RESEARCH PAPER



Asymmetric transcriptomic signatures between the cob and florets in the maize ear under optimal- and low-nitrogen conditions at silking, and functional characterization of amino acid transporters ZmAAP4 and ZmVAAT3

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Received 31 January 2015; Revised 20 May 2015; Accepted 3 June 2015

Editor: Dabing Zhang

Abstract

Coordinated functioning of the cob and florets of the maize ear confers grain yield. The cob is critical for carbon partitioning and assimilated nitrogen (N) supply for grain development. However, molecular recognition of the cob and peripheral florets, characterization of genes mediating translocation of N assimilates, and responses of these two tissues to low N (LN) remain elusive. Transcriptional profiling of the ear of a maize hybrid at silking in the field revealed 1864 differentially expressed genes between the cob and florets, with 1314 genes up-regulated in the cob and 550 genes up-regulated in florets. The cob was characterized by striking enrichment of genes that are involved in carbon/N transport and metabolism, consistent with the physiological role of the cob in carbon/N storage and transfer during ear development. The florets were characterized by enrichment of hormone signalling components and development related genes. We next examined the response of the cob and florets to LN stress. LN caused differential expression of 588 genes in the cob and only 195 genes in the florets, indicating that the cob dominated the response of the ear to LN at the transcriptional level. LN caused comprehensive alterations such as carbon/N metabolism or partitioning, hormone signalling and protein phosphorylation in terms of gene expression in the cob and/or florets. Fourteen genes responsive specifically to LN provided potential molecular markers for N-efficient maize breeding. We further functionally characterized two newly identified broad-spectrum amino acid transporters, ZmAAP4 and ZmVAAT3, that showed distinct expression patterns in the cob and florets and potentially important roles in amino-N mobilization in the ear. While both proteins could transport various amino acids into yeast or Arabidopsis cells, ZmAAP4 appeared to have higher efficiencies than ZmVAAT3 in transporting seven out of 22 examined amino acids.

Key words: Cob, florets, maize, nitrogen, transcriptome, ZmAAP4, ZmVAAT3.

Abbreviations: C, carbon; DGE, digital gene expression; FDR, false discovery rate; GO, gene ontology; LK, low potassium; LN, low nitrogen; LP, low phosphorus; ON, optimal nitrogen; RT-qPCR, reverse transcription-quantitative PCR.

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Introduction

Maize (Zea mays L.) is one of the most important food and feed crops worldwide, and its yield is mostly derived from mature kernels whose production is closely correlated with ear growth at the silking stage (Cantarero et al., 1999). Ear sink capacity, determined by the kernel number and kernel size, is established during a critical period of 2-3 weeks around silking (Schussler and Westgate, 1991; Cantarero et al., 1999). Rather than being merely a supportive tissue of peripheral florets or resulting kernels after pollination, the maize cob serves as a critical sink, providing carbohydrates and nitrogen (N) nutrients for bearing florets and kernels during the reproductive stage (Crawford et al., 1982). Abundant amino acids in the cob are important for carbohydrate portioning in the ear; amino acid interchange and metabolism within the cob at the silking stage plays crucial roles in determining sink strength of the ear and preconditions assimilated-N supply for grain development (Singletary *et al.*, 1990; Below, 1997; Seebauer et al., 2004; Cañas et al., 2009). The soft cob accounts for a significant part of the ear shoot at silking, after which the cob proportion gradually decreases with ear development (Jacobs and Pearson, 1992; Seebauer et al., 2004). Pollination also triggers a local hardening process immediately beneath the pollinated ovule in the cob, and within 5 d after pollination, the outer cob parenchyma gradually develops into hard sclerenchymal tissue (Lenz, 1948). Kernel development and growth depends on the availability of carbon (C) and N assimilates supplied by the cob and the capacity of the kernel to utilize them (Cazetta *et al.*, 1999). Collectively, coordinated functioning of the cob and florets of the maize ear determines grain yield. Insufficient supply of C/N assimilates from the cob may result in kernel abortion and severe yield reduction.

Several genes have been well known for their fundamental functions in mediating ear and/or grain development in maize, such as Fasciated Ear2, Branched Silkless1, Glutamine Synthetase (Gln1-3 and Gln1-4), Opaque-7, Shrunken-2, and Empty Pericarp5 (Taguchi-Shiobara et al., 2001; Chuck et al., 2002; Martin et al., 2006; Wang et al., 2011; Hannah et al., 2012; Bommert et al., 2013; Liu et al., 2013). Along with global dissection of different tissues in maize at the metabolic and transcriptomic levels (Kaeppler, 2013; Monaco et al., 2013; Sekhon et al., 2013; Li et al., 2014; Liu et al., 2015), many transcription factors, protein kinases, hormones, and genes associated with starch and storage protein syntheses were shown to be involved in regulation of ear development at the transcriptional level (Liu et al., 2008; Zhu et al., 2009; Wang et al., 2010; Cañas et al., 2011; Amiour et al., 2012; Chen et al., 2014). However, only four cob-specific genes are identified out of 863 organ-specific genes (Sekhon et al., 2011), and molecular characterization of the cob and peripheral florets remains extremely limited. Although it is well known that amino-N interconversion and remobilization in the maize cob is highly dynamic and that amino acid transporters play essential roles in fruit development in plants (Hirner et al., 1998; Okumoto et al., 2002; Rolletschek, et al., 2005; Hammes et al., 2006; Schmidt et al., 2007; Sanders *et al.*, 2009), no genes mediating transport of amino acids within and/or between the cob and florets have been functionally characterized in maize.

At the silking stage, a variety of abiotic stresses negatively affect ear development, resulting in lower fertilization and seed setting rates, reduced assimilate partitioning to the developing ear, and decreased final grain yield (Kiniry and Ritchie, 1985; Schussler and Westgate, 1991; Cantarero et al., 1999; Andrade et al., 2002). N is one of the most important nutrients for maize growth and grain formation, in part by controlling the ability of the kernel to utilize C (Below et al., 2000). N uptake at silking determines kernel number (Andrade et al., 2002; D'Andrea et al., 2008). N deficiency inhibits ear growth and biomass accumulation, and leads to fewer kernels due to reduced cell division, less distal silks, and imbalanced C/N metabolism (Mozafar, 1990; Jacobs and Pearson, 1992; Lemcoff and Loomis, 1994; Seebauer et al., 2004). Previously we showed that low N (LN) affected production of numerous proteins involved in C/N metabolism and hormonal metabolism in young ears at the silking stage (Liao et al., 2012a). However, it is not clear how the cob and florets respond to LN at the transcriptional level. Here, we used the digital gene expression (DGE) analysis to determine gene expression levels in the maize ear grown in the field at the silking stage, and found 1864 differentially expressed genes between the cob and florets. The cob had 1314 up-regulated genes, which were enriched with genes involved in transport facilitation and energy metabolism. We next functionally characterized two broad-spectrum amino acid transporters ZmAAP4 and ZmVAAT3, and found that the former had higher efficiencies in transport of a subset of amino acids. Under LN, the cob dominated the ear response at the transcriptional level, as indicated by 588 and 195 genes differentially expressed in the cob and florets, respectively. Additionally, LN specifically caused differential expression of 14 genes compared to that under phosphorus or potassium deficiency.

Materials and methods

Plant materials and growth conditions

Field experiments with a widely grown, high yield maize hybrid DH3719 were conducted following a well established protocol at the Shangzhuang Experimental Station, China Agricultural University, Beijing (Liao et al., 2012a). Sixteen adjacent plots with the same fertility were used for control (optimal N fertilization, ON: 250kg ha⁻¹), LN (zero N fertilization), low phosphorus (without application of 135 kg P₂O₅ ha⁻¹), and low potassium (without application of 120 kg K₂O ha⁻¹) treatments with four replicates (a total of 16 plots comprising 4 replicates of 4 treatments) (Supplementary Table S1). Control and LN samples were harvested for genome-wide transcriptomic analyses. In addition, low phosphorus and low potassium samples were harvested for identification of genes that were specifically responsive to LN. Soil properties were the same as previously described (Liao et al., 2012a). Three plants with newly emerging silks (~1-5 cm) were randomly selected from each plot and harvested early in the morning (6-7 a.m.) at the silking stage, a short period of time when $\sim 50\%$ of plants in the field had silks growing out of the husks. The cob (the central white tissue of the ear) and peripheral florets (the peripheral light yellow tissue of the ear) of the maize ear were harvested separately, flash frozen and stored at -80°C.

RNA isolation, DGE library construction and sequencing

Total RNA was extracted from the cob and florets of maize plants that were grown under control and LN conditions, respectively, using TRIZOL reagent (Invitrogen). Three biological replicates were used, giving a total of 12 samples (2 tissues \times 2 treatments \times 3 replicates). From each sample, 6 µg total RNA was used for library construction according to the standard DGE protocol (Eveland *et al.*, 2010). Briefly, mRNA was purified by oligo(dT) magnetic beads adsorption (Invitrogen) and reverse transcribed into cDNAs. The cDNAs were digested with an anchoring restriction enzyme NlaIII, which recognizes and cuts cDNAs at the 'CATG' site, and ligated to an Illumina-specific adapter (Adapter 1) at the 5' end. The junction of Adapter 1 and the CATG site is the recognition site of MmeI, which cuts DNA 17bp downstream of this CATG site. Following MmeI digestion, cDNAs were purified and a second Illumina adapter, Adapter 2, was ligated to the 3' ends. After 15 cycles of linear PCR amplification, 95 bp fragments containing the cDNA tag and both adapter sequences were purified by 6% TBE PAGE gel electrophoresis and then sequenced by an Illumina HiSeq 2000 sequencer for 49 cycles. Sequencing data (accession no: GSE59613) were deposited in the Gene Expression Omnibus (GEO) database at the National Center for Biotechnology Information.

Bioinformatics analysis of DGE data

Raw reads (without the 'CATG' tag) were first subject to 3' adapter removal and 'CATG' was electronically added to the 5' end. Only clean reads that contained no ambiguous nucleotide ('N'), were exactly 21 nt long (CATG + 17 bp sequenced cDNA), and had a read count of at least two in a library were retained for further analysis. Clean reads were clustered into unique tags and then mapped to the maize reference genome (v2, www.maizesequence.org) using Bowtie (Langmead et al., 2009), allowing up to one mismatch. Remaining tags were then mapped to the annotated maize transcripts to recover reads that were located at the exon-intron boundaries. Since UTR regions were not well annotated in the maize genome, gene boundaries were extended by 300 nt at both 5' and 3' ends to maximize the capture of complete UTRs (Eveland et al., 2010). Tags that were mapped to more than three genes in the genome were removed from consideration. If a tag was mapped to two or three genes, the tag count was divided by the number of mapped genes. The expression value of a gene was calculated as the total count of all tags that were mapped to the sense strand of the gene region.

EdgeR (Robinson *et al.*, 2010) was used to identify the differentially expressed genes between the cob and florets. Differentially expressed genes between LN and control in the cob and florets respectively were also identified to obtain the LN response genes. Data normalization was performed using the trimmed mean of M-values (TMM) method (Robinson and Oshlack, 2010). The method of Benjamini and Hochberg (1995) was used for adjustment for multiple comparisons. A gene was identified as differentially expressed if the false discovery rate (FDR) is <0.05 and its expression level showed at least 2-fold change. A gene was identified as 'preferentially expressed' if its normalized expression level was at least 10 TPTM (transcripts per ten million reads) in one sample but was zero TPTM in another sample.

The Gene Ontology (GO) term enrichment of differential expressed genes was conducted using the R package TopGO (Alexa *et al.*, 2006), which included an improved weighted scoring algorithm and Fisher test that was used to determine the significance of enrichment. The functional categorization of differentially expressed genes was visualized using MapMan (Thimm *et al.*, 2004), a Java software used to display genomics datasets onto diagrams of metabolic pathways and other biological processes. The heat map figure was generated with the R package pheatmap (Kolde, 2012) using the normalized gene expression values in each sample.

Reverse transcription-quantitative real time PCR (RT-qPCR)

RNA purification, cDNA synthesis and RT-qPCR analysis followed previous descriptions (Liao et al., 2012a), with primers listed in Supplementary Table S2. The relative expression level was calculated using the comparative C_T method (Livak and Schmittgen 2001) with ZmUbiquitin (for maize) and AtTub4 (for gene overexpression lines) as the internal control. Each treatment had three biological replicates.

Transient expression of GFP tagged ZmAPP4 and ZmVAAT3

The coding sequences of ZmAPP4 and ZmVAAT3 without stop codon were fused to the N-terminus of GFP under the control of the CaMV 35S promoter using the pUC-GFP vector (Takara Bio). The primers for cloning were as follows: ZmAPP4-GFP-F (TCTAGAATGGCGGAGAACAACGTC) ZmAPP4and GFP-R (GGATCCGGACTTGAACGGG); ZmVAAT3-GFP-F (TCTAGAATGGCGGCGG CGGAGGAG) and ZmAPP4-GFP-R (GGATCCCTAGTAGCTTTCGGCTATCTTG- G). The onion epidermis was placed on the Murashige and Skoog medium [per litre: 4.2 g Murashige and Skoog salts (Gibco-BRL), 2% (w/v) Phytablend (Caisson Laboratories), 50 mg ampicillin, 30 g sucrose, pH 5.7]. Gold particles were coated with 5–10 µg plasmids, washed, resuspended and bombarded into the onion epidermal cells three times (as replicates) for each gene using the Biolistic PDS-1000/He Particle Delivery System (Bio-Rad) according to the manufacturer's instructions. Transformed cells were incubated at 28°C in the dark for 18h before observation. A confocal microscope (Nikon Eclipse TE2000-E) was used to visualize GPF control and GFP tagged ZmAPP4 and ZmVAAT3. The EZ-C1 (for Nikon C1 Confocal microscope) image software was used for optical sections and Z-stack processing.

Functional characterization of ZmAPP4 and ZmVAAT3 using the $22\Delta 8AA$ yeast strain

The Saccharomyces cerevisiae mutant $22\Delta 8AA$ (gap1-1, put4-1, uga4-1, can1, apl1, lyp1, hip1, dip5), unable to absorb arginine, aspartate, citrulline, GABA, glutamate or proline (Fischer et al., 2002), was used for functional characterization of ZmAPP4 and ZmVAAT3. Coding sequences of ZmAPP4 and ZmVAAT3 were cloned into the pDR195 yeast expression vector (with PMA1 promoter and ADH terminator) and transformed into the 22A8AA strain, and named as ZmAPP4-pDR-22A8AA and ZmVAAT3pDR- 22A8AA, respectively. The primers ZmAPP4-pDR-L (CTCGAGA-TGGCGGAGAACAACGTC) and ZmAPP4pDR-R (TCAGTAGGACTTGAACGG-G); ZmVAAT3-pDR-L (CTCGAGATGGCGGCGGCGGAGGAG) and ZmVAAT3pDR-R (GGATCCCTAGTAGCTTTCGGCTATCTTGG) were used for subcloning of ZmAPP4 and ZmVAAT3 into the pDR195 vector. The restriction sites used for gene subcloning were XhoI and BamHI. The empty vector pDR195 was transformed into the 22A8AA strain (as pDR-22A8AA) and the EUROFAN (http:// mips.gsf.de/proj/eu- rofan/) wild-type (WT) yeast strain 23344c (MAT α , ura3) (as pDR-23344c) as negative and positive controls. The heat shock method was used for yeast transformation (Suga and Hatakeyama, 2005). Transformants were selected on solid agar with yeast N base (YNB) media (6.7g l⁻¹) without uracil, supplemented with 0.5 g l⁻¹ ammonium sulphate and 20 g l⁻¹ D-glucose. Growth assays were performed in uracil-free YNB media containing 1 or 3 mM of Asp, Glu, Pro, Arg, GABA or citrulline as sole N source and 2% D-glucose as C source. The yeast culture with the OD600 value of 1.0 was diluted as 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} solutions. 10 µl droplets of yeast culture and diluted solutions were spotted onto YNB synthetic media, observed after 3-d incubation at 30°C, and photographed with a digital camera. All experiments were repeated three times with independent colonies of different transformation events.

Generation and phenotyping of gene overexpression plants

The coding sequence of *ZmAAP4* was amplified with primers ZmAPP4-OE-F (TCTAGAATGGCGGAGAACAACGTC) and

ZmAPP4-OE-R (AAGCTTTCAGT-AGGACTTGAACGGG) and that of ZmVAAT3 was amplified with primers ZmVAAT3-OE-F (TCTAGAATGGCGGCGGCGGAGGAG) and ZmVAAT3-OE-R (AAGCTTCTAGTAGCTTTCGGCTATCTTGG) (Supplementary dataset 1). The PCR products were cloned into pMD19-T vector (Takara Bio), sequenced, digested and ligated into the pSuper1300⁺ vector (with a modified 35S promoter; Yang et al., 2010) using XbaI and HindIII sites. The recombinant plasmids pSuper1300⁺-ZmAAP4 and pSuper1300⁺-ZmVAAT3 were then transformed into the Agrobacterium tumefaciens strain GV3101. Transformation of Arabidopsis thaliana (Col-0) was performed via the floral dip method (Clough and Bent, 1998). Transgenic plants were selected using 35 μ g ml⁻¹ hygromycin. Resistant T₁ plants were transferred into the soil (perlite:vermiculite, 1:2), and seeds were harvested from individual plants at maturity for further screen. T₂ seedlings with ~3:1 segregation ratio (alive:dead) on the selective media were transferred for seed harvest. Transgenic lines were screened until all seedlings were alive on the selective growth media. Three independent homozygous lines (T₃ in our studies) were selected for further analyses according to the expression level of the transformed gene.

Arabidopsis seeds were surface sterilized, germinated, and grown on ATS agar medium (Schofield et al., 2009) with a day/night period of 16/8h (70 µmol m⁻² s⁻¹) at 23°C±1 after stratifying 48h at 4°C in the dark. After 4 days' growth, uniform ZmAPP4-OE, ZmVAAT3-OE and Col-0 seedlings were transformed on a square petri dish (13×13 cm²) containing ATS medium (Schofield et al., 2009) with 1% sucrose, 3mM NO3, and one of 22 selected amino acids in appropriate concentrations as the N source. The fresh weight (g), number of leaves per plant, rosette diameter (cm) and primary root length (cm) of Arabidopsis seedlings were measured 12 d after transfer. Three petri dishes for each treatment were considered as three independent biological replicates. For each measurement, the average value of four seedlings from a single petri dish was considered as a single replicate. The primary root length was measured using a ruler and expressed in cm. The number of leaves per seedling was counted manually. The rosette diameter of each seedling was measured diagonally twice using a ruler, recording the average value of two measurements on the same rosette in cm. The fresh weight was measured using an electronic balance with 0.1 mg resolution (AE 163, Mettler Instrument Corp, Hightstown, NJ).

Results

A total of >54 million high quality reads were generated by digital gene expression (DGE) to transcriptomically profile the cob and florets of the maize ear at the silking stage under optimal nitrogen (ON) and low nitrogen (LN) conditions. The vast majority of tags (52 340 577) were mapped to protein encoding genes, with others to pseudogenes (1 065 100 tags) and transposable elements (694 843 tags) (Supplementary Table S3). To examine the gene expression with a second approach, we randomly selected 20 genes with various expression levels and differential expression (2-fold change and FDR<0.05) between the cob and florets for RT-qPCR analysis and found the exact same expression patterns for all re-examined genes using these two independent approaches (Supplementary Table S4), indicating that our DGE data were highly reliable.

Gene expression in the cob and florets at the silking stage

Using FDR<0.05 and 2-fold change as thresholds, we found 1864 genes that were differentially expressed between the

cob and florets at the silking stage: 1314 up-regulated in the cob and 550 up-regulated in florets (Table 1; Supplementary Table S5). The differentially expressed genes between the cob and florets were next subject to gene ontology (GO) term enrichment analysis. The up-regulated genes in the cob were enriched with genes involved in transport facilitation, C/N metabolism, and photosynthesis (Fig. 1A). In contrast, most up-regulated genes in florets were involved in cellular processes and lipid biosynthesis (Fig. 1B). Genes related to biotic responses were enriched in both tissues (Fig. 1).

Genes involved in metabolic pathways, hormone and cellular response processes were further analysed using the MapMan programme. Strikingly, genes involved in photosynthesis such as chlorophyll binding (photosystem II light harvesting complex gene B1B2 and light-harvesting chlorophyll b-binding protein 3) and photosystem subunit (photosystem II subunit P-2, photosystem I subunit O) genes (Fig. 2A; Supplementary Table S5) were unanimously upregulated in the cob. Amino acid metabolism related gens GAD2 (Glutamate decarboxylase 2, GRMZM2G355906), a sarcosine oxidase (GRMZM2G052266) and a carboxylyase (GRMZM2G159149) were up-regulated 8.2-, 5.6-, and 10.2fold, respectively, in the cob (Fig. 2A; Supplementary Table S5). Genes mediating secondary metabolism such as flavonoid and phenylpropanoid metabolism were also generally up-regulated in the cob (Fig. 2A). Up-regulation of these three categories of genes suggested more active C/N metabolism in the cob tissue than in florets at the silking stage. Notably, several genes regulating ABA synthesis and signalling were up-regulated in the cob, indicating that ABA may be an important regulator for cob development during this stage (Fig. 2B). Different from those up-regulated in the cob, a subset of genes regulating cell wall modification, cell wall cellulose synthesis and cell wall degradation were up-regulated in florets (Fig. 2A). Genes involved in lipid degradation such as glycerophosphoryl diester phosphodiesterase (GRMZM2G058-227) and fatty acid reductase 5 (GRMZM2G120938) were up-regulated 7.2- and 6.7-fold in florets, respectively (Fig. 2A; Supplementary Table S5). In addition, a gene mediating cell division (GRMZM2G166684) was also up-regulated 2.7-fold in florets. Together with enhanced transcription of SWITCH1 (GRMZM2G300786) and cyclin d5;1 (GRMZM2G047637), these results suggested more dynamic cell division, cell wall and membrane formation in florets supporting female flower development in maize (Fig. 2C; Supplementary Table S5).

Table 1. The number of differentially expressed genes in ears of maize hybrids at silking under different N conditions, revealed by transcriptional profiling

	Cob vs floret		LN vs ON	
	ON	LN	Cob	Floret
Up-regulated genes	1153	920	287	106
Down-regulated genes	442	427	244	63
Differentially expressed genes	1864	1594	588	195
(≥2-fold change)				

LN, low nitrogen; ON, optimal nitrogen fertilization.



Fig. 1. Significantly enriched GO terms in up-regulated genes in (A) the cob versus (B) the florets, under sufficient N supply.

Of particular interest were genes only detected in the cob or florets. In total, 63 out of 161 cob-specific genes (Supplementary Table S6) and 45 out of 108 floret-specific genes are functionally unknown genes (Supplementary Table S7), indicating that DGE may identify previously uncharacterized transcripts. We analysed tissue-specific genes with proper annotation using manual functional classification and heat map illustration (Supplementary Tables S6, S7; Fig. 3). First, 18 genes involved in transport facilitation were only detected in the cob, including two carbohydrate transporters, four amino acid transporters, four nutrient transporters and eight other transporters, suggesting the cob's unique function to transport carbohydrates, amino acids and nutrients for bearing florets (Fig. 3A). Second, 21 genes involved in metabolism also showed cob-specific expression pattern. Third, certain cytokinin and ethylene signalling components, such as cytokinin dehydrogenase (CKX) and ethylene-insensitive 3 (EIN 3), were only detected in the cob (Fig. 3B). Fourth, 14 transcription factors were only expressed in the cob. In addition, genes involved in cell rescue, signal transduction and proteolytic degradation were also preferentially expressed in the cob during this phase. In sharp contrast, only a few floret-specific genes were related to transport facilitation; three major groups of floret-specific genes were involved in development, transcription and metabolism (Fig. 3). Thirteen development related genes, 21 transcription factors, and 15 metabolism related genes (such as hydrolyzing O-glycosyl compounds, crinkly 4 related 4 (CR4), pectate lyase family protein and subtilase family protein) co-modulated floret development (Fig. 3). 3-ketoacyl-coa synthase 6 and strictosidine synthase family proteins were essential for protein synthesis during floret growth. As expected, hormone signalling components isopentenyl transferase 5, gibberellin 2-oxidase 6, gibberellin

20 oxidase 2, and auxin response factor 16 were specifically expressed in florets (Fig. 3). In summary, the cob and florets showed contrasting gene expression profiles, underpinning their tissue identities and distinct biological functions at a critical developmental stage in maize.

Effects of low nitrogen supply on global gene expression in the cob and florets

LN resulted in differential expression (2-fold cutoff) of 588 genes in the cob and 195 genes in florets at the silking stage (Table 1). For genes with enhanced expression under LN, 258 genes were only detected in the cob, 64 genes only in florets, and 61 genes in both tissues (Fig. 4); for those with reduced expression under LN, 224 genes were detected only in the cob, 25 genes only in florets, and 45 in both tissues (Fig. 4).

GO enrichment analysis showed that among 319 genes up-regulated in the cob in response to LN, genes involved in 15 biological processes were enriched; and among 269 genes down-regulated in the cob, genes involved in 14 biological processes were enriched (Fig. 5A). Among 125 genes upregulated in the florets in response to LN, genes involved in 15 biological processes were enriched; and among 70 genes down-regulated in the florets, genes involved in six biological processes were enriched (Fig. 5B). The most enriched categories in the cob under LN were carbohydrate and nitrogen metabolic processes (Fig. 5A). Although less significant than those in the cob, genes involved in carbohydrate and nitrogen metabolic processes were still enriched in florets (Fig. 5B), indicating that LN affected these two biological processes in both the cob and florets although different number of genes were affected.



Fig. 2. Functional categorization of the differentially expressed genes (FDR<0.05) between the cob and florets under sufficient N supply. Each coloured dot represented one gene. Red colour indicates an increased expression in the cob relative to florets whereas the blue colour indicates an increased expression in florets relative to the cob. (A) Metabolism overview of the differentially expressed genes between the cob and florets. (B) Hormone related genes. (C) Cellular response related genes.

Further analysing expression of genes mediating C/N metabolism by MapMan visualization showed differential responses of the cob and florets to LN stress (Supplementary Fig. S1; Supplementary Table S8). In the cob, the expression of two genes involved in starch degradation (GRMZM2G34770 and GRMZM2G082034) was repressed. Similarly, *methionine*

adenosyltransferase (GRMZM2G117198) was downregulated 2.3-fold, although 3-phosphoglycerate dehydrogenase (GRMZM2G073814) was up-regulated 2.5-fold. Genes mediating minor CHO metabolism were differentially regulated in the cob. *Hydrolyzing O-glycosyl compound* (GRMZM2G340656) mediating raffinose synthesis and

c.b. real Column	A. Transport	D. Metabolism
Image: Selection of the selection	Cob Floret	Cob Floret
Image: Status (Status (GRMZM2G180080(cytochrome BC1 synthesis)	GRMZM2G091643(AMP-dependent synthetase and ligase)
	GRMZM2G434557(Vacuolar iron transporter (VIT))	GRMZM2G302002(nydroxycinnamoyi-coa) snikimate/quinate nydroxycinnamoyi transferase)
	GRMZM2G08/901(sucrose transporter z	RC209013.3_FG003(O-methylicalise)
	GRMZM2G05761(Maior facilitator, protein)	GRMZN2G179679(Nodulin MtN3)
Image: District Distrit District District District District District District District D	GRMZM2G155370(chloroplast import apparatus 2)	GRMZM5G822100(Cytochrome P450 protein)
	GRMZM2G457523(cation/hydrogen exchanger 28)	GRMZM2G444560(P-loop containing nucleoside triphosphate hydrolases protein)
	GRMZM2G145989(amino acid permease 1)	GRMZM2G097981(NAD(P)-binding Rossmann-fold protein)
•••••••••••••••••••••••••••••	GRMZM2G020859(potassium transport 2/3)	GRMZM2G150289907(Ankyrin repeat)
GRAZQ201926(g)thmine dumped year GRAZQ201926(g)thmine dumped year GRAZQ200926(g)thmine dumped year GRAZQ200926(g)thmine dumped year GRAZQ200926(g)thmine dumped year GRAZQ200927(g)thmine dumped year GRAZQ200927(g)thmine dumped year GRAZQ200927(g)thmine dumped year GRAZQ201927(g)thmine dumped year GRAZQ201927(g)thmine dumped year	GRMZM2G101376(zinc transporter 1 precursor)	GRMZM2G367668(laccase 2)
Basel Acade Sections Extra ANTHANS is relating in the section in the sect	GRMZM2G175425(glutamine dumper 4)	GRMZM2G094955(homolog of Medicago truncatula MTN3)
Image: Selective Selectiv	GRMZM2G021909(ENTH/ANTH/VHS protein)	GRMZM2G110511(UDP-glucosyl transferase 73B5)
Image: Production of the second product of the second pro	GRMZM2G030216(nodulin MtN21 /EamA-like transporter)	GRMZM2G123654(HXXXD-type acyl-transferase)
Chronic Discrete Statution and Discrete Statution Chronic Discrete Statution Chronic Discrete Statution Chronic Discrete Statution Chronic Discrete Statution Chronic Discrete Statution Chronic Discrete Statution Chronic Discrete Statution Chronic Discrete Statution Chronic Discrete Statution Chronic Discrete Statution Chronic Discrete Statution Chronic Discrete Statution Chronic Discrete Statution Chronic Discrete Statution Chronic Discrete Statution Chronic Discrete Statution Chronic Discrete Statution Chronic Discrete Statution Chronic Discrete Statution Chronic Discrete Statution Chronic Discrete Statution Chronic Discrete Statution Chronic Discrete Statution Chronic Discrete Statution Chronic Discrete Statution Chronic Discrete Statution Chronic Discrete Statution Chronic Discrete Statution Chronic Discrete Statution Chronic Discrete Statution Chronic Discrete Statution Chronic Discrete Statution Chronic Discrete Statution Chronic Discrete Statution Chronic Discrete Statution Chronic Discrete Statution Chronic Discrete Statution Chronic Discrete Statution Chronic Discrete Statution Chronic Discrete Statution Chronic Discrete Statution Chronic Discrete Statution </td <td>GRMZM2G000730(P-glycoprotein 11) GRMZM2G310758(Peptatricopentide repeat (PBP) protein)</td> <td>GRMZNOG13322(Nodulin MtN3)</td>	GRMZM2G000730(P-glycoprotein 11) GRMZM2G310758(Peptatricopentide repeat (PBP) protein)	GRMZNOG13322(Nodulin MtN3)
CHARLAGE (1) Stability of the membrane lipbochem) CHARLAGE (1) Stability of the membrane lipbochem) CHARLAGE (1) Stability of the membrane lipbochem) CHARLAGE (1) Stability of the membrane lipbochem) CHARLAGE (1) Stability of the membrane lipbochem) CHARLAGE (1) Stability of the membrane lipbochem) CHARLAGE (1) Stability of the membrane lipbochem) CHARLAGE (1) Stability of the membrane lipbochem) CHARLAGE (1) Stability of the membrane lipbochem) CHARLAGE (1) Stability of the membrane lipbochem) CHARLAGE (1) Stability of the membrane lipbochem) CHARLAGE (1) Stability of the membrane lipbochem) CHARLAGE (1) Stability of the membrane lipbochem) CHARLAGE (1) Stability of the membrane lipbochem) CHARLAGE (1) Stability of the membrane lipbochem) CHARLAGE (1) Stability of the membrane lipbochem) CHARLAGE (1) Stability of the membrane lipbochem) CHARLAGE (1) Stability of the membrane lipbochem) CHARLAGE (1) Stability of the membrane lipbochem) CHARLAGE (1) Stability of the membrane lipbochem) CHARLAGE (1) Stability of the membrane lipbochem) CHARLAGE (1) Stability of the membrane lipbochem) CHARLAGE (1) Stability of the membrane lipbochem) CHARLAGE (1) Stability of the membrane lipbochem) CHARLAGE (1) Stability of the membrane lipbochem) CHARLAGE (1) Stability of the membrane lipbochem) CHARLAGE (1) Stability of the membrane lipbochem) CHARLAGE (1)	GRMZM5G830545(amino acid permease 8)	GRMZM2G034958(GDSL-like Lipase/Acylhydrolase protein)
Image: Set 2014 S	GRMZM2G102968(Putative membrane lipoprotein)	GRMZM2G057963(nudix hydrolase homolog 12)
Image: Set UNL/Control (Set Unit Unit Unit Unit Unit Unit Unit Uni	GRMZM2G004694(polyol/monosaccharide transporter 5)	GRMZM2G001938(Exostosin)
Localization of the second problem in the dual transported in the second transported in the seco	GRMZM2G1/5140(ammonium transportier 1;1)	GRMZM2G338865(Glycosyltransferase family 61 protein)
Burdingment Burdingme	GRM2M2G000645(Iransmembrane amino acid transporter)	GRMZM2G019619(Glycosyl bydrolase_protein)
B. Development File GRUZZQC11233GP(FT:1:is 6) GRUZZQC1123GP(FT:1:is 6) GRUZZQC1123GP(FT:1:i		GRMZM2G380286(Long-chain fatty alcohol dehydrogenase)
BRXZA02013SSIP(PK1-like 0) Comparison (Comparison 0) Comparison (Comparin) (Comparison 0) Comparison (Comparison 0) C	B. Development	GRMZM5G870067(UDP-glucosyl transferase 85A2)
Chrozyze (Composition 1) Composition 1) Composition 1) Composition 1) Composition 1) Composition 1) Composition 1) Composition 1) Composition 1) Composition 1) Composition 1) Composition 1) Composition 1) Composition 1) Co	GRMZM2G112538(PVR1-like 6)	GRMZM2G027130(Chalcone and stillene synthase)
Image: Accesses J. FCool (ROTUNDE/CLAW Be 15) Image: Accesses J. Cool (ROTUNDE/CLAW Be 15) Image: Accesses J. Cool (ROTUNDE/CLAW Be 15) Image: Accesses J. Cool (ROTUNDE/CLAW Be streamburg by complexity insisted-anchord) Image: Accesses J. Cool (ROTUNDE/CLAW Be streamburg by complexity insisted-anchord) Image: Accesses J. Cool (ROTUNDE/CLAW Be streamburg by complexity insisted-anchord) Image: Accesses J. Cool (ROTUNDE/CLAW Be streamburg by complexity insisted-anchord) Image: Accesses J. Cool (ROTUNDE/CLAW Be streamburg by complexity insisted-anchord) Image: Accesses J. Cool (ROTUNDE/CLAW Be streamburg by complexity insisted-anchord) Image: Accesses J. Cool (ROTUNDE/CLAW Be streamburg by complexity insisted-anchord) Image: Accesses J. Cool (ROTUNDE/CLAW Be streamburg by complexity insisted-anchord) Image: Accesses J. Cool (ROTUNDE/CLAW Be streamburg by complexity insisted-anchord) Image: Accesses J. Cool (ROTUNDE/CLAW Be streamburg by complexity insisted-anchord) Image: Accesses J. Cool (ROTUNDE/CLAW Be streamburg by complexity insisted-anchord) Image: Accesses J. Cool (ROTUNDE/CLAW Be streamburg by complexity insisted-anchord) Image: Accesses J. Cool (ROTUNDE/CLAW Be streamburg by complexity insisted-anchord) Image: Accesses J. Cool (ROTUNDE/CLAW Be streamburg by complexity insisted-anchord) Image: Accesses J. Cool (ROTUNDE/CLAW Be streamburg by complexity insisted-anchord) Image: Accesses J. Cool (ROTUNDE/CLAW Be streamburg by complexity insisted-anchord) Image: Accesses J. Cool (ROTUNDE/CLAW Be streamburg by complexity insisted-anch	GRMZM2G112524(PYR1-like 6)	GRWZM2G144 Ib0(embryo delective 2423)
Cranscription GRX2X20217722(cyclustene magnetic dehydrogenae 1) GRX2X2021772(cyclustene magnetic syntase - like 3) GRX2X2021772(cyclustene magnetic syntase - like 4) GRX2X2021772(cyclustene magnetic syntase - l	AC203957.3_FG001(ROTUNDIFOLIA like 15)	GRMZN2G162755(UDP-Glycosyltransferase protein)
Lindu/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Starsen/Lindu/Star	GRMZM2G167220(cytokinin oxidase/dehydrogenase 1)	GRMZM2G101142(strictosidine synthase-like 4)
CHUZUZGG 112407 (C) C) ERA - Ensaturational and protein () CHUZUZG 112407 (C) ERA - Ensaturational and protein () CHUZUZG 112407 (C) ERA - Ensaturational and protein () CHUZUZG 112407 (C) ERA - Ensaturational and protein () CHUZUZG 112407 (C) ERA - Ensaturational and protein () CHUZUZG 112407 (C) ERA - Ensaturational and protein () CHUZUZG 112407 (C) ERA - Ensaturational and protein () CHUZUZG 112407 (C) ERA - Ensaturational and protein () CHUZUZG 112407 (C) ERA - Ensaturational and protein () CHUZUZG 112407 (C) ERA - Ensaturational and protein () CHUZUZG 112407 (C) ERA - Ensaturational and protein () CHUZUZG 112407 (C) ERA - Ensaturational and protein () CHUZUZG 112407 (C) ERA - Ensaturational and ensaturation and ensaturational and ensaturational and ensatura	GRMZM2G338454(centroradialis)	GRMZM2G395508(UDP-glucosyl transferase 88A1)
AC207043.3 FG002V0G molf-containing profein () FG002V0G molf-containing profein () AC207043.3 FG002V0G molf-containing profein () FG002V0G molf-containing profein () AC207043.3 FG002V0G molf-containing profein () FG002V0G molf-containing profein () AC207043.3 FG002V0G molf-containing profein () FG002V0G molf-containing profein () AC207043.3 FG002V0G molf-containing profein () FG002V0G molf-containing profein () AC207043.3 FG002V0G molf-containing profein () FG002V0G molf-containing profein () AC207043.3 FG002V0G molf-containing profein () FG002V0G molf-containing profein () AC207043.3 FG002V0G molf-containing profein () FG002V0G molf-containing profein () AC207043.3 FG002V0G molf-containing profein () FG002V0G molf-containing profein () AC207043.3 FG002V0G molf-containing profein () FG002V0G molf-containing profein () AC207043.3 FG002V0G molf-containing profein () FG002V0G molf-containing profein () AC207043.3 FG002V0G molf-containing profein () FG002V0G molf-containing profein () AC207043.3 FG002V0G molf-containing profein () FG002V0G molf-containing profein () AC207043.3 FG002V0G molf-containing profein () FG002V0G molf-containing pr	GRMZW2G151611(Entylene insensitive 3) GRMZW2G15747(COBRA-like extracellular glycosyl-phosphatidyl inositol-anchored)	GRMZM2G092621(Galactose mutarotase-like protein)
C. Transcription GRMZM2G57364(pr2A)-bas suburit 8) GRMZM2G57364(pr2A)-bas suburit 8) GRMZM2G57364(pr2A)-bas suburit	AC207043.3 FG002(VQ multi-containing protein)	GRMZM2G060/481(3-kotagul-coA sumbase 6)
 GRAZMAG172053(vacoular related MAC-domain protein 1) GRAZMAG172053(vacoular related PAC-domain protein 1) GRAZMAG172053(vacoular related PAC-domain protein 1) GRAZMAG11911(citulase 2) GRAZMAG11931(citular response fuctors) GRAZMAG11932(citular response fuctors) GRAZMAG11933(citular response fuctors	GRMZM2G134040(RPA70-kDa subunit B)	GRMZM2G008064(Nucleotide=dinbosho=sugar transferase)
Comparison of the second	GRMZM2G172053(vascular related NAC-domain protein 1)	GRMZM2G136571(alpha/beta-Hydrolases protein)
 Convertigion Conve	GRMZM2G12/121(PEBP (phosphatidylethanolamine-binding protein))	GRMZM2G067049(OSBP(oxysterol binding protein)-related protein 4C)
C:NUZW2C021051 (jibberdin 20 oxidase 2) C:NUZW2C021051 (jibberdin 20 oxidase 2) C:NUZW2C021051 (jibberdin 20 oxidase 2) C:NUZW2C012051 (jibberdin 20 oxidase protein 3) C:NUZW2C012051 (jibberdin 20 oxidase	GRMZM20000709(peluxin Tb)	
GRNZ2N2C055434[early noclulin-like protein] GRNZ2N2C055434[early noclulin-like protein] GRNZ2N2C055434[early noclulin-like protein] GRNZ2N2C054342[NZN2S11432] GRNZ2N2C054342[NZN2S11432] GRNZ2N2C054342[NZN2S11432] GRNZ2N2C054342[NZN2S11432] GRNZ2N2C054342[NZN2S11432] GRNZ2N2C054342[NZN2S11432] GRNZ2N2C054342[NZN2S11432] GRNZ2N2C051432] GRNZ2N2C051432] GRNZ2N2C051432] GRNZ2N2C051432] GRNZ2N2C051432] GRNZ2N2C0515344[NZN2S1145] GRNZ2N2C0515344[NZN2S1145] GRNZ2N2C0515344[NZN2S1145] GRNZ2N2C0566102[Nz-harpin glycosidases protein] GRNZ2N2C051534[Nz-harpin glycosidases protein] GRNZ2N2C0515354[Nz-harpin glycosidases protein] GRNZ2N2C011255[CF12+drd2C417038](Potentily associates 6] GRNZ2N2C011255[CF22+drd2C42:rsr fingers protein] GRNZ2N2C011255[CF22+drd2C42:rsr fingers protein] GRNZ2N2C011255[CF22+drd2C42:rsr fingers protein] GRNZ2N2C011355[Cr22+rsr finger finder 4] GRNZ2N2C011455[CF22+rsr finger finder 4] GRNZ2N2C011455[CF22+rsr finger finder 4] GRNZ2N2C011455[CF22+rsr finger finder 4] GRNZ2N2C011455[CF22+rsr finger finde	GRMZM2G021051(gibberellin 20 oxidase 2)	E. Cell rescue and defense
GRNZ242313398(Pecin lyase-like protein) GRNZ24231398(Pecin lyase-like protein) GRNZ2423138(Pecin lyase-like protein) GRNZ2423148(Pecin lyase-like protein) GRNZ2423148(Pecin lyase-like protein) GRNZ2423148(Pecin lyase-like protein) GRNZ2423148(Pecin lyase-like protein) GRNZ243348(Pecin lyase-like protein) GRNZ243348(Pecin lyase-like protein) GRNZ243348(Pecin lyase-like protein) GRNZ243348(Pecin lyase-like protein) <td>GRMZM2G055434(early nodulin–like protein 3)</td> <td>GRMZM2G433184(Disease resistance-responsive (dirigent-like protein))</td>	GRMZM2G055434(early nodulin–like protein 3)	GRMZM2G433184(Disease resistance-responsive (dirigent-like protein))
C-MUZM22119895(CHNML+ Teplet 4) C-MUZM22119895(CHNML+ Teplet 4) C-MUZM2211985(CHNML+ Teplet 4) C-MUZM2211918(CHNML+ Teplet 4)	GRMZM2G139396(Pectin lyase-like protein)	GRMZM2G014055(thioredoxin H-type 7)
CRNZ2X2000442(2):operating/times/tenses	GRMZM2G116685(CRINKLY4 related 4)	GRMZM2G039502(Chaperone Dhap-domain protein)
CRNZ2M22003284[autin regionse factor 16] CRNZ2M20127028(expansin A7) GRNZ2M20127028(expansin A7) GRNZ2M20127028(expansin A7) GRNZ2M20127028(expansin A7) GRNZ2M20127028(expansin A7) GRNZ2M201210018(expansin A7) GRNZ2M201210018(expansin A7) GRNZ2M20121018(expansin A7) GRNZ2M2012018(expansin A7) GRNZ2M2012018(expansin A7) GRNZ2M2012018(expansin A7) GRNZ2M2012018(expansin A7) GRNZ2M2012018(expansin A7) GRNZ2M2014(expansin A7) GRNZ2M2014(expansin A7) GRNZ2M2011999(homebox protein 16) GRNZ2M2011998(RNA) GRNZ2M2011998(RNA) GRNZ2M20119	GRMZN2G08462(isopenenvirtalsferase 5)	GRMZM2G126763(glutathione S-transferase phi 8)
GRNZM2G1252[gebpania] GRNZM2G1255[gebpania] GRNZM2G1255[g	GRMZM2G005284(auxin response factor 16)	GRMZM2G333659(NB-ARC domain-containing disease resistance protein)
CRWZM2203125(protokarmal ractor 1) GRWZM220328(plotheraing ractor 1) GRWZM2203125(plotheraing ractor 1) GRWZM2203125(plotheraing ractor 1) GRWZM22031125(plotheraing ractor 1) GRWZM220111125(plotheraing ractor 1)	GRMZM2G127029(expansin A7)	GRMZM2G125918(Pathogenesis-related thaumatin protein)
LinkWardsuberta2(sinc-marpin givossidase protein) GRW2M2G128586(myb domain protein 20) GRW2M2G1285566(myb domain protein 20) GRW2M2G1285566(myb domain protein 20) GRW2M2G1285567(CH2) and C2HC zinc finger) GRW2M2G128567(CH2) and C2HC zinc finger) GRW2M2G0128(1-2ncmark) GRW2M2G0148(2-2ncmark) GRW2M2G0148(1-2ncmark) GRW2M2G0148(1-2ncmark) GRW2M2G0148(1-2ncmark) GRW2M2G0148(1-2ncmark) <td>GRMZM2G141325(protodermal factor 1)</td> <td>GRMZM5G860607(Thioredoxin protein)</td>	GRMZM2G141325(protodermal factor 1)	GRMZM5G860607(Thioredoxin protein)
Conversion C	GRMZM2G066162(Six-harpin glycosidases protein)	GRMZM2G12959904(Low temperature and sait responsive)
C. Transcription CFMZZU26126566(myb domain protein 20) CFMZZU26126566(myb domain protein 20) GRMZZM220125(C2H2-type zinc finger) GRMZZM220125(C2H2-type zinc finger) GRMZZM2200125(C2H2-type zinc finger) GRMZZM2200455(C2H2-type zinc finger) GRMZZM2200455(C2H2-type zinc finger) GRMZZM2200455(C2H2-type zinc finger) GRMZZM2200455(C2H2-type zinc finger) GRMZZM2200455(C2H2-type zinc finger) GRMZZM2200455(C2H2-type zinc finger) GRMZZM2200455(C2H2-type zinc finger) GRMZZM2200455(C2H2-type zinc finger) GRMZZM2200455(C2H2-type zinc finger) GRMZZM2200455(C2H2-type zinc finger) GRMZZM2200455(C2H2-type zinc finger) GRMZZM2200455(C2H2-type zinc finger) GRMZZM2200455(C2H2-type zinc finger) GRMZZM2200455(C2H2-type zinc finger) GRMZZM220462(Conce-type zinc finger) GRMZZM220452(1968/LSD1 zinc finger) GRMZZM220462(Conce-type zinc finger) GRMZZM220462(Conce-type zinc finger) GRMZZM220462(C2NE) GRMZZM220462(C2NE) GRMZZM220462(C2NE) GRMZZM220462(C2NE) GRMZZM220464(C2HZ) GRMZZM220464(CAM2-type zinc finger) GRMZZM220464(CAM2-type zinc finger) GRMZZM220464(CAM2-type zinc finger) GRMZZM2201458(C2NE) GRMZZM220464(C2H2-type zinc finger) GRMZZM220464(CAM2-type zinc finger) GRMZZM2204642(C2H2-type zinc finger) GRMZZM2201458(C2H2-tinclin contant intel) GRMZZM2204	GRMZM2G410783(Pertin lyase-like protein)	GRMZN2G023827(Ankyrin repeat)
C. Transcription GRMZJW2G125668(myb domain protein 20) GRMZJW2G1205(CH2-Vpg Price finger) GRMZJW2G1080(CH2-Vpg Price finger) GRMZJW2G1080(SH2) GRMZJW2G10849(NAC-like, activated by AP3/PI) GRMZJW2G10849(NAC-like, activated by AP3/PI) GRMZJW2G10849(NAC-like, activated by AP3/PI) GRMZJW2G10849(NAC-like, activated by AP3/PI) GRMZJW2G1082(SH2) GRMZJW2G1082	Contraction of the protein of the pr	GRMZM2G420758(Disease resistance-responsive (dirigent-like protein))
 Infanseription RMXZM2G1252656(myb domain protein 20) GMXZM2G0125(5242-type zinc finger) GRMZM2G0125(5242-type zinc finger) GRMZM2G0125(5244442542437444444444444444444444444444		GRMZM2G371486(Heavy metal transport/detoxification protein)
GRMZM2G125656(myb domain protein 20) GRMZM2G0125(C2H2-ype zinc finger) GRMZM2G0125(C2H2-ype zinc finger) GRMZM2G0125(C2H2-ype zinc finger) GRMZM2G0125(C2H2-ype zinc finger) GRMZM2G012207(Heavy metal transport/detoxfication protein) GRMZM2G012215(homeobox 1) GRMZM2G012215(homeobox 1) GRMZM2G012215(homeobox 1) GRMZM2G012215(homeobox 1) GRMZM2G012215(homeobox 1) GRMZM2G01215(homeobox 1) GRMZM2G01215(homeobox 1) GRMZM2G12855(C2H2 and homeobox 1) GRMZM2G12855(C2H2 and homeobox 1) GRMZM2G019957(C2H2 and homeobox 1) GRMZM2G01957(C2H2 and home 1 GRMZM2G01957(CH2 and home 1 GRMZM2G01957(CH2 and home 1 GRMZM2G01957(CH2 and home 1 GRMZM2G01957(CH2 and	C. Transcription	GRMZM2G108090(HSP20-like chaperones protein)
GRMZM2G001205(2H2-type zinc finger) GRMZM2G008583(mt) domain protein 4) GRMZM2G008583(mt) domain protein 4) GRMZM2G008583(mt) domain protein 4) GRMZM2G00857(2H2 and C2HC zinc finger) GRMZM2G00858(mt) chorns (inter) GRMZM2G00858(mt) chorns (inter) GRMZM2G0090528(cyclic nucleotide-gated cation channel 4) GRMZM2G01878(h2KE times; times times) GRMZM2G01878(h2KE times; times times) GRMZM2G01878(h2KE times; times times) GRMZM2G01878(h2KE times; times times) GRMZM2G01873(h3KE times; times times) GRMZM2G01873(h3KE times; times) GRMZM2G018748(h2KE times)	GRMZM2G126566(myb domain protein 20)	GRWZWZG036427(Adenine nucleotide alpha bydrolase 2)
GRMZ/M2G044985 GRMZ/M2G049849KAC-like, activated by AP3/P1) GRMZ/M2G002915(homeobox 1) GRMZ/M2G002915(homeobox 1) GRMZ/M2G012915(homeobox 1) GRMZ/M2G009528(cyclic nucleotide-gated cation channel 4) GRMZ/M2G012915(homeobox 1) GRMZ/M2G009528(cyclic nucleotide-gated cation channel 4) GRMZ/M2G014952(hasic helix-loop-helix (bHLH) DNA-binding protein) GRMZ/M2G0149346(sarchochate peroxidases 1) GRMZ/M2G01491243(Catinod-helix (bHLH) DNA-binding protein) GRMZ/M2G0197837(heavy metal transport/detoxification 'protein) GRMZ/M2G01981(sb) Lix (higher) GRMZ/M2G0197837(heavy metal transport/detoxification 'protein) GRMZ/M2G01981(sb) Lix (higher) GRMZ/M2G0197837(heavy metal transport/detoxification 'protein) GRMZ/M2G01981(sb) Lix (higher) GRMZ/M2G0197837(heavy metal transport/detoxification 'protein) GRMZ/M2G019787(heavy metal transport/detoxification 'protein) GRMZ/M2G0197837(heavy metal transport/detoxification 'protein) GRMZ/M2G019787(heavy metal transport/detoxification 'protein) GRMZ/M2G019787(heavy metal transport/detoxification 'protein) GRMZ/M2G01981(sb) Lix (higher) GRMZ/M2G019787(heavy metal transport/detoxification 'protein) GRMZ/M2G019787(heavy metal transport/detoxification 'protein) GRMZ/M2G019787(heavy metal transport/detoxification 'protein) GRMZ/M2G019787(heavy metal transport/detoxification 'protein) GRMZ/M2G019787(heavy metal transport/det	GRMZM2G001205(C2H2-type zinc finger)	GRWZW266858417(nucleonae-ascorbate transporter 7)
GRWZM2G002315(Intervalues up And on 1) GRWZM2G0093346(ascorbate peroxidase 1) GRWZM2G009557(CH2 and C2H2 cation fingers protein) GRWZM2G0193546(ascorbate peroxidase 1) GRWZM2G019557(CH2) GRWZM2G0114252(basic helix-loop-helix (bHLH) DNA-binding protein) GRWZM2G0114252(basic helix-loop-helix (bHLH) DNA-binding protein) GRWZM2G011388(ANA/P2H3-like transcription activator) GRWZM2G0114550(Core-2I)-branching beta-1.6-N-acetylglucosaminyltransferase) GRWZM2G01737(Home of the first of th	GRMZM2G084583(myb domain protein 4) GRMZM2G4398498(NAC-like activited by AB3/Pl)	GRMZM2G032807(Heavy metal transport/detoxification protein)
GRMZM22G089557/C2H2 and c2HC zinc fingers protein) GRMZM2G4425(basic bick/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/coup-telix/c	GRMZN2G002915(homeohox 1)	GRMZM2G093346(ascorbate peroxidase 1)
GRMZM2G414252[basic helix-loop-helix (bit-lu) DNA-binding protein) GRMZM2G414252[basic helix-loop-helix (bit-lu) DNA-binding protein 16) GRMZM2G414252[basic helix-loop-helix (bit-lu) DNA-binding protein 16] GRMZM2G478042[Core-2/l-branching beta-1,6-N-acetylglucosaminyltransferase) GRMZM2G477806[ARE hiding factor 4) GRMZM2G477806[ARE hiding factor 4) GRMZM2G40302551[WRKY family transcription factor) GRMZM2G403983[Seinefiltreceptor-like protein kinase) GRMZM2G403983[Seinefiltreceptor-like protein kinase) GRMZM2G403983[WhAG domain containing protein 11] GRMZM2G403983[WhAG domain containing protein 11] GRMZM2G403983[Whod momin protein 11] GRMZM2G404284[WIAF] GRMZM2G404284[WIAF] GRMZM2G404284[WIAF] GRMZM2G404282[WIAF] GRMZM2G404284[WIAF] GRMZM2G40484[WIAF] GRMZM2G40484[WIAF] GRMZM2G40484[WIAF] GRMZM2G40484[WIAF] </td <td>GRMZM2G089557(C2H2 and C2HC zinc fingers protein)</td> <td>GRMZM2G090528(cyclic nucleotide-gated cation channel 4)</td>	GRMZM2G089557(C2H2 and C2HC zinc fingers protein)	GRMZM2G090528(cyclic nucleotide-gated cation channel 4)
Image: CRMZ/2G431445(Acyl-CoA N=acyt(ransferases (NAT) protein) Image: CRMZ/2G431445(Acyl-CoA N=acyt(ransferases) Image: CRMZ/2G431243(Calmodulin-binding transcription activator) Image: CRMZ/2G431243(Calmodulin-binding transcriptional factor) Image: CRMZ/2G478760(ABRE binding factor 4) Image: CRMZ/2G478760(ABRE binding protein 1)	GRMZM2G414252(basic helix-loop-helix (bHLH) DNA-binding protein)	E Signal transduction
GRMZ/M2G119999(nomeodox protein 10 GRMZ/M2G119999(nomeodox protein 10 GRMZ/M2G19999(nomeodox protein 10 GRMZ/M2G19999(nomeodox protein 10 GRMZ/M2G198041243(Calmodulin -beginnermic 10 GRMZ/M2G198041243(Calmodulin -beginnermic 10 GRMZ/M2G198041243(Calmodulin -beginnermic 10 GRMZ/M2G198041243(Calmodulin -beginnermic 10 GRMZ/M2G198041745 GRMZ/M2G19804174 GRMZ/M2G198174	GRMZM2G211445(Acyl-CoA N-acyltransferases (NAT) protein)	1. Signar transutcitori
GRNZZNZG088413()/EPJ03-ilike initiating tentoriphonal factor) GRNZZNZG088413()/EPJ03-ilike initiating tentoriphonal factor) GRNZZNZG088413()/EPJ03-ilike initiating tentoriphonal factor) GRNZZNZG088413()/EPJ032(core-2/l-branching beta-1,6-N-acetylglucosaminyltransferase) GRNZZNZG088413()/EPJ0351(WRK/Hamily transcription factor) GRNZZNZG002551(WRK/Hamily transcription factor) GRNZZNZG002551(WRK/Hamily transcription factor) GRNZZNZG01283(RNA helicase) GRNZZNZG01283(RNA helicase) GRNZZNZG0128443(Ar2/B3-like transcriptional factor) GRNZZNZG0128443(Ar2/B3-like transcriptional factor) GRNZZNZG01284443(Ar2/B3-like transcriptional factor) GRNZZNZG01283(FineCeptor kinase) GRNZZNZG01083(myk domain protein 1) GRNZZNZG01083(myk domain protein 11) GRNZZNZG01083(myk dotalit nuclear-localized protein 20) GRNZZNZG011236(righter protein 1) GRNZZNZG011236(righter protein 1) GRNZZNZG011236(righter protein 20) GRNZZNZG011236(righter protein 20)	GRMZM2G119999(nomeobox protein 16) GRMZM2G431242(Calmedulin-binding transcription activator)	GRMZM2G01973(Reticulon)
GRMZM2G089106[LSD1 zinc finger) GRMZM2G178062(core-21/branchinger) GRMZM2G178062(core-21/branchinger) GRMZM2G178062(core-gto-like protein kinase) GRMZM2G178062(core-gto-like protein kinase) GRMZM2G12828(Leucine-rich repeat protein kinase) GRMZM2G12883(RNA helicase) GRMZM2G013283(RNA helicase) GRMZM2G12883(RNA helicase) GRMZM2G013283(RNA helicase) GRMZM2G013283(RNA helicase) GRMZM2G013283(RNA helicase) GRMZM2G013283(RNA helicase) GRMZM2G013283(RNA helicase) GRMZM2G1714850(NAC domain containing protein 1) GRMZM2G013283(Senine(threonine-protein kinase) GRMZM2G013283(RNA helicase) GRMZM2G013283(Colleun-delume-binding protein n) GRMZM2G013283(RNA helicase) GRMZM2G013283(Na helicine-ceptor-like protein kinase) GRMZM2G013283(RNA helicase) GRMZM2G013787(Leucine-rich receptor-like protein kinase) GRMZM2G013787(Homeobox-leucine z)poer) GRMZM2G038437(receptor kinase 3) GRMZM2G046252(PHD finger protein) GRMZM2G088816(Leucine-rich reseptor like protein kinase) GRMZM2G011236(related to AP2 11) GRMZM2G01282(related to AP2 11) GRMZM2G011236(related to AP2 11) GRMZM2G0488816(Leucine-rich repat protein) GRMZM2G011236(related to AP2 11) GRMZM2G01236(serine carboxypeptidase-like 3) GRMZMZG0488816(C2H2-like zinc finger protein)	GRMZM2G088413(AP2/B3-like transcriptional factor)	GRMZM2G349655(calmodulin like 42)
GRMZM2G178062(20cre-2/L-braiching beta-1,6-M-acetylglucosaminyltransferase) GRMZM2G47806(ARBE binding factor 4) GRMZM2G478760(ARBE binding factor 4) GRMZM2G478760(ARBE binding factor 4) GRMZM2G478760(ARBE binding factor 4) GRMZM2G478760(ARBE binding factor 4) GRMZM2G478760(ARBE binding factor 4) GRMZM2G47320(Maletelin/receptor-like protein kinase) GRMZM2G47320(Maletelin/receptor-like protein kinase) GRMZM2G47320(Maletelin/receptor-like protein kinase) GRMZM2G473780(ARBE binding factor) GRMZM2G47323(Maletelin/receptor-like protein kinase) GRMZM2G43781(BNR/Agp-box repeat) GRMZM2G43781(BNR/Agp-box repeat) GRMZM2G43781(BNR/Agp-box repeat) GRMZM2G4308074(Leucine=rich receptor-like protein kinase) GRMZM2G43781(BNR/Agp-box repeat) GRMZM2G4308074(Leucine=rich receptor-like protein kinase) GRMZM2G430781(bromebox-leucine zpper) GRMZM2G4308074(Leucine=rich receptor-like protein kinase) GRMZM2G46252(PHD longer protein 111) GRMZM2G4308074(Leucine=rich receptor-like protein kinase) GRMZM2G406825(PHD longer protein 20) GRMZM2G430902(217454)(Eucine=rich repeat (LRR)) GRMZM2G445648(C2HZ-Like zinc finger protein 20) GRMZM2G430902(2126)(Eucine=rich repeat (LRR)) GRMZM2G445648(21126) GRMZM2G430902(2126)(Eucine=rich repeate) GRMZM2G44554(PHD lonker) GRMZM2G44446(Eucine=rich repeate) <	GRMZM2G089106(LSD1 zinc finger)	GRMZM2G128644(calmodulin (CAM)-binding protein of 25 kDa)
GRMZM2G479760(ABRE binding factor 4) AC209739.3 - FC003(Calcium-dependent ipld-Dinding (CaLB domain)) GRMZM2G03551(WRXP family transcription factor) GRMZM2G03551(WRXP family transcription factor) GRMZM2G03253(RNA helicase) GRMZM2G042323(Senie(threonine-protein kinase) GRMZM2G012283(RNA helicase) GRMZM2G1283(Collame-dependent ipld-Dinding (CaLB domain)) GRMZM2G012283(RNA helicase) GRMZM2G1283(Senie(threonine-protein kinase) GRMZM2G1485(NAC domain containing protein 1) GRMZM2G307397(Homeobox-leucine zpper) GRMZM2G307397(Homeobox-leucine zpper) GRMZM2G307397(Homeobox-leucine zpper) GRMZM2G30837(seceptor kinase 3) GRMZM2G30837(seceptor kinase 3) GRMZM2G178435(chromain remodeling 40) GRMZM2G048252(rich fuger protein 1) GRMZM2G19786(ICu-domain 26) GRMZM2G11236(related to AP2 11) GRMZM2G138485(serine carboxypeptidase-like 33) GRMZM2G138445(serine carboxypeptidase-like 33) GRMZM2G13844564(C2H2-like zinc finger protein 2) GRMZM2G138445(serine carboxypeptidase-like 33) GRMZM2G138445(serine carboxypeptidase-like 33) GRMZM2G13844564(C2H2-like zinc finger protein 2) GRMZM2G138445(serine carboxypeptidase-like 33) GRMZM2G138445(serine carboxypeptidase-like 33) GRMZM2G13537(rdetber) GRMZM2G13845(serine carboxypeptidase-like 40) GRMZM2G13845(serine carboxypeptidase-like 40) GRMZM2G1335(rdetber) GRMZM2G0	GRMZM2G178062(Core-2/I-branching beta-1,6-N-acetylglucosaminyltransferase)	GRMZM2G162829(Leucine-rich repeat protein kinase)
GRMZ/M2G03521(WRKY taminy transcription flactor) GRMZ/M2G01323(RKM helicase) GRMZ/M2G01323(RKM helicase) GRMZ/M2G01323(RKM helicase) GRMZ/M2G01323(RKM helicase) GRMZ/M2G01323(RKM helicase) GRMZ/M2G0348(APZ) GRMZ/M2G048(APZ) GRM	GRMZM2G479760(ABRE binding factor 4)	AC209/39.3 FG003(Calcium-dependent lipid-binding (CaLB domain))
GRNZZW2G114850(NAC domain containing protein 1) GRNZZW2G114852(noline calcium-binding protein 1) GRNZZW2G127184(BNZ/B2-like transcriptional factor) GRNZZW2G127714(BNIXAg-box repeat) GRNZZW2G701083(myb domain protein 111) GRNZZW2G701083(myb domain protein 111) GRNZZW2G701083(myb domain protein 111) GRNZZW2G70108(myb domain protein 120) GRNZZW2G701083(myb domain protein 111) GRNZZW2G70108(myb domain protein 120) GRNZZW2G701082(myb domain protein 11) GRNZZW2G70108(myb domain protein 120) GRNZZW2G701082(myb domain protein 120) GRNZZW2G70108(myb domain protein 120) GRNZZW2G701082(myb domain protein 11) GRNZZW2G70108(myb domain protein 120) GRNZZW2G701082(myb domain protein 11) GRNZZW2G70108(myb domain protein 120) GRNZZW2G701082(myb domain protein 11) GRNZZW2G70108(myb domain protein 120)	GRMZM2G003551(WRKY family transcription factor)	GRMZM2G302293(Serine//hecepior=protein kinase)
GRMZM2G024948/kP2/B3-like transcriptional factor) GRMZM2G1744[BNK/Ap-box repeat] GRMZM2G17744[BNK/Ap-box repeat] GRMZM2G307397[Homeobox-leucine zipper] GRMZM2G17744[BNK/Ap-box repeat] GRMZM2G307397[Homeobox-leucine zipper] GRMZM2G17744[BNK/Ap-box repeat] GRMZM2G307397[Homeobox-leucine zipper] GRMZM2G17744[BNK/Ap-box repeat] GRMZM2G307397[Homeobox-leucine zipper] GRMZM2G17744[BNK/Ap-box repeat] GRMZM2G01785[(Cyclin] GRMZM2G177445[chromain remodeling 40] GRMZM2G17746[(Leucine-rich repeat (LRR)) GRMZM2G11726[strating beta-1,6-N-acetylglucosaminyltransferase] GRMZM2G101766[(Q-domain 26) GRMZM2G11236[related to AP2 11] GRMZM2G138495[serine carboxypeptidase-like 33] GRMZM2G1588816[c2H2-like zinc finger protein] GRMZM2G138495[serine carboxypeptidase-like 33] GRMZM2G1588816[c2H2-like zinc finger protein] GRMZM2G138495[serine carboxypeptidase-like 33] GRMZM2G1588816[c2H2-like zinc finger protein] GRMZM2G138495[serine carboxypeptidase-like 31] GRMZM2G11384684(c2H2-type zinc finger) GRMZM2G13849[serine carboxypeptidase-like 40] GRMZM2G430902[zinc finger (J3H24-type Zinc finger) GRMZM2G07222(soltaise) GRMZM2G430902[zinc finger (J3H24-type Zinc finger) GRMZM2G138495[serine carboxypeptidase-like 40] GRMZM2G4264584(22H2-type Zinc finger) GRMZM2G138495[serine c	GRMZM2G114850(NAC domain containing protein 1)	GRMZM2G419452(pollen calcium-binding protein 1)
GRMZM2G30807427714{BNR/Asp-box repeat) GRMZM2G307397(homebox-leucine zipper) GRMZM2G307397(homebox-leucine zipper) GRMZM2G402652(PHD finger protein) GRMZM2G4652(PHD home modeling 40) GRMZM2G178435(chromatin remodeling 40) GRMZM2G178435(chromatin remodeling 40) GRMZM2G011236(related to AP2 r1) GRMZM2G011236(related to AP2 r1) GRMZM2G011236(related to AP2 r1) GRMZM2G011236(related to AP2 r1) GRMZM2G115388(1ab(C2H2-like zinc finger protein) GRMZM2G115388(2Hz-time zinc finger protein) GRMZM2G115388(2Hz-time zinc finger protein) GRMZM2G011236(related to AP2 r1) GRMZM2G011236(related to AP2 r1) GRMZM2G011236(related to AP2 r1) GRMZM2G011236(related to AP2 r1) GRMZM2G011238(related to AP2 r1) GRMZM2G01238(serine carboxypeptidase-like 5) GRMZM2G01238(serine carboxypeptidase-like 6) GRMZM2G01238(serine carboxypeptidase-like 40) GRMZM2G01238(subliase) GRMZM2G01238(subliase) GRMZM2G01238(subliase) GRMZM2G01238(subliase) GRMZM2G01238(subliase) GRMZM2G01238(subliase) GRMZM2G01238(subliase) GRMZM2G01238(subliase) GRMZM2G01238(subliase) GRMZM2G01238(subliase) GRMZM2G01238(subliase) GRMZM2G01238(subliase) GRMZM2G01238(subliase) GRMZM2G01238(subliase) GRMZM2G01238(subliase) GRMZM2G01238(subliase) GRMZM2G01238(subliase) GRMZM2G01238(subliase) GRMZM2G01238(subliase) GRMZM2G01238(subliase) GRMZM2G01238(subliase) GRMZM2G01238(subliase) GRMZM2G01238(subliase) GRMZM2G03828(subliase) GRMZM2G03828(subliase) GRMZM2G03828(subliase) GRMZM2G03828(subliase) GRMZM2G03828(subliase) GRMZM2G0388(subliase) GRMZM2G03828(subliase) GRMZM2G0388(subliase) GRMZM2G03	GRMZM2G024948(AP2/B3–like transcriptional factor)	GRMZM2G430780(Protein kinase protein)
GRMZ/M2G30/39/(Homedox-leucine zipper) GRMZ/M2G1135(index discussion in protein 111) GRMZ/M2G03125(PHD finger protein) GRMZ/M2G04252(PHD finger protein) GRMZ/M2G1135(index discussion in trunclear-localized protein 20) GRMZ/M2G11358(add 148(core-21-branching beta-1,6-N-acetylglucosaminyltransferase) GRMZ/M2G01258(zilac finger protein 1) GRMZ/M2G002822(zinc finger protein 1) GRMZ/M2G01258(zilac finger protein 2) GRMZ/M2G01258(zilac fing	GRMZM2G127714(BNR/Asp-box repeat)	GRMZM2G308074(Leucine-rich receptor-like protein kinase)
GRMZM2G04225(PK) Grant Protein 111) GRMZM2G04225(PK) Grant Protein 111) GRMZM2G04225(PK) Grant Protein 20) GRMZM2G04225(PK) Grant Protein 20) GRMZM2G135495(serine carboxypeptidase-like 33) GRMZM2G135495(serine carboxypeptidase-like 31) GRMZM2G135445684(C2H2-like zinc finger protein 20) GRMZM2G135445684(C2H2-like zinc finger protein 20) GRMZM2G13544584(C2H2-like zinc finger protein 20) GRMZM2G13544584(C2H2-like zinc finger protein 20) GRMZM2G1354(Serine carboxypeptidase-like 40) GRMZM2G032227(AP2/B3-like transcriptional factor) GRMZM2G1357(Indeterminate(IID-Onain 12) GRMZM2G11357(Indeterminate(ID-Onain 12) GRMZM2G11357(Indeterminate(ID-Ona	GRMZM2G30/39/(Homeobox-leucine zipper)	GRMZM2C9304375(receptor kinase 3)
GRMZM22G178435(chromatin remodeling 40) GRMZM22G178435(chromatin remodeling 40) GRMZM22G178435(chromatin remodeling 40) GRMZM2G0178435(chromatin remodeling 40) GRMZM2G0178464184(Core 21-branching beta-1,6-N-acetylglucosaminyltransferas) GRMZM2G01785(refeature 40 F2 11) GRMZM2G01785(refeature 40 F2 11) GRMZM2G178485(serine carboxypeptidase-like 33) GRMZM2G138485(serine carboxypeptidase-like 33) GRMZM2G138485(serine carboxypeptidase-like 33) GRMZM2G138485(serine carboxypeptidase-like 31) GRMZM2G138445644(C2H2-like zinc finger protein) GRMZM2G138445644(C2H2-like zinc finger protein) GRMZM2G138445644(C2H2-like zinc finger) GRMZM2G138445644(C2H2-like zinc finger) GRMZM2G138445644(C2H2-like zinc finger) GRMZM2G1385(indetminiate(ID-domain 12) GRMZM2G415454644(C2H2-like zinc finger) GRMZM2G411545644(C2H2-like zinc finger) GRMZM2G4115454(Serine carboxypeptidase-like 40) GRMZM2G4115454(Serine carboxypeptidase-like 20) GRMZM2G4115454(Serine carboxypeptidase-like 20) GRMZM2G411545454(Serine Serine Carboxypeptidase-like 20) GRMZM2G411545454(Serine	GRMZA/26/06/25/2/PHD (incer protein)	GRMZM2G088861(Leucine-rich repeat (LRR))
GRMZM2G119168/AT-hook motif nuclear-localized protein 20) GRMZM2G404184/Core-2I-branching beta-1,6-N-acetylglucosaminyltransferase) GRMZM2G40184/Core-2I-branching beta-1,6-N-acetylglucosaminyltransferase) GRMZM2G102822/inc finger protein 11) GRMZM2G1028582196/C2H2-like zinc finger protein) GRMZM2G1538456967(phytochrome interacting factor 3-like 5) GRMZM2G153845684196/C2H2-like zinc finger protein) GRMZM2G1538456847(phytochrome interacting factor 3-like 5) GRMZM2G1538456847(2H2-Like zinc finger protein) GRMZM2G1538465847(2H2-Like zinc finger protein) GRMZM2G153835(zinc finger protein) GRMZM2G153835(zinc finger protein) GRMZM2G153835(zinc finger protein) GRMZM2G153835(zinc finger protein) GRMZM2G113537(indeterminate(ID-domain 12) GRMZM2G4201357(indeterminate(ID-domain 12) GRMZM2G42110(basic hike 20)-holding) GRMZM2G42110(basic hike 20)-holding)	GRMZM2G178435(chromatin remodeling 40)	GRMZM2G170766(IQ-domain 26)
I GRMZM2G404184(Core-2/l-branching beta-1,6-N-acetylglucosaminyltransferase) G. Proteolytic degradation GRMZM2G005828/zicn finger protein 1) GRMZM2G011236(related to AP2 11) GRMZM2G011236(related to AP2 11) GRMZM2G011236(related to AP2 11) GRMZM2G011236(related to AP2 11) GRMZM2G011236(related to AP2 11) GRMZM2G111236(related to AP2 11) GRMZM2G11236(related to AP2 11) GRMZM2G111236(related to AP2 11) GRMZM2G11236(serine carboxypeptidase-like 31) GRMZM2G111236(related to AP2 11) GRMZM2G11236(serine carboxypeptidase-like 31) GRMZM2G111358(related to AP2 11) GRMZM2G11236(serine carboxypeptidase-like 31) GRMZM2G111358(related to AP2 11) GRMZM2G11236(serine carboxypeptidase-like 31) GRMZM2G111358(related to AP2 11) GRMZM2G11236(serine carboxypeptidase-like 31) GRMZM2G111357(indetrimetratilin) GRMZM2G11357(indetrimitate(ID-domain 12) GRMZM2G111357(indetrimitate(ID-domain 12) GRMZM2G11344464(Eukaryotic aspartyl protease) GRMZM2G111357(indetrimitate(ID-domain 12) GRMZM2G134446(Eukaryotic aspartyl protease) GRMZM2G11357(indetrimitate(ID-domain 12) GRMZM2G134446(Eukaryotic aspartyl protease) GRMZM2G1342411(basic helix-loop-helix (bHL-H) DNA-binding) GRMZM2G134446(Eukaryotic aspartyl protease)	GRMZM2G119168(AT-hook motif nuclear-localized protein 20)	
UKMZ/M2G008282(znnc tinger protein 11) GRMZ/M2G012659(serine carboxypeptidase–like 33) GRMZ/M2G11236(related to AP2 11) GRMZ/M2G136495(serine carboxypeptidase–like 33) GRMZ/M2G11236(related to AP2 11) GRMZ/M2G136495(serine carboxypeptidase–like 31) GRMZ/M2G113644564(C2H2-like zinc finger protein) GRMZ/M2G136495(serine carboxypeptidase–like 31) GRMZ/M2G1153845(serine carboxypeptidase–like 31) GRMZ/M2G136495(serine carboxypeptidase–like 31) GRMZ/M2G1153844564(C2H2-Lype zinc finger) GRMZ/M2G032323(Subliase) GRMZ/M2G032227(AP2/B3-like transcriptional factor) GRMZ/M2G072240(serine carboxypeptidase–like 40) GRMZ/M2G01357(indeterminate(ID-domain 12) GRMZ/M2G01357(vindeterminate(ID-domain 12) GRMZ/M2G042110(basic helix-loop-helix) (bHLH) DNA-binding) GRMZ/M2G0308(Subliase)	GRMZM2G404184(Core-2/I-branching beta-1,6-N-acetylglucosaminyltransferase)	G. Proteolytic degradation
University of the set of	GRMZM2G006282(zinc finger protein 11)	GPM7M2C022650(serine carboy/nentidase-like 33)
GRMZM263686967(pt)ytochrome interacting factor 3–like 5) GRMZM26158666967(pt)ytochrome interacting factor 3–like 5) GRMZM26115386(apt)ablet=http://dtolases.protein) GRMZM2615886(apt)ablet=http://dtolases.protein) GRMZM2615886(apt)ablet=http://dtolases.protein) GRMZM2615886(apt)ablet=http://dtolases.protein) GRMZM2611538(abplablet=http://dtolases.protein) GRMZM2611538(abplablet=http://dtolases.protein) GRMZM2611538(abplablet=http://dtolases.protein) GRMZM2611538(abplablet=http://dtolases.protein) GRMZM2611357(interminate(IID-domain 12) GRMZM2611357(interminate(IID-domain 12) GRMZM2611357(interminate(IID-domain 12) GRMZM2611357(interminate(IID-domain 12) GRMZM2611358(ablet=http://dtolases.protein) GRMZM263(ablet=http://dtolases.protein) GRMZM263(ablet=http://dtolases.protein) GRMZM263(ablet=http://dtolases.protein) GRMZM263(ablet=http://dtolases.protein) GRMZM263(ablet=http://dtolases.protein) GRMZM263(ablet=http://dtolases.protein) GRMZM263(ablet=http://dtolases.protein) GRMZM263(ablet=http://dtolases.protein) GRMZM263(ablet=http://dtolases.protein) GRMZM263(ablet=http://dtolases.protein) GRMZM263(ablet=http://dtolases.protein) GRMZM263(ablet=http://dtolases.protein) GRMZM263(ablet=http://dtolases.protein) GRMZM263(ablet=http://dtolases.protein) GRMZM263(ablet=http://dtolases.protein) GRMZM263(ablet=http://dtolases.protein) GRMZM263(ablet=http://dtolases.protein) GRMZM263(ablet=http://dtolases.protein) GRMZM263(ablet=http://dtolases.protein) GRMZM263(ablet=http://dtolases.protein) GRMZM263(ablet=http://dtolases.protein) GRMZM263(ablet=http://dtolases.protein) GRMZM263(ablet=http://dtolases.protein) GRMZM263(ablet=http://dtolases.protein) GRMZM263(ablet=http://dtolases.protein) GRMZM263(ablet=http://dtolases.protein) GRMZM263(ablet=http://dtolases.protein) GRMZM263(ablet=http://dtolases.protein) GRMZM263(ablet=http://dtolases.protein) GRMZM263(ablet=http://dtolases.protein) GRMZM263(ablet=http://dtolases.protein) GRMZM263(ablet=http://dtolases.protein) GRMZM263(ablet	GRMZM62888196(22H2-like zinc finner protein)	GRMZM2G136495(serine carboxypeptidase=like 53)
GRMZM2G115388(zinc finger protein 2) GRMZM2G048161(Eukaryotic aspartyl protease) GRMZM2G486484(2EUL+2ype zinc finger) GRMZM2G052223(Subtilase) GRMZM2G01357(indeterminate(ID-domain 12) GRMZM2G073224(serine carboxypeptidase-like 40) GRMZM2G411357(indeterminate(ID-domain 12) GRMZM2G11357(indeterminate(ID-domain 12) GRMZM2G42011357(indeterminate(ID-domain 12) GRMZM2G11357(indeterminate(ID-domain 12) GRMZM2G42011357(indeterminate(ID-domain 12) GRMZM2G1038(Subtilase) GRMZM2G4201155(indeterminate(ID-domain 12) GRMZM2G1155(Subtilase) GRMZM2G4201155(indeterminate(ID-domain 12) GRMZM2G1155(Subtilase) GRMZM2G4211(basic hikk (bulk), DNA-binding) GRMZM2G45(Subtilase)	GRMZM5G865967(phytochrome interacting factor 3-like 5)	GRMZM2G152895(alpha/beta-Hydrolases protein)
Image: CRMZM2G445684(C2H2-type zinc finger) Image: CRMZM2G073223(Subtliase) Image: CRMZM2G073223(Subtliase) Image: CRMZM2G073223(Subtliase) Image: CRMZM2G07323(Subtliase) Image: CRMZM2G07323(Subtliase) Image: CRMZM2G074304(Subtliase) Image: CRMZM2G074304(Subtliase)	GRMZM2G115388(zinc finger protein 2)	GRMZM2G048161(Eukaryotic aspartyl protease)
UKMZ/MZGV224/(kP2/K3-like transcriptional tactor) UKMZ/MZGV/224/(serine carboxypeptidase-like 40) GRMZ/MZG11357(indeterminate(ID-domain 12) GRMZ/MZG11357(indeterminate(ID-domain 12) GRMZ/MZG11357(indeterminate(ID-domain 12) GRMZ/MZG114241(serine carboxypeptidase-like 20) GRMZ/MZG11357(indeterminate(ID-domain 12) GRMZ/MZG1144464(Eukaryotic aspartyl protease) GRMZ/MZG411(basic helix-loop-helix (bHLH) DNA-binding) GRMZ/MZG408(Subliase)	GRMZM2G445684(C2H2-type zinc finger)	GRMZM2G073223(Subtilase)
GRMZ/M2G042010(/size helix-log-helix (bHLH) DNA-binding)	GRMZM2G082227(AP2/B3-like transcriptional factor)	GRMZM2GU/2240(serine carboxypeptidase-like 40)
GRMZM26042101(basic helix-loop-helix (bHLH) DNA-binding)	GRMZM26011337(indeterminate(iD)=domain 12)	GRMZM2G144464(Eukarvotic aspartyl protease)
	GRMZM2G042101(basic helix-loop-helix (bHLH) DNA-binding)	GRMZM2G029780(Subtilase)

log10(TPTM+1)





Fig. 4. Venn diagram of up-regulated and down-regulated genes in the cob versus the florets, under LN.



Fig. 5. Significantly enriched biological process (BP) GO terms in up-regulated (red) and down-regulated (green) genes in (A) the cob versus (B) the florets, under LN.

trehalose phosphate synthase 11 (GRMZM2G122231) were down-regulated and a trehalose-6-phosphate phosphatase (GRMZM2G174396) was up-regulated 3.2-fold. Likewise, secondary metabolism related genes had differential response to LN in the cob. A flavonoid related gene (GRMZM2G099420) was up-regulated 3.5-fold in the cob under the LN condition and the lignin biosynthesis related gene (GRMZM2G443445) was down-regulated 8.4-fold. Notably, many genes regulating photosynthetic light reactions had substantial down-regulation in the cob (Supplementary Fig. S1A), in contrast to unanimous up-regulation of genes in the same process in florets in response to LN (Supplementary Fig. S1B). Transcription of many genes in growth-related processes such as cell wallmodification enzymes MERISTEM-5 (GRMZM2G392125) and TOUCH4 (AC210669.3_FG001), and regulator of chromosome condensation 1 family genes (GRMZM2G337819 and GRMZM2G302245) were also up-regulated in florets under LN. Genes implicated in nucleotide salvage and nucleotide degradation were down-regulated by LN in florets. Most genes involved in minor CHO metabolism, secondary metabolism and amino acid metabolism, except glutamate synthesis and proline degradation, remained unchanged in their expression levels in florets (Supplementary Fig. S1B). Notably, 49 genes had a presence/absence expression pattern in the cob and/or florets under LN (Fig. 6), which were involved in carbohydrate metabolism, nitrogen metabolism, transport facilitation, transcription regulation and cell rescue and defence.

Lastly, LN specifically responsive genes (P<0.01) were defined as those with significantly differential expression under LN compared to control, while having opposite expression patterns or no differential expression under low

phosphorus or low potassium. We comparatively analysed expression levels of a subset of genes closely related to C/N metabolism, hormonal signalling, plant development and defence in the ear using RT-qPCR, and identified six genes with specific up-regulation and eight genes with specific down-regulation under LN in the ear (Fig. 7).

Functional characterization of two amino acid transporters

Consistent with dynamic assimilated-N translocation and amino acid interconversion in the ear, numerous C/N transport and metabolism genes, including 21 putative amino acid transporters, were found with differential expression in the cob and florets (Supplementary Table S5). To bridge gene expression with physiological functions of amino acid transporters in the ear and comparatively analyse their transport capabilities, we cloned GRMZM2G110195_T01 and GRMZM2G080843_T01 for functional characterization. GRMZM2G110195_T01 (ZmAAP4) encodes the putative amino acid permease 4, with 12 transmembrane domains (Supplementary Dataset S1), and had balanced expression in the cob and florets according to the DGE data. GRMZM2G080843_T01 (ZmVAAT3) encodes the putative vacuolar amino acid transporter 3, with 11 transmembrane domains (Supplementary Dataset S1), and was preferentially expressed in florets. At the silking stage, RT-qPCR showed that ZmAAP4 had higher expression levels in the ear (a similar level in the cob and florets) and stem than in the root, leaf and tassel (Fig. 8A). ZmVAAT3 was expressed in the root, tassel and ear, but not in the leaf and stem (Fig. 8A).

log10	log₁₀(TPTM+1)						
0.0 0.5	5 1.0 1.5 2.0	2.5					
	Cob		Floret				
I NI		LN					
				GRMZM2G045154(letratricopeptide repeat(IPR)-like supertamily)			
				GRMZM2G162749(cycling DOF factor 2)			
				GRMZM2G168252(small nuclear ribonucleoprotein F)			
				GRMZM2G000743(KNOTTED-like from Arabidopsis thaliana)			
				GRMZM2G011357(indeterminate(ID)–domain 12)			
				GRMZM2G018314(P-loop nucleoside triphosphate hydrolases protein)			
				GRMZM2G042756(C-repeat/DRE binding factor 2)			
			+++	GRMZM2G069370(AGAMOUS-like 12)			
				GRMZM2G076345(SAUR-like auxin-responsive protein family)			
				GRMZM2G081192(plasma membrane intrinsic protein 2)			
				GRMZM2G093346(ascorbate peroxidase 1)			
				GRMZM2G104626(3-ketoacyl-CoA synthase 1)			
				GRMZM2G108723(Plant protein 1589 of unknown function)			
				GRMZM2G116685(CRINKLY4 related 4)			
				GRMZM2G144166(embryo defective 2423)			
				GRMZM2G170388(Pectin lyase-like)			
				GRMZM2G304091(dentin sialophosphoprotein-related)			
				GRMZM2G386995(ARM repeat)			
				GRMZM2G395429(rapid alkalinization factor 1)			
				GRMZM5G872141(Nodulin MtN3)			
				GRMZM2G007729(HSP20–like chaperones)			
				GRMZM2G087901(sucrose transporter 2)			
				GRMZM2G089106(LSD1 zinc finger)			
				GRMZM2G092363(CONSTANS–like 3)			
				GRMZM2G112205(Eukaryotic aspartyl protease)			
				GRMZM2G118657(UDP-glucosyl transferase 85A7)			
				GRMZM2G127404(Nucleotide–diphospho–sugar transferases superfamily)			
				GRMZM2G131055(Glycosyltransferase family 61 protein)			
				GRMZM2G180080(cytochrome BC1 synthesis)			
				GRMZM2G301877(UDP-Glycosyltransferase)			
				GRMZM2G349655(calmodulin like 42)			
				GRMZM2G400929(GDSL-like Lipase/Acylhydrolase)			
				GRMZM2G042125(Protein of unknown function (DUF1278))			
				GRMZM2G045431 (basic helix-loop-helixDNA-binding)			
				GRMZM2G072322(late embryogenesis abundant domain-containing protein)			
				GRMZM2G081099(receptor like protein 29)			
				GRMZM2G084583(myb domain protein 4)			
				GRMZM2G087590(Mog1/PsbP-like photosystem II PsbP)			
				GRMZM2G099984 (Low temperature and salt responsive protein)			
				GRMZM2G110511(UDP-glucosyl transferase 73B5)			
				GRMZM2G158636(pentatricopeptide (PPR) repeat-/CBS containing protein)			
				GRMZM2G170577(Voltage-gated chloride channel)			
				GRMZM2G322844 (natural resistance-associated macrophage protein 1)			
				GRMZM2G379053(zeaxanthin epoxidase (ZEP) (ABA1))			
				GRMZM2G425629(Late embryogenesis abundant)			
				GRMZM2G161905(Glutathione S-transferase)			
				GRMZM2G171548(UDP-glucosyltransferase 74F2)			
				GRMZM2G353091(RING/Ŭ-box)			
				GRMZM2G432926(plasma membrane intrinsic protein)			

Fig. 6. Expression pattern of preferentially expressed genes in the cob or florets under LN.

It showed ~70-fold higher expression in the ear compared to that in the root. In the ear, its expression level in the florets is nearly 170-fold higher than in the cob (Fig. 8A). Next, we used GFP tagged constructs to examine where ZmAAP4 and ZmVAAT3 are expressed in the cell. We found that when expressed in the onion epidermal cells, ZmAAP4 was mainly located on the plasma membrane and in the nucleus, whereas ZmVAAT3 was mainly located on the plasma and nuclear membranes (Fig. 8B).

To investigate the transport functionality of ZmAAP4 and ZmVAAT3, we cloned each gene into a pDR195 yeast expression vector and transformed into the yeast mutant strain $22\Delta 8AA$, which is deficient in absorbing arginine, aspartate, citrulline, GABA, glutamate or proline. In sharp contrast to the mutant strain $22\Delta 8AA$ itself, the mutant complemented with ZmAAP4 displayed a similar and robust growth pattern on the media with proline and glutamate and dramatically weaker growth on the media with citrulline, GABA, aspartate or arginine (Fig. 9); the yeast mutant expressing ZmVAAT3 showed vigorous growth on the six different media specified by the individual amino acid (Fig. 9), indicating that both ZmAAP4 and ZmVAAT3 transported multiple amino acids in the yeast system, although there was a large variation in transport efficiencies.

To further characterize their functions in a plant system, we generated transgenic *Arabidopsis* seedlings overexpressing *ZmAAP4* (ZmAAP4-OE) or *ZmVAAT3* (ZmVAAT3-OE), which were grown on a series of ATS media (Schofield *et al.*, 2009) containing different basic amino acids for 12 d for morphological examination. These seedlings showed dramatic alterations in primary root length in many media supplemented by individual amino acids (Fig. 10; Supplementary Tables S10, S11). Compared to WT plants, ZmAAP4-OE seedlings showed reduced primary root growth on media with 17 amino acids except Ala, Ser, Tyr, Asp and Asn (Fig. 10A; Supplementary Tables S9, S11); however, ZmVAAT3-OE



GRMZM2G112377(phosphate transporter 1;4) GRMZM2G400156(SAUR-like auxin-responsive protein family) GRMZM2G062129(senescence-associated family protein) GRMZM2G001799(indole-3-acetic acid inducible 14) GRMZM2G076345(SAUR-like auxin-responsive protein family) GRMZM2G076345(SAUR-like auxin-responsive protein family) GRMZM2G072322(LEA domain-containing protein) GRMZM2G072322(LEA domain-containing protein) GRMZM2G081192(plasma membrane intrinsic protein 2) GRMZM2G142680(NB-ARC domain-containing disease resistance protein) GRMZM2G437575(Protein phosphatase 2C family protein) GRMZM2G051619(gibberellin 2-oxidase 1) GRMZM2G051619(gibberellin 2-oxidase 1) GRMZM2G161905(Glutathione S-transferase family protein) GRMZM2G432926(plasma membrane intrinsic protein 2)

Fig. 7. RT-qPCR analysis of selected genes that showed response to LN, but not to low P or low K stress.

seedlings had enhanced primary root length on media supplemented with Leu, Ala, Val, Phe, Met, Arg, Asn or Glu and reduced growth on media with Ser, Cys, Thr, Trp, Lys, Gln, Cit, GABA or His (Fig. 10B; Supplementary Tables S10, S11). Either stimulatory or inhibitory effects of amino-N on primary root growth in ZmAAP4-OE and ZmVAAT3-OE lines suggested substantial uptake of corresponding amino acids mediated by ZmAAP4 and ZmVAAT3 in vivo. In addition to alterations in primary root growth, the number of rosette leaves in ZmAAP4-OE and ZmVAAT3-OE seedlings also varied compared to WT seedlings: ZmAAP4-OE seedlings had more rosette leaves on media with Met, Tyr, Asp, Glu or Asn and less leaves on media containing 17 out of 22 tested amino acids except Met, Tyr, Asp, Glu and Asn (Supplementary Tables S9, S11); ZmVAAT3-OE seedlings had more rosette leaves on media containing Leu, Phe, Tyr or Gln, and less leaves on media with 16 amino acids except Leu, Phe, Tyr, Asp, Gln and GABA (Supplementary Tables S10, S11). The rosette diameter of ZmAAP4-OE seedlings significantly increased on the medium with Ala and decreased on media with 14 amino acids except Ala, Leu, Thr, His, Asp, Asn, Gln and Cit (Supplementary Tables S9, S11); the rosette diameter of ZmVAAT3-OE seedlings significantly increased on media supplemented with Ala and decreased on media containing Gly, Val, Ile, Ser, Tyr, Trp, His, Lys, Glu, Gln or Cit (Supplementary Tables S10, S11). Not surprisingly, the fresh weight of ZmAAP4-OE seedlings increased on the medium supplemented with Ala or Leu and decreased on media with 17 amino acids except Ala, Leu, Asp, Asn or Gln (Supplementary Tables S9, S11); while the fresh weight of ZmVAAT3-OE seedlings significantly increased on media with Met, Pro, Phe or Asn and decreased on media containing Ile, Ser, Cys, Trp, His, Lys, Arg, Glu, Gln or GABA (Supplementary Tables S10, S11).

Discussion

Asymmetric transcriptomic signatures of the cob and florets in the maize ear

To date, there has been no in-depth characterization of the cob and peripheral florets at the molecular level. Predominant enriched GO terms were related to transport facilitation in the cob, underpinning the nature of the cob as a source tissue providing C and N for floret development and growth during the silking stage (Fig. 1). Sucrose is a main carbohydrate intermediate in maize (Hofstra and Nelson, 1969). Different from strong expression of sucrose transporter 2 in sink leaves in tomato (Barker et al., 2000), specific expression of sucrose transporter 2 (GRMZM2G087901) may promote carbon transport from the cob to florets at the silking stage (Fig. 3). Peptide transport is a more efficient means of N allocation than amino acid transport (Higgins and Payne, 1982). The peptides can be rapidly hydrolysed by peptidases as a source of amino acids, N or C in plants (Koh et al., 2002). PTR1 is a crucial high affinity peptide transporter in Arabidopsis (Komarova et al., 2008). ZmPTR1 mediates small peptide transport from the endosperm into the embryo in maize, and its overexpression promotes plant growth when grown on a medium with Ala-Ala dipeptide as the unique N source (Tnani et al., 2013). Specific expression of *PTR1* in the cob at the silking stage indicated dynamic amino acid and peptide translocation towards florets (Fig. 3). Serine carboxypeptidase-like proteins play a role in mobilization of seed storage reserves in barley (Degan et al., 1994). Serine-type carboxypeptidases (GRMZM2G057611 and GRMZM2G101576) may promote nutrient mobilization from the cob to florets in maize (Fig. 3). Additionally, potassium and zinc transport from the cob to florets, mediated by potassium transporter



Fig. 8. (A) Gene expression levels of ZmAAP4 and ZmVAAT3 in different maize tissues. The gene expression level in the root was normalized to 1 when compared with the other four tissues or organs, and that of the cob was normalized to 1 when compared with that of florets within the ear. (B) Subcellular localization of ZmAAP4 and ZmVAAT3 by transient expression of GFP fusion proteins in onion epidermal cells.

2/3, zinc induced facilitator-like 2 and zinc transporter 12 precursor, were also indispensible for floret development (Fig. 3; Kim et al., 1998; Haydon and Cobbett, 2007). The most enriched biological process GO term was photosynthesis in the cob, with a significant P-value of 9.60e-12 (Fig. 1). Our TopGO analysis showed that out of 1314 up-regulated genes in the cob, 21 were involved in photosynthesis. Photosynthesis genes may be involved in energy transfer or metabolism, supporting highly dynamic C and N transport activities within the cob ('source') or towards florets ('sink') (Thomas and Rodriguez, 1994; Rajcan and Tollenaar, 1999). Alternatively, these genes may have previously uncharacterized functions in the cob, as many genes have multiple functions beyond original annotation. Indeed, a large number of photosynthesis genes were expressed in the root in Arabidopsis and rice (Himanen et al., 2004; Lian et al., 2006) although their biological functions remain to be elucidated.

Two major types of genes preferentially expressed in florets were hormone signalling components and developmental regulators, which collectively regulated floret development and growth (Fig. 3). Cytokinin plays essential roles in determining sink strength and grain production in crop plants (Ashikari et al., 2005). IPT was specifically expressed in florets, which regulated floret development and kernel formation via controlling cytokinin biosynthesis (Medford et al., 1989; Gan and Amasino, 1995; Brugiere et al., 2008). Specific expression of gibberellin 2-oxidase 6 and gibberellin 20 oxidase 2, auxin response factor 16, coordinated to promote floret growth (Dellaporta and Calderon-Urrea, 1994; Sasaki et al., 2002; Wang et al., 2005). Interestingly, a series of genes related to reproductive development were specifically expressed in florets. The maize CR4 gene encodes a serine/threonine receptorlike kinase that controls a series of developmental processes in seed formation and embryo morphogenesis (Becraft et al., 1996). ASL plays a role in cell fate determination in flowers



Fig. 9. Functional characterization of ZmAAP4 and ZmVAAT3 in the 22 Δ 8AA yeast system. The 23344c and 22 Δ 8AA served as positive and negative controls, respectively, with the (NH₄)₂SO₄ treatment as a control of N source.

(Li *et al.*, 2005). Genes encoding plastocyanin-like domaincontaining protein and subtilase family protein mediate seed development (Haldrup *et al.*, 1999; D'erfurth *et al.*, 2012). *AP2/ERF* family genes act as transcription activators in promoting cell proliferation, differentiation, and morphogenesis, especially during embryogenesis in *Arabidopsis* (Yamaguchi-Shinozaki and Shinozaki, 2006; Iwase *et al.*, 2011). Floret specific expression of *RAP2.11* might suggest its role in mediating floret differentiation in maize. Twenty-one transcription factors, especially bZIP and MYB family proteins, might respond to hormone signalling and play a significant role in regulating grain filling (Fig. 3; Jakoby *et al.*, 2002; Dubos *et al.*, 2010).

Taken together, our results suggested that tissue-specific gene expression played fundamental roles in mediating tissue differentiation and development. Consistent with their developmental functions at the silking stage, the cob was characteristic of preferential expression of genes related to C/N transport and metabolism, serving as a major C/N source for floret development; while florets favoured preferential expression of hormone signalling components and development related genes, supporting floret post-initiation development. Preferential expression of certain C/N metabolism related genes in florets indicated that these genes were necessary for floret buildup following initial differentiation. Our finding also substantially expanded the current spectrum of how to define the cob and florets at the transcriptional level, providing valuable tissue-specific marker genes for further functional characterization.



Fig. 10. Phenotypes of (A) ZmAAP4-OE and (B) ZmVAAT3-OE Arabidopsis seedlings 12 d after transfer onto ATS medium supplemented with different amino acids. The NO₃⁻ treatment served as a control of N source. OE1, OE2 and OE3 represent three independent gene overexpression lines.

The cob had a dominant role at the transcriptomic level in the ear at the silking stage

The cob plays fundamental roles during ear development by providing C and N nutrients or assimilates for floret development and grain filling, and sink strength of the ear is closely associated with amino acid interchange and metabolism in the cob at the silking stage (Cliquet et al., 1990; Ma and Dwyer, 1998; Seebauer et al., 2004; Cañas et al., 2009). Despite the functional importance of the maize cob, only four cob-specific genes were identified in maize transcriptomic profiling, in sharp contrast to the other 859 organ-specific genes (Sekhon et al., 2011). We found 1314 genes with significant (\geq 2-fold change) up-regulation in the cob with sufficient nutrient supply, which was ~2.3-fold as many as those up-regulated in florets (Table 1), together with more tissue specifically expressed genes in the cob (Supplementary Tables S6, S7), suggesting obvious dominance of the cob in the ear in terms of gene activation at the silking stage. Dominance of the cob in gene activation at the silking stage was in agreement with its function as a crucial source tissue supporting floret development during a critical developmental stage. Further, 3-fold genes (588 genes) had differential expression in the cob under LN, compared with 195 differentially expressed genes in florets (Table 1; Fig. 4), indicating that the cob dominated the ear response to N limitation at the transcriptomic level at the

same critical developmental stage. Even for GO enrichment analysis, enriched biological processes in the cob were significantly more than those in florets (Fig. 5), corroborating that the cob responded to N limitation in a dominant manner, with florets as a secondary player in terms of gene regulation. One possible reason was that the cob, as the crucial tissue supporting differentiation and development of florets, was much more sensitive to LN than the florets. Given the dominant role of the cob in the response to LN in the ear at silking, we expected more genes involved in metabolic processes identified in the cob with differential expression under the LN condition. Indeed, we found many genes involved in starch cleavage, minor CHO metabolism and secondary metabolic processes in the cob under LN, with few identified in florets under LN during this stage (Supplementary Fig. S1). Also, less differentially expressed genes regulating amino acid metabolism and biotic stress processes were detected in florets than in the cob under the LN condition. In plants, the cell wall plays an important role in various physiological processes such as growth, intercellular communication and interaction with the environment (Cosgrove, 2005). Genes regulating macromolecule catabolic and anabolic processes in the cell wall were differentially regulated in the cob rather than in florets under LN. It is well known that LN significantly reduces ear growth, biomass accumulation in the ear after silking and the kernel number by reducing sink strength and altering C metabolism (Uhart and Andrade, 1995a, b; Andrade et al., 2002; Paponov et al., 2005; Méchin et al., 2007; Liao et al., 2012a, b). Misregulation of numerous genes mediating C/N metabolism and transport in the cob (Fig. 5, Supplementary Fig. S1; Supplementary Table S8) perfectly interpreted negative physiological effects of LN on ear growth and kernel development. Likewise, more genes involved in the cell rescue and response such as farred light photo-transduction and oxidation reduction processes were detected in the cob (Fig. 5; Supplementary Table S8). Consistent with these observations, ribosomal protein family genes were differentially regulated in the cob under LN (Supplementary Table S8). Further, expression alterations of genes involved in transcription facilitation and signal transduction in the cob represented a tissue-preferential primary response of the regulatory machineries to LN. Taken together, our results suggest that the cob is the dominant tissue in the ear in terms of gene transcription at the silking stage, and it at least numerically dominates the ear response to LN as well.

Low nitrogen had comprehensive impacts on gene expression in the ear

LN severely inhibits maize growth and significantly reduces grain yield. Under LN, genes involved in the glutamate biosynthetic process, proline catabolic process, lipid catabolic process, homoiothermy, response to freezing, regulation of DNAdependent transcription and twocomponent signal transduction were up-regulated (Fig. 5); and genes mediating protein dephosphorylation and proline biosynthesis were down-regulated in both tissues (Fig. 5), indicating complicated and concerted responses of the cob and florets to the LN stress. N deficiency affects N and sugar metabolism and/ or partitioning between source and sink tissues (Hermans et al., 2006). Glutamine (Gln), glutamate (Glu), and 2-oxoglutarate are major components of the N-sensor system to monitor accumulation of different 'checkpoint' molecules of NO_3^- and NH_4^+ in higher plants (Kang and Turano, 2003). Proline biosynthesis is regulated by the N flux and functions in osmo-regulation and other stress responses by controlling the level of P5CS (Kishor et al. 1995; Székely et al., 2008). Up-regulation of glutamate biosynthesis, proline catabolic process and down-regulation of proline biosynthesis may indicate internal N status, similar to the indicative role of Asn/Gln in the cob (Seebauer et al., 2004), or serve as a potential mechanism to alleviate N deficiency by accelerating amino acid metabolism and internal N exchange under LN in the ear during the silking stage. In particular, Glu is among those amino acids critical for carbohydrate partitioning in the ear at the silking stage, and its metabolism in the cob partially preconditions assimilated-N supply for grain development and growth (Seebauer et al., 2004; Cañas et al., 2009). LN may negatively affect C/N allocation towards the developing florets by altering glutamate and proline metabolism in this study. Glutamate and proline metabolism might also have a similar role in mediating kernel set and filling as Gln1-3 and Gln1-4 do (Martin et al., 2006); thus, LN-directed abnormal glutamate and proline metabolism partially contributed

to reduction in the kernel size and number. Alternatively, amino acids may form conjugates with IAA to regulate ear growth and development under the low N condition as they function to stimulate cell or tissue elongation (Feung et al., 1977; Staswick et al., 2005). The lipid catabolic process was also up-regulated in both tissues under LN (Fig. 5), similar to previous report in Arabidopsis (Martin et al., 2002). Protein phosphorylation plays an important regulatory role in signal transduction and control of enzyme activities under changing N conditions (Engelsberger and Schulze, 2012). Enrichment of 'protein phosphorylation' terms suggested that protein phosphorylation may activate certain N assimilatory pathways for ear development under the LN condition, and dephosphorylation may inactivate downstream targets (Fig. 5). The NR (nitrate reductase) activity is post-translationally regulated by a phosphorylation-dephosphorylation mechanism (Kaiser and Huber, 1994; Bachmann et al., 1996). CHL1, a dual affinity nitrate transporter and a nitrate sensor, also functions depending upon phosphorylation by the protein kinase CIPK23 under low nitrate conditions (Ho et al., 2009). In this study, the phosphatase 2C (GRMZM2G159811) was significantly repressed in the cob and florets; the calciumdependent phosphotriesterase (AC233955.1 FG008) was upregulated 10.7-fold in florets and a phosphatase-related gene (GRMZM2G149704) was upregulated 2.9-fold in the cob (Supplementary Table S8). Therefore, protein dephosphorylation may be very important to activate N assimilation supporting ear development in maize under LN.

Importantly, we identified 14 genes specifically responsive to LN compared to the low phosphorus or potassium treatment (Fig. 7), indicating direct effects of LN on N metabolism and allocation. Glutathione S-transferases (GSTs) function in regulation of cellular metabolism and are involved in a variety of stress responses (Marrs, 1996). Specific down-regulation of the glutathione s-transferase in florets probably indicated reduced nitrogen metabolism as an adaptive response to insufficient N supplies (Fig. 7). Closely related to N nutritional status is carbohydrate partitioning that modulates plant growth and development over the entire life cycle (Chiou and Bush, 1998; Hermans et al., 2006). LN generally promotes carbon allocation towards the root to stimulate lateral root emergence and overall root growth in contrast to suppressive effects of K deficiency on root growth (Hermans et al., 2006). The sucrose synthase 3 plays a major role in sucrose entry into diverse pathways involved in cellular processes (Fu and Park, 1995). Up-regulation of two sucrose synthase 3 genes in the cob in response to N limitation suggested enhanced sucrose synthesis and metabolism probably towards florets. A previous systemic screen of N nutritional biomarkers revealed 112 genes in maize, with no hormone biosynthesis related genes (Yang et al., 2011). However, we found that auxin and GA signalling components were also specifically responsive to LN, indicating that auxin and GA may be closely linked to N signalling in the maize ear. Potentially, coordinated modulation of auxin and GA signalling may reprogramme cell division or expansion in the maize ear under LN at the silking stage. Notably, phosphate transporter 1;4 may be an biomarker indicating N status in maize (Yang et al., 2011). N

deficiency also induced up-regulation of the senescence-associated gene, similar to previous report in *Arabidopsis* (Peng *et al.*, 2007), suggesting that the senescence-associated gene may have novel functions in plant response to N deficiency or somehow promote ear premature development under LN. Other non-nutrient stress related genes either have multiple functions in diverse environmental stresses, or indicate that the LN stress triggers a N specific response, together with a general stress response applied to a wide array of other biotic and abiotic stresses (Fujita *et al.*, 2006; Atkinson and Urwin, 2012; Liao *et al.*, 2012a, b). Identification of 14 LN specifically responsive genes provided putative molecular markers for N deficiency diagnosis in the field and N efficient maize breeding in the long run.

ZmAAP4 and ZmVAAT3 were broad-spectrum amino acid transporters with differential substrate selectivity and transport

Dynamic amino acid metabolism is a crucial feature of the quickly developing maize ear at the silking stage (Mozafar, 1990; Jacobs and Pearson, 1992; Lemcoff and Loomis, 1994; Seebauer et al., 2004). Amino acid transporters may be responsible for amino-N transfer from the vegetative tissues towards the developing ear; or more importantly, they operate downstream of amino acid metabolism to mediate N allocation and carbon partitioning during ear development and growth as a fundamental biological process (Seebauer et al., 2004; Cañas et al., 2009, 2011). However, no amino acid transporters have been functionally characterized in maize. We demonstrated that ZmAAP4 and ZmVAAT3 were broad-spectrum amino acid transporters with different transport efficiencies, as indicated by large phenotypic variations among gene overexpression lines (Fig. 10). This conclusion was consistent with the notion that AAP and VAAT members are broad-spectrum amino acid transporters (Fischer et al., 1995; Su et al., 2004). Among 22 amino acids transported by ZmAAP4 and ZmVAAT3, Asp, Asn, Glu, Gln, Arg, Ala, Val, Ser and His are the most abundant amino acids in the cob; Asp, Asn, Glu, Gln, Arg, Ala, Ser and Pro are critical for carbohydrate partitioning in the ear at the silking stage (Seebauer et al., 2004). Enzymatic interconversions among Gln, Ala, Asp and Asn in the cob precondition assimilated-N supply for grain development and growth (Seebauer et al., 2004; Cañas et al., 2009). Essential physiological or biological functions of amino acids transported by ZmAAP4 and ZmVAAT3 in the ear suggested that ZmAAP4 and ZmVAAT3 played important roles in promoting amino-N mobilization in and between the cob and florets during tissue differentiation and embryo and organ genesis in the ear. Floret-preferential expression of ZmVAAT3 (Fig. 8A) further indicated its potential functions in mediating amino acid fluxes, storage or other metabolic processes during kernel development. Notably, both ZmAAP4 and ZmVAAT3 were able to transport nine amino acids essential for human development, including Lys, Trp, Met, Phe, Val, Thr, Leu, Ile and His (Fürst, 1998). Most cereal grains contain very limited amount of Lys, Trp and Met (Galili et al., 2002). High-Lys

LY038 maize is grown in a number of countries and quality protein maize, enriched in Lys and Trp, is under development through genetic engineering (Glenn, 2007). Transport of Lys, Trp and Met by ZmAAP4 and ZmVAAT3 is potentially important for enhancing nutritional values of maize grains as a staple food and feed resource. ZmAAP4 and ZmVAAT3may therefore serve as valuable target genes for maize breeding given large genetic variations in gene expression and regulation in maize germplasms across the world.

Importantly, ZmAAP4 may have higher efficiencies than ZmVAAT3 in transporting seven amino acids, including five nonpolar amino acids (Phe, Pro, Met, Leu and Val), basic amino acid Arg (pH 6-7), and acidic amino acid Glu (pH 6-7), given similar gene expression levels in different transgenic lines (Supplementary Fig. S2). Negative effects of Phe, Pro or Met on fresh weights of ZmAAP4-OE seedlings were in sharp contrast to positive effects of these three amino acids on the same measurement of ZmVAAT3-OE seedlings (Supplementary Tables S9, S10), implying over-uptake of Phe, Pro or Met due to a higher transport efficiency of ZmAAP4 than that of ZmVAAT3, given the consistent results from three representative biological replicates. Phe, Met, Leu, Val, Arg or Glu stimulated longitudinal growth of the primary root of ZmVAAT3-OE seedlings; by contrast, they reduced primary root length of ZmAAP4-OE seedlings probably due to amino-N over-uptake and consequent disruption of C/N metabolic homeostasis, corroborating the higher transport efficiency of ZmAAP4 (Supplementary Tables S9, S10). Consistently, Phe or Leu reduced the rosette leaf number in ZmAAP4-OE seedlings, in contrast to stimulation of rosette leaf proliferation in ZmVAAT3-OE seedlings (Supplementary Tables S9, S10). One possible interpretation could be that relatively preferential expression of ZmVAAT3in the ear (Fig. 8A) made it more substrate selective, resulting in low efficiencies in mediating transport of a subset of amino acids. On the other hand, ZmAAP4 had more comparable expression in all five tissues (Fig. 8A), indicating less substrate selectivity and higher transport efficiencies. This observation was also consistent with a previous report that AAP members were more efficient than VAATs (Su et al., 2004).

Although, as previously discussed, functional investigation using gene overexpression lines showed larger transport capacities of ZmAAP4 under certain circumstances, ZmVAAT3 was a stronger transporter than ZmAAP4 in terms of Cit, GABA, Asp and Arg uptake according to yeast complementation data (Fig. 9). More robust growth of the ZmVAAT3 complemented $22\Delta 8AA$ strain than that of the ZmAAP4 complemented strain on media with Cit, GABA, Asp or Arg also implied ZmVAAT3's higher affinity to these very different amino acids in a yeast system. Indeed, ZmAAP4 and ZmVAAT3 had quite different substrate selectivity as well as transport efficiencies as shown by their differential phenotypes (Fig. 10). Downregulation of fresh weights of ZmVAAT3-OE seedlings (by Gln) and ZmAAP4-OE seedlings (by Gly, Val, Ile, Ser, Cys, Thr, Met, Pro, Phe, Tyr, His or Cit) were likely derived from variations in substrate affinity or transport efficiencies of these two transporters confronting different amino-N cargoes (Fig. 10; Supplementary Tables S9, S10). Similarly, Ser inhibited primary root growth of ZmVAAT3-OE seedlings,

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while Gly, Ile, or Pro inhibited that of ZmAAP4-OE seedlings (Fig. 10; Supplementary Tables S9, S10), indicating substrate differentiation and efficiency variation of two transporters. This argument was also well supported by contrasting effects of many other tested amino acids on the leaf number and rosette diameter of ZmVAAT3-OE and ZmAAP4-OE seedlings (Fig. 10; Supplementary Tables S9, S10).

Supplementary data

Supplementary data are available at JXB online.

Supplementary Figure S1. Metabolism overview of the differentially expressed genes in the cob and florets under LN.

Supplementary Figure S2. Transcription levels of ZmAAP4 and ZmVAAT3 in gene overexpression lines.

Supplementary Table S1. Fertilization practice among different nutrient treatments.

Supplementary Table S2. Primer sequences used for RT-qPCR.

Supplementary Table S3. Total number of tags and mapped genes from the DGE sequencing data.

Supplementary Table S4. Validation of the expression fold change of selected genes from DGE data by RT-qPCR.

Supplementary Table S5. List of differentially expressed genes between the cob and florets under sufficient nitrogen supply.

Supplementary Table S6. List of genes that were preferentially expressed in the cob with sufficient nitrogen supply.

Supplementary Table S7. List of genes that were preferentially expressed in florets with sufficient nitrogen supply.

Supplementary Table S8. List of differentially expressed genes in the cob and florets under LN.

Supplementary Table S9. Quantification of phenotypes of ZmAAP4-OE *Arabidopsis* seedlings 12 d after transfer onto ATS medium supplemented with 3mM NO₃⁻ and different amino acids.

Supplementary Table S10. Quantification of phenotypes of ZmVAAT3-OE *Arabidopsis* seedlings 12 d after transfer onto ATS medium supplemented with 3 mM NO₃⁻ and different amino acids.

Supplementary Table S11. Summary of effects of 22 amino acids on growth parameters of ZmAAP4-OE and ZmVAAT3-OE *Arabidopsis* seedlings.

Supplementary Dataset S1. Coding sequences and functional domains of ZmAAP4 and ZmVAAT3.

Acknowledgements

We thank the 863 High-tech grant (2012AA10A306), the NSFC grant (31172016), the programme for New Century Excellent Talents in University (NCET-12–0521), the Innovative Group Grant of the NSFC (31421092) and the 100-Talent Program from the Chinese Academy of Sciences for financial support. We thank Prof. Daniel Wipf for providing the 22 Δ 8AA yeast strain.

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