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RESEARCH ARTICLE

Differential global gene expression changes in response to low nitrogen stress in two maize inbred lines with contrasting low nitrogen tolerance

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Abstract

The global gene expression changes in response to low nitrogen stress in leaf tissues of two maize inbred lines(Mo17 and Hz4) with contrasting low nitrogen tolerance was analyzed using the Affymetrix maize genome array. Our results showed 887 genes involved in a variety of biological processes were responsive to low nitrogen treatment in Mo17, out of which 384 were up-regulated and 503 were down-regulated. In Hz4 the expression of 1108 genes was changed, of which 696 were up-regulated and 412 genes were down-regulated. The gene expression profiles also revealed 1799 genes differentially expressed (910 and 889 genes with higher level of expression in Hz4 and Mo17, respectively) between the two lines under low nitrogen conditions. These results explain at the transcript level why Mo17 is more sensitive than Hz4 under low nitrogen stress. In addition to previously reported nitrogen response genes (nitrate transporter, nitrite reductase, nitrate reductase, ferredoxin) we found many new nitrogen response genes (early light-inducible protein, uroporphyrinogen methyltransferase, phosphoenolpyruvate carboxylase, tonoplast intrinsic protein, sesquiterpene cyclase). Our results not only provide new insights on the molecular mechanisms of nitrogen stress, but also serve as a valuable resource to researchers who aim to improve the efficiency of maize nitrogen use.

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Introduction

Nitrogen (N) is the most important macronutrient and nitrate is the main inorganic nitrogen source for plant growth and development. In the past several decades, the increasing use of nitrogen fertilizer in crop production has played a major role in improving grain yield with the goal of meeting the increasing demand for food in the world (Frink, 1999). Associated with the increase of crop production is the serious problems of pollution of freshwater (London, 2005), marine ecosystems (Beman et al., 2005) and the atmosphere (Ramos, 1996; Stulen et al., 1998) resulting from intensification of the use of N fertilizers in agriculture. The heavy reliance on fertilization increase for improving crop yield is associated with more pressures on environment protection, low cost-efficiency and sustainable agriculture development. This situation is currently very serious in developing countries like China (Yan, 2006).

The development of plant varieties with high nitrogen use efficiency from diverse germplasm is the best economic strategy to solve this problem. Some efforts have been focused on genetic dissection of plant nitrogen utilization by the mapping of relevant quantitative trait loci (QTL) in a number of plants, including *Arabidopsis* (Olivier, 2003), rice (Lian, 2005), maize (Agram, 1999) and wheat (Laperche, 2007). Although these studies have provided valuable information to guide the manipulation of desirable genes through breeding, the understanding of the molecular basis of plant responses to nitrogen is still limited. This is in part due to the complex properties and the involvement of numerous genes in N uptake and assimilation mechanisms.

With the advent of technology that is both large-scale and high-throughput, the dissection of gene expression changes in

response to nitrate, from among thousands of genes, or the whole genome scale became feasible (Rensink et al., 2005). In *Arabidopsis*, whole-genome gene expression changes throughout the whole genome in response to nitrate for both short-term and long-term treatment have been analyzed, and numerous unknown genes were revealed to be involved in nitrogen responses besides the known genes (Wang et al., 2000, 2003; Price et al., 2004; Peng, 2007). In rice, Lian et al. (2006) reported that significant expression differences could be detected in root tissue rather than leaf tissue after short-term treatment with low nitrogen stress compared to the normal nitrogen supply. In wheat under long-term nitrogen limitation, numerous genes, especially for fructan accumulation in stems, were revealed to be involved in adaptation to the stress conditions (Ruuska et al., 2007). These results, based on microarray chip techniques brought new insights to the study of the genetic basis of nitrate responses in plants. However, gene expression in response to nitrate in maize has not yet been investigated on a large-scale or whole-genome transcription analysis.

Maize is the one of the most important staple cereals worldwide that is economically important both as animal feed and as a potential bio-fuel source. However, the grain yield of maize is highly dependent on the input of nitrogen fertilizer. It was estimated that five million tons of N fertilizer were applied annually to fields of maize production in the industrialized world and higher amounts in developing nations, especially in China (FAO, 2004). Thus, it is essential to dissect the molecular basis of nitrogen responses to facilitate the improvement of maize varieties with high nitrogen use efficiency (NUE).

Mo17 is a major maize inbred line representing the 'Lancaster' heterotic group in China that has shown a strong response to nitrogen stress during shoot to silk period. Hz4 is a major maize inbred line representing the 'Tangsansipingto' heterotic group, that it doesn't get very stressed in response to low N concentration (Cao et al., 2000; Agrama et al., 1999). Both inbred lines are used extensively in breeding (Pan et al., 2002) based on their physiological traits including yield, plant height, etc. However, the higher sensitivity of Mo17 to nitrogen stress treatment relative to that of Hz4 remains unexplained. The sensitivity difference may be better understood through a thorough study of gene expression patterns and their relationship to nitrate metabolism in these two genotypes. Any findings could have important implications for inbred line development and hybrid combination (Pan et al., 2002; Li et al., 2002; Xie et al., 2007).

Mo17 is sensitive to nitrogen stress during the shoot to spin silk period. Meanwhile Hz4 is tolerant to nitrogen stress levels and does not show altered yield, plant height, etc., under nitrogen stress conditions (Cao et al., 2000; Agrama et al., 1999). Therefore, the purpose of our research was to investigate the global gene expression pattern of these two inbred lines under long-term treatment with nitrogen stress, and to identify the relevant genes involved in different physiological and metabolic pathways related to the nitrate response. It is expected the results of this study will provide fundamental information that will lead to the development of an efficient strategy for manipulating genes for NUE improvement of maize variety in combination of other physiological and agronomic information.

Materials and Methods

Maize inbred lines

The two maize inbred lines used in this experiment, Mo17 and Hz4, are the key maize inbred lines representing the two most important heterotic groups in China. Hz4 represents the 'Tangsansipingto' group, and Mo17 represents the 'Lancaster' group. Both lines have been used very extensively in breeding programs (Pan et al., 2002). According to previous phenotypic analyses of yield and other agronomic traits, Mo17 is sensitive to nitrogen stress during the shoot to spin silk period while Hz4 is tolerant to nitrogen stress levels and doesn't show altered physiological properties under nitrogen stress conditions (Cao et al., 2000; Agrama et al., 1999).

Maize growth media

The growth medium contained the following components (final concentrations):40μM KH_2PO_4 (pH6.5), 130μM K_2SO_4, 110μM $MgSO_4{\cdot}7H_2O$, 170μM KCL, 0.16μM EDTA-Fe, 0.17 μM H_3BO3,0.02μM $MnSO_4{\cdot}H_2O$, 0.02μM Zn $SO_4{\cdot}7H_2O$, 8.33E-05μM Cu $SO_4{\cdot}5H_2O$, 0.167μM $H_2MoO_4{\cdot}4\ H_2O$. 100μM $Ca(NO_3)_2$. Nitrogen stress media is the same as the growth medium without 100μM $Ca(NO_3)_2$. Component solutions were added separately to deionized water before use. Quartz sand was sterilized with formalin and subjected to open-air drying before use.

Maize growth conditions

Seeds of Mo17 and Hz4 were germinated at 30°C in an illumination incubator with constant deionized water for 7 d. Then tender seedlings were transplanted to a flower pot filled with sterilized quartz sand in a controlled environment, where temperature was held at 25°C to 30°C and a fresh 40-mℓ aliquot of liquid growth media was added to each flowerpot daily.

Nitrogen stress treatment

At the two-leaf stage half of plants were transferred to the nitrogen stress media for stress treatment. At least three flowerpots were used for each treatment. For both nutrient regimens, the leaves were excised and frozen in liquid nitrogen for total RNA preparation when the sixth leaf was visible.

Isolation of total RNA

Total RNA was initially isolated using a TRIzol kit (TaKaRa),

an aliquot of the recovered total RNA was used for quantification and then total RNA concentration was adjusted to 1 $\mu g.\mu\ell^{-1}$ prior to use.

Microarray analysis

Gene expression patterns related to the nitrate response in the two inbred lines were analyzed using the Maize Genome Array Affymetrix chip which contains 17,734 probe sets to monitor approximately 14,850 maize transcripts representing 13,339 known or predicted genes from Maize (http://www.affymetrix.com/products/arrays/specific/maize.affx). Isolated total RNA samples were treated as described in the Affymetrix instruction manual (Affymetrix Gene Chip Expression Analysis Technical Manual). In brief, an aliquot of 5 μg of total RNA was used to synthesize double-stranded cDNA using the Super Script Double-Stranded cDNA Synthesis Kit (Invitrogen) and poly (T)-nucleotide primers that contained a sequence recognized by T7 RNA polymerase. A portion of the resulting double-stranded cDNA was used as a template to generate biotin-tagged cRNA from an *in vitro* transcription reaction (IVT), using the Bio-Array High-Yield RNA Transcript Labeling Kit. 15μg of the resulting biotin-tagged cRNA was fragmented to strands of 35 to 200 bases in length according to the protocols from Affymetrix. 10 μg of this fragmented target cRNA was hybridized to an Affymetrix Maize array containing 17,734 transcripts. Hybridization was performed at 45°C with rotation for 16 hr. The Gene Chip arrays were washed and then stained on an Affymetrix Fluidics Station 400, followed by scanning on a Gene Array scanner. The hybridization data were analyzed using Gene Chip Operating software GCOS 1.4. A logistic scaling procedure and comparison analysis were performed on different arrays with GCOS 1.4 and Excel. Normalization was performed as suggested by Irizarry (2003). Differential gene expression was identified using significance analysis by unpaired Student's t-test between appropriate pairwise comparison of different samples under consideration. Benjamini and Hochberg false discovery rate (FDR) multiple testing corrections was applied to the differentially expressed genes ($P < 0.05$) (Benjamini and Hochberg, 1995).

Table 1. Sequence of primers used for qRT-PCR

GB accession no.	Strand	Primer sequence(5'-to-3')
BM331860	F	GGTAACACAAATAACACGAGTCAAG
	R	GCTCTGGAGTGAAGAATAATGTGAA
U44087.1	F	CACGACACCACCTATCTTCTTCACC
	R	AAGATGAAGTCAGAGGGGAACCAGT
BM337131	F	TAGAATACACGAAACGGATTGCAAC
	R	AGGACAGACAAAGGCTACAGATACC
CD443909	F	GGTGCGTGCCCTGTTGA
	R	ACACACGCACTCCATTACCA
AW927712	F	CCTCGCCTTCCTTGACG
	R	AGCAGCCGGTTTTATCG
BM072990	F	AGGGTAAGATGAAATAGACCACGAG
	R	TGTTTTTCGAGACAATACAGTGCAG
CK370542	F	GGACCCTGGAGGAAGCACA
	R	AGCATGGACGCCCTGAACT
CO526660	F	TCGCAGTCGCAGCCAAAT
	R	ATGCCCTACCTCAACGAATACA
CO519149	F	CAGCAACAACGGGCAGAG
	R	TTTGAGGCGAACGAGTGG
13397940	F	CTGTCTACTATCCAGCGAAACCA
	R	GCTCCCACTTATCCTACACCTC
BM334482	F	AGGCAAGAGCATAAACGAGA
	R	CATCAACAAAGTGGAGGGAC
BM379264	F	CAGACAGACCCAGATAGATGAGA
	R	ATGCTGTTGACGAGGACTGAT
BM380179	F	ATGAATGCACAACCCACAGCT
	R	AGTGCCCGTAATCACATCAGC
Actin	F	5'-GTGACCTTACCGACAACC
	R	5'-CCAATACCAGGGAACATAG

Quantitative real-time RT-PCR

Real-time PCR was performed using an iQ™5 Optical System (Bio-Rad). Template cDNA samples were prepared using the PrimeScript First Strand Synthesis System Kit (TaKaRa,) for reverse transcriptase-PCR with 500 ng of total RNA. Primers (Table 1) were designed with the software Primer Expression 3.0 (Applied Biosystems). The actin gene was used as internal control. Each PCR reaction contained 2 μℓ of cDNA and 0.5 μM of each of the primers. The SYBR PrimeScriptTM RT-PCR Kit from TaKaRa was used for the PCR reactions. The initial denaturing time was 2 min., followed by 40 PCR cycles consisting of 94°C for 10 sec, 56~62°C for 20 sec, and 72°C for 10 sec. A melting curve was run after the PCR cycles followed by a cooling step. Quantification was performed with Bio-Rad iQ5 Relative Quantification Software 2.0.

Results

Overall view of microarray data

The results showed that after the long nitrogen stress treatment, 887 genes involved in a variety of biological processes were responsive to low nitrogen treatment in maize inbred line Mo17, out of which 384 were up-regulated and 503 were down-regulated. In line Hz4 the expression of 1108 genes also changed, out of which 696 were up-regulated and 412 genes down-regulated. There are more genes in response to low nitrogen stress treatment in Hz4 (1108 genes) than in Mo17 (887 genes) indicating that Hz4 may have higher tolerance to low nitrogen stress than Mo17 by expressing more genes. The gene expression profiles also revealed 1799 differential expression genes (910 and 889 high level expression genes in Hz and Mo17, respectively) between these two lines under low nitrogen conditions.

Nitrate transporter and assimilation genes

Microarray results for known nitrate transporter and assim-

Table 2. Nitrate transporter and assimilation genes.

GenBank Accession	Signal (M0)	Signal (Mn)	Signal (H0)	Signal (Hn)	Signal Ratio (Hn/H0)	Signal Ratio (Mn/M0)	Gene
AY129953.1	2.3*	35.8*	3.7*	14.4*	NC	4.2	nrt2.1
AW461127	3532.7	3744.8	4669.6	5044.3	NC	NC	NAR1S
AY109418.1	518	1221.5	369.8	470.5	NC	1.4	NiR
AY187878.1	529	578.9	1225.4	844.9	0.5	NC	Nrt1.1
M27821.1	13.5	54.7	72.6	81.5	NC	NC	NR
X64446.1	26.4	65.9	46.9	69.5	NC	1.3	NAD(P)H
CF349220	43.9	25.2*	37.9	58.5	NC	NC	NTL1
AB001385.1	357.2	510.9	249.8	489.4	1.1	1.2	FdVI
AB035645.1	11214.1	15818.4	13283.4	13129	0.4	NC	L-FNRII
CF624577	1319.4	961.9	626.9	347.1	-0.6	-1.1	pFD1
AB016810.1	6947.3	10728.7	7535.6	6706.4	0.5	NC	pFD2

Note: Table showing average signal ratios and signal intensities for the nitrate transport and assimilation genes. The signal ratios were determined by the Affymetrix GCOS software by comparing each probe pair on the induced array with the corresponding probe pair on the control array and, thus, are not simply the ratio of the average signal intensities. NC: no change as determined by the Affymetrix GCOS software. A value is given for the ratio only if both RNA samples had a call of "I" or "D". *An asterisk by a signal value indicates an "Absent" call.

ilation genes are highlighted in Table II. Of 11 genes, five showed significant induction in Mo17 (*Z. mays* putative high affinity nitrate transporter (nrt2.1), nitrite reductase (NiR), nitrate reductase (NAD(P)H), ferredoxin (Fd VI) and Maize ferredoxin I isoproteinp(FD1)). The nrt2.1 gene showed the most dramatic induction ratio, however, because the signals from the control sample and nitrogen stress treatment sample for this gene were called as absent ("A"), the induction ratio cannot be considered reliable. Conversely, NAD(P)H, FdVI, FD1 and NiR were induced under nitrogen stress conditions, similar to the results reported for maize (Matsumura et al., 1997) but the response was small. In Hz4, there were six nitrate transporter and assimilation genes that showed a response to nitrogen stress (Table 2), however, the response was small. Compared to the changes observed in Hz4, all the response of NiR, NAD(P)H, FD1 and FdVI were stronger in Mo17. Overall, nitrate transporter and assimilation genes showed no or very little change following nitrogen stress treatment in both M017 and Hz4 lines, but the magnitude of gene induction or repression in response to nitrogen stress conditions was greater in Mo17 than that in Hz4 (e.g. signal log2 Ratio: 1.2 versus 1.1; for Fd VI, -1.1 versus -0.6 for FD1).

Novel nitrogen stress response genes

To gain additional insights from the *Z. mays* chip data, a subset of genes/ESTs with large signals and strong induction ratios in both maize inbred lines (Mo17 and Hz4) grown under nitrogen stress conditions were selected for further analysis (Table 3). These were identified from the set of genes, which had been induced/repressed in pooled RNA from three biology replicates of each group (nitrate-treated and control) were selected.

Early light-inducible protein (*ELIP*)

Early Light-Inducible Protein is a member of a family of organelles found in the cytoplasm of plants, which is membrane-bounded. In our *Z. mays* array data, the ELIP gene had reliable signal intensities (in nitrogen treated and control plants) and showed a strong response to nitrogen stress in samples of both Mo17 and Hz4 lines. This gene was repressed in both lines, but the magnitude of repression was larger in Mo17 than in Hz4 (-4 versus -2.9).

Uroporphyrinogen III methyltransferase (*UPM1*)

Uroporphyrinogen III methyltransferase (*UPM1*) catalyzes the branch point step in the biosynthesis of siroheme, an essential cofactor for NiR. This gene displayed strong induction in both Mo17 and Hz4. UPM1 was strongly induced in maize within 2 hr of 16 mM nitrate treatment (Sakakibara et al., 1996). Our array data showed it was strongly induced at 10μM calcium nitrate for more than one month (before flowering) and showed different induction levels in the two inbred lines.

Phosphoenolpyruvate carboxylase (*pepcZm3B*)

Phosphoenolpyruvate carboxylase (PEPC) catalyzes a reaction in the tricarboxylic acid cycle of photosynthesis, which promotes carbon utilization through the fixation of carbon. But, it has both catalytic activity and lyase activity. PEPC is involved in organic acid metabolism, and were response to nitrate (Scheible et al., 1997; 2004). In our array, *pepcZm3*B showed good induction. This gene showed strong induction in both Mo17 and Hz4. It was interesting that *pepcZm3B* showed opposite responses to the nitrate treatment in the two lines: namely, *pepcZm3B* was up-regulated in Mo17 but down-regulated in Hz4.

***Z. mays* tonoplast intrinsic protein (*ZmTIP1*)**

Three clones corresponding to *ZmTIP1* were present in the array, but only one clone showed different expression both

Table 3. Maize genes responding to nitrate.

GenBank Accession	Signal (M0)	Signal (Mn)	Signal (H0)	Signal (Hn)	Signal Ratio (Hn/H0)	Signal Ratio (Mn/M0)	Gene
U44087.1	165.5	1637.1	476.7	3737.6	3	3.5	beta-D-glucosidase precursor
AW288498	5535.4	1187	4447.8	2128.4	-1.1	-2.1	Chalcone synthase
BM075175	1181.8	1739.9	4242.1	807.5	-2.4	0.5	cysteine protease
L38424.1	233.4	494	268.4	1007.7	1.9	1.2	dapB
AF365951.1	1710.5	141.1	3614	461	-2.9	-4	ELIP
AF136823.1	1255.3	6419	4944.7	3349.9	-0.6	2.5	Elongation factor
BQ619268	459.7	1339.4	652.3	185.4	-2.1	1.7	GT1
CO519149	284.7	7003.6	1810.4	3820.2	1	4.5	kin1
D83391.1	49.3	244.7	425.7	1619	2	1.8	Uroporphyrinogen III methyltransferase
11990232-88	1640.2	974.2	538	4754.3	3.1	-0.6	NADH dehydrogenase
X63871.1	103	1283.6	3724.2	1163.9	-1.6	3.8	pepcZm3B
BQ539201	795.5	1550.2	516.3	2181.9	2.2	0.8	pheA
AY105086.1	691.7	9462.3	2448	8020.6	1.4	3.3	Protein kinase
11990232-22	2516.7	10613.2	9620.4	12760.6	0.4	2.2	psaA
BM380325	115.9	435.5	115.7	761.8	2.3	1.8	Putative zinc finger protein
CF629317	82.5	345.8	153.5	348.6	1.9	2.4	serine carboxypeptidase
AF296122.1	507.2	150.9	623.5	128.7	-2.2	-1.2	stc1
CA829088	296.7	1034.7	206.2	1148.4	2.4	2	uracilphosphoribosyl transferase
CF637986	81.4	387.1	3993.1	1632.5	-1	2	ZmTIP1

Note: Table shows average signal ratios and signal intensities for genes induced/depressed by nitrate. Signals from array data were detected from fixed RNA of three biology repetitions. H0 was Hz4 treated with control. Hn was Hz4 treated with nitrate; M0 was Mo17 treated with control, and Mn was Mo17 treated with nitrate.

in Mo17 and Hz4. However, the long-term nitrogen stress treatment prompted different changes in the expression of *ZmTIP1* in Mo17 and Hz4.

Z. mays sesquiterpene cyclase 1 (*stc1*)

Stc1 catalyzes metabolic process and magnesium ion binding, it has lyase activity. Data in our array showed that this gene was down-regulated in response to nitrogen stress levels in both Mo17 and Hz4. The magnitude of the down-regulation was bigger in Hz4 than that in Mo17.

Additional genes

In addition to genes described above, several other genes showed changes in expression patterns both in Mo17 and Hz4, including a putative zinc finger protein, a Bacillus subtilis dihydropicolinate reductase(*dapB*)-like gene, chalcone synthase, an elongation factor, and genes for unknown proteins.

Confirmation of microarray data by qRT-PCR

To verify the data from the Gene Chip experiments, some genes were selected randomly to confirm their expression differences with qRT-PCR. Reactions were performed using pooled cDNA from three biological replicates of maize plants subjected to each treatment, and the results were compared with the results obtained using the chip (Table 4). Although the microarray value of some probe differed somewhat from the corresponding value of qRT-PCR, the changed trend was same between the two sets of data. Genes that were more sensitive to nitrate (e.g. CD443909, BM380179, and CO526660) displayed stronger expression changes in both methods of microarray and qRT-PCR, whereas genes that were least induced (e.g. BM331860) gave the lowest ratios in both methods. The results matched well with each other for both Mo17 and Hz4 grown under prolonged low-nitrate-treatments. The results demonstrate that genetically distinct maize inbred lines have differential global gene expression responses to low nitrogen stress treatment.

Discussion

Different responses to nitrogen stress treatment in Mo17 and Hz4 lines

Le Gouis (2000) confirmed that there is a genetic variability for grain yield at a low nitrogen levels and that the genotype × nitrogen (N) level interaction is significant. Bertin and Gallais (2001) found that most of the chromosomal regions for yield, grain composition, and traits related to N uptake efficiency detected at low N input corresponded to quantitative trait loci (QTLs) detected at high N input, while Agrama et al. (1999)

Table 4. Comparison of qRT-PCR and Zea mays array data.

Sequence ID	Signal value(H0)	Signal value (Hn)	Signal value (M0)	Signal value (Mn)	Signal Ratio (Hn/H0)	RT-PCR (Hn/H0)	Signal Ratio (Mn/M0)	qRT-PCR (Mn/M0)
CD443909	302.3	1939.6	524.8	7840.5	6.416143	6.30407	14.93998	25.87407
BM380179	47.9	752.5	47.9	1157.7	15.70981	16.6475	24.1691	45.12932
BM331860	529.2	12	2.2	1	0.022676	0.48179	0.454545	0.431739
BM072990	905.2	9146.7	2963.5	8912.7	10.10462	1.63655	3.007491	5.733399
U44087.1	476.7	3737.6	165.5	1637.1	7.840571	1.22524	9.891843	1.630148
BM337131	5.3	56.5	30.7	2790.4	10.66038	1.62762	90.89251	1.368589
AW927712	234.6	1878.2	122.7	507.5	8.005968	1.30981	4.136104	1.141151
BM379264	61.5	5041.4	151.4	8518.8	81.97398	11.17618	56.26684	1.349872
CK370542	29.3	528.7	14.9	1549.7	18.04437	3.40029	104.0067	2.518838
CO526660	110.5	2077	1	61.5	18.79638	171.8544	61.5	10.16107
CO519149	1810.4	3820.2	284.7	7003.6	2.110141	7.00401	24.59993	98.30669
13397940	298.7	4237.5	11013.9	976.8	14.18647	19.13778	0.088688	4.752148
BM380179	101.1	752.5	47.9	1157.7	7.443126	2.21242	24.1691	1.260161

Note: Signals from array data were detected from pool RNA of three biology repetitions.H0 was Hz4 treated with control. Hn was Hz4 treated with nitrate; M0 was Mo17 treated with control, and Mn was Mo17 treated with nitrate.

detected more QTLs at low N input. However, knowledge about the underlying mechanisms responsible for good nitrogen use efficiency is limited in plants (Stitt, 1999), especially in maize. In addition, the differences in nitrate metabolism between maize and other model plants including *Arabidopsis* are poorly understood. Previous research only served to identify some genes whose expression levels change in response to different nitrate concentrations. But little was known about the genetic expression patterns found among different genotypes of the same species, especially in maize. Our analysis showed differences in the genetic expression patterns in response to nitrogen stress treatments between the key maize inbred lines of Mo17 and Hz4. Microarray analyses demonstrates that more genes show a greater change in expression levels in Hz4 than in Mo17 following a prolonged nitrate treatment (1108 in Hz4 versus 887 in Mo17). These genes may be related to the nitrate metabolic process and their functions need to be investigated further. All the results (including genes directly and indirectly involved in nitrate transport and assimilation) proved that Mo17 was more sensitive than Hz4 under nitrogen stress-treated in nucleic acid level.

Genes involved in nitrate transport and assimilation indirectly showed good induction in response to long-term nitrogen stress treatment in maize

Using the maize array that contains probes for the maize genome genes/ESTs selected from NCBI's GeneBank (up to September 29, 2004), has lead to the most extensive analysis of nitrate-induced genes so far in maize. With this method, the response to prolonged nitrogen stress condition (prior to flowering) was measured for more than 5,000 genes. Many genes directly involved in nitrate absorption, transport and assimilation have not been discovered, but expression analysis should help to identify more. An interesting feature of the nitrate regulation observed in these experiments is that the NAR1S, L-FNRII, pFD2 genes had high signal intensities but no/little response to nitrate. NR, previously reported to have a strong response to nitrate, didn't show induction in our experiment. The differences in these findings may result from the different lengths of the experimental treatments. The changes in expression of NR and other nitrogen metabolism genes observed in other experiments may be rapid and transient (Wang et al., 2003), but these changes would not be observed over a longer time period. Conversely, many genes not directly involved in nitrate metabolism (e.g. *UPM1*, *pepcZm3B*, *ZmTIP1*, etc.) showed both high signal intensities and a strong response to nitrate (Table 3). In addition, the magnitude of change in the expression of these genes differed between the two genotypes with varying degrees of tolerance to nitrogen stress conditions. While maize seems to have a typical nitrate metabolism process, it appears that many genes indirectly involved in nitrate metabolism that respond to an extended nitrogen stress treatment are important for the observed differences in nitrate between two key inbred lines of maize.

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