1	GRMZM2G065640	GRMZM2G065640_P01	1	W	287188063..287188991	2	CX{9}CX{14}CCX{12}CXCX{25}CX{9}C	24	175	17239.55	8.21	S	SbLTPg2	OsLTPg9	-
ZmLTPg8.2		GRMZM2G065640_P02	1	W	287188063..287188991	2	CX{9}CX{14}CCX{12}CXCX{25}CX{9}C	24	158	15377.38	4.66	S	SbLTPg2	OsLTPg9	-
ZmLTPg9	GRMZM2G168833	GRMZM2G168833_P01	1	W	289786005..289788499	2	CX{9}CX{16}CCX{12}CXCX{26}CX{8}C	-	-	-	-	-	SbLTPg1	OsLTPg10	-
ZmLTPg10	GRMZM2G414620	GRMZM2G414620_P01	2	C	41484533..41485576	1	CX{9}CX{14}CCX{12}CXCX{24}CX{10}C	35	180	18249.23	9.95	S	SbLTPg19	-	-
ZmLTPg11	GRMZM2G151021	GRMZM2G151021_P01	2	W	47422297..47424522	2	CX{12}CX{8}CCX{14}CXCX{24}CX{13}C	27	190	19051.12	6.55	S	SbLTPg18	OsLTPg29	-
ZmLTPg12	AC194203.3_FG003	AC194203.3_FGP003	2	C	212487102..212487524	0	CX{9}CX{16}CCX{14}CXCX{22}CX{9}C	22	118	11730.54	7.99	S	SbLTPg13	OsLTPg21	-
ZmLTPg13	GRMZM2G176347	GRMZM2G176347_P01	2	C	233632142..233633151	2	CX{10}CX{18}CCX{12}CXCX{24}CX{8}C	25	170	16664.09	11.1	S	SbLTPg11	OsLTPg17	-
ZmLTPg14	GRMZM2G071575	GRMZM2G071575_P01	4	C	45262758..45263990	2	CX{9}CX{14}CCX{12}CXCX{29}CX{9}C	25	160	16219.39	6.97	S	SbLTPg21	OsLTPg22	AtLTPg4
ZmLTPg15	GRMZM2G145054	GRMZM2G145054_P01	4	W	126473476..126474249	1	C{6}CX{13}CCX{12}CXCX{25}CX{5}C	-	-	-	-	-	SbLTPg16	-	-
ZmLTPg16	GRMZM2G130454	GRMZM2G130454_P03	5	W	4429841..4430924	2	CX{9}CX{14}CCX{12}CXCX{41}CX{20}C	32	160	16243.38	11.34	S	-	-	-
ZmLTPg17.1	GRMZM2G141858	GRMZM2G141858_P01	5	W	49545870..49548495	2	CX{6}CX{13}CCX{12}CXCX{25}CX{8}C	25	124	12692.44	6.93	S	SbLTPg23	OsLTPg13	AtLTPg7
ZmLTPg17.2		GRMZM2G141858_P02	5	W	49545872..49548469	1	CX{6}CX{13}CCX{12}CXCX{25}CX{8}C	25	115	11827.49	6.93	S	SbLTPg23	OsLTPg13	AtLTPg7
ZmLTPg18	GRMZM2G116167	GRMZM2G116167_P01	5	W	59978801..59980128	2	CX{6}CX{15}CCX{12}CXCX{25}CX{8}C	26	149	14486.34	10.8	S	SbLTPg24	OsLTPg24	-
ZmLTPg19	GRMZM2G089400	GRMZM2G089400_P01	6	C	89065818..89068077	2	CX{5}CX{13}CCX{12}CXCX{25}CX{8}C	25	124	12805.72	8.27	S	SbLTPg23	OsLTPg13	AtLTPg7
ZmLTPg20.1	GRMZM2G089288	GRMZM2G089288_P03	7	W	8901782..8903227	2	CX{9}CX{14}CCX{12}CXCX{25}CX{9}C	46	140	13977	4.27	S	-	OsLTPg15	-
ZmLTPg20.2		GRMZM2G089288_P01	7	W	8901856..8903227	2	CX{9}CX{14}CCX{12}CXCX{25}CX{9}C	29	140	13977	4.27	S	-	OsLTPg15	-
ZmLTPg21	GRMZM2G046750	GRMZM2G046750_P01	7	W	9201072..9202357	2	CX{10}CX{14}CCX{12}CXCX{24}CX{8}C	33	187	17984.37	5.04	C	SbLTPg10	-	-
ZmLTPg22	GRMZM2G170044	GRMZM2G170044_P01	7	W	16096163..16099019	2	CX{9}CX{16}CCX{12}CXCX{26}CX{8}C	28	178	17674.11	9.05	S	SbLTPg8, SbLTPg12	OsLTPg2, OsLTPg19	AtLTPg6, AtLTPg13, AtLTPg14
ZmLTPg23	GRMZM2G171597	GRMZM2G171597_P01	7	C	167020090..167021470	2	CX{9}CX{16}CCX{14}CXCX{22}CX{9}C	24	145	14127.38	10.37	S	SbLTPg13	OsLTPg21	-
ZmLTPg24	GRMZM2G379035	GRMZM2G379035_P01	8	C	171016014..171017268	2	CX{9}CX{16}CCX{12}CXCX{25}CX{9}C	27	167	16275.83	5.14	S	SbLTPg15, SbLTPg22	OsLTPg1, OsLTPg12	AtLTPg21
ZmLTPg25	GRMZM2G004466	GRMZM2G004466_P01	9	W	149245864..149247159	2	CX{9}CX{16}CCX{12}CXCX{24}CX{9}C	28	175	17097.42	8.13	S	SbLTPg7	OsLTPg3	AtLTPg11, AtLTPg31
ZmLTPg26	GRMZM2G140646	GRMZM2G140646_P01	10	C	15003990..15007027	2	CX{9}CX{21}CCX{18}CXCX{24}CX{20}C	51	183	18124.19	5.51	S	SbLTPg20	OsLTPg28	-
Single															
ZmLTPx1.1		GRMZM2G036063_P01	7	W	45409454..45410733	1	CX{9}CX{16}CCX{13}CXCX{26}CX{12}C	24	144	14941.3	4.87	S	SbLTPx3	-	-
ZmLTPx1.2	GRMZM2G036063	GRMZM2G036063_P02	7	W	45409455..45410645	1	CX{9}CX{16}CCX{13}CXCX{26}CX{12}C	24	145	15097.49	5.14	S	SbLTPx3	-	-
ZmLTPx1.3		GRMZM2G036063_P03	7	W	45409465..45410441	0	CX{9}CX{16}CCX{13}CXCX{26}CX{12}C	24	130	13711.83	6.68	S	SbLTPx3	-	-
ZmLTPx2	GRMZM2G166484	GRMZM2G166484_P01	10	C	118762389..118764275	2	CX{12}CX{8}CCX{14}CXCX{24}CX{13}C	21	170	17367.16	11.39	S	-	-	-

Table S3. Designations used for nsLTP sequences in this report, and their corresponding database accession numbers.

Arabidopsis			Rice			Sorghum		
Name	Locus	Group	Name	Locus	Group	Name	Locus	Group
AtLTP1.1	AT2G15050.3	Type 1	OsLTP1.1	LOC_Os01g12020.1	Type 1	SbLTP1.1	Sb03g001580.1	Type 1
AtLTP1.2	AT2G15325.1	Type 1	OsLTP1.2	LOC_Os01g60740.1	Type 1	SbLTP1.2	Sb03g038280.1	Type 1
AtLTP1.3	AT2G18370.1	Type 1	OsLTP1.3	LOC_Os03g59380.1	Type 1	SbLTP1.3	Sb07g002600.1	Type 1
AtLTP1.4	AT2G38530.1	Type 1	OsLTP1.4	LOC_Os05g40010.1	Type 1	SbLTP1.4	Sb08g002660.1	Type 1
AtLTP1.5	AT2G38540.1	Type 1	OsLTP1.5	LOC_Os06g06340.1	Type 1	SbLTP1.5	Sb08g002670.1	Type 1
AtLTP1.6	AT3G08770.1	Type 1	OsLTP1.6	LOC_Os06g34840.1	Type 1	SbLTP1.6	Sb08g002680.1	Type 1
AtLTP1.7	AT3G51590.1	Type 1	OsLTP1.7	LOC_Os08g03690.1	Type 1	SbLTP1.7	Sb08g002690.1	Type 1
AtLTP1.8	AT3G51600.1	Type 1	OsLTP1.9	LOC_Os11g02350.1	Type 1	SbLTP1.8	Sb08g002700.1	Type 1
AtLTP1.9	AT4G33355.1	Type 1	OsLTP1.12	LOC_Os11g02400.1	Type 1	SbLTP1.9	Sb10g021170.1	Type 1
AtLTP1.10	AT5G01870.1	Type 1	OsLTP1.13	LOC_Os11g02424.2	Type 1	SbLTP2.1	Sb01g017700.1	Type 2
AtLTP1.11	AT5G59310.1	Type 1	OsLTP1.14	LOC_Os11g24070.1	Type 1	SbLTP2.2	Sb01g017710.1	Type 2
AtLTP1.12	AT5G59320.1	Type 1	OsLTP1.16	LOC_Os12g02300.1	Type 1	SbLTP2.3	Sb01g049830.1	Type 2
AtLTP1.13	AT4G28395.1	Type 1	OsLTP1.17	LOC_Os12g02310.1	Type 1	SbLTP2.4	Sb03g031720.1	Type 2
AtLTP2.2	AT1G43666.1	Type 2	OsLTP1.18	LOC_Os12g02320.1	Type 1	SbLTP2.5	Sb09g027590.1	Type 2
AtLTP2.3	AT1G43667.1	Type 2	OsLTP1.19	LOC_Os12g02330.2	Type 1	SbLTP2.6	Sb09g027600.1	Type 2
AtLTP2.4	AT1G48750.1	Type 2	OsLTP1.20	LOC_Os12g02340.1	Type 1	SbLTP2.7	Sb10g029330.1	Type 2
AtLTP2.5	AT1G66850.1	Type 2	OsLTP1.21	LOC_Os11g02369.1	Type 1	SbLTPc1	Sb02g030420.1	Type C
AtLTP2.6	AT1G73780.1	Type 2	OsLTP1.22	LOC_Os11g02389.1	Type 1	SbLTPc2	Sb07g025160.1	Type C
AtLTP2.7	AT2G14846.1	Type 2	OsLTP2.1	LOC_Os01g49640.1	Type 2	SbLTPd1	Sb01g026220.1	Type D
AtLTP2.8	AT3G12545	Type 2	OsLTP2.2	LOC_Os01g49650.1	Type 2	SbLTPd2	Sb01g026225.1	Type D
AtLTP2.9	AT3G18280.1	Type 2	OsLTP2.3	LOC_Os03g02050.1	Type 2	SbLTPd3	Sb01g026230.1	Type D
AtLTP2.10	AT3G57310.1	Type 2	OsLTP2.4	LOC_Os05g47700.1	Type 2	SbLTPd4	Sb02g009300.1	Type D
AtLTP2.11	AT5G38160.1	Type 2	OsLTP2.5	LOC_Os05g47730.1	Type 2	SbLTPd5	Sb02g009320.1	Type D
AtLTP2.12	AT5G38170.1	Type 2	OsLTP2.6	LOC_Os06g49190.1	Type 2	SbLTPd6	Sb02g009340.1	Type D
AtLTP2.13	AT5G38180.1	Type 2	OsLTP2.7	LOC_Os10g36070.1	Type 2	SbLTPd7	Sb03g037210.1	Type D
AtLTP2.14	AT5G38195.1	Type 2	OsLTP2.8	LOC_Os10g36090.1	Type 2	SbLTPd8	Sb03g037220.1	Type D
AtLTPc1	AT5G07230.1	Type C	OsLTP2.9	LOC_Os10g36100.1	Type 2	SbLTPd9	Sb03g039880.1	Type D
AtLTPc2	AT5G52160.1	Type C	OsLTP2.10	LOC_Os10g36110.1	Type 2	SbLTPd10	Sb03g043610.1	Type D
AtLTPc3	AT5G62080.1	Type C	OsLTP2.11	LOC_Os10g36160.1	Type 2	SbLTPd11	Sb06g016160.1	Type D
AtLTPd1	AT5G48485.1	Type D	OsLTP2.12	LOC_Os10g36170.1	Type 2	SbLTPd12	Sb06g016170.1	Type D
AtLTPd2	AT5G48490.1	Type D	OsLTP2.13	LOC_Os11g40530.1	Type 2	SbLTPd13	Sb08g005360.1	Type D
AtLTPd3	AT5G55410.1	Type D	OsLTPc1	LOC_Os08g43290.1	Type C	SbLTPg1	Sb01g004610.1	Type G
AtLTPd4	AT5G55450.1	Type D	OsLTPc2	LOC_Os09g35700.1	Type C	SbLTPg2	Sb01g005410.1	Type G
AtLTPd5	AT5G55460.1	Type D	OsLTPd1	LOC_Os01g68580.1	Type D	SbLTPg3	Sb01g011270.1	Type G
AtLTPd6	AT2G37870.1	Type D	OsLTPd2	LOC_Os01g68589.1	Type D	SbLTPg4	Sb01g005420.1	Type G
AtLTPd7	AT3G53980.1	Type D	OsLTPd3	LOC_Os07g18750.1	Type D	SbLTPg5	Sb01g033830.1	Type G
AtLTPd8	AT5G05960.1	Type D	OsLTPd4	LOC_Os07g18990.1	Type D	SbLTPg6	Sb01g036750.1	Type G
AtLTPd9	AT1G32280.1	Type D	OsLTPd5	LOC_Os01g62980.1	Type D	SbLTPg7	Sb01g044330.1	Type G
AtLTPd10	AT4G30880.1	Type D	OsLTPd6	LOC_Os04g33920.1	Type D	SbLTPg8	Sb01g046080.1	Type G
AtLTPd11	AT4G33550.2	Type D	OsLTPd7	LOC_Os04g33930.2	Type D	SbLTPg9	Sb02g004130.1	Type G

AtLTPd12	AT5G56480.1	Type D	OsLTPd8	LOC_Os05g06780.1	Type D	SbLTPg10	Sb02g004200.1	Type G
AtLTPe1	AT3G07450.1	Type E	OsLTPd9	LOC_Os01g58650.1	Type D	SbLTPg11	Sb02g004210.1	Type G
AtLTPe2	AT3G52130.1	Type E	OsLTPd10	LOC_Os01g58660.1	Type D	SbLTPg12	Sb02g005900.1	Type G
AtLTPg1	AT1G03103.1	Type G	OsLTPd11	LOC_Os10g05720.2	Type D	SbLTPg13	Sb02g039860.1	Type G
AtLTPg2	AT1G05450.2	Type G	OsLTPd12	LOC_Os11g29420.1	Type D	SbLTPg14	Sb02g041710.1	Type G
AtLTPg3	AT1G18280.1	Type G	OsLTPd13	LOC_Os03g25350.1	Type D	SbLTPg15	Sb03g037810.1	Type G
AtLTPg4	AT1G27950.1	Type G	OsLTPd14	LOC_Os11g03870.1	Type D	SbLTPg16	Sb04g024055.1	Type G
AtLTPg5	AT1G36150.1	Type G	OsLTPg1	LOC_Os01g59870.1	Type G	SbLTPg17	Sb05g022630.1	Type G
AtLTPg6	AT1G55260.1	Type G	OsLTPg2	LOC_Os03g07100.1	Type G	SbLTPg18	Sb06g017510.1	Type G
AtLTPg7	AT1G62790.1	Type G	OsLTPg3	LOC_Os03g09230.1	Type G	SbLTPg19	Sb06g019070.1	Type G
AtLTPg8	AT1G73550.1	Type G	OsLTPg4	LOC_Os03g20760.1	Type G	SbLTPg20	Sb06g020910.1	Type G
AtLTPg9	AT1G73560.1	Type G	OsLTPg5	LOC_Os03g26800.1	Type G	SbLTPg21	Sb07g026120.1	Type G
AtLTPg10	AT1G73890.1	Type G	OsLTPg6	LOC_Os03g26820.1	Type G	SbLTPg22	Sb09g023870.1	Type G
AtLTPg11	AT2G13820.1	Type G	OsLTPg7	LOC_Os03g57970.1	Type G	SbLTPg23	Sb10g027810.1	Type G
AtLTPg12	AT2G27130.1	Type G	OsLTPg8	LOC_Os03g57980.1	Type G	SbLTPg24	Sb10g029740.1	Type G
AtLTPg13	AT2G44290.1	Type G	OsLTPg9	LOC_Os03g57990.1	Type G	SbLTPx1	Sb01g005430.1	single
AtLTPg14	AT2G44300.1	Type G	OsLTPg10	LOC_Os03g58940.1	Type G	SbLTPx2	Sb02g011135.1	single
AtLTPg15	AT2G48130.1	Type G	OsLTPg11	LOC_Os04g38840.1	Type G	SbLTPx3	Sb02g011205.1	single
AtLTPg16	AT2G48140.1	Type G	OsLTPg12	LOC_Os05g41030.1	Type G			
AtLTPg19	AT3G22600.1	Type G	OsLTPg13	LOC_Os06g47200.1	Type G			
AtLTPg20	AT3G22620.1	Type G	OsLTPg14	LOC_Os07g07790.1	Type G			
AtLTPg21	AT3G43720.1	Type G	OsLTPg15	LOC_Os07g07870.1	Type G			
AtLTPg22	AT3G58550.1	Type G	OsLTPg16	LOC_Os07g07860.1	Type G			
AtLTPg23	AT4G08670.1	Type G	OsLTPg17	LOC_Os07g07920.1	Type G			
AtLTPg24	AT4G12360.1	Type G	OsLTPg18	LOC_Os07g07930.1	Type G			
AtLTPg25	AT4G14805.1	Type G	OsLTPg19	LOC_Os07g09970.1	Type G			
AtLTPG26	AT4G14815.1	Type G	OsLTPg20	LOC_Os07g30590.1	Type G			
AtLTPG27	AT4G22630.1	Type G	OsLTPg21	LOC_Os07g43290.1	Type G			
AtLTPG28	AT4G22666.1	Type G	OsLTPg22	LOC_Os08g42040.1	Type G			
AtLTPG29	AT5G09370.1	Type G	OsLTPg23	LOC_Os11g37320.1	Type G			
AtLTPG30	AT5G13900.1	Type G	OsLTPg24	LOC_Os06g49770.1	Type G			
AtLTPG31	AT5G64080.1	Type G	OsLTPg25	LOC_Os11g37280.1	Type G			
AtLTPg17	AT3G22570.1	single	OsLTPg28	LOC_Os01g42210.1	Type G			
AtLTPg18	AT3G22580.1	single	OsLTPg29	LOC_Os03g46110.1	Type G			
AtLTPG33	AT4G22640.1	single	OsLTPx1	LOC_Os03g44000.1	single			
AtLTPG34	AT4G22650.1	single	OsLTPx2	LOC_Os07g27940.1	single			
AtLTPx1	AT1G52415.1	single	OsLTPx3	LOC_Os11g34660.1	single			
AtLTPx2	AT1G64235.1	single						
AtLTPx3	AT4G08530.1	single						

Table S4. Sorghum genes encoding proteins with a Pfam domain PF00234 which belong to hybrid proline-rich proteins, alpha-amylase/trypsin inhibitors, prolamin storage proteins and 2S albumin storage proteins.

hybrid proline-rich proteins			
Sb02g032910	Sb02g032920	Sb02g037735	Sb02g037740
Sb09g024070	Sb02g041700	Sb05g000960	Sb02g011200
Sb02g011170	Sb02g011190	Sb04g032620	Sb04g032650
Sb06g024800	Sb10g025210	Sb01g029450	Sb10g000510
Sb06g028200	Sb01g029630	Sb01g029620	Sb06g030490
Sb05g003860	Sb01g009990	Sb06g030500	Sb10g004790
Sb01g050420	Sb06g024770	Sb04g032640	Sb04g032670
Sb01g029610	Sb04g032660	Sb01g050360	Sb04g032680
Sb06g024780	Sb06g024790		
alpha-amylase/trypsin inhibitors			
Sb02g006470	Sb02g006550	Sb02g006480	Sb02g002495
Sb02g006570	Sb09g024570	Sb02g006560	Sb02g002500
prolamin storage proteins			
Sb02g025490	Sb02g025510	Sb09g000360	
2S albumin storage proteins			
Sb02g004220	Sb01g012630	Sb01g012620	

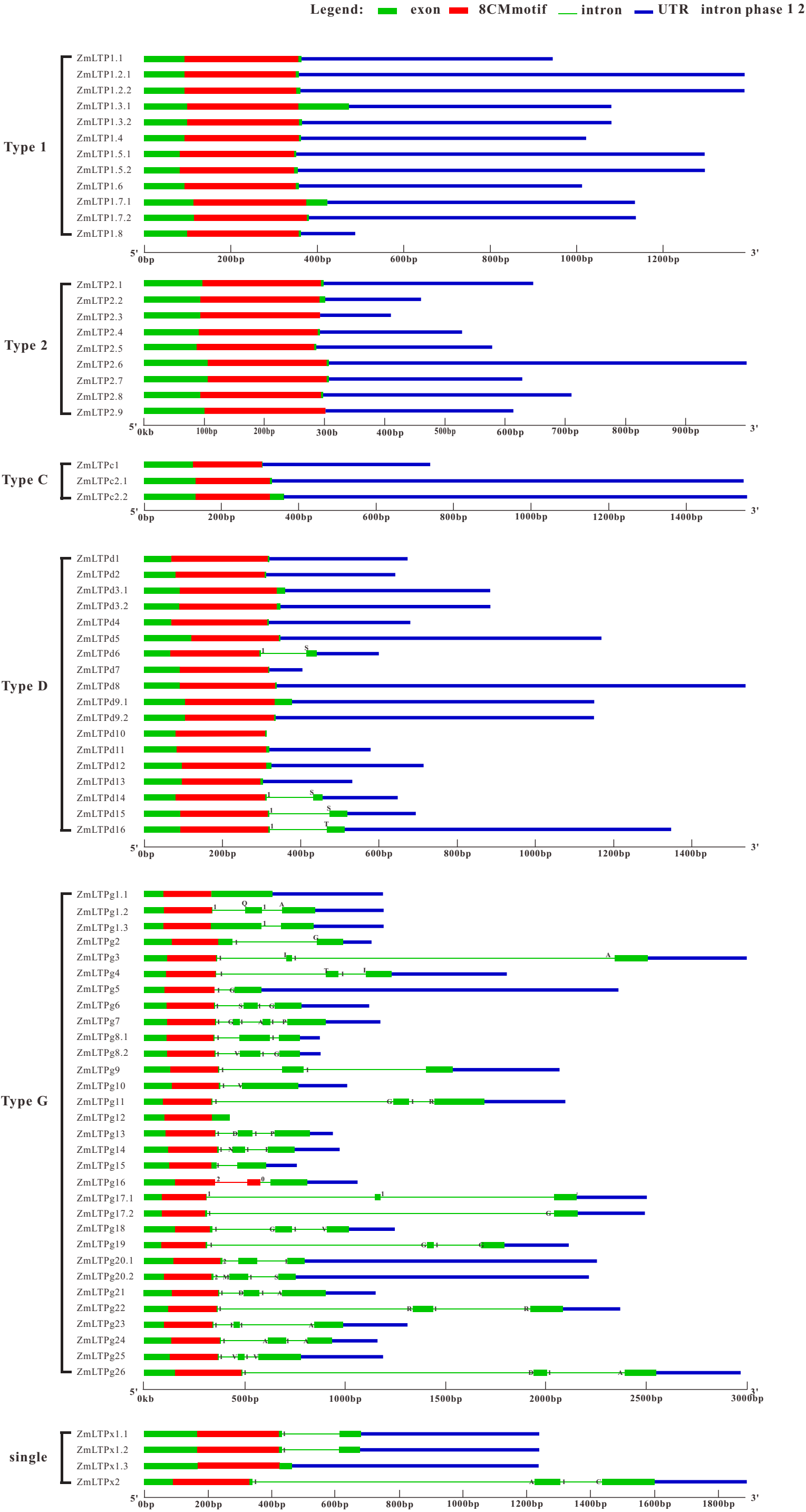
Table S5. A summary of nsLTP genes in maize, sorghum, rice and Arabidopsis.

Group	<i>Zea mays</i>	<i>Sorghum bicolor</i>	<i>Oryza sativa</i>	<i>A. thaliana</i>
Type 1	8	9	18	13
Type 2	9	7	13	13
Type C	2	2	2	3
Type D	16	13	14	12
Type E	0	0	0	2
Type G	26	24	27	29
single	2	3	3	7
Tota	63	58	77	79

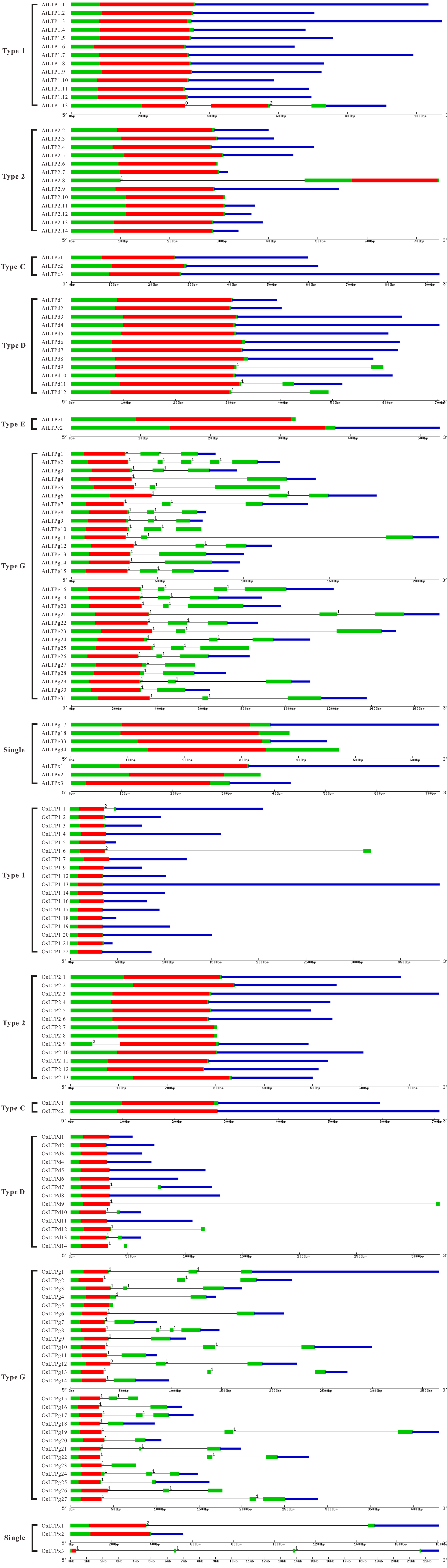
[illegible]

Table S6. Predicted phosphorylation sites in ZmLTPs.¹phosphorylation site.²phosphorylated residue.

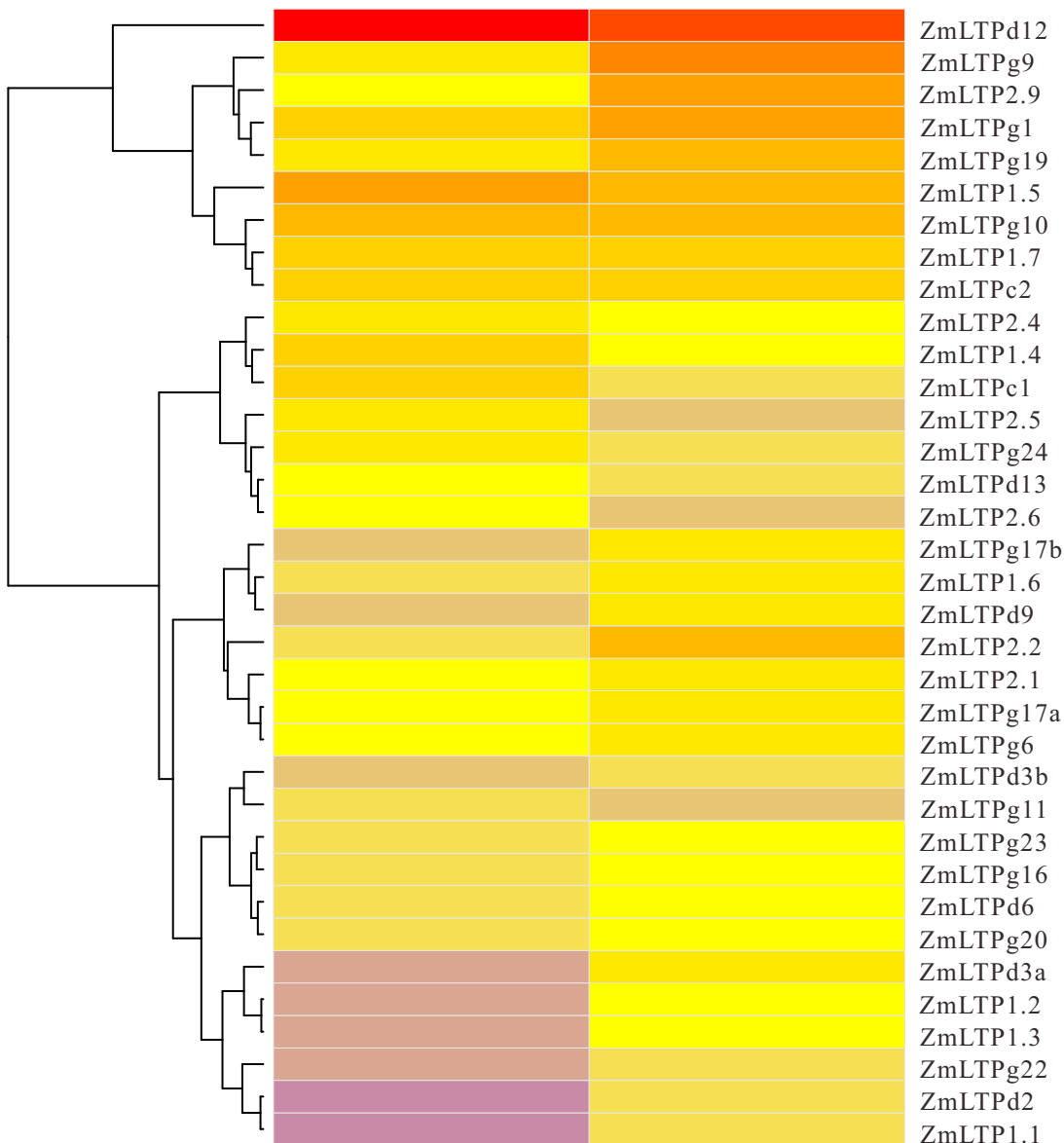
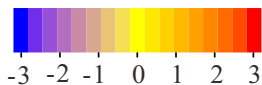
Name	Full length	Position ¹	Amino acide ²	Name	Full length	Position ¹	Amino acid ²
ZmLTP1.5.2	117	110	theonine	ZmLTPg13	195	59	serine
ZmLTP2.4	96	79	serine			122	serine
ZmLTPd1	111	49	theonine			132	theonine
ZmLTPd4	111	49	theonine	ZmLTPg14	185	144	theonine
ZmLTPg1.1	211	126	serine			150	serine
		183	serine	ZmLTPg15	166	11	serine
ZmLTPg1.2	192	128	serine			17	serine
ZmLTPg1.3	247	126	serine	ZmLTPg16	192	84	serine
		183	serine	ZmLTPg17.1	149	106	serine
ZmLTPg2	189	153	theonine			110	serine
ZmLTPg3	181	148	serine			114	serine
		156	serine	ZmLTPg19	149	106	serine
ZmLTPg5	161	130	theonine			110	serine
ZmLTPg6	182	84	serine			114	serine
		127	theonine	ZmLTPg21	220	131	serine
		130	theonine	ZmLTPg23	169	136	theonine
ZmLTPg7	201	11	serine			148	serine
		162	serine	ZmLTPg24	194	166	serine
ZmLTPg8.1	199	124	serine	ZmLTPg25	203	30	theonine
		158	serine			33	serine
		163	serine			116	theonine
		170	serine			148	serine
		175	serine			150	theonine
ZmLTPg8.2	182	124	serine			155	theonine
		147	theonine			173	serine
		158	serine	ZmLTPg26	234	183	serine
ZmLTPg10	215	178	serine			211	theonine
		185	theonine	ZmLTPx1.2	169	161	serine
ZmLTPg11	217	114	serine	ZmLTPx2	191	141	theonine
		188	theonine				



Legend: exon 8CMmotif intron UTR intron phase 1 2



log_{2.8}-fold change



ETH-DL3

ETH-DH7

Table S9. Analysis of *cis*-elements in the 1 kb sequence upstream of the translation initiation codon in ZmLTP genes.

<i>cis</i> -lement	Function	ZmLTP1.1	ZmLTP1.2	ZmLTP1.3	ZmLTP1.4	ZmLTP1.5	ZmLTP1.6	ZmLTP1.7	ZmLTP1.8	ZmLTP2.1
RY-element	<i>cis</i> -acting regulatory element involved in seed-specific regulation									
O2-site ^[18]	<i>cis</i> -acting regulatory element involved in zein metabolism regulation				1		1			
CAT-box	<i>cis</i> -acting regulatory element related to meristem expression							1	1	1
CCGTCC-box	<i>cis</i> -acting regulatory element related to meristem specific activation				4		1			1
NON-box	<i>cis</i> -acting regulatory element related to meristem specific activation									
OCT	<i>cis</i> -acting regulatory element related to meristem specific activation									
Skn-1_motif	<i>cis</i> -acting regulatory element required for endosperm expression	1	1	2	1	2	1	1	3	2
AACA_motif	involved in endosperm-specific negative expression									
GCN4_motif ^[25]	<i>cis</i> -regulatory element involved in endosperm expression					1	1			1
circadian ^[9]	<i>cis</i> -acting regulatory element involved in circadian control		1	2					2	
MSA-like	<i>cis</i> -acting element involved in cell cycle regulation						1		1	
as-2-box ^[8]	involved in shoot-specific expression and light responsiveness							1		
ACE ^[15]	<i>cis</i> -acting element involved in light responsiveness								1	
C-box	<i>cis</i> -acting regulatory element involved in light responsiveness				1					
G-Box ^[10]	<i>cis</i> -acting regulatory element involved in light responsiveness				1		3	6	2	
G-box	<i>cis</i> -acting regulatory element involved in light responsiveness	1	1		6	3	4	10	5	2
ATCC-motif	part of a conserved DNA module involved in light responsiveness									
ATCT-motif	part of a conserved DNA module involved in light responsiveness						1			
Box 4 ^[12]	part of a conserved DNA module involved in light responsiveness					1	1	2		2
AE-box ^[19]	part of a module for light response	1								
AT1-motif	part of a light responsive module									
Box II	part of a light responsive element									
CATT-motif	part of a light responsive element	1						2	1	
chs-CMA2a	part of a light responsive element									
chs-Unit 1 m1	part of a light responsive element									
GAG-motif ^[11]	part of a light responsive element				1		2	2		1
GA-motif ^[26]	part of a light responsive element			1			1			
Gap-box	part of a light responsive element									
GATA-motif ^[1]	part of a light responsive element	1	1					1	1	1
GATT-motif	part of a light responsive element					1				1
GTGGC-motif	part of a light responsive element									
I-box ^[4]	part of a light responsive element	1				1	1	1	2	2
LAMP-element	part of a light responsive element									
L-box	part of a light responsive element		1					1		
LS7	part of a light responsive element									
rbcs-CMA7a	part of a light responsive element									
TCCC-motif ^[20]	part of a light responsive element					1				
TCT-motif ^[27]	part of a light responsive element						1			
TGG-motif	part of a light responsive element									
3-AF1	light responsive element									
4cl-CMA2a	light responsive element									
AAAC-motif	light responsive element									
Box I ^[23]	light responsive element	1								
GT1-motif ^[2]	light responsive element		3							
MNF1	light responsive element		1	1		2				
Sp1	light responsive element	4	1		4		2	2	5	
TGA-element ^[21]	auxin-responsive element								1	
AuxRR-core	<i>cis</i> -acting regulatory element involved in auxin responsiveness	1	1	1					1	
CGTCA-motif	<i>cis</i> -acting regulatory element involved in the MeJA-responsiveness		1	3	3			2	1	2
TGACG-motif	<i>cis</i> -acting regulatory element involved in the MeJA-responsiveness		1	3	3			2	1	2
TCA-element ^[24]	<i>cis</i> -acting element involved in salicylic acid responsiveness	1		1	1			1		
SARE	<i>cis</i> -acting element involved in salicylic acid responsiveness									
ABRE ^[6]	<i>cis</i> -acting element involved in the abscisic acid responsiveness	2				1		1	2	
motif IIb	abscisic acid responsive element								1	
CE3	<i>cis</i> -acting element involved in ABA and VP1 responsiveness									
TATC-box	<i>cis</i> -acting element involved in gibberellin-responsiveness					1				45
GARE-motif ^[3]	gibberellin-responsive element			1		1				2
P-box ^[22]	gibberellin-responsive element				1					
ERE ^[28]	ethylene-responsive element									
Box-W1 ^[16]	fungai elicitor responsive element									1
EIRE	elicitor-responsive element									
WUN-motif	wound-responsive element									
TC-rich repeats ^[17]	<i>cis</i> -acting element involved in defense and stress responsiveness	2	1							
HSE ^[24]	<i>cis</i> -acting element involved in heat stress responsiveness					1	1			
LTR ^[7]	<i>cis</i> -acting element involved in low-temperature responsiveness				1	1				1
C-repeat/DRE	regulatory element involved in cold- and dehydration-responsiveness				1					
ARE ^[13]	<i>cis</i> -acting regulatory element essential for the anaerobic induction	1								1
GC-motif ^[13]	enhancer-like element involved in anoxic specific inducibility	1			2			1	1	
MBS ^[5]	MYB binding site involved in drought-inducibility		2		2	1	1	1		3
MBSII	MYB binding site involved in flavonoid biosynthetic genes regulation	1								
MBSII	MYB binding site involved in flavonoid biosynthetic genes regulation	1								
MRE	MYB binding site involved in light responsiveness			1						1
CCAAT-box	MYBHv1 binding site		1	1	1		1			
Box III	protein binding site	1								
AT-rich element	Binding site of AT-rich DNA binding protein (ATBP-1)									
A-box	<i>cis</i> -acting regulatory element				2	1	5	9		
OBP-I site	<i>cis</i> -acting regulatory element									
ATGCAAAT motif	<i>cis</i> -acting regulatory element associated to the TGAGTCA motif									
TA-rich region	enhancer					33				
5UTR Py-rich stretch ^[14]	<i>cis</i> -acting element conferring high transcription levels				4		2	1	2	1
CAAT-box	common <i>cis</i> -acting element in promoter and enhancer regions	8	15	6	8	12	6	14	7	15
TATA-box	core promoter element around -30 of transcription start	18	19	22	5	109	30	30	10	45

ZmLTP2.2	ZmLTP2.3	ZmLTP2.4	ZmLTP2.5	ZmLTP2.6	ZmLTP2.7	ZmLTP2.8	ZmLTP2.9	ZmLTPc1	ZmLTPc2	ZmLTPd1	ZmLTPd2	ZmLTPd3	ZmLTPd4	ZmLTPd5	ZmLTPd6	ZmLTPd7	ZmLTPd8	ZmLTPd9
	1		1											1	1	1		
		1	1		1	2			1		1			2		1		
1					2	2				2		1		2		3		
1		1				1		4		2			1	2		3		
																	1	
	1	1	1	1		2	3		1		3		1	1	1	3	2	1
															1			
1		1		1	2	1	1			1	1				1			
			2			1			3	2		4			1	2		
							1											
1	1		1	1	2		1				1						2	2
1		2			1	3	2	1	2		1	1	1	2	1	1	6	1
6		5		2	2	7	3	2	2		1	2	1	4	2	3	10	1
						1												
	1		1								1			1	1			
	2		1	1						1	3				1			
1											3				1	1	1	1
								1			1							
		5	1											1		1		
													1					
1	1				1	1		2	1				1	1	1			1
							1								2			
			1		1							1						
1	1				2	1								1				2
			1															
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	1		1								1				1			
					1										1			
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2	1		1	1					1	2				1	1	2		1
		1	2		1			1			2			2				2
		1	1	2	2	4	4	4	1	2		1	1	4	1			
	1	1	1				1			1	1		1	2		1	1	1
1				2						2								3
2			1			3	3		1		2	2	1			1	1	
2			1		3	3	3		1		2	2	1			3	1	
1	2	1		1	1	1	1		1	3	1		1			1		1
1	5	1	2		1	1				1		1		2	1			1
1		1	1		1	1	1		1			1						
							2								1		3	
		1	1		1													
				1						1				1		1		1

ZmLTPd10	ZmLTPd11	ZmLTPd12	ZmLTPd13	ZmLTPd14	ZmLTPd15	ZmLTPd16	ZmLTPg1	ZmLTPg2	ZmLTPg3	ZmLTPg4	ZmLTPg5	ZmLTPg6	ZmLTPg7	ZmLTPg8	ZmLTPg9	ZmLTPg10	ZmLTPg11	ZmLTPg12
1				1	2	1	1	1						2	1	1		1
	1			2			2							1				
	3						2			2			1		1		1	
3	2	2		3	2	3	2	1	2	2	1		2	1	1	1		1
1					1	1	2	1		2	2		1					
				1	3	2		1	1					1				
								1	1									
1							1				1	1				1		
1			1	2		1	2	2		2	3	2		1	3	1		
1	1	2	2	2	2	3	4	5		6	4	4	3	3	7	3	3	2
1	2	1		1								1				1		
3			1	1	1	3		2					2	1				
3		1		1				1							1		1	
									1									
		1								1					1			
	1						1		1									
	1	3	1			1	1	1			1	1	1					
				1						1	1	1						1
		1				1						1				1		
							3											
		2		1												1		
			1				1							1				
					1													
1	1	1		1					1			1			1		1	
												1		1				
			1			1					1	1	1					
2		1				1			2		1	1	1				1	
			1		1				2	1		1				2		
		1	3	2	1	1	2		1	6				1	3		1	1
1	2		1								1							1
											1						2	
2	5	1	6		1	1	3	3		3		1	5		2	2		
2	5	1	6		1	1	3	3		3		1	5		2	2		
								1				1						
1		1	1	1	2	2	1	1						1			1	
							1											
	1				1	1												
		1										1	1			1		
			1									1	1	1	2			3
									1					1	1			
2				1							2	2					2	1
		1			1	1						3	2			1	1	
	1							2					2					1
												1						1
		0	1							2			1					2
2		1	2	3	2		1		1	2		2	1	1	2			5
	1		1															
	1							3	1	2			1			2		2
		1		1	1	1												
											1	1						
		2		3			2	2		2	2	2	1	1	7	1	1	
1																		
	3						2			2			1		1		1	
12	11	10	8	21	22	23	13	19	16	10	28	14	19	15	8	14	17	8
39	18	35	10	47	26	26	4	28	32	1	26	40	13	22	9	47	13	15

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Table S10. Average log2 signal intensity values and CV values of 75 detected ZmLTP transcripts in sixty distinct tissues representing eleven major organ systems of inbred line B73 ordered by three clusters.

Cluster	Probe ID	Name	Germinating	Root	Whole Seedling			Stem and SAM			
			Seed	Primary Root	Primary Root	Primary Root	Coleoptile	Whole Seedling	Stem and SAM	Stem and SAM	Stem and SAM
			24H_Germinating Seed	6DAS_GH_Primary Root	VE_Primary Root	V1_GH_Primary Root	6DAS_GH_Coleoptile	VE_Whole Seedling	V1_Stem and SAM	V3_Stem and SAM	V4_Stem and SAM
I	GRMZM2G027378_T01	E2enzyme	14.99108344	14.96681372	15.08497063	15.13858015	14.64734925	14.68300126	14.91667599	14.89353998	14.76298105
	GRMZM2G083725_T01	ZmLTPg4	13.87081295	9.798044608	9.566718994	9.279310397	12.54429042	12.66984775	11.66176408	11.23747209	11.00543328
	GRMZM2G101958_T01	ZmLTP1.6	15.43595056	5.944157934	5.86597871	5.708280789	14.77260169	13.97230106	11.98962401	12.1247562	11.8650346
	GRMZM2G137329_T01	ZmLTP2.1	9.781391871	6.10518166	6.04857669	5.614069729	11.70236284	11.72127898	8.27527778	9.169359349	9.801450538
	GRMZM2G107839_T02	ZmLTP1.3.2	7.930266572	4.960581101	7.751873576	6.385911515	10.39280923	13.25931411	11.11643015	11.19503787	11.56415078
	GRMZM2G107839_T01	ZmLTP1.3.1	8.260831797	5.422447829	8.148904024	6.857657216	11.26990716	13.91562197	11.72152189	11.89933689	12.25289454
	GRMZM2G010868_T01	ZmLTP1.2.1	12.83178981	5.120302147	4.953554627	5.310347513	13.52072236	13.44654956	11.87526323	11.24417945	11.33791002
	GRMZM2G010868_T02	ZmLTP1.2.2	13.36298633	5.573491295	5.299871143	5.628925855	13.57608914	13.40283343	11.49475772	10.91574486	10.99955154
	GRMZM2G170969_T01	ZmLTPd13	7.83956002	11.72300744	12.17967237	10.76136752	7.348852847	7.63068494	10.5212676	10.33023919	10.84936804
	GRMZM2G126397_T01	ZmLTP1.1	8.465328269	5.585514215	5.78419275	5.621128317	13.25788126	12.82102273	12.99856183	12.48069183	11.88653622
	GRMZM2G136364_T02	ZmLTPd3.1	8.55897357	9.594155846	10.68928615	9.106544672	9.542046517	9.649223042	10.6516256	10.95219133	14.3996985
	GRMZM2G136364_T01	ZmLTPd3.2	8.55897357	9.594155846	10.68928615	9.106544672	9.542046517	9.649223042	10.6516256	10.95219133	14.3996985
	GRMZM2G164440_T01	ZmLTPd5	9.275555412	11.16326919	12.70851287	11.54977129	9.804099033	10.99006754	11.26394203	11.16719111	11.53672245
	GRMZM2G141858_T02	ZmLTPg17.2	9.183614447	12.57051706	11.33685615	11.75255043	11.34452014	11.25550541	11.74505377	11.31408924	11.78429808
	GRMZM2G141858_T01	ZmLTPg17.1	9.863915432	13.06191773	11.8169141	12.24608882	11.74752236	11.73746803	12.10576216	11.81137787	12.23999155
	GRMZM2G089400_T01	ZmLTPg19	11.08186433	10.4815189	11.64035122	10.80695422	10.69425675	10.61462221	10.75967658	10.75008117	10.86104227
	GRMZM2G078876_T01	ZmLTPg3	11.39754703	11.25258837	10.75483925	10.46094757	11.90724091	11.35134605	11.3210038	10.93393667	10.40344711
	GRMZM5G898755_T02	ZmLTP1.7.1	5.922265114	9.35679242	6.406865643	7.292075182	13.07291472	12.19344665	12.56366231	12.84180539	13.20454547
	GRMZM5G898755_T01	ZmLTP1.7.2	6.031675328	9.644585551	6.672254382	7.532602634	13.61430747	12.68239662	12.9602683	13.23674861	13.69946677
	GRMZM5G850455_T02	ZmLTPg1.2	10.84871682	8.502349853	7.157336315	6.691846906	12.5643128	12.23680715	13.34330358	13.35090871	13.22172759
GRMZM5G850455_T01	ZmLTPg1.1	10.45803785	8.432620369	7.128376993	6.662665768	12.53459291	12.18733932	13.22578827	13.31221039	13.18817246	
GRMZM5G850455_T03	ZmLTPg1.3	10.45803785	8.432620369	7.128376993	6.662665768	12.53459291	12.18733932	13.22578827	13.31221039	13.18817246	
II	GRMZM2G091054_T01	ZmLTPd14	7.546653517	4.711569209	4.757126488	4.766745108	4.671617883	4.856082958	4.634610935	4.767328062	4.737055439
	GRMZM2G071575_T01	ZmLTP2.3	6.211689595	6.448964505	5.46784283	5.698830261	5.614353809	6.035973217	5.523002235	5.153593929	5.35924308
	GRMZM2G087413_T01	ZmLTPd6	6.36506539	5.294239939	5.147739929	5.068861703	5.20272888	5.297904354	5.34691446	5.205323888	5.286879765
	GRMZM2G081464_T01	ZmLTP2.2	12.33738346	4.551674596	4.730742698	4.695722129	4.99370791	5.254608636	4.892007412	4.963768331	4.747189336
	GRMZM2G063375_T01	ZmLTPd16	5.311377292	4.782482261	4.723191578	4.823482248	4.790297653	4.827412215	4.94069201	4.755430377	4.646265421
	GRMZM2G155555_T01	ZmLTPd15	5.274446575	4.751429433	4.699864146	4.812150202	4.755888265	4.936002419	4.909747552	4.762361727	4.697828079
	GRMZM2G387360_T01	ZmLTP2.4	4.984559403	5.469276357	5.489543749	5.257845594	5.524424717	8.272927672	8.090119231	6.640191783	5.860390759
	GRMZM2G166484_T01	ZmLTPx2	8.00434085	7.605477457	7.463015478	7.619487551	7.091376343	8.378530977	7.052680961	7.92469698	7.639205208
	GRMZM2G071575_T01	ZmLTPg14	12.31359798	6.355488282	6.147973867	5.944248849	8.171251756	8.638227938	7.811545734	7.325482095	7.348346796
	GRMZM2G403007_T01	ZmLTP2.6	6.725901712	14.4260496	13.16111882	13.53123194	11.78822413	8.490444606	10.63449006	10.84334673	11.94154429
	GRMZM2G004909_T01	ZmLTP2.7	6.134896778	13.65041712	12.71530221	12.96139907	9.95799559	9.00642108	9.565376503	9.233501145	10.8395306
	GRMZM2G031102_T01	ZmLTPd1	6.042274091	13.80285917	14.52636537	14.12600422	9.233038111	11.41613854	10.47228365	9.150929803	11.05294268
	GRMZM2G099867_T01	ZmLTPd4	6.042274091	13.80285917	14.52636537	14.12600422	9.233038111	11.41613854	10.47228365	9.150929803	11.05294268
	GRMZM2G168833_T01	ZmLTPg9	6.472667529	6.253107803	6.300233929	6.1326723	8.675208247	9.899354262	6.645632074	6.489101947	6.595204149
	GRMZM2G005991_T01	ZmLTPg7	5.926548638	7.51703008	11.33375848	12.35132163	6.621747027	11.82066366	7.118810064	7.336635911	6.890032281
	GRMZM2G006047_T02	ZmLTPg6	6.710132523	9.190946032	12.0556046	12.85110679	7.931172207	12.03817705	9.038675772	8.910163487	7.445716305
	GRMZM2G170044_T01	ZmLTPg22	11.75291457	9.843314368	10.3980977	8.940283468	8.044709355	10.1112996	9.078827159	9.567440717	10.2298226
	GRMZM2G004466_T01	ZmLTPg25	6.271925412	9.147292831	9.865381436	8.275933343	8.251338097	9.758383976	9.49743278	10.35568197	10.97779353
	GRMZM2G065557_T01	ZmLTPd8	6.924854401	10.84611322	10.79655138	9.393593067	8.869856983	9.334800181	8.606041287	9.91331258	11.33182535
	GRMZM2G320373_T01	ZmLTP2.8	11.45190881	10.17273512	10.33634988	8.624651011	8.821365324	8.035352868	7.959556661	8.38290461	10.37181735
GRMZM2G116167_T01	ZmLTPg18	6.548395756	9.690543281	8.633749113	8.763806117	10.59921311	8.711253352	8.377524889	8.733767386	8.248962733	
III	GRMZM2G097137_T01	ZmLTPg5	7.784534579	8.091670568	9.597044029	10.70686402	7.873424248	8.148832988	7.825260982	8.03332879	7.794217118
	GRMZM2G140646_T01	ZmLTPg26	7.593281673	8.024727445	7.878006909	8.093285737	8.099376552	8.206925531	7.515060076	8.11234466	7.876561108
	GRMZM2G046750_T01	ZmLTPg21	5.165703919	6.00009625	8.91596149	10.05785895	5.383756125	9.978171506	6.275136072	6.353476447	5.614176371
	GRMZM2G130454_T03	ZmLTPg16	5.095868854	5.683813072	8.783022239	9.908405032	5.458236712	8.913644004	6.1672417	5.832798339	5.458973505
	GRMZM2G176347_T01	ZmLTPg13	5.286333506	5.875321566	7.						

Internode				Cob		Leaves				
V5_Shoot Tip	V5_First Internode	V7_First Internode	V9_Fourth Internode	V18_Immature Cob	R1_Pre-pollination Cob	V1_Pooled Leaves	V3_First Leaf and Sheath	V3_Topmost Leaf	V5_Base of stage-2 Leaf	V7_Base of stage-2 Leaf
14.95020591	14.89897196	14.91088606	15.04541993	14.81239968	15.00153645	14.71295858	15.03668792	14.62578307	14.67739792	14.96059379
10.48052697	10.28007168	10.1151912	8.836060239	11.74875268	13.04109927	12.65255558	12.36844789	12.92337686	12.56785184	12.77825897
11.03278346	11.71327151	11.0524337	9.006933633	7.655210343	10.42019268	14.42517946	14.81542795	14.40973837	13.59933077	13.19408424
7.130579871	8.792656321	8.700491951	6.071929818	5.769905034	7.457539422	13.65724689	13.92123685	13.11526829	7.911805615	8.450275743
9.520982661	10.61646727	11.40746325	5.150949396	5.23497808	6.145949042	13.50689077	12.35579622	13.72178919	12.56161769	10.78143201
10.2378934	11.47776862	12.10474889	5.454935074	5.521947505	6.489718001	14.0458245	13.16810619	14.22777303	13.25224884	11.46380992
10.79465057	10.48193261	10.50397454	6.986419886	6.269061292	11.61996859	13.37141649	12.90597293	13.62763889	13.48794969	13.09027857
10.2687132	10.43065173	10.49393478	7.548207696	6.398919755	11.3718515	13.45311547	12.99677114	13.59918424	13.63774325	13.18508001
10.94057629	11.70765276	11.37493071	9.415123152	9.486233756	12.4489527	5.91967844	5.844246193	6.068672619	7.994276367	7.274500144
12.07188453	10.07509537	8.246652692	10.83745442	12.45991551	13.80326823	7.161891387	6.715038033	10.27268263	12.93734133	13.60531302
12.19085846	13.31584142	15.08267137	13.44075495	8.993323844	11.25885523	8.879254467	7.869386749	9.898978483	10.73426128	12.67139392
12.19085846	13.31584142	15.08267137	13.44075495	8.993323844	11.25885523	8.879254467	7.869386749	9.898978483	10.73426128	12.67139392
11.42155473	11.79181895	12.41704	12.19282838	11.11218503	11.23109305	11.44935747	9.15215861	10.81143068	11.93924078	11.89859715
11.85311192	11.15796888	11.89329658	12.15153007	11.20337445	11.64681413	11.28841134	10.69828841	11.25471985	10.8589648	11.30182754
12.28750011	11.59609885	12.34354059	12.57979732	11.73426785	12.1239076	11.69188504	10.95559518	11.70438844	11.26006841	11.72400438
11.05192881	11.36282798	11.53991697	11.20531801	9.17523721	10.52659417	10.35263552	11.85817469	10.26834441	10.01745309	10.19582093
11.13145825	11.26918055	11.40136195	11.89382604	11.15100229	12.69101951	10.8880534	9.781712723	11.28433569	12.71637928	12.89550714
13.57587849	13.4404095	13.6623195	13.50367008	11.64875825	13.7445634	6.424127023	6.974334019	10.35645012	14.20775913	14.23142664
14.02891706	13.87379553	14.14303967	14.04701297	11.94954179	14.1010431	6.199333066	6.854699382	10.67463744	14.61343432	14.64247716
13.64251999	12.91446719	13.16087093	12.73428929	13.23669274	13.91211896	6.610086052	8.229420646	9.621090103	13.46953517	13.9828609
13.61302192	12.89857199	13.14081774	12.70393553	13.22638707	13.88239907	6.718888869	8.194145134	9.49213269	13.43841779	14.06123125
13.61302192	12.89857199	13.14081774	12.70393553	13.22638707	13.88239907	6.718888869	8.194145134	9.49213269	13.43841779	14.06123125
4.797696856	4.775335135	4.702723746	4.773306485	5.02047952	4.886266696	5.01192913	4.831634901	5.113073322	4.736243409	4.738897878
5.158114317	5.225662272	5.168299251	5.292529617	5.341899576	5.262169663	5.330350594	5.246578262	5.311106519	5.188291667	5.233150973
5.236543287	5.374672973	5.091698518	5.293560189	5.873982073	5.399780862	5.243669721	5.043074295	5.37476538	5.12275425	5.111526656
4.997114915	4.745030078	4.700633144	4.892736209	5.019592243	4.684129397	5.452693087	5.407119679	5.153783155	4.86564864	4.948041999
4.947797273	4.587165699	4.70961427	4.564402364	4.73756265	4.559742545	4.955493323	5.053042908	5.06193027	4.636646382	4.963134297
4.858322261	4.627827836	4.737038949	4.723633386	4.746058311	4.570433381	4.993802858	4.82721898	5.047533654	4.631735685	4.850063779
5.781635541	5.633862953	5.548935147	6.227085889	6.106370709	5.936291499	7.174089047	11.41261035	6.471979852	5.879578943	5.69883129
8.397908167	6.933333385	7.117966813	8.633840518	9.254362124	7.87732225	7.820832211	7.755638768	6.932764458	6.818594178	6.926467459
6.778222061	6.641739093	6.691646104	5.882548491	7.301218632	7.452686268	7.812169014	7.751414094	8.150954367	8.794525299	8.9843958
9.887366763	11.99377713	13.07525448	10.23903152	6.189676925	10.57411641	7.04015668	7.043765894	7.560912722	8.644236833	8.929111774
8.015683441	9.879275563	11.8497032	10.18705417	6.826245699	8.977724119	8.261458674	7.639709085	8.180706253	8.353842117	8.713681182
8.374159259	10.94027721	12.70311159	9.230503026	6.408597919	8.714803114	11.71834394	7.362699397	11.94589693	11.45069487	12.02565907
8.374159259	10.94027721	12.70311159	9.230503026	6.408597919	8.714803114	11.71834394	7.362699397	11.94589693	11.45069487	12.02565907
6.928821127	7.217318804	7.678893793	7.01848526	6.794092456	6.903946509	9.580442021	8.655654248	10.73418329	7.874054383	6.382065089
6.101143867	7.626299638	10.39906406	6.4400009	5.820604156	6.376709102	11.24552894	7.883274537	11.63915268	9.092086056	7.275790726
7.008533954	9.304317226	12.06165882	6.169550325	5.795414961	5.414101162	11.91800498	10.11774873	12.51957381	10.19037154	7.356592131
10.03639427	11.06587336	11.57420893	9.157825652	7.835035046	9.070447807	10.55619935	8.827227535	10.97862882	9.605181998	9.204400101
10.47371668	11.46383398	11.81474509	9.548046854	8.519921189	9.186678467	9.163779479	7.844792569	9.372104355	9.063073788	8.931783795
10.1584471	11.74050735	13.40707133	10.59923097	7.174444283	8.98512949	9.964084667	9.400848742	9.227108319	8.986673713	9.856919121
8.812525163	10.33284528	12.86476602	9.852140247	6.268938529	7.468480723	6.904337297	6.632895524	7.900348423	9.871923165	9.341822101
8.628532728	8.49142056	9.416018279	10.04315043	7.917781403	9.708236012	7.188452931	6.484104835	7.617226242	9.332144617	10.26622706
7.82449345	7.790061383	8.967702264	7.852551625	7.435115273	7.371908846	8.529449148	7.732769267	8.697718365	7.726869649	7.832157088
8.693541328	7.904814385	8.30593707	8.860353229	8.350406574	8.145301515	8.959293004	8.15579566	8.175484613	8.567003759	7.78394216
5.64593778	6.35575016	10.10295707	4.179508224	5.116813904	5.003573365	10.50286754	6.910012167	10.87502435	7.052531735	4.925351167
5.389683214	5.973963694	8.381752355	5.659389559	5.266093829	5.090652939	8.264560237	6.519168139	9.45356578	7.684602939	5.953086515
5.493579871	6.194203146	8.040042189	6.168164589	5.740544373	5.571078585	7.1020399	6.690361764	8.18512495	6.629423436	5.951870785
6.457076442	6.104944575	6.309741674	6.665678565	6.460448381	6.404406114	6.958101215	6.216069784	6.547658733	6.437125979	6.154541533
6.808282203	6.46529511	7.159131986	6.650586731	8.252537696	7.03341495	8.008466833	8.273639854	8.915287929	7.055244417	6.903665991
7.13667614	6.419703263	7.632856401	7.343191678	8.741218418	7.87803013	8.719221378	8.561859692	9.320715007	7.621782031	7.244183317
5.695353996	5.907429494	5.840944486	5.346397102	5.50026178	6.085558895	5.716101076	6.328072395	5.743325573	5.620611076	6.091472097
5.448671895	5.371011146	5.333610661	5.414477312	5.547811825	5.39772526	5.9305726	7.105365718	6.101525306	5.287296625	5.241945788
5.974527954	5.983754212	5.946945133	6.608651721	6.36942628	6.400525595	6.464973728	6.355860776	6.015483397	6.446411792	5.797965503
6.214756457	5.656789362	5.982096968	5.863962224	5.426481179	6.648391628	5.308555445	5.154149833	5.323294543	5.478010444	5.508552997
6.214756457	5.382695449	5.90038085	5.845807069	5.361201486	6.648391628	6.02012797	5.320745834	5.412251653	5.160986954	5.349217191
5.547062957	5.355639715	5.569605681	5.944408789	6.206964204	5.262243184	5.597333294	5.174534756	5.372799536	5.132766655	5.721927193
5.391254309	5.283060745	5.387175526	5.437182469	5.395002504	5.501397751	5.652266653	5.222030732	5.252888843	5.457361462	5.288612315
5.391254309	5.281962185	5.435525138	5.455057758	5.58991697	5.537729538	5.92423551	5.332298465	5.471220939	5.457361462	5.330998361
5.391254309	5.281962185	5.435525138	5.455057758	5.58991697	5.537729538	5.92423551	5.332298465	5.471220939	5.457361462	5.330998361
5.288609652	5.419023798	5.489966196	5.582738392	5.17779869	5.355971969	5.911251615	5.05243864	5.193739307	5.761752115	5.324800247
5.39499302	5.236427767	5.070467477	5.630583639	6.963003392	5.639931858	5.436271551	5.347346143	5.053776464	5.972022271	5.393672132
5.853557447	6.080665502	6.3630761	6.689525611	5.822327939	6.328788931	6.239878477	6.329265873	6.049807971	6.247901295	6.347820769
6.555458869	6.218072516	6.243385297	7.04294987	6.86356995	7.138555731	6.998237079	6.833996999	6.945616606	6.531227065	6.654026521
6.669781474	7.5725396	7.294092449	6.898877063	6.440307993	6.529212682	6.638277202	6.680958678	6.136198534	6.353166465	6.535327279
5.570000019	5.515730297	5.78189192	5.989113508	5.657568917	5.7723695	5.569777729	5.547765634	5.345432969	5.522220694	5.735138278
5.851830077	5.705079845	6.360222271	6.466468212	5.683117847	6.246175149	6.154345119	5.878598834	5.606187828	5.871282416	5.797992852
5.12088855	4.729663188	4.750048425	5.195364236	5.232984683	4.997376133	6.567248023	6.665871258	7.725411244		

Tassel and Anthers											
V5_Tip of stage-2 Leaf	V7_Tip of stage-2 Leaf	V9_Immature Leaves	V9_Eleventh Leaf	V9_Thirteenth Leaf	VT_Thirteenth Leaf	R2_Thirteenth Leaf	V9_Eighth Leaf	V13_Immature Tassel	V18_Meiotic Tassel	R1_Silks	R1_Anthers
14.9597247	14.93030013	14.71119165	14.98487679	14.76304437	14.99438767	15.11359589	15.1201473	15.06172001	14.81218859	14.90280327	14.60959308
11.83977831	10.70141189	12.82964421	12.37583469	12.39140257	11.65915384	11.38570891	11.31130257	13.07146913	14.40486644	14.96811654	13.45873038
14.17365429	12.9997975	13.4688125	14.38538395	14.67669803	13.66598284	13.75736399	13.9236243	9.357628774	12.94911972	15.103246	13.2691526
13.53070748	12.82397698	9.446569296	13.66669373	13.80330407	13.34174241	12.94435379	13.10737729	7.567943842	9.752526862	9.515747237	12.20620577
12.65365465	12.30542211	13.3240019	14.34252694	14.35508976	12.00784129	12.22562225	12.39983157	6.091749759	14.82119682	13.40817808	13.80026292
13.29565894	12.95944117	14.02280114	14.82776609	14.88685895	12.80153651	12.8590679	12.96540307	6.466846922	15.12580974	14.05248273	14.35060524
11.95014789	10.79809806	13.95107309	13.7468713	14.22067797	11.50047445	10.92381175	11.13907023	9.2309582	14.14598967	14.89998938	13.24548023
12.42758497	11.09020155	14.00116719	13.86861496	14.3712011	11.63156466	11.14464814	11.28656626	8.839798998	14.21773499	15.25473371	13.32755426
5.812260725	6.355135592	5.73256136	8.252107156	5.899061014	6.06236394	6.053685986	8.037859167	9.91357998	7.216920431	5.721762175	6.210742536
6.588328382	6.71379561	12.398633	6.513061389	6.947419616	9.184493648	8.692411258	7.981012055	13.51069158	12.75276362	14.68211991	10.35604821
11.107863	10.73614633	10.9902875	13.61495349	12.52043872	10.12434403	8.938618311	10.95241068	10.59375669	12.31242556	8.96329943	8.898158013
11.107863	10.73614633	10.9902875	13.61495349	12.52043872	10.12434403	8.938618311	10.95241068	10.59375669	12.31242556	8.96329943	8.898158013
9.005973447	8.062749214	12.52350583	11.93601858	12.48705643	7.626942864	6.831221892	6.853133544	11.46298768	10.87724628	9.596807908	7.302348091
8.87155161	8.232275954	11.60473195	10.54444248	10.21421132	9.348507726	9.753086707	10.15683321	11.28592106	11.91402669	13.19283978	12.47827088
9.337485195	8.902403284	12.02590836	11.13670214	10.71490593	9.988879016	10.24310767	10.66269173	11.84291049	12.41873441	13.5073827	12.96802246
10.36632241	9.171418934	9.802910419	10.38898175	10.15157196	9.031325848	10.99356851	9.313481325	9.488889142	11.36312624	10.68727245	11.85611912
10.28722919	9.85439412	13.03715329	10.83200804	11.72338817	10.30132528	10.11706527	9.674548245	11.77721965	12.00999188	13.41851879	11.14138363
5.915741526	5.768598408	13.92974871	9.973874903	10.64491679	6.162470984	6.896647397	6.244511439	12.40242385	13.4584896	12.23229191	12.71823903
5.95561301	5.661939488	14.46484117	10.17662269	10.99017194	6.115468581	6.678634565	6.276133002	12.80328206	13.93396083	12.63957776	13.16924277
6.771310989	6.067589753	13.01138213	12.03823599	11.35391953	6.744236669	7.417718434	7.856280428	13.89026025	13.09864222	13.39845606	10.63603262
6.74263329	6.293008174	13.02163649	11.85005773	11.17427377	6.723356882	7.379544372	7.389239655	13.8359659	13.05830152	13.49419596	10.55581679
6.74263329	6.293008174	13.02163649	11.85005773	11.17427377	6.723356882	7.379544372	7.389239655	13.8359659	13.05830152	13.49419596	10.55581679
5.029469174	4.826531647	4.847846771	4.819739848	4.889385638	4.829258953	5.126378448	5.022806093	4.930214044	4.810540732	4.949311537	4.81694193
5.547689713	5.202995939	5.417545991	5.422047448	5.216547539	5.459661314	5.596569509	5.454131763	5.608555894	5.460591614	5.574978922	5.474335803
5.13357015	5.609932633	5.6461326	5.128370768	5.095303306	5.537012251	5.432468196	5.61182309	5.53278803	5.177711292	5.705518059	5.200973444
5.565236349	4.893234237	4.799097538	4.875252334	4.924237238	5.210743912	4.969193522	5.069548344	4.83055188	4.80033712	5.532767704	5.049923279
5.010983879	4.749885814	4.703716804	4.672974036	4.563992043	4.88610728	5.259788533	5.135080436	4.620389874	4.575763193	4.573674166	4.584433717
4.997572404	4.70846165	4.738643959	4.669611896	4.740271021	4.741842568	5.34847632	5.034279628	4.585980486	4.658523225	4.715571066	4.653444625
5.962353071	6.563164517	5.919655784	5.613718227	5.534085101	7.659491746	9.495593471	6.332742752	5.981089654	9.157938671	6.912492121	13.56167982
7.540533317	8.2078455	9.567262246	8.576513418	7.651901479	9.050081237	7.950534483	8.009501811	10.79115411	8.787803074	9.056929623	8.421544813
7.118371665	6.825196083	7.726532432	6.968100669	7.43162373	6.796191954	6.515637859	6.879229023	7.649272387	10.40596003	8.33827039	8.743904406
8.053591714	8.510853551	7.16335552	10.27873848	9.511780431	8.338076401	7.833152184	9.501598371	6.231585204	10.1670937	5.939551371	5.942168906
9.8239361	9.776399057	8.254082611	10.95668634	11.15257643	11.28972099	11.50621365	11.83756881	7.402658096	10.9771497	6.045319598	7.54364466
12.08796786	9.966553633	11.68615779	13.22397304	13.51178985	6.419383911	6.612686419	6.79401451	6.88807812	13.27103099	10.63908808	7.461660137
12.08796786	9.966553633	11.68615779	13.22397304	13.51178985	6.419383911	6.612686419	6.79401451	6.88807812	13.27103099	10.63908808	7.461660137
9.593862673	9.334656897	7.565226985	10.97587562	11.60422397	9.790749749	9.190770076	8.988457575	6.561188377	11.69157853	7.801616656	11.75880254
7.343590297	6.512571459	12.72540383	9.491077255	12.22539015	6.496466509	6.258928056	6.528069871	6.011110377	11.59826076	6.956372123	9.846144429
8.090150684	7.776532075	12.7201065	9.295509773	12.11130246	7.827688172	7.03268172	7.866732065	5.773638699	11.75454862	6.25216231	11.69484509
8.667984538	9.317219865	9.736064807	10.87682603	10.56247961	8.815080658	8.398891639	9.039336438	8.831706706	11.36706424	7.818791598	11.01846172
9.883152125	9.969704543	9.516998302	10.67231889	11.36889907	8.287307824	7.408203459	8.346187707	8.606145003	9.391726064	7.973704593	7.255445807
9.243943444	9.122099636	8.856165543	11.45131609	12.01056375	9.244985328	9.324459369	9.578122303	7.265975995	10.03805419	8.80359866	7.587384809
7.133135148	6.023808633	8.803823257	11.01400701	10.31427457	6.58332883	6.55561132	8.29748431	6.335043028	8.179251486	8.707606332	6.275402565
7.243883646	7.402754689	9.297818702	6.802565297	6.819742673	7.203582935	6.810178297	6.701466105	7.924784268	7.343680788	11.48756504	7.092442388
7.660852493	7.169780133	7.901736304	7.511025833	7.705585413	7.436392888	7.51502176	7.419506328	7.482579773	8.024094365	7.849474564	7.970379028
8.496591169	9.072477472	8.903650415	8.604569464	7.921665384	8.889201535	8.113768954	7.71843301	8.498005077	7.706467416	8.388693892	8.067358178
5.607226294	5.023116265	11.52149855	8.698438211	11.69118764	5.151794144	5.300104995	5.208948571	5.098003082	10.44236155	4.784881892	8.319840541
5.65910216	6.039762542	9.241686249	6.57146251	8.538415953	5.65806661	5.710851635	4.903431342	5.049392827	7.413654599	5.816959232	7.133588966
6.067628777	6.574073085	9.132303924	6.550597234	8.624346835	6.280258772	5.823613236	5.520536855	5.771344901	7.605836846	5.756536904	6.755167242
6.395721912	6.188388498	6.73838947	6.783280551	6.35904485	11.52113785	10.45458217	8.404449833	6.391104518	12.71128907	6.952205245	6.103173137
7.136305745	7.869168128	7.009943416	7.299664587	7.14960928	7.526165863	7.627150478	8.710147379	8.057898805	15.36595562	7.458226004	6.905407414
7.372036147	8.448778087	7.956565585	7.914898658	7.589778629	8.092587796	8.584288605	9.27655723	8.828002207	15.66203004	8.255164842	7.449369828
6.366031559	5.362909191	5.356163487	5.772022313	5.71320101	5.841812411	7.36191216	6.945555071	5.55744959	14.83454246	5.973387046	6.162505108
6.497724877	6.179846884	5.558943028	5.504743855	5.419029279	6.165406403	5.943359803	7.573643765	5.381181999	13.445679	5.835223015	5.471668817
6.795318993	7.123370377	6.255519949	6.1016178	5.915193428	7.232618012	6.767177073	6.448748842	6.311787751	12.71599437	7.069270087	6.097095638
5.578319038	5.489883368	5.271082526	5.488659262	5.18456911	5.265108517	5.869499268	5.913360316	5.559878101	15.54068988	5.245787062	5.264925993
5.818074569	5.949398797	5.283873557	5.53595749	5.372648523	5.537357876	5.85352681	5.985003042	5.720522315	15.54068988	5.245622653	5.295077769
5.324411824	5.2943752	5.490746078	5.246918397	6.344461281	5.567075166	6.009556532	5.559167524	5.782825793	11.75000723	5.229016636	5.346293203
5.409365566	5.438920088	5.663093333	5.395028333	5.320009612	5.490675322	5.371656319	5.317031381	5.258560273	10.0845582	5.669606724	5.208729703
5.409365566	5.438920088	5.693906425	5.462140111	5.214755647	5.627837377	5.371656319	5.286419575	5.221725834	9.134622849	5.669606724	5.169465323
5.409365566	5.438920088	5.693906425	5.462140111	5.214755647	5.627837377	5.371656319	5.286419575	5.221725834	9.134622849	5.669606724	5.169465323
5.675912984	5.978790584	5.598984623	5.599481411	5.097635955	5.817478534	5.055661982	5.11945189	5.196430363	4.885853252	5.293544151	5.568485007
5.450588627	5.787251109	5.501901882	5.405029281	5.243716194	6.343874871	5.496642513	5.754203926	9.213993519	6.77666946	6.515636091	5.469283286
6.630812956	6.97736962	6.069781839	5.								

Husk		Whole Seed							
R1_Innermost Husk	R2_Innermost Husk	R2_Outer Husk	2DAP_Whole Seed	4DAP_Whole Seed	6DAP_Whole Seed	8DAP_Whole Seed	10DAP_Whole Seed	12DAP_Whole Seed	14DAP_Whole Seed
14.85820025	14.87294354	15.07281125	14.97340243	14.7738447	15.12955781	14.86449684	14.88814484	15.07322062	14.93413719
13.78050577	12.83318937	12.63798483	13.63128377	14.5036069	14.6534721	14.56176277	14.15834008	13.76500721	13.22457499
13.6654973	14.26010892	14.24028648	11.32644985	11.86529675	12.36856391	12.35428705	12.50563679	12.71321473	12.53803751
10.92979227	10.47891867	12.72569177	9.147549857	9.843473681	11.06417842	11.73541433	11.86872342	12.04703535	11.47495771
14.28113388	15.14775821	15.22979689	10.05687744	11.74600577	12.69207674	13.28811939	13.11770181	12.48217207	12.25401142
14.85653631	15.26209023	15.53339491	10.85781574	12.32758746	13.33839359	13.95039446	13.811861	13.31499892	12.87590967
14.62729072	14.96903419	14.42720869	12.27235914	12.90552099	13.3117262	13.37958226	13.33363093	12.46645322	12.10076969
14.96104023	14.93652623	14.63528528	11.76929884	12.84574458	12.80515181	13.09525095	13.16553744	12.50861712	11.88419179
6.206010788	6.118903933	5.999877403	12.651856	12.35496527	12.12285402	11.48951604	11.26994096	11.28572168	9.985186377
12.25584442	6.893531653	6.933167215	13.87676091	14.09557506	14.2427099	13.71932993	12.69046996	11.21377752	9.704733046
10.19944984	8.810616526	8.035759591	11.37190865	13.34448329	15.18255748	15.32303111	14.79876203	13.85806815	12.15234875
10.19944984	8.810616526	8.035759591	11.37190865	13.34448329	15.18255748	15.32303111	14.79876203	13.85806815	12.15234875
11.79418431	10.878331	9.638054262	11.53489707	11.4603532	12.30235401	12.1279318	11.92695757	12.84789896	12.0458376
12.74620905	12.52651327	11.97872937	11.81679176	11.77006923	12.48452107	12.03732772	11.87782708	11.70008398	11.36497242
13.19583492	13.22397093	12.41082346	12.29212147	12.29126749	13.00186889	12.52219689	12.45691707	12.16060705	11.81285388
11.63439069	10.80232752	11.71745344	10.93154359	11.23425757	11.87220695	11.33888446	10.96164581	11.60271627	11.87295055
12.40567837	11.51893938	11.09300609	12.59475654	12.94377991	13.19176339	13.10620687	12.89637633	13.02390086	12.9039104
14.25919387	9.995887882	7.369760944	13.95260464	14.57536376	14.52921567	14.33467049	14.33467049	13.66179205	13.15847481
14.45335425	10.25366885	7.110926588	14.32469214	14.94488441	14.84519565	14.67523008	14.39810587	14.20122123	13.65817594
14.03739625	12.97850612	12.53724068	13.9367448	14.00432664	13.84015457	13.48692685	13.24903124	13.18944185	12.95881872
14.19414976	12.89792368	12.47397671	14.17958296	13.97102252	13.83773624	13.47011026	13.20792216	13.12080865	12.87191122
14.19414976	12.89792368	12.47397671	14.17958296	13.97102252	13.83773624	13.47011026	13.20792216	13.12080865	12.87191122
4.808163758	5.155443686	4.875266885	4.782827969	4.911815864	5.709304491	13.27767497	14.63191088	15.12494954	15.14461667
5.23045036	6.456201834	5.654366093	5.314698164	5.574952751	8.264388297	12.78073441	14.25897644	14.97693347	14.73787558
5.313835076	5.037659634	5.824501108	5.175531277	5.286027962	6.180794373	12.88224081	14.81483844	15.24908061	15.05239123
4.966452081	4.906648697	5.033444488	5.208263901	4.867586585	4.849570337	5.066453239	5.218925441	9.076859911	11.14547682
4.743949133	5.577881621	5.14314077	5.15408403	4.845958142	5.121358452	4.84072766	10.08647269	11.9483811	11.83126031
4.690961627	5.27743193	5.22868372	5.005590008	4.799912573	5.015140175	4.970995959	10.0520633	11.91450034	12.15282113
8.130278497	10.88473363	12.59099362	7.14294201	9.160954739	11.43913846	10.92355729	9.612398164	9.03835389	9.440896378
7.791445441	7.491187745	7.975577533	8.241422718	7.748852595	7.533594617	7.910034056	9.50503509	9.775345898	9.548788862
7.784912654	8.06630025	8.035380413	7.634580278	7.928638051	8.345260827	8.608817375	8.552435663	9.885322573	9.190933044
8.867910735	6.10127079	6.062137559	10.92850939	11.31533397	11.46460567	10.0273366	8.004942765	6.153838844	5.473785694
8.019095044	6.437359141	6.299656521	9.137242651	9.436138601	9.735730199	8.34863157	7.317110304	6.439568186	5.737784722
11.16566309	9.313036052	7.634585456	9.155102818	9.796715866	10.01299639	8.695056098	7.552991311	6.613546136	6.799124289
11.16566309	9.313036052	7.634585456	9.155102818	9.796715866	10.01299639	8.695056098	7.552991311	6.613546136	6.799124289
9.434543461	9.600091176	11.10553501	6.987917146	7.487451297	6.967611725	7.533775756	7.716473831	7.169207993	8.242348239
10.83843785	6.476732954	8.031278522	5.680483406	6.373750368	9.087144588	9.609257074	10.37260407	9.336099963	7.987305564
11.8913404	8.574122691	10.91142213	6.126712258	6.639336992	8.644331736	9.202778892	9.475875477	8.437267336	7.013702486
10.23265268	10.77834886	10.62401027	9.583088969	9.778545031	10.01043562	9.746896605	9.748402432	9.814301823	9.596928507
8.611495062	6.928648593	7.58883393	9.021222095	8.965422526	9.339940169	9.02235004	8.597023492	7.966395895	7.494507805
9.323288415	8.170655016	7.646497428	8.300708897	8.808216609	10.00355779	8.232687016	8.379098044	7.405962559	7.576121154
7.317208284	7.036882389	6.314887472	7.631017876	7.909234553	8.459554071	7.53398325	6.701820702	5.945916202	5.831862785
9.289936031	7.181017309	6.922061761	9.588429261	9.810413274	9.59321106	9.017454576	8.469521533	7.607117545	7.45285606
7.953708922	7.72732645	8.008437865	7.588406245	7.413728415	7.708353883	7.924109391	7.889866537	7.822176913	7.729507487
7.853667232	7.734787781	7.877530214	8.173222549	7.725313681	7.537657348	7.712037711	8.371639962	8.101646412	8.321666243
10.24911956	5.485020806	7.890442031	4.937391666	5.685793535	8.250922665	8.416998682	8.893979324	7.051782126	5.807812294
8.500357535	5.246769608	6.339749058	5.312608621	5.414945494	5.827557481	5.641937638	5.541967929	5.599966529	5.125074808
7.696525193	5.3000528	6.413723153	5.79311149	5.296457863	6.276961898	6.402592942	6.621715554	6.422650423	6.140402669
6.127516543	5.848873612	6.388246937	6.235970786	6.286802313	6.325486031	6.44549271	6.286581866	6.269732746	6.137067544
7.045661656	7.4845278	8.421433896	7.393136834	8.052511179	8.552090455	7.523376057	6.927169026	6.632114176	6.707883775
8.006166638	8.159595184	9.218896848	7.897943631	8.674237334	8.828333566	7.986473705	7.321936483	6.972997215	7.324904778
5.794456084	6.594257948	6.417301692	5.873513265	6.28719129	6.705536503	6.654061981	6.460594009	6.363451971	6.749216145
5.354171375	5.111109975	5.46434985	5.170290449	5.074922223	5.180055829	5.015612786	5.272150834	5.418162683	6.136278537
5.890616175	5.270539028	6.146517855	5.721077559	5.633434852	5.50260986	5.666063034	5.794459213	6.261131564	6.506010197
6.857256151	5.476094281	5.330174958	6.777629327	6.894763493	6.173397542	6.567124183	6.13682518	5.494381879	5.22010872
6.857256151	5.324273996	5.497614678	6.663501347	6.655976181	6.21804988	5.908369986	6.213524328	5.193912944	5.231917352
5.290923268	5.403747978	5.590590145	5.2138827	5.402969258	5.26757479	5.331975228	5.056915139	5.909793434	5.854085314
5.268929241	5.100752277	5.446319265	5.330367784	4.97015546	5.087993561	5.470820758	5.343750796	5.409145397	5.767244364
5.217383994	4.90336552	5.556624511	5.42465923	5.033153323	5.083245043	5.324040263	5.508619267	5.582562211	5.625214281
5.217383994	4.90336552	5.556624511	5.42465923	5.033153323	5.083245043	5.324040263	5.508619267	5.582562211	5.625214281
5.193418927	4.98848755	5.34259685	5.192484461	5.032085688	5.034960575	5.191708202	5.043301065	5.470668693	5.74714308
5.515460847	5.125269985	5.385200704	5.686155117	5.77776766	5.53087883	5.563566615	7.353052	8.49175628	7.426877326
5.936111351	5.537735368	6.172955649	6.100788471	5.386093748	5.802118638	5.574654407	5.96612026	5.711794362	5.897046818
6.700695577	6.115019978	6.710474601	7.227444077	6.708515531	6.583591874	6.218524665	6.2408575	6.086743496	6.025683649
6.160161836	5.838491188	6.18093035	6.691284037	6.904136353	6.375719095	6.690427603	6.398014999	6.141728226	5.875576261
5.248920393	4.921844882	5.31152655	5.303522611	5.101402581	5.084893291	5.135627114	5.371189497	5.371007826	5.700818251
5.550980204	5.177831973	5.422037427	5.317887667	5.250549912	5.093917329	5.177612317	5.561250377	5.528222262	5.913886437
5.292060906	5.382451311	5.310653218	5.360660446	5.355304388	7.259632584	7.870687297	5.94151111	5.69891225	5.485447187
5.42564279	4.846695149	5.402227379	4.917858131	4.743920404	4.866073368	4.782334648	5.179271947	5.341616734	5.273531794
4.799427895	4.766788005	4.872656375	4.876430892	4.763107889	4.936670205	5.004614594	4.718334808	4.793620761	4.893510013
5.360281261	4.877991907	4.953010984	4.906737617	4.825328351	5.131472212	4.859136642	4.95748265	4.922941504	5.266987183
5.573287075	4.915439237	4.916345191	5.239529276	5.063680324	4.918039686	4.803830523	4.711299373	5.144604397	4.866770777
5.686374858	5.691397006	6.15099357	6.104425532	5.44545869	5.664049053	5.597647286	5.920409652	5.582590395	5.894463265
5.292511274	5.027606501	5.557970336	5.253234051	5.143279304	5.010217964	5.055085134	5.848149862	5.355202806	5.332646003
5.656197946	5.287843862	5.514746849	5.311735868	5.356769755					

Endosperm										
16DAP_Whole Seed	18DAP_Whole Seed	20DAP_Whole Seed	22DAP_Whole Seed	24DAP_Whole Seed	12DAP_Endosperm	14DAP_Endosperm	16DAP_Endosperm	18DAP_Endosperm	20DAP_Endosperm	22DAP_Endosperm
15.06384415	14.83026451	15.15673103	15.0266319	15.09183031	15.02821342	14.98584308	15.09509072	15.18066035	15.22506189	15.17645698
13.34486485	12.74903539	12.99354799	12.71473945	13.26806529	13.10055179	12.35614274	11.49721888	12.80589958	10.16701224	9.588437202
13.09499874	13.1878255	14.02126036	13.86045715	14.54153162	10.14042469	10.17091552	11.04598426	13.42902448	12.01843176	11.76811975
11.6329387	11.26651555	12.49275006	12.29710991	12.89744356	9.682572983	8.86574951	8.473634069	11.00396757	10.19484216	9.635453675
11.74765887	11.6183333	12.16193528	11.2816733	11.16160083	7.921376894	7.87151928	8.813876787	11.56092381	7.910352913	6.877561421
12.59917398	12.32927903	12.86613678	11.95736589	11.82806659	8.417356854	8.418542048	9.332852887	12.26637557	8.30617093	7.224429444
11.90665849	11.52594825	11.51954875	10.16028608	10.705642	8.773374095	8.367598345	8.472591555	11.24401898	6.65348555	5.86531087
12.1566726	11.60931032	11.7465065	11.14644557	11.54620989	8.477065496	8.351018905	8.578594662	11.41797171	7.996534003	7.833575876
9.22456944	9.279315733	10.05110155	9.580566077	9.979793942	9.351711956	8.72292134	8.160730189	9.288182739	6.673814506	6.658896789
9.458784953	8.99000785	9.976544582	9.96422162	10.78729812	8.867721351	7.780529436	7.767944735	7.465783831	7.298271705	6.837419636
10.30880985	8.517449522	8.470109663	8.262841166	10.05440006	11.88478618	8.852310648	5.567275626	6.211534299	5.783857484	5.710149274
10.30880985	8.517449522	8.470109663	8.262841166	10.05440006	11.88478618	8.852310648	5.567275626	6.211534299	5.783857484	5.710149274
12.14683819	11.31939364	11.85225586	11.5456905	11.81088363	10.71594333	9.767633942	9.334928318	10.90331779	7.593719466	7.542929145
11.06166048	11.08657892	11.31948609	11.07382227	11.00401486	10.76203071	9.740699168	9.63095775	9.971504705	9.566848522	9.391911597
11.41785806	11.51045505	11.75356761	11.55364609	11.48790168	11.15058432	10.11913513	10.03280127	10.36664705	9.858186569	9.913309865
11.84976773	11.23812714	11.47335762	11.23519012	11.61448589	12.1955701	12.11652861	11.53555214	11.70936653	11.41379774	11.32932142
13.17126872	12.24093556	12.47188052	12.2012656	12.43648006	12.80123157	12.30094041	12.79957967	12.48535345	12.59127248	12.63007902
13.05352087	12.70722354	13.04456751	12.55390024	12.8000097	11.79659073	11.23837213	11.50630409	12.9286909	10.00150116	9.990865787
13.52977666	13.19019605	13.6149214	13.02068912	13.30382333	12.1520866	11.62431196	11.85082333	13.38022867	10.21727266	10.31985983
12.7650619	12.71054285	12.89021968	12.42335173	12.74413512	12.97187922	12.61782924	12.33291905	12.84644997	12.504155	12.07395189
12.88210211	12.59784404	12.86376875	12.41732519	12.73538717	12.98249403	12.61248573	12.27900234	12.84489944	12.23018251	12.04093552
12.88210211	12.59784404	12.86376875	12.41732519	12.73538717	12.98249403	12.61248573	12.27900234	12.84489944	12.23018251	12.04093552
14.9888324	14.99328766	14.47280062	14.01742834	13.6152201	15.26018153	14.86262184	14.47647755	15.06412292	12.70161089	12.30534356
14.5499082	14.06798495	13.76874863	13.79814854	13.97649035	14.47604732	13.23894482	13.09275998	12.41494666	13.26834629	13.59116498
15.23444645	14.98010616	14.37909323	14.61508001	14.2827855	15.04142752	14.10615163	13.71165917	13.16002921	13.90185412	14.23679341
12.37752197	12.05071159	13.1813748	12.80632073	13.39114791	8.29943112	10.42191814	11.09028429	12.73105436	10.53753846	10.60545212
11.00165657	11.31718087	8.646537614	8.961461752	8.852193591	10.56278024	10.02970301	7.968824197	9.194986642	7.149065588	6.724355045
11.62170048	11.33119927	9.026632651	9.592509588	9.164037121	11.11711785	10.14758981	8.945144364	9.477524936	7.474405862	7.418839926
10.09286641	8.833261185	9.762469599	9.128262852	9.736703031	9.10524876	8.813818475	8.332898423	9.986872286	7.791351285	8.248428946
9.783910581	9.247628516	9.453467174	9.392155055	9.897784426	10.54862863	9.797960233	9.585669752	9.330071641	9.423619973	10.41768369
8.933259566	9.767411267	10.26635512	10.09996289	10.65972257	9.327885178	9.203506499	8.648916211	9.677310315	9.44036251	9.640822195
6.545180239	5.303418861	5.524957127	5.573702147	5.380032665	5.24446322	5.452761098	5.7323788	5.227756866	6.103214259	6.17406857
5.538932759	5.526925916	5.584140218	5.556731368	5.383750507	5.490968567	5.609391128	5.920944983	5.755029409	6.432445087	5.710015616
5.743478157	5.505936456	5.580560878	5.459354013	5.827981267	6.229132455	6.114060567	6.148688259	5.203773008	5.439483251	5.721168741
5.743478157	5.505936456	5.580560878	5.459354013	5.827981267	6.229132455	6.114060567	6.148688259	5.203773008	5.439483251	5.721168741
7.957993335	7.558909445	8.258226661	7.484601539	7.874461875	6.240557957	6.38234447	6.383612407	6.711978026	6.518591404	6.066376979
7.261281783	6.83277921	5.960795743	6.007511207	6.366108046	5.892016766	6.016301346	6.50357235	6.162209952	6.285462154	6.007428006
7.079524053	6.242208805	5.563762077	5.912772969	5.802036448	5.92738933	5.778275514	6.051077486	5.519638316	6.704928863	6.32886268
9.364367666	8.876930175	9.152361902	8.902016266	9.119767155	8.900718612	7.983698779	7.869147743	8.488222289	6.933572126	7.358553722
7.696507575	6.850310908	6.911116382	6.812276658	6.69809812	8.127879727	7.054558154	7.173565844	7.327269922	7.3237515	6.368782543
7.46150395	7.479660721	7.682732767	7.718589096	7.968045329	7.908421599	7.904922157	7.849488732	7.724821543	7.475012364	8.016580566
6.204350065	5.703327717	5.83082585	5.827309306	6.012455113	5.526974964	5.757436778	6.400907902	5.834874117	6.313167609	5.756924354
7.470132999	7.163425105	7.257725354	7.145286723	7.247263585	7.60750934	7.40047528	7.674902961	7.223641217	8.188771354	7.434024976
7.513112535	7.242882366	7.008189638	7.110119167	7.271765855	7.500514993	7.581745522	7.408350685	7.235909082	7.094423676	7.19451287
8.101080777	7.982844695	7.731643757	7.74135294	7.79502329	7.497258706	7.808037772	7.781778008	7.802412471	7.254772892	7.704713122
5.991832974	5.592417908	5.275108815	5.152354602	5.013949583	5.004202527	5.080610501	5.259362606	5.022380096	5.050145476	5.23641272
5.552687563	5.155231492	5.196669762	5.409806025	5.368370848	5.600363616	5.023334857	5.267553962	5.339321114	6.011350704	5.246153724
5.795984036	5.580716305	5.513478166	5.599112889	5.648412383	5.599121838	5.915689103	6.262617583	5.496694919	7.442955315	5.295256434
6.427392999	6.261837923	6.415310365	6.19331136	6.155499257	6.276339922	6.079083433	6.707927159	6.370417217	6.742907383	6.405649885
6.417075794	6.393554434	6.743809083	6.091159259	6.311837796	6.783973673	6.275468439	6.79711062	6.501089818	6.504395547	6.638346991
6.848790219	6.401108846	6.876572087	6.287156532	6.371035635	6.973534016	6.526121033	7.211343173	6.873301967	6.67541412	6.842218984
6.983257153	6.332298028	6.596720815	6.214790307	6.311363467	7.127248119	7.200756004	6.927083252	7.031301063	6.976660334	7.106788121
6.884534766	5.833009367	6.510630369	5.726294108	5.793501698	6.358445169	6.88550407	7.469874578	6.853483689	6.583373163	6.566932284
7.378665354	5.970044015	6.481111948	6.095073589	6.253389105	6.4616864	6.543061959	6.689413854	6.467652562	6.650781016	6.706099259
5.314190261	5.179903975	5.373879501	4.900348772	5.140147757	5.589944234	4.989615999	6.248350539	5.065884069	5.341384813	5.328305165
5.122460967	5.189884912	5.291390353	5.419743289	5.140147757	5.419743289	4.989615999	5.930005946	5.119542588	5.166695049	5.328305165
6.560979539	5.920328474	6.933804831	6.45408032	6.748498691	7.152162703	6.539932711	6.233049195	7.136832439	5.491923517	6.304010268
5.500303593	5.401794395	5.61033671	5.479268775	5.309290822	5.78815856	5.338591716	5.644992895	5.405266748	5.922160218	5.522700882
5.338389067	5.631910913	5.501103058	5.175926088	5.305705387	5.540412447	5.441957811	5.275040827	5.227154447	6.107412368	5.435216169
5.338389067	5.631910913	5.501103058	5.175926088	5.305705387	5.540412447	5.441957811	5.275040827	5.227154447	6.107412368	5.435216169
7.262387456	7.192305777	9.194011238	9.054987309	10.49990529	5.260382816	5.860071175	6.029111672	8.322877169	6.608152691	6.751602225
8.106834324	6.938341791	7.404493025	7.407460283	7.733644137	9.319500641	8.41420488	7.230425807	6.971114193	7.846472726	8.198990134
5.9143326541	5.889762636	5.745756315	6.074139255	5.956812309	6.06182531	6.264520628	6.09486283	6.09402656	5.950152778	5.85996571
5.926991011	6.19781124	5.776577779	5.927854219	5.917678324	6.134111114	6.237198738	5.802202807	5.855825589	6.134086795	5.980543853
6.055876351	6.49646397	6.580245427	6.261979654	6.300181217	6.416468106	6.480816109	6.400002174	6.349855638	7.350058454	6.430361579
4.900405321	5.199272064	5.169597308	5.299817643	5.228993871	5.167864431	5.738801548	5.28287424	5.083993391	5.309487752	5.180299553
4.967069639	5.547606174	5.223199232	5.46280305	5.384504966	5.153180496	5.888793705	5.730154527	5.36373686	5.485434094	5.298228632
5.714424275	5.157243597	5.209725349	5.035609234	5.032757842	6.962439868	5.885859973	5.545270993	5.		

Embryo							S	X _{mean}	CV
24DAP_Endosperm	16DAP_Embryo	18DAP_Embryo	20DAP_Embryo	22DAP_Embryo	24DAP_Embryo	18DAP_Pericarp			
14.94703959	15.03198922	15.08184243	14.73671161	15.02423301	15.04119612	15.00942463	0.115394704	14.98177666	0.007702338
9.401823552	14.15612887	14.00205385	13.96791036	14.2600858	14.50015141	13.78329141	1.794835356	13.43877789	0.133556442
11.75265706	14.42498441	14.37591012	14.07704121	14.08988464	14.09174551	14.21648515	0.940478207	13.86124401	0.067849481
9.063590147	7.141998758	6.61305679	7.440721921	8.276597869	9.328756254	13.2560256	2.229562387	8.731535334	0.255345973
5.553393352	7.96758116	8.035620577	8.747323041	9.117634798	9.919393423	12.68498715	2.169545583	8.860847643	0.24484628
6.04973631	8.402530217	8.581082257	9.365684779	9.869644189	10.65917809	13.38275932	2.255446617	9.472945023	0.238093498
5.441297813	12.30193327	11.97403847	11.27875736	10.92414346	11.06122394	12.63121334	2.450035263	10.80180109	0.226817291
7.38083957	12.41159247	12.21197404	11.58576441	11.41844265	11.44478392	12.66006361	1.797848363	11.30192295	0.159074555
6.293953392	11.34602941	11.4717509	11.04681393	11.61703017	12.27074272	10.47547824	1.995187267	10.64597125	0.187412423
7.034584662	13.13368253	13.01098817	12.72180366	12.65230454	12.76370479	8.8623705	2.45804486	11.45420555	0.214597586
6.079507501	10.6953785	10.78174792	11.2964738	11.80799992	12.83183728	9.425192656	2.181278392	10.4168768	0.209398502
6.079507501	10.6953785	10.78174792	11.2964738	11.80799992	12.83183728	9.425192656	2.181278392	10.4168768	0.209398502
7.025953463	11.58447582	11.56429975	11.56868234	12.11731633	12.42952827	12.67298168	1.928587205	11.28046252	0.170967033
9.160123637	11.32924458	11.21370915	11.20383552	11.16651133	11.66772647	13.22115786	1.185875267	11.28032979	0.105127712
9.525704544	11.78220931	11.67713832	11.6709376	11.61883203	12.1386841	13.55430711	1.181278705	11.70968757	0.100880463
10.8837724	11.21856916	11.26856015	11.09313332	11.08958932	11.12685582	11.32022181	0.144731116	11.14295743	0.012988573
12.05440926	12.73534051	12.42118161	12.30599131	12.4361335	12.1403503	12.88576821	0.300355522	12.42559639	0.024172322
8.620921617	11.29737306	11.28285453	11.8846959	12.60748991	13.05088922	13.37706106	1.597129361	11.73161219	0.13613895
8.595925693	11.63062527	11.69768259	12.38193491	13.09376998	13.60968698	13.9961316	1.806879872	12.14367957	0.148791794
11.89068736	13.09897839	12.98057904	13.01259308	12.94852935	13.20834793	13.37506671	0.482010777	12.93068312	0.037276513
11.78381491	13.08468525	12.95054222	12.96626124	12.92440422	13.17617107	13.34615896	0.510259227	12.89029112	0.039584771
11.78381491	13.08468525	12.95054222	12.96626124	12.92440422	13.17617107	13.34615896	0.510259227	12.89029112	0.039584771
11.56011976	12.29896012	8.555209895	10.74004992	10.66270429	10.91808253	14.05003078	1.683919814	11.25502247	0.149614967
13.26376641	10.21215528	8.839935819	10.90162707	11.72830528	10.23743666	12.54637549	1.519213347	11.10422886	0.136813944
13.67170577	11.26044904	10.32070059	12.20727779	12.6423669	11.13020371	13.41137352	1.24542096	12.09201105	0.102995354
10.44152129	9.079706172	5.662080985	8.384113529	8.364120103	8.416924495	10.98236478	1.726336627	8.761547336	0.197035588
6.168667128	6.132868899	5.335564866	6.050392717	5.565244515	4.921690023	7.298260675	0.76241276	5.924669832	0.12868443
6.281820216	6.690728552	5.189331527	6.256817701	5.62930139	5.21707255	8.142394458	1.027693179	6.201066628	0.165728453
7.606991638	7.31279099	6.316128766	6.39422423	6.300855907	6.90374737	8.315096367	0.766513694	7.021405038	0.109168135
10.44058463	8.179194383	8.020824782	7.678156731	7.834394119	7.597638952	8.463311173	0.982872901	8.316300681	0.118186311
8.538186612	11.54045884	11.23310272	11.37728269	11.71826846	11.90860367	7.937183684	1.642847161	10.60758381	0.154874775
5.695704268	5.211531916	5.593303015	5.243717795	5.974198216	5.510073233	5.927378572	0.300571327	5.593701002	0.053733892
5.660867041	6.09371668	5.811478723	5.74098779	5.947171534	5.868328101	5.817031401	0.141059263	5.84851161	0.024118831
5.893952439	6.546490896	6.366300699	6.361918857	6.517153786	6.313792918	6.167790298	0.222874615	6.309628556	0.035322937
5.893952439	6.546490896	6.366300699	6.361918857	6.517153786	6.313792918	6.167790298	0.222874615	6.309628556	0.035322937
6.133191731	6.105348384	6.152435112	6.080662701	6.254875944	6.197796872	10.43990986	1.62094476	6.766317229	0.239560858
6.310616387	5.707181301	5.621327483	5.627967014	5.989881926	5.870966777	6.648050553	0.385914427	5.967998777	0.064663959
6.336771774	6.019272093	5.22171043	5.295656943	5.853993359	5.905461223	6.062783946	0.409628024	5.813664253	0.070459525
7.116457313	6.686772425	6.636613818	6.257971249	6.765865194	6.752845282	10.23811194	1.359737852	7.207805317	0.188647971
6.691416029	7.518601153	7.123480241	7.102818863	7.298209286	6.937618487	7.037111653	0.262146409	7.101322245	0.036915155
8.164936411	7.046745156	7.058444618	6.66992008	7.121730568	7.56067226	7.02252527	0.48560316	7.23495738	0.067119008
5.745351813	6.67672837	6.323142678	6.804374048	7.1838014	7.818686536	6.07469044	0.698889713	6.660967898	0.104923147
7.416173845	7.07559081	7.093664443	6.966569336	7.315228456	7.545385457	8.010909613	0.35788552	7.346217423	0.048716979
7.190019183	7.490249602	7.231445378	7.111184111	7.388643611	7.286377317	7.425482972	0.136841072	7.303343168	0.018736771
7.8955003	8.283698233	8.557005652	8.455441118	8.312106124	8.050859977	7.862757944	0.271086793	8.202481335	0.033049364
5.095074691	4.996412057	4.920181678	4.975908395	4.986448986	4.773549379	5.430961601	0.203568674	5.025505255	0.040507106
5.281907268	5.04327994	5.500670059	5.009079652	5.208290305	5.305550126	5.108710026	0.171849908	5.208212482	0.032995948
5.683116543	5.614775098	5.499604344	5.651731994	5.872858435	5.524795453	6.046828717	0.196170731	5.699101512	0.034421344
6.419687623	6.177480677	6.569837376	6.225919362	6.337324204	6.380440793	6.653573778	0.17198803	6.39489483	0.026894583
6.083651595	6.696573066	6.79261534	6.576825732	6.545405769	6.925159983	7.426696175	0.408910415	6.720989666	0.060840804
6.270975823	6.984370042	7.375547272	6.769599944	7.014740549	7.437243612	8.285426871	0.630421404	7.16255773	0.08801624
6.364044223	6.574064994	5.97271328	6.36180109	6.079917783	6.172891602	7.023032336	0.353267168	6.364066473	0.05550966
5.981232244	5.457542777	5.852957007	5.002380931	5.347353609	5.234716143	5.748804204	0.35565404	5.517855274	0.064455123
6.038650701	6.01016739	6.164571805	5.493528446	5.955745505	5.809433955	6.559069131	0.325547736	6.004452419	0.054217723
5.197794594	5.265556406	5.503175858	5.299251536	5.300949955	5.30635422	5.134100706	0.114924935	5.286740468	0.021738335
5.254645349	5.25657887	5.404174552	5.321425533	5.301709001	5.30635422	5.283485763	0.050752406	5.304053327	0.009568608
5.445751474	6.037945251	5.841021069	6.181617909	5.904800606	6.409270977	5.33382547	0.384069418	5.879176108	0.065327082
5.665814409	5.148493685	5.647705668	5.338112913	5.326939373	5.323439362	5.741172326	0.225382215	5.455953962	0.041309406
5.593302314	5.264376054	5.640022394	5.511534862	5.417813958	5.460210178	5.649799846	0.138169495	5.505294229	0.025097568
5.593302314	5.264376054	5.640022394	5.511534862	5.417813958	5.460210178	5.649799846	0.138169495	5.505294229	0.025097568
5.89970022	6.077556705	5.493864613	5.989704638	5.851179041	7.570281833	6.210135007	0.6625657	6.156060294	0.107628202
8.065297697	5.825395736	5.362863901	5.13352167	5.565198963	4.994131128	5.593821737	1.041957712	5.791461547	0.179912739
6.229863424	6.069656423	6.05600932	6.671862654	6.358518139	8.36751342	6.028756297	0.836974007	6.540311382	0.127971584
6.482467778	5.807648768	6.238543709	6.256388088	6.067484061	6.500145446	6.15825092	0.240147744	6.215846967	0.038634758
6.290137732	6.97288059	6.794940781	6.443355323	7.124753302	7.0695342	6.566392639	0.324918694	6.75171351	0.048123886
5.538291892	5.473019108	5.675862486	5.520834098	5.469197778	5.301311506	5.187170155	0.161182022	5.452241003	0.029562527
5.686614087	5.546508464	5.894096345	5.646051577	5.60289397	5.362548289	5.325936193	0.194877005	5.580664132	0.034920038
5.219397374	5.074365814	4.813357808	4.98794355	4.892195191	5.196880825	5.287212713	0.177849232	5.067336182	0.035097184
5.226139733	4.843225936	5.093193103	4.911464891	5.45865399	5.003875358	5.488279184	0.255276197	5.146404599	0.049602823
4.731905123	4.856176352	4.814057321	5.047672297	4.858063639	4.823626206	4.937772108	0.100650392	4.867039007	0.020680005
4.894903536	4.919694111	5.003307158	4.998214193	5.007421853	4.886485609	4.859899429	0.062787749	4.938560841	0.012713774
4.691898198	4.869510279	5.03120718	4.655736654	5.062644544	5.997411773	5.093372292	0.450158725	5.057397274	0.089009959
5.865606064	5.780784958	5.777822817	5.861992921	5.731377708	5.658663686	5.86070455	0.078326758	5.790993243	0.013525617
5.385296807	5.21931812	5.008141488	5.344709998	5.215722577	5.186039928	5.300370267	0.124731479	5.237085598	0.023816964
5.234990709	5.415332925	5.322615971	5.252797322	5.381316785	5.374220657	5.829599377			

Table S11. The probe sets of ZmLTP genes on the maize 18k GeneChip in this study.

Gene ID	Name	Probe Set ID	Match
GRMZM2G126397	ZmLTP1.1	Zm.7127.1.A1_at	15/15
GRMZM2G010868	ZmLTP1.2	Zm.714.1.A1_at	15/15
GRMZM2G107839	ZmLTP1.3	Zm.4168.1.A1_at	15/15
GRMZM2G096234	ZmLTP1.4	Zm.3909.1.A1_at	15/15
GRMZM2G101958	ZmLTP1.6a	Zm.2085.1.S1_s_at	15/15
GRMZM2G101958	ZmLTP1.6b	Zm.19.2.S1_x_at	10/15
GRMZM5G898755	ZmLTP1.7	Zm.16488.1.A1_at	15/15
GRMZM2G137329	ZmLTP2.1	Zm.930.1.A1_at	15/15
GRMZM2G406552	ZmLTP2.3	Zm.11801.1.S1_at	15/15
GRMZM2G387360	ZmLTP2.4	Zm.17087.1.S1_at	15/15
GRMZM2G403007	ZmLTP2.6	Zm.13789.1.A1_at	9/15
GRMZM2G004909	ZmLTP2.7	Zm.8222.1.A1_at	8/15
GRMZM2G320373	ZmLTP2.8	Zm.17726.1.A1_at	8/15
GRMZM2G393150	ZmLTP2.9	Zm.9640.1.A1_at	15/15
GRMZM2G073377	ZmLTPc2	Zm.10322.1.S1_at	11/15
GRMZM2G031102	ZmLTPd1	Zm.3374.1.S1_at	10/15
GRMZM2G071771	ZmLTPd2	Zm.11586.1.A1_at	15/15
GRMZM2G136364	ZmLTPd3	Zm.16476.1.A1_at	15/15
GRMZM2G099867	ZmLTPd4	Zm.3374.1.S1_at	10/15
GRMZM2G164440	ZmLTPd5	Zm.1090.2.A1_a_at	15/15
GRMZM2G087413	ZmLTPd6	Zm.253.1.A1_at	14/15
GRMZM2G065557	ZmLTPd8	Zm.4529.1.A1_a_at	15/15
GRMZM2G094632	ZmLTPd9	Zm.3825.1.A1_at	15/15
GRMZM2G170969	ZmLTPd13a	Zm.4302.2.A1_x_at	15/15
GRMZM2G170969	ZmLTPd13b	Zm.4302.2.A1_a_at	15/15
GRMZM2G170969	ZmLTPd13c	Zm.4302.1.A1_x_at	11/15
GRMZM2G091054	ZmLTPd14	Zm.8180.1.S1_at	14/15
GRMZM5G850455	ZmLTPg1	Zm.5674.1.S1_at	15/15
GRMZM2G078876	ZmLTPg3	Zm.1503.2.S1_at	15/15
GRMZM2G083725	ZmLTPg4	ZmAffx.191.1.A1_at	15/15
GRMZM2G005991	ZmLTPg7a	Zm.3313.1.A1_a_at	11/15
GRMZM2G005991	ZmLTPg7b	Zm.3313.2.A1_x_at	9/15
GRMZM2G005991	ZmLTPg7c	Zm.3313.1.A1_x_at	7/15
GRMZM2G168833	ZmLTPg9	Zm.2246.1.A1_at	14/15
GRMZM2G130454	ZmLTPg16	Zm.3724.3.A1_a_at	15/15
GRMZM2G141858	ZmLTPg17	Zm.1477.1.S1_at	15/15
GRMZM2G116167	ZmLTPg18	Zm.1046.1.S1_at	15/15
GRMZM2G089400	ZmLTPg19	Zm.14674.1.S1_at	15/15
GRMZM2G089288	ZmLTPg20	Zm.2305.1.S1_at	15/15
GRMZM2G170044	ZmLTPg22	Zm.10158.1.A1_at	15/15
GRMZM2G171597	ZmLTPg23	Zm.16112.1.S1_at	13/15

log₂-fold change



-6 -4 -2 0 2 4 6

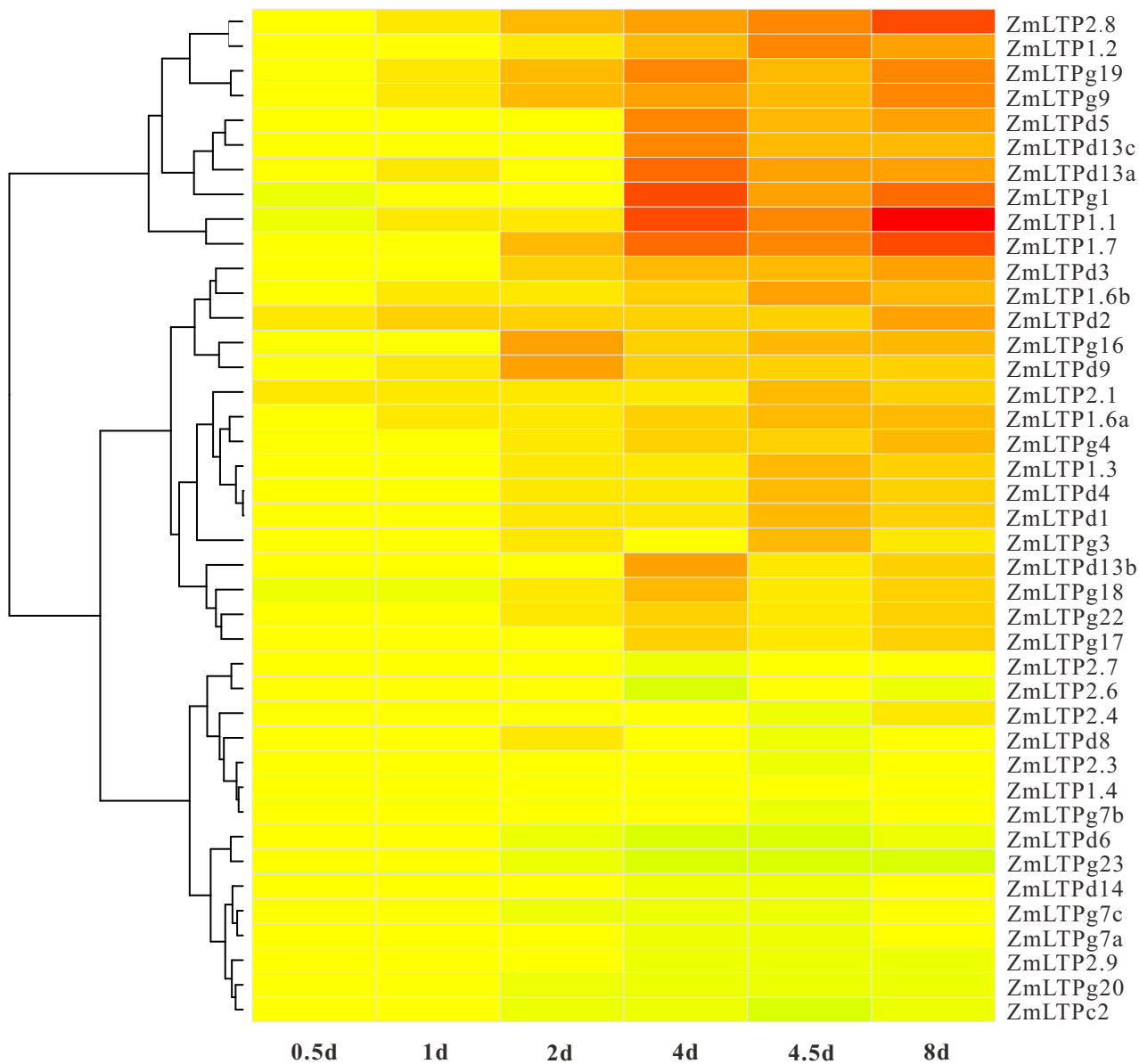


Table S12. Primers used for real time PCR analysis.

Gene name	Forward primer	Reverse primer
<i>ZmLTP2.4</i>	5’ GGCGATCATCAGCGGGTC 3’	5’ CGTTGGGGCTGTTGATGTAG 3’
<i>ZmLTP2.6</i>	5’ CCATCATCATCGGGAGGTCG 3’	5’ GAGTTGACGTAGCGCTGAAG 3’
<i>ZmLTP2.8</i>	5’ GTGTCGCGCTGTTCCCTGG 3’	5’ GCTGGGGTTCCTCTTGTACTION 3’
<i>ZmLTPd2</i>	5’ GTCTGCAACCTGTCGAGC 3’	5’ TGTAGAACCTCACCCACACG 3’
<i>ZmLTPd8</i>	5’ CTTCGCGGGGCTGGATAC 3’	5’ TTGGGGATGGTCATGGCC 3’
<i>ZmLTPd9</i>	5’ GGACTGCTGCAAAACCATCA 3’	5’ CAGTCCTGCTTTGATCCGC 3’
<i>ZmLTPg16</i>	5’ GTGCCTCAACTACATCAGCG 3’	5’ GCGTCCTGTCGATGGTGA 3’
<i>ZmLTPg18</i>	5’ CTGCATCTTCAGCTTCCACC 3’	5’ GCCCACCACCAGATGAAACT 3’

Table S13. List of FPKM values of 42 ZmLTP genes in maize reproductive (ovary) and vegetative tissue (leaf) under both drought and well-watered conditions. MCC and MCD stand for maize ovary, well watered and drought, respectively; MLC and MLD stand for maize basal leaf meristem, well watered and drought, respectively. Numbers 1 and 2 indicate the two biological replicates. The extent of differential expression is measured in terms of fold change and (-) indicates failure to calculate or undetected values. Values in red and blue indicate the fold increase and decrease in expression in the drought-stressed tissue, respectively.

Name	Gene ID	MCC.1	MCC.2	Average	MCD.1	MCD.2	Average	Ovaries (Fold Change)	Name	Gene ID	MLC.1	MLC.2	Average	MLD.1	MLD.2	Average	Leaf (Fold Change)
ZmLTP2.8	GRMZM2G320373	10.426	8.22402	9.32501	293.913	176.666	235.2895	25.2320	ZmLTPd3	GRMZM2G136364	98.5154	32.6996	65.6075	226.254	468.784	347.519	5.2969
ZmLTPg14	GRMZM2G071575	9.68297	16.8128	13.24789	61.5871	103.104	82.34555	6.2158	ZmLTP2.8	GRMZM2G320373	23.0335	10.6743	16.8539	39.3189	86.4044	62.86165	3.7298
ZmLTP2.2	GRMZM2G081464	1.17237	0.748169	0.96027	3.5746	1.58045	2.577525	2.6842	ZmLTPd4	GRMZM2G099867	10.7975	2.7424	6.76995	12.1264	35.7739	23.95015	3.5377
ZmLTPd4	GRMZM2G099867	2.14948	0.981653	1.565567	4.88947	0.547447	2.718459	1.7364	ZmLTPd1	GRMZM2G031102	10.6147	2.69596	6.65533	11.9211	35.1681	23.5446	3.5377
ZmLTPd1	GRMZM2G031102	2.11308	0.96503	1.539055	4.80667	0.538177	2.672424	1.7364	ZmLTP2.6	GRMZM2G403007	0.895399	0.06069	0.4780445	1.40634	1.94226	1.6743	3.5024
ZmLTPc2	GRMZM2G073377	1.56115	1.01596	1.288555	0.980828	2.97719	1.979009	1.5358	ZmLTPg2	GRMZM2G174680	1.6429	1.15136	1.39713	4.15883	5.15967	4.65925	3.3349
ZmLTP2.6	GRMZM2G403007	20.1625	24.8437	22.5031	0.39234	0.198247	0.295294	76.2060	ZmLTP1.1	GRMZM2G126397	43.3601	5.88486	24.62248	42.5902	111.67	77.1301	3.1325
ZmLTP2.1	GRMZM2G137329	20.9869	53.3202	37.15355	1.43846	0.464549	0.951505	39.0470	ZmLTPg3	GRMZM2G078876	30.2099	19.885	25.04745	64.7171	63.4785	64.0978	2.5591
ZmLTP1.2	GRMZM2G010868	595.094	1436.18	1015.637	43.0125	36.1625	39.5875	25.6550	ZmLTPg1	GRMZM5G850455	3.75822	3.0752	3.41671	7.42871	9.44568	8.437195	2.4694
ZmLTP1.1	GRMZM2G126397	1342.75	1562.15	1452.45	95.4805	59.8011	77.6408	18.7070	ZmLTPd5	GRMZM2G164440	25.2971	39.8123	32.5547	58.0493	75.1908	66.62005	2.0464
ZmLTPg9	GRMZM2G168833	3.19474	4.07053	3.632635	0.239117	0.202985	0.221051	16.4335	ZmLTP1.5	GRMZM2G025026	1.27297	0.214966	0.743968	1.39798	1.15311	1.275545	1.7145
ZmLTPg6	GRMZM2G006047	5.36824	7.43594	6.40209	0.359445	0.443662	0.401554	15.9430	ZmLTP1.2	GRMZM2G010868	1156.78	330.585	743.6825	1042.53	1330.33	1186.43	1.5953
ZmLTPg24	GRMZM2G379035	48.1625	51.1694	49.66595	3.98622	3.2204	3.60331	13.7830	ZmLTP2.7	GRMZM2G004909	128.523	72.6659	100.59445	146.805	167.643	157.224	1.5629
ZmLTPg21	GRMZM2G046750	2.39596	1.93677	2.166365	0.539786	0.122738	0.331262	6.5397	ZmLTP1.3	GRMZM2G107839	2207.42	689.465	1448.4425	1911.63	2503.43	2207.53	1.5241
ZmLTPg1	GRMZM5G850455	184.23	207.755	195.9925	33.3676	26.5791	29.97335	6.5389	ZmLTPg19	GRMZM2G089400	11.3913	8.56868	9.97999	13.4208	14.3896	13.9052	1.3933
ZmLTPg3	GRMZM2G078876	176.717	208.607	192.662	29.2066	29.9628	29.5847	6.5122	ZmLTPg22	GRMZM2G170044	67.4672	24.2184	45.8428	52.8522	73.1059	62.97905	1.3738
ZmLTPg2	GRMZM2G174680	6.97953	10.3239	8.651715	2.00723	1.43356	1.720395	5.0289	ZmLTPd8	GRMZM2G065557	9.94042	10.1019	10.02116	9.41453	14.1537	11.784115	1.1759
ZmLTPg17	GRMZM2G141858	56.2329	63.9697	60.1013	12.3026	17.8129	15.05775	3.9914	ZmLTPd10	AC233926.1_FG002	37.4067	26.9737	32.1902	41.5912	29.5362	35.5637	1.1048
ZmLTPd9	GRMZM2G094632	11.0245	10.6958	10.86015	2.95937	3.5894	3.274385	3.3167	ZmLTPg17	GRMZM2G141858	27.0536	18.9292	22.9914	23.715	26.1727	24.94385	1.0849
ZmLTPg19	GRMZM2G089400	16.672	17.0799	16.87595	5.23999	5.11543	5.17771	3.2593	ZmLTPg25	GRMZM2G004466	22.973	22.0804	22.5267	23.6736	23.6156	23.6446	1.0496
ZmLTPg16	GRMZM2G130454	3.44783	4.29984	3.873835	1.0493	1.33798	1.19364	3.2454	ZmLTP2.9	GRMZM2G393150	0.593746	0.845037	0.7193915	0.381498	0.210728	0.296113	2.4294
ZmLTPd3	GRMZM2G136364	14.2103	13.8827	14.0465	8.36008	0.513462	4.436771	3.1659	ZmLTPg24	GRMZM2G379035	8.41691	0	8.41691	3.01347	5.1377	4.075585	2.0652
ZmLTPg5	GRMZM2G097137	1.00325	0.586983	0.795117	0.211924	0.428934	0.320429	2.4814	ZmLTPd11	GRMZM2G000221	34.5622	11.3914	22.9768	20.9225	4.41849	12.670495	1.8134
ZmLTPd12	GRMZM2G471051	0.702159	0.983819	0.842989	0.650837	0.287756	0.469297	1.7963	ZmLTPg10	GRMZM2G414620	0.451229	0.76671	0.6089695	0.371987	0.320803	0.346395	1.7580
ZmLTPg10	GRMZM2G414620	0.847816	0.277165	0.562491	0.351432	0	0.351432	1.6006	ZmLTPg18	GRMZM2G116167	3.82673	0.101742	1.964236	0.837906	1.46976	1.153833	1.7024
ZmLTPg4	GRMZM2G083725	346.72	771.911	559.3155	357.561	454.026	405.7935	1.3783	ZmLTPg7	GRMZM2G005991	2.94005	2.43093	2.68549	2.28582	1.00237	1.644095	1.6334
ZmLTPd5	GRMZM2G164440	57.0507	54.5583	55.8045	32.5263	51.9309	42.2286	1.3215	ZmLTPg6	GRMZM2G006047	19.8552	16.8587	18.35695	17.5124	6.08085	11.796625	1.5561
ZmLTP2.7	GRMZM2G004909	5.39505	4.01455	4.7048	5.7845	1.54254	3.66352	1.2842	ZmLTPg16	GRMZM2G130454	5.92326	3.69522	4.80924	5.12351	1.08849	3.106	1.5484
ZmLTPg20	GRMZM2G089288	5.96294	5.88883	5.925885	3.01775	6.73541	4.87658	1.2152	ZmLTPg21	GRMZM2G046750	8.18335	7.51481	7.84908	8.41245	1.96057	5.18651	1.5134
ZmLTPg22	GRMZM2G170044	8.03274	6.02072	7.02673	6.88194	5.5397	6.21082	1.1314	ZmLTPg13	GRMZM2G176347	6.45889	4.34133	5.40011	5.60318	1.89512	3.74915	1.4404
ZmLTP1.3	GRMZM2G107839	261.266	559.841	410.5535	0	0	0	-	ZmLTPg5	GRMZM2G097137	3.19798	1.96057	2.579275	2.80344	0.788911	1.7961755	1.4360
ZmLTPd13	GRMZM2G170969	118.475	45.6443	82.05965	0	0	0	-	ZmLTPg9	GRMZM2G168833	179.275	96.4799	137.87745	111.426	89.6491	100.53755	1.3714
ZmLTPg18	GRMZM2G116167	151.749	170.997	161.373	0	0	0	-	ZmLTPg14	GRMZM2G071575	3.66903	2.20221	2.93562	2.55683	1.79477	2.1758	1.3492
									ZmLTPg4	GRMZM2G083725	161.271	100.659	130.965	123.116	103.977	113.5465	1.1534
									ZmLTPd9	GRMZM2G094632	6.11464	4.13128	5.12296	4.58564	4.76498	4.67531	1.0957
									ZmLTPd2	GRMZM2G071771	82.2042	56.1774	69.1908	80.5053	54.7451	67.6252	1.0232

log₂-fold change



-2 0 2

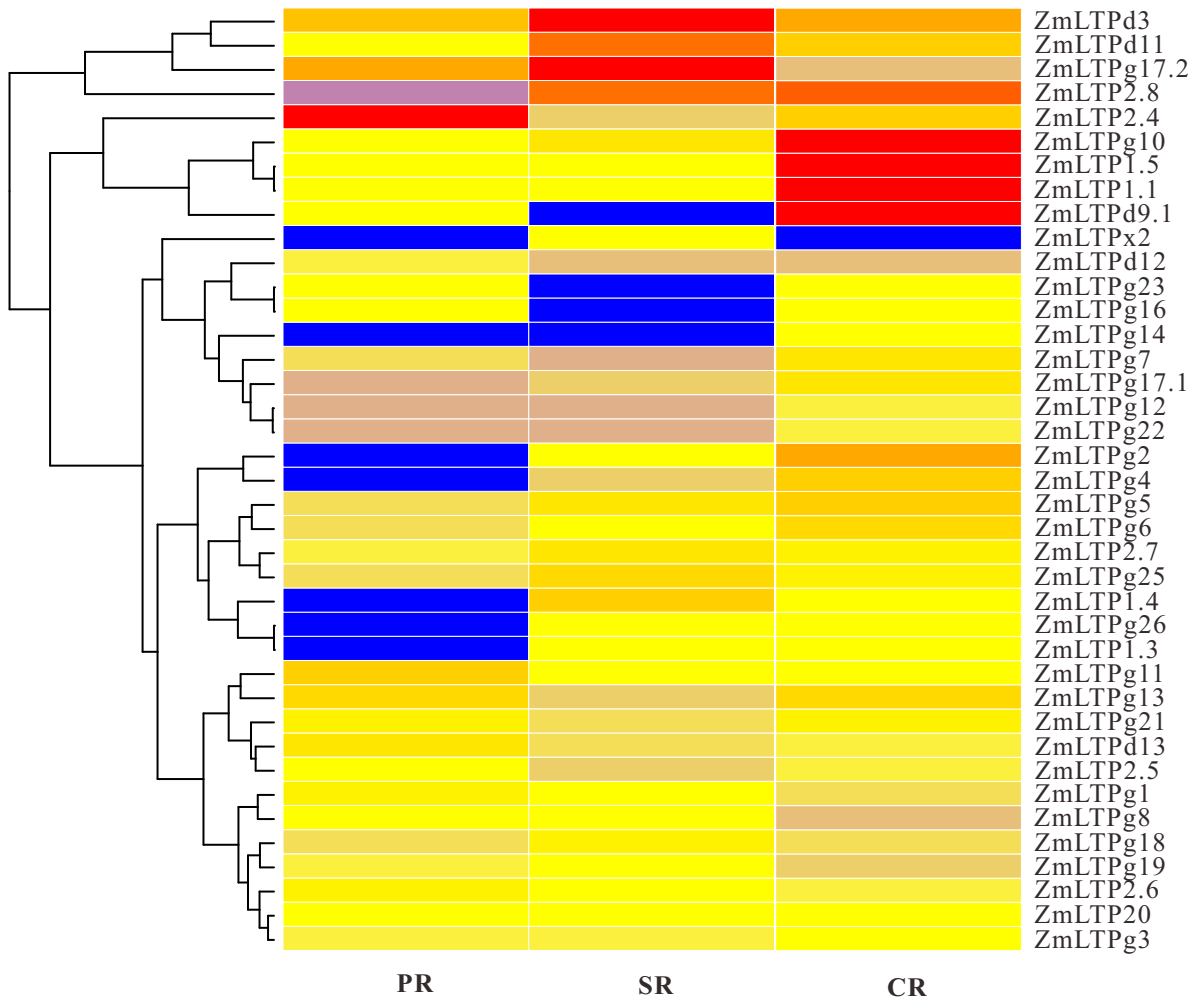


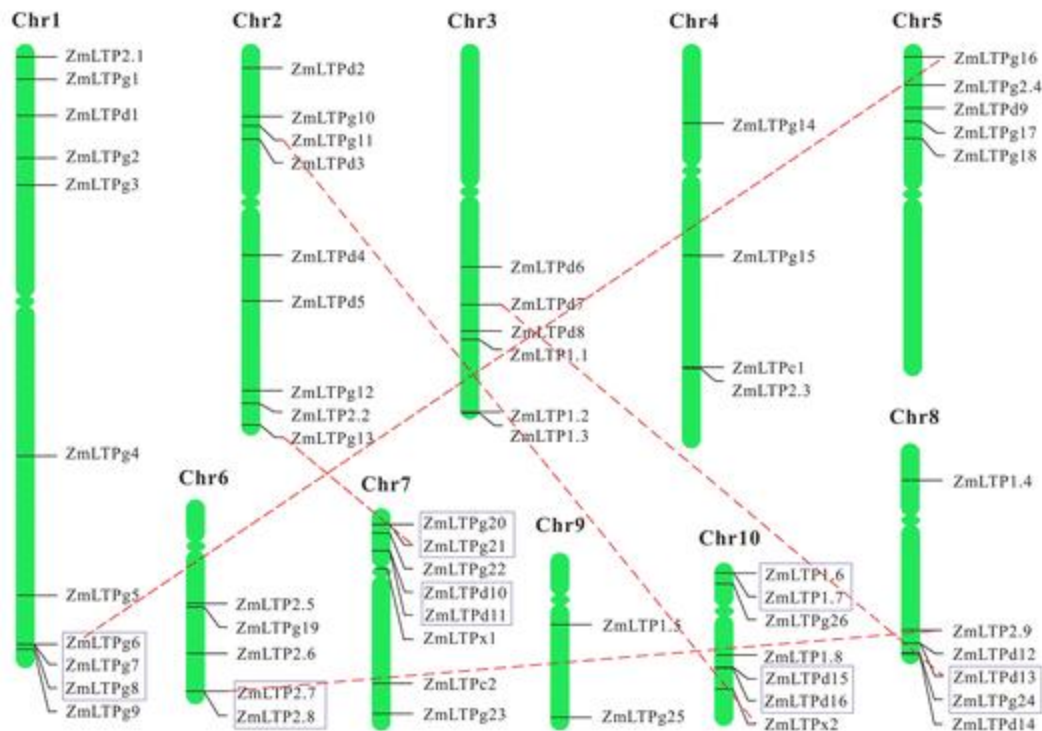
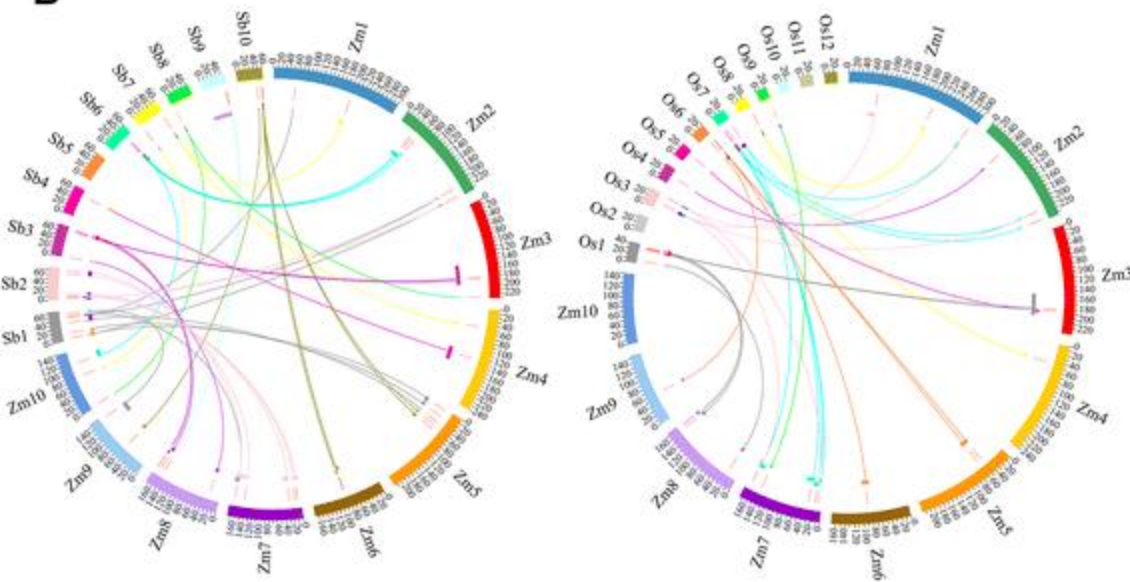
Table S14. The probe sets of ZmLTP genes on the maize 58k NSF array in this study.

Gene ID	Name	Probe Set ID	Expect	Identities
GRMZM2G126397	ZmLTP1.1	MZ00057079	0	100.00%
GRMZM2G010868	ZmLTP1.2	MZ00056626	0	100.00%
GRMZM2G107839	ZmLTP1.3	MZ00041203	0	100.00%
GRMZM2G096234	ZmLTP1.4	MZ00001642	2.00E-99	91.90%
GRMZM2G025026	ZmLTP1.5	MZ00024911	0	100.00%
GRMZM2G101958	ZmLTP1.6	MZ00041611	1.00E-149	98.30%
GRMZM5G898755	ZmLTP1.7	MZ00040001	0	100.00%
GRMZM2G137329	ZmLTP2.1	MZ00026029	0	100.00%
GRMZM2G081464	ZmLTP2.2	MZ00021772	1.00E-135	97.10%
GRMZM2G387360	ZmLTP2.4	MZ00024305	0	100.00%
GRMZM2G039383	ZmLTP2.5	MZ00048959	2.00E-86	100.00%
GRMZM2G403007	ZmLTP2.6	MZ00028450	0	100.00%
GRMZM2G393150	ZmLTP2.9	MZ00019363	0	99.90%
AC225127.3_FG003	ZmLTPc1	MZ00023792	0	97.90%
GRMZM2G073377	ZmLTPc2	MZ00044799	0	96.30%
GRMZM2G071771	ZmLTPd2	MZ00031966	0	100.00%
GRMZM2G136364	ZmLTPd3a	MZ00056914	1.00E-167	100.00%
GRMZM2G136364	ZmLTPd3b	MZ00015921	1.00E-167	100.00%
GRMZM2G087413	ZmLTPd6	MZ00015063	1.00E-177	99.10%
GRMZM2G094632	ZmLTPd9	MZ00015293	0	97.90%
GRMZM2G471051	ZmLTPd12	MZ00022218	0	100.00%
GRMZM2G170969	ZmLTPd13	MZ00041900	0	100.00%
GRMZM5G850455	ZmLTPg1	MZ00023652	0	99.40%
GRMZM2G006047	ZmLTPg6	MZ00057049	0	100.00%
GRMZM2G168833	ZmLTPg9	MZ00029700	0	93.30%
GRMZM2G414620	ZmLTPg10	MZ00021034	0	99.20%
GRMZM2G151021	ZmLTPg11	MZ00051921	0	100.00%
GRMZM2G071575	ZmLTPg14	MZ00046275	0	100.00%
GRMZM2G130454	ZmLTPg16	MZ00030532	1.00E-179	100.00%
GRMZM2G141858	ZmLTPg17a	MZ00025985	0	99.90%
GRMZM2G141858	ZmLTPg17b	MZ00043905	0	100.00%
GRMZM2G089400	ZmLTPg19	MZ00018623	0	98.10%
GRMZM2G089288	ZmLTPg20	MZ00015331	0	98.90%
GRMZM2G170044	ZmLTPg22	MZ00019645	0	99.80%
GRMZM2G171597	ZmLTPg23	MZ00044499	0	99.00%
GRMZM2G379035	ZmLTPg24	MZ00057310	0	100.00%
GRMZM2G036063	ZmLTPx1.3	MZ00051527	0	100.00%

Table S7. Syntenic sites harboring nsLTP genes in maize, sorghum and rice.

Block ID	Gene	Chr	Start	End	Gene	Chr	Start	End
33	ZmLTPg6	1	287110656	287111891	ZmLTPg16	5	4429841	4431021
93	ZmLTPg11	2	47422297	47424522	ZmLTPx2	10	118762389	118764275
261	ZmLTPg13	2	233632142	233633151	ZmLTPg21	7	9201072	9202357
269	ZmLTPd7	3	162372093	162372586	ZmLTPd13	8	162756043	162756672
246	ZmLTP2.7	6	162646836	162647576	ZmLTP2.9	8	152130321	152131030
36	OsLTP1.1	1	6541005	6543076	ZmLTP1.4	8	25946151	25947246
43	OsLTPd9	1	33906370	33909592	ZmLTPd14	8	171565757	171566487
43	OsLTPg1	1	34626184	34629997	ZmLTPg24	8	171016014	171017268
11	OsLTP1.2	1	35129858	35130917	ZmLTP1.1	3	184434264	184435351
11	OsLTPd5	1	36483566	36484818	ZmLTPd8	3	179414637	179416401
44	OsLTPd1	1	39836639	39837257	ZmLTPd12	8	162752046	162752849
180	OsLTPg5	3	15300737	15301156	ZmLTPg23	7	167020090	167021470
134	OsLTPg5	3	15300737	15301156	ZmLTPg3	1	66023613	66026784
165	OsLTPg5	3	15300737	15301156	ZmLTPg12	2	212487102	212487524
135	OsLTPg10	3	33563702	33566773	ZmLTPg9	1	289786005	289788499
179	OsLTPg10	3	33563702	33566773	ZmLTPg22	7	16096163	16099019
207	OsLTPd6	4	20534150	20535514	ZmLTPd3	2	55687675	55688855
228	OsLTP1.4	5	23508004	23509669	ZmLTP1.1	3	184434264	184435351
327	OsLTP1.6	6	20254443	20257626	ZmLTP1.5	9	61863515	61865024
313	OsLTPg13	6	28617447	28620321	ZmLTPg19	6	89065818	89068077
292	OsLTPg13	6	28617447	28620321	ZmLTPg17	5	49545870	49548495
291	OsLTPg24	6	30113940	30115510	ZmLTPg18	5	59978801	59980128
348	OsLTPg17	7	3976186	3977717	ZmLTPg13	2	233632142	233633151
362	OsLTPg17	7	3976186	3977717	ZmLTPg21	7	9201072	9202357
334	OsLTPg19	7	5313788	5318164	ZmLTPg9	1	289786005	289788499
359	OsLTPg19	7	5313788	5318164	ZmLTPg22	7	16096163	16099019
361	OsLTPd3	7	11090520	11091267	ZmLTPd10	7	30954067	30954378
357	OsLTPg21	7	25920677	25922871	ZmLTPg23	7	167020090	167021470
343	OsLTPg21	7	25920677	25922871	ZmLTPg12	2	212487102	212487524
372	OsLTPg22	8	26554150	26557081	ZmLTPg4	1	194657095	194659202
396	OsLTPg22	8	26554150	26557081	ZmLTPg14	4	45262758	45263990
447	OsLTPc2	9	20534165	20534954	ZmLTPc2	7	141644107	141645776
46	SbLTPx1	1	4463665	4464105	ZmLTPg16	5	4429841	4431021
40	SbLTPg4	1	4460355	4461248	ZmLTPg13	2	233632142	233633151
47	SbLTP2.1	1	18272382	18273147	ZmLTP2.4	5	23838144	23838818
57	SbLTPd1	1	43752841	43754087	ZmLTPd9	5	39411646	39412902
58	SbLTPg5	1	57231144	57233750	ZmLTPg23	7	167020090	167021470
43	SbLTPg5	1	57231144	57233750	ZmLTPg12	2	212487102	212487524
63	SbLTPg7	1	67453781	67454898	ZmLTPg25	9	149245864	149247159
8	SbLTPg6	1	60370302	60371426	ZmLTPg2	1	52625970	52627201
172	SbLTPg9	2	4685954	4687002	ZmLTPg20	7	8901782	8904176
172	SbLTPg10	2	4753198	4754293	ZmLTPg21	7	9201072	9202357
153	SbLTPg10	2	4753198	4754293	ZmLTPg13	2	233632142	233633151
180	SbLTPg12	2	7100123	7103454	ZmLTPg22	7	16096163	16099019
176	SbLTPd5	2	13351451	13352128	ZmLTPd10	7	30954067	30954378
178	SbLTPc1	2	65488459	65488989	ZmLTPc2	7	141644107	141645776
171	SbLTPg13	2	74005754	74008032	ZmLTPg23	7	167020090	167021470
146	SbLTPg13	2	210788762	212649098	ZmLTPg12	2	212487102	212487524
237	SbLTP1.1	3	1446324	1447200	ZmLTP1.4	8	25946151	25947246
209	SbLTP1.2	3	66219316	66220110	ZmLTP1.1	3	184434264	184435351

209	SbLTPd9	3	67522375	67523472	ZmLTPd8	3	179414637	179416401
235	SbLTPd7	3	65227168	65227630	ZmLTPd14	8	171565757	171566487
235	SbLTPg15	3	65758095	65759880	ZmLTPg24	8	171016014	171017268
234	SbLTPd10	3	70899337	70899654	ZmLTPd12	8	162752046	162752849
271	SbLTPg16	4	53770386	53770992	ZmLTPg15	4	126473476	126474249
391	SbLTPd11	6	44576681	44577477	ZmLTPd3	2	55687675	55688855
365	SbLTPg18	6	46903482	46905662	ZmLTPx2	10	118762389	118764275
383	SbLTPg18	6	46903482	46905662	ZmLTPg11	2	47422297	47424522
383	SbLTPg19	6	48688841	48689610	ZmLTPg10	2	41484533	41485576
421	SbLTP1.3	7	2833270	2834356	ZmLTP1.8	10	84608056	84608542
412	SbLTPg21	7	61309583	61311559	ZmLTPg4	1	194657095	194659202
438	SbLTPg21	7	61309583	61311559	ZmLTPg14	4	45262758	45263990
471	SbLTP1.4	8	2779571	2780575	ZmLTP1.6	10	4165442	4166593
486	SbLTP1.4	8	2779571	2780575	ZmLTP1.2	3	230606444	230608235
549	SbLTPg22	9	53507894	53509662	ZmLTPg24	8	171016014	171017268
564	SbLTP2.5	9	56662520	56663221	ZmLTP2.9	8	152130321	152131030
125	SbLTP1.9	10	46718450	46719021	ZmLTP1.5	9	61863515	61865024
107	SbLTPg23	10	57671753	57674547	ZmLTPg19	6	89065818	89068077
81	SbLTPg23	10	57671753	57674547	ZmLTPg17	5	49545870	49548495
105	SbLTP2.7	10	59161545	59162227	ZmLTP2.5	6	86281901	86282589
79	SbLTPg24	10	59476463	59477591	ZmLTPg18	5	59978801	59980128

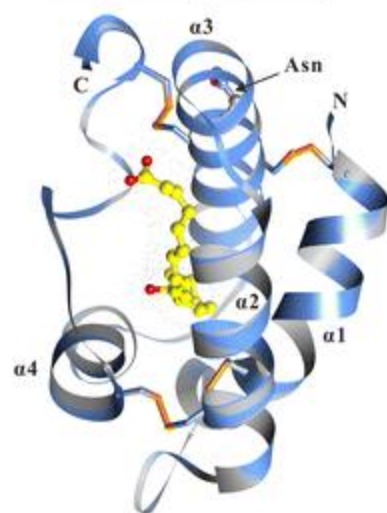
A**B**

A

ZmLTP1.6(1FK7)	-AISTGQVASAIAPPLISYARG---	QDSGPASGTSQVKS LNNAARTTADRAKNC---	LKNAAAVSGLNAGNNAISFSKGGVS---	TYTISTDTSRVN
ZmLTP1.2(29-121)	AITGGQVSSAIAPLISYARG---	TGSGPASCQGVRLKSAASTAQAQRAINC---	LKNAAAVSGLNAGNNAISFSKGGVS---	TYTISTDTSRVN
nsLTP2(1TUK)	---AQAS---QLAVCASAILS---	GAKPSGECGNLR-----	QQ---GDFQCYAKDPTTYQYIIRSH---	RDTLTSCGLA-V-----H---
ZmLTP2.1(32-98)	---SINAG---QLAACAPAITA---	GAPSSASCENLK-----	QQ---GDFQCYVKNPTTYRYINSEN---	AKVVASGGVS-V-----RC-----
DIR1(2RKN)	AIDLQMSQDELNECKPAVSK---ENFTSP	SQPCETALQH-----	AG---FACLCQYKNSPWLSFGVDPEL	SALAKQGLANAT-----T-----
ZmLTPd5(37-115)	AAVYCDMSNEQFMSCQFAAKTTDPPAASQAK	EDALAG-----	AD---LKGCGYKNSRMMGVYNIDKSR	MELEAKQGLATPP-----DC-----

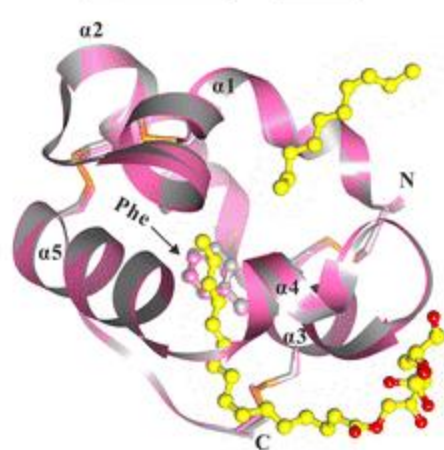
B

... C ... C ... CC ... CNC ... C ... C ...



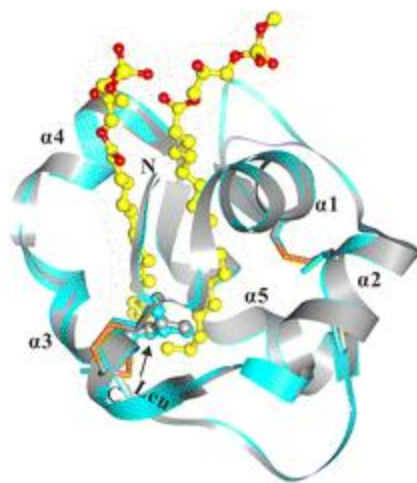
C

... C ... C ... CC ... CFC ... C ... C ...

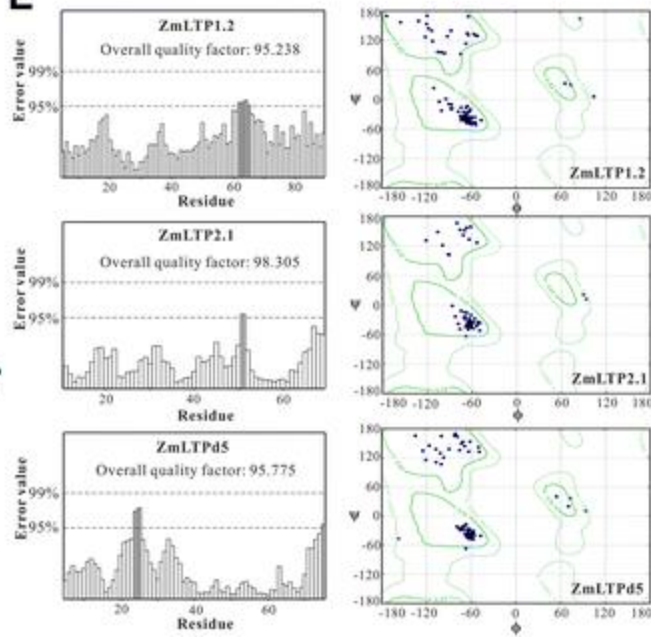


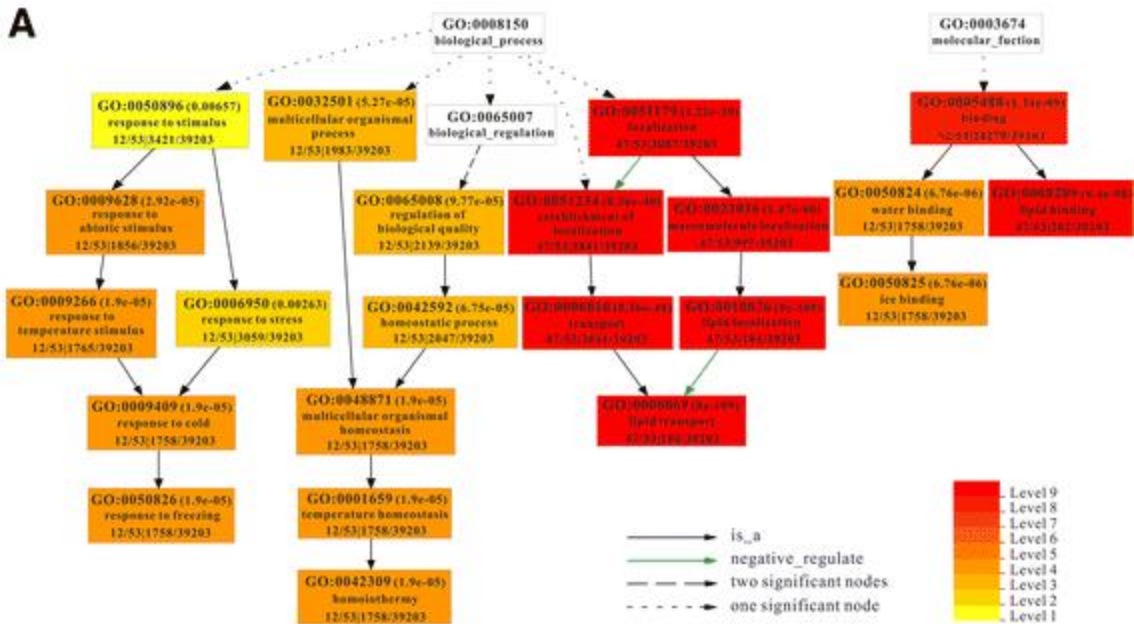
D

... C ... C ... CC ... C L C ... C ... C ...



E

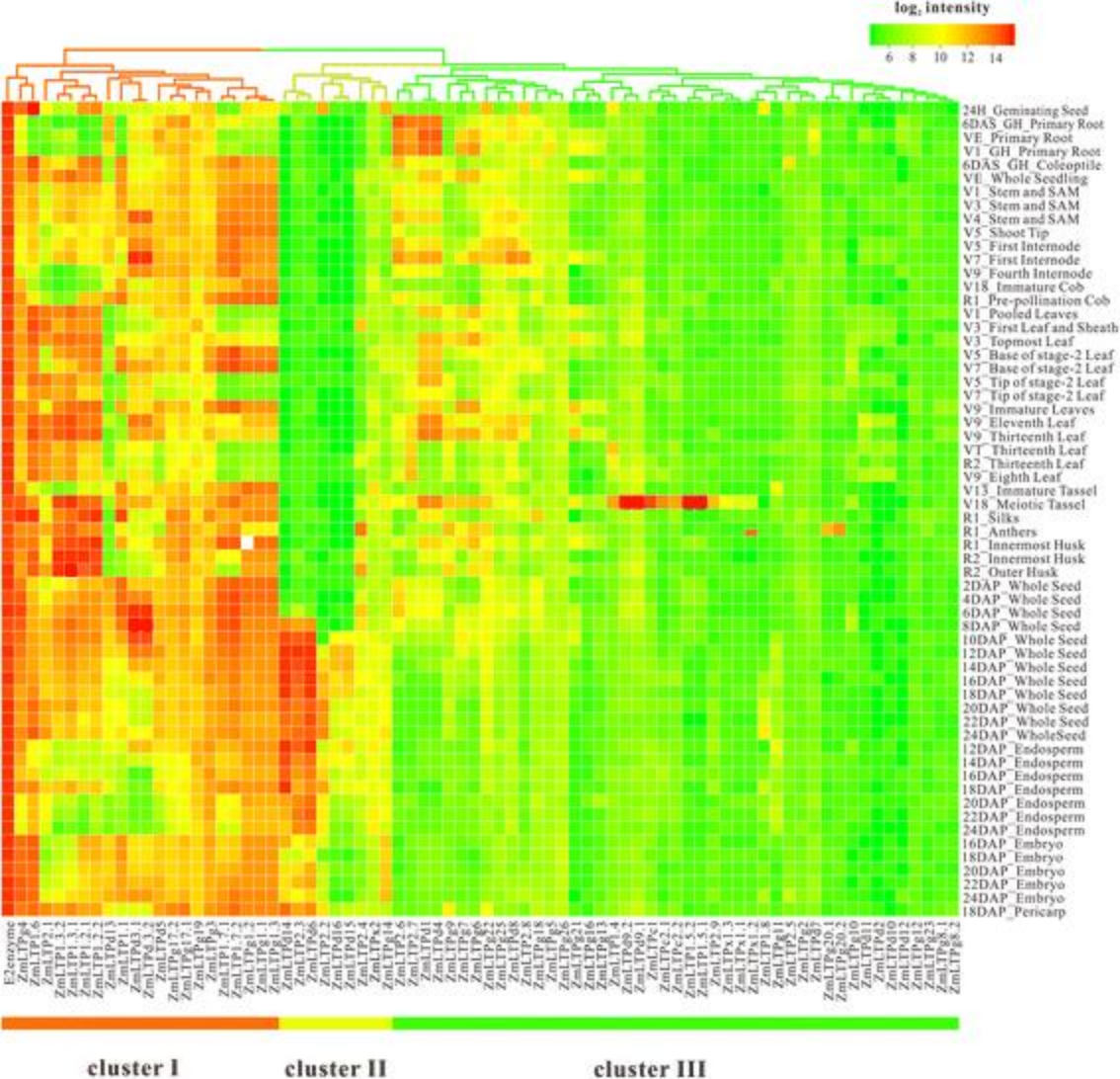


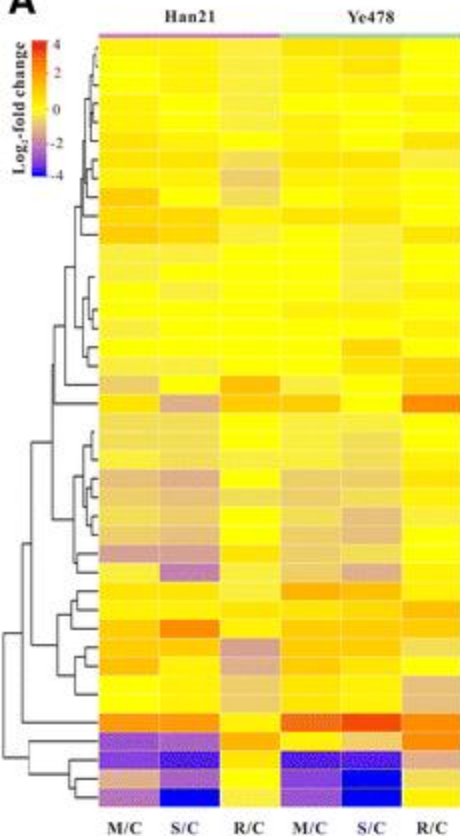
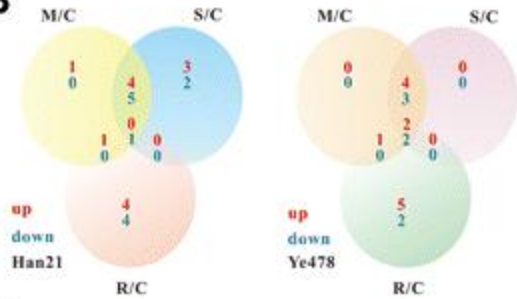


B

■ *Cis*-element present (≥ 1)
 □ *Cis*-element absent (0)





A**B****C**