ORIGINAL PAPER

Genomewide computational analysis of nitrate response elements in rice and *Arabidopsis*

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Abstract Nitrate response element (NRE) was originally reported to be comprised of an Ag/cTCA core sequence motif preceded by a 7-bp AT rich region, based on promoter deletion analyses in nitrate and nitrite reductases from Arabidopsis thaliana and birch. In view of hundreds of new nitrate responsive genes discovered recently, we sought to computationally verify whether the above motif indeed qualifies to be the cis-acting NRE for all the responsive genes. We searched for the specific occurrence of at least two copies of the above motif in and around the nitrate responsive genes and elsewhere in the Arabidopsis and rice (Oryza sativa) genomes, with respect to their positional, orientational and strand-specific bias. This is the first comprehensive analysis of NREs for 625 nitrate responsive genes of Arabidopsis and their rice homologs, representing dicots and monocots, respectively. We report that the above motifs are present almost randomly throughout these genomes and do not reveal any specificity or bias towards nitrate responsive genes. This also seems to be true for

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D. Choudhury (⊠) Centre for Biotechnology, Jawaharlal Nehru University, New Delhi 110 067, India e-mail: devapriyach@yahoo.co.in smaller subsets of nitrate responsive genes in Arabidopsis, such as the 21 early responsive genes, 261 and 90 genes for root-specific and shoot-specific response, respectively, and 25 housekeeping genes. This necessitates a fresh search for candidate sequences that qualify to be NREs in these and other plants.

Keywords Motifs · Nitrate · Promoters · Response elements · Signaling · Transcriptional regulation

Introduction

Nitrate response at the plant level is mediated by the transcriptional regulation of several hundreds of genes, but the mechanism(s) of nitrate signaling is not known (Raghuram et al. 2006). The identification of cis-acting nitrate-responsive element (NRE) provides an end point for nitrate signal transduction and paves the way for unraveling the preceding steps of the signaling pathway. Several attempts have been made to delineate the regulatory regions of nitrate and nitrite reductases (NR and NiR) in transgenic plants but none of them identified the minimum consensus sequence specific to nitrate response (Dorbe et al. 1992; Vaucheret et al. 1992; Neininger et al. 1993; Rastogi et al. 1993; Vincentz et al. 1993; Lin et al. 1994). Sivasankar et al. (1998) reported that a 30 bp upstream sequence (-230 to -200)and an untranslated leader sequence (+5 to +67) are responsible for coregulating nitrate induction of spinach NiR, but their relevance to other nitrate responsive genes or other plants remains to be established. Sequence motifs that are targets for GATA family of transcription factors have been reported in the regulatory regions of NR and NiR in higher plants and their possible role in nitrate-stimulated gene expression has been suggested (Jarai et al. 1992;

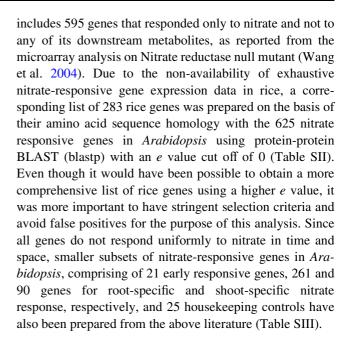


Daniel-Vedele and Caboche 1993; Rastogi et al. 1997). But GATA-mediation is also known for other signals like light and conclusive evidence for their involvement in nitratespecific response is lacking. Using linker-scanning analysis, Hwang et al. (1997) identified the cis-acting sequence elements that are essential for nitrate-dependent transcription of NR in Arabidopsis thaliana. These elements are composed of several copies of a core Ag/cTCA sequence motif preceded by a \sim 7-bp AT-rich sequence present in the 5' flanking regions of NR1 and NR 2 genes. This particular sequence [(a/t)₇Ac/gTCA] motif was also found to be present in the 5' flanking regions of NR and NiR genes from eight other plants at various locations, indicating that it is very well conserved. Using gel-shift assays, it was also demonstrated that this sequence motif is essential for binding to proteins in the nuclear extracts of Arabidopsis (Hwang et al. 1997), though nitrate-dependence of the binding could not be established. Subsequently, deletion analyses of birch promoter regions revealed the presence of the same sequence motifs in NR (Hachtel and Strater 2000) and NiR (Warning and Hatchel 2000). However, experimental approaches to delineate NREs were so far restricted to NR from Arabidopsis and birch, and NiR from birch alone. Also, it has never been demonstrated clearly that the motif was sufficient by itself for nitrate-induced transcription by showing cis-activation of a minimal promoter. Even the sequence comparisons with NR and NiR promoters from other plants were restricted to very few plant species (Hwang et al. 1997). In order to establish the true relevance of the proposed sequence motif to qualify as NRE, its specific occurrence and role needs to be demonstrated in all the nitrate-responsive genes known in plants. The list runs into several hundreds, as revealed by microarray analyses in Arabidopsis (Wang et al. 2000, 2003, 2004; Scheible et al. 2004) and tomato (Wang et al. 2001) and by subtractive hybridization analyses in rice (Wang et al. 2002), making it difficult to validate the NRE in each of them experimentally. We describe here an attempt to validate the relevance of the [(a/t)₇Ac/gTCA] motif as NRE in the post-genomic model plants Arabidopsis and rice using a computational approach.

Materials and methods

Compilation of nitrate responsive gene sets

A shortlist of 625 unique nitrate-responsive genes confirmed by microarray, RT-PCR and other studies was compiled from published data on *Arabidopsis*, (Wang et al. 2000, 2003, 2004; Scheible et al. 2004). The list was non-overlapping and redundancies were eliminated on the basis of existing annotations available for the genes that were reported to be nitrate responsive (Table SI). This list



Sequence retrieval

Two sets of sequences were drawn for both Arabidopsis and rice consisting of nitrate responsive and non-nitrate responsive genes. The sequences were extracted from their respective chromosomes according to the annotations downloaded from NCBI in the case of Arabidopsis and TIGR in the case of rice, using the program Extractseq from the EMBOSS suite (Rice et al. 2000). The annotations for Arabidopsis genes (.ptt files) were downloaded from NCBI ftp site (ftp://ftp.ncbi.nih.gov/). Since the .ptt files for rice were unavailable on the web at the time of this study, they were generated using the annotation information for Oryza sativa on the TIGR web site (ftp://ftp.tigr.org/pub/ data/Eukaryotic_Projects/o_sativa/annotation_dbs/). In addition to this, flanking sequences of 1 kb on either side of each of the genes were also extracted similarly. This list was used for all the further analysis.

Determination of the optimum distance between two consecutive Ag/cTCA motifs

For this purpose, 100 random sequences were generated for each of the chromosomes of *Arabidopsis thaliana* and *Oryza sativa* (cv. Japonica) having the same length and base compositions. Using FUZZNUC program from the EMBOSS suite (Rice et al. 2000), the occurrence of the Ag/cTCA motif was searched in all the randomly generated sequences as well as the original chromosomes in all possible orientations. An optimum distance range of 0–35 was calculated on the basis of a plot of the expectation values as a function of the distance between two consecutive motifs (Fig. 1) and used for further analysis in both plants.



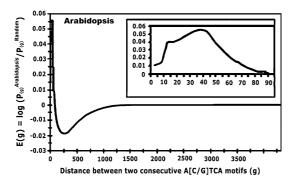


Fig. 1 Optimum distance between two consecutive Ag/cTCA core motifs. The optimum distance was calculated using the formula $E(g) = log(P(g)^{Genome}/P(g)^{random})$, where $P(g)^{Genome}$ is the probability of finding a pair of Ag/cTCA motifs separated by 'g' bases. P (g) ^{Genome}

Search criteria for the occurrence of the Ag/cTCA core motif

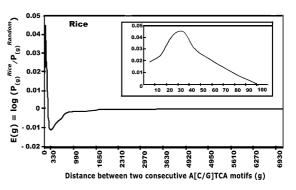
A genome wide search for pair wise occurrence of the Ag/cTCA motifs was conducted in the regions spanning -1,000 to +1,000 nucleotides of every gene in all 27 possible combinations and orientations on both strands. The raw data thus obtained was further categorized on the basis of position (upstream, downstream or within the ORF), distance from the start site, major and minor grooves of the DNA double helix, +/- strands and association with upregulated and down-regulated nitrate responsive genes. The statistical significance of these results was tested against randomized sequences by Chi-square tests.

Determination of the AT richness of the region preceding the Ag/cTCA core motif

The flanking sequences of 25 nucleotides on either side of the motif were extracted using the program Extractseq from the EMBOSS suite (Rice et al. 2000) and a pictorial representation of the base composition was generated using WEBLOGO Version 2.8.2 (Crooks et al. 2004).

Results

A list of 625 unique nitrate-responsive genes was compiled from the published data on *Arabidopsis* and 283 of their rice homologs were generated as described in Materials and methods and considered for the current analysis (Tables SI and SII). In order to search for the distribution of the Ag/cTCA core motifs of the proposed NRE in multiple copies, it was essential to determine the optimum distance range between adjacent copies. A statistical determination (see Materials and methods) revealed that copies of the core motif separated by a distance of upto 35 nucleotides are



was obtained by calculating the number of pairs of Ag/cTCA separated by 'g' bases and dividing the result by the genome length. P (g)^{random} was obtained similarly with 100 randomly generated sequences

over represented in the *Arabidopsis* and rice genomes, as compared to randomized genomic sequences (Fig. 1). Accordingly, we searched for pairs of Ag/cTCA motifs separated by 35 nucleotides or less in the genomes of *Arabidopsis* and rice.

Core motifs and preceding sequences around nitrate responsive genes

Initial search for the pair-wise occurrence of the proposed Ag/cTCA core motifs in all possible combinations and orientations within the coding regions and 1 kb of flanking regions on either side of the known nitrate responsive genes revealed that the motif occurred in 542 (86.7%) out of the 625 nitrate responsive genes tested in *Arabidopsis*, and 112 (39.58%) out of 283 genes in rice. An analysis of the base composition of 25 nucleotides of flanking sequences on either side of the core motif did not reveal any AT:GC bias in both organisms (Fig. 2).

Distribution of the core motif around nitrate responsive genes

The distribution of pairs of the proposed Ag/cTCA core motif was compared in terms of their occurrence either within the coding regions of nitrate responsive genes or their 1,000-bp upstream and downstream regions, with respect to their randomized controls for both *Arabidopsis* and rice. The data (Fig. 3) clearly indicates that the motifs were randomly dispersed throughout and did not show any absolute specificity for either upstream, downstream or the coding regions of the genes in both the organisms.

While there were some differences in the % distribution between these regions, they were not statistically significant by Chi-square test with respect to their randomized controls in any of the regions either in *Arabidopsis* or in rice. A similar analysis for the distribution of the core motifs in terms



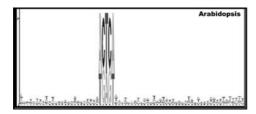


Fig. 2 Base composition around the proposed Ag/cTCA core motif. The flanking sequences of 25 nucleotides on either side of the motif around the nitrate responsive genes of *Arabidopsis* and rice were



analyzed for their base composition and graphically represented using WEBLOGO

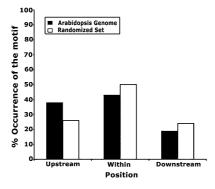
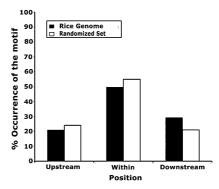


Fig. 3 Positional distribution of the proposed Ag/cTCA core motif. The distribution of the core motif among upstream, downstream or within the coding regions of the nitrate responsive genes was not very distinct from their respective randomized controls for *Arabidopsis* and



rice. There are no significant differences between % occurrence of the motifs in rice or *Arabidopsis* versus their randomized controls as per Chi-square test ($P \ge 0.037$ for *Arabidopsis* and $P \ge 0.02$ for rice; degrees of freedom = 2)

of their distance from the translational start site of nitrate responsive genes also revealed more or less uniformly high occurrence over a wide region spanning -1 to 2 kb (Fig. 4).

Positional and strand bias evaluation of the core motif

The possibility of strand specific distribution of copies of the Ag/cTCA core motif was also tested with the nitrate

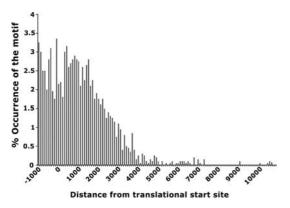


Fig. 4 Distribution of the Ag/cTCA core motif around translational start site. The occurence of the core motifs was plotted against their distance, spanning regions of -1 to +10 kb from the translational start site of the nitrate responsive genes in *Arabidopsis*. Note the more or less uniformly high occurrence in the -1 to +2 kb region

responsive gene set (and their flanking sequences) only in *Arabidopsis*, as the annotations required for this analysis in rice were not available at the time of this analysis. The data presented (Fig. 5) clearly reveals that the motifs were dispersed on both strands and did not show any statistically significant strand bias with respect to their randomized controls by Chi-square test.

The possibility of positional/strand bias in a smaller subset of nitrate responsive genes in *Arabidopsis* was also tested, both with respect to up-regulated and down-regulated genes (Figures S1 and S2, respectively), as well as to their differential expression in time and space, such as the 21 early responsive genes, 261 and 90 genes for root-specific and shoot-specific response, respectively, and 25 housekeeping genes (Figure S3). These analyses were done at all positions viz. upstream, downstream and within the coding regions of the genes. However, there were no significant differences in the strand-wise distribution, either within or between these subsets.

Spacing in motif pairs with respect to DNA helicity

An analysis for possible differential spacing between members of a pair of core motifs was also done, in terms of the major and minor grooves of the DNA double helix representing the nitrate responsive genes (and their flanking



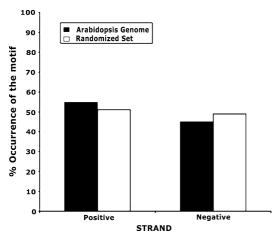


Fig. 5 Strand-wise distribution of the proposed Ag/cTCA core motif. The occurence of the core motifs on each of the strands of the nitrate responsive genes of *Arabidopsis* was not very distinct from their randomized controls. There are no significant differences between % occurrence of the motifs in the + and - strands of Arabidopsis versus their randomized controls as per Chi-square test ($P \ge 0.062$; degrees of freedom = 1)

regions) in *Arabidopsis*. No significant preference for any specific distance between members of the motif pair was found, with respect to their position, either upstream, downstream or within the coding regions of the genes. (Figures S4, S5 and S6, respectively)

Random distribution of the proposed NRE

The absence of even a single pair of the proposed NRE core motif in 83 nitrate responsive genes in Arabidopsis and at least 171 such genes in rice as per the search criteria adopted, and the lack of any particular pattern in their distribution raised questions of specificity. This was further verified by a search for pair-wise occurrence of the core motif around genes that are nitrate non-responsive in both the organisms. It was found that the Ag/cTCA core motifs are present in 22,927 out of 29,235 (78.42%) of the nonresponsive gene set in Arabidopsis and in 28,324 out of 60,174 (47.07%) in rice (Fig. 6). The randomness of the motif was further confirmed in 25 housekeeping genes of Arabidopsis, which revealed 60 and 80% occurrence of the core motif in the positive and negative strands (Figure S3D). These results indicate that the motif is neither specific to nitrate responsive genes, nor common to all nitrate responsive genes.

Discussion

Response elements can be identified by their essential characteristics such as: specific presence in all responsive genes,

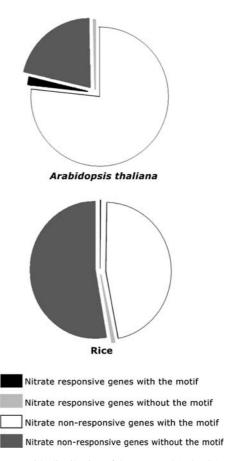


Fig. 6 Genomewide distribution of the proposed Ag/cTCA motif. The relative pair wise occurrence values of the core motif in the genomes of *Arabidopsis* and rice were categorized in terms of nitrate responsive or non-responsive genes with or without the motif. Note the lack of specificity in distribution

occurrence in multiple copies (Shinozaki and Shinozaki 2005), coexistence with other regulatory elements (Shen and Ho 1995 and Shen et al. 1996) and existence of a clear positional, strand or orientation bias. They may be located either in the region upstream or downstream of the gene or within the gene, especially in introns (Levine and Tjian 2003; Arnone and Davidson 1997; Davidson 2001; Carey and Smale 2000; Mancini-DiNardo et al. 2003; Adlam and Siu 2003; Lee et al. 2003). The knowledge of the above principles, coupled with the increasing availability of genomic sequences and high throughput gene expression data has made it possible to computationally analyze the promoter regions of co-regulated genes and predict candidate sequences for response element(s). In the case of NRE, which has been experimentally attempted for few of a large set of co-regulated genes in a few plants, validation of the proposed [(a/t)₇Ac/gTCA] motif upstream of NR gene in Arabidopsis by Hwang et al. (1997) has not progressed beyond NR and NiR in birch (Hachtel and Strater 2000; Warning and Hatchel 2000). Moreover, it has never been conclusively demonstrated that the motif was



sufficient by itself for nitrate-induced transcription by showing cis-activation of a minimal promoter. The expanding list of nitrate responsive genes (Wang et al. 2000, 2001, 2002, 2003, 2004; Scheible et al. 2004) and the increasing availability of their sequences from model plants has prompted the computational re-evaluation of these elements as NREs in the present study. This is the first comprehensive analysis of NREs for 625 nitrate responsive genes of *Arabidopsis* and their rice homologs, representing dicots and monocots, respectively.

Arabidopsis has been used as a reference point for the preparation of a nitrate responsive gene list in the present study, as microarray analyses and other published studies in this plant have revealed the largest number of nitrate responsive genes known so far. A unique and confirmed set of 625 genes had been short listed for the present study (Table SI), which includes 595 genes that responded only to nitrate and not to any of its downstream metabolites, as reported from the microarray analysis on a nitrate reductase null mutant of Arabidopsis (Wang et al. 2004). As complete transcriptome data for nitrate responsive gene set in rice is not yet available and a subtractive hybridization study identified only 37 genes (Wang et al. 2002), extrapolation from the Arabidopsis nitrate responsive gene set by identifying their corresponding rice sequence homologs was the best option to ensure comprehensive coverage of the rice genes for the current analysis. Using the highest stringency cut off at an e value of ~ 0 yielded only 283 rice homologs of Arabidopsis nitrate responsive genes (Table SII). However, the compilation and analysis of smaller subsets of nitrate-responsive genes was restricted to Arabidopsis, based on the experimental evidence for response types reported in literature, in terms of upregulated/downregulated, or early, shoot-specific and root-specific nitrateresponsive genes (Table SIII).

Based on a statistically determined optimal distance of 35 nucleotides between two members of a pair of Ag/cTCA core sequence motifs in Arabidopsis and rice (Fig. 1), a genome wide search for pair-wise occurrence of the motifs was conducted in the regions spanning -1,000 to +1,000nucleotides of every gene. This revealed that the motif occurs in 86.72% (542) of the 625 known nitrate responsive genes in Arabidopsis, but also in 78.42% of the non-responsive gene set. Similar distribution was obtained in rice at 39.58 and 47.07%, respectively, indicating that the motif is neither specific to nitrate responsive genes, nor common to all nitrate responsive genes. The higher % occurrence of the core motif in *Arabidopsis* (regardless of nitrate response) is more likely to be due the AT rich nature of its genome (66 %), as compared to that of rice (56.46%). Indeed, this also seems to be the reason why a \sim 7 bp AT rich sequence preceding the core motif was found to be a necessary component of the experimentally proposed cis-acting sequence

(Hwang et al. 1997). However, our analysis of the base composition of the -25 to +25 nucleotides flanking the core Ag/cTCA motif does not reveal any significant AT:GC bias among the nitrate responsive genes that contain the motif in both *Arabidopsis* and rice (Fig. 2).

Within the nitrate responsive gene set, there were also no significant positional differences in the occurrence of the core motif, either upstream, downstream or within the coding region (Figs. 3, 4). The positional differences in the occurrence of the motif were also analyzed for the *Arabidopsis* gene set with respect to any bias between the positive and negative strands (Fig. 5), up-regulated and down-regulated subsets of genes (Figs. S1 and S2), other response subsets such as early, root-specific and shoot-specific genes (Fig. S3) as well as major and minor grooves (Figs. S4–S6), but no significant differences were observed. The information on translational start sites and strand specifications for rice genes was not available in public domain at the time of the analysis.

Finally, an analysis of the distribution of the core motif throughout the genomes of *Arabidopsis* and rice categorized in terms of nitrate responsive or non-responsive genes with or without the Ag/cTCA core motif was very revealing (Fig. 6). The absence of even a single pair of the core motif in a large number of nitrate responsive genes, as well as its presence in even larger number of nitrate non-responsive genes clearly indicates that the motif is neither specific to nitrate responsive genes, nor common to all nitrate responsive genes and is randomly distributed throughout the genomes in both *Arabidopsis* and rice.

Thus, the occurrence and distribution of the $[(a/t)_7Ac/$ gTCA] motif in Arabidopsis and rice is not in accordance with the established common characteristics of known response elements (Shinozaki and Shinozaki 2005) and therefore, do not qualify to be cis-acting NREs. However, this does not exclude the possibility that this motif may operate in combination with another sequence motif(s) to qualify as a specific NRE. It is more likely that an entirely new sequence motif will emerge on a more detailed analysis of the nitrate responsive genes for flanking sequences that are specific and conserved. A search for the GATA binding elements in the promoter regions of the 625 nitrate responsive genes and 25 housekeeping genes revealed their lack of specificity in occurrence and distribution (data not shown). We are currently analyzing the results of our motif sampling to identify alternative candidate sequences that may qualify to be NREs for experimental validation.

The robustness of our results is amply indicated by the fact that our computational analysis is based on the 625 nitrate responsive genes (or their smaller subsets) of *Arabidopsis* and 283 genes of rice and their non-nitrate responsive controls, as compared to the experimentally proposed [(a/t)₇Ac/gTCA] motif based on one gene in *Arabidopsis*



(Hwang et al. 1997) and two genes in birch (Hachtel and Strater 2000 and Warning and Hatchel 2000). While the non-availability of the birch genome does not permit a similar verification of the experimental birch data, it is important to point out that both *Arabidopsis* and birch represent dicots, whereas our analysis includes monocots (rice) to enable generalization across the two phylogenetic groups, using the best characterized post-genomic data available so far.

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Table SI. Locus IDs of Arabidopsis Nitrate Responsive Genes used in the present study

			1			
At1g01190	At1g26665	At1g61660	At1g80310	At2g33550	At3g10960	At3g50970
At1g01530	At1g26670	At1g61740	At1g80380	At2g33710	At3g11410	At3g51860
At1g02040	At1g27100	At1g62180	At1g80440	At2g33720	At3g12670	At3g52360
At1g02230	At1g27170	At1g62660	At1g80460	At2g33830	At3g13110	At3g52930
At1g02310	At1g28330	At1g63010	At2g01140	At2g35930	At3g13560	At3g52950
At1g02340	At1g29050	At1g63090	At2g01530	At2g36090	At3g13730	At3g52960
At1g03080	At1g29390	At1g63940	At2g05540	At2g36580	At3g13930	At3g54140
At1g04770	At1g30260	At1g64140	At2g07180	At2g38170	At3g14205	At3g54900
At1g05300	At1g30510	At1g64170	At2g14920	At2g38180	At3g14940	At3g55740
At1g05340	At1g30820	At1g64190	At2g15320	At2g38340	At3g15630	At3g55980
At1g07610	At1g31180	At1g64370	At2g15620	At2g38640	At3g15650	At3g56400
At1g08090	At1g31200	At1g64590	At2g15830	At2g39200	At3g16560	At3g56620
At1g08100	At1g31230	At1g65690	At2g15890	At2g39250	At3g17610	At3g57040
At1g08650	At1g31320	At1g66140	At2g15970	At2g39570	At3g18560	At3g57630
At1g09780	At1g31770	At1g66200	At2g16005	At2g39920	At3g18820	At3g57920
At1g10120	At1g32450	At1g66380	At2g16060	At2g40140	At3g19030	At3g58610
At1g10470	At1g32920	At1g66390	At2g16660	At2g40750	At3g19710	At3g58990
At1g12110	At1g35140	At1g66930	At2g17060	At2g40970	At3g20340	At3g60160
At1g12820	At1g35560	At1g67110	At2g17500	At2g41310	At3g21510	At3g60300
At1g12940	At1g36370	At1g67810	At2g17710	At2g41560	At3g21700	At3g60330
At1g13300	At1g36640	At1g67870	At2g17820	At2g41660	At3g21710	At3g60690
At1g13420	At1g43650	At1g67910	At2g18280	At2g41730	At3g22550	At3g60750
At1g13440	At1g43710	At1g67920	At2g18300	At2g42060	At3g22840	At3g61060
At1g14340	At1g44100	At1g68190	At2g18690	At2g42070	At3g22850	At3g61190
At1g14780	At1g44160	At1g68520	At2g19810	At2g42350	At3g22890	At3g61460
At1g14870	At1g47128	At1g68600	At2g21210	At2g42600	At3g23570	At3g61820
At1g15290	At1g48320	At1g68670	At2g22480	At2g42890	At3g24520	At3g61830
At1g15550	At1g48600	At1g68880	At2g22500	At2g43100	At3g25780	At3g61890
At1g16170	At1g49000	At1g69040	At2g24290	At2g43500	At3g25790	At3g62650
At1g17060	At1g49160	At1g70410	At2g24550	At2g43550	At3g26210	At3g63110
At1g18470	At1g49500	At1g70780	At2g25680	At2g44380	At3g26280	At3g63260
At1g18590	At1g49860	At1g71030	At2g26650	At2g44940	At3g26510	At4g00630
At1g19020	At1g50110	At1g71970	At2g26820	At2g45340	At3g27170	At4g01440
At1g19050	At1g51800	At1g72160	At2g26980	At2g46130	At3g28510	At4g01460
At1g19250	At1g52200	At1g72200	At2g27510	At2g46620	At3g28690	At4g01840
At1g19330	At1g52870	At1g72220	At2g27830	At2g47160	At3g28740	At4g02380
At1g19440	At1g53180	At1g73600	At2g28250	At2g47190	At3g28910	At4g02810
At1g19450	At1g53510	At1g73870	At2g28550	At2g48080	At3g30260	At4g02920
At1g19660	At1g53885	At1g73920	At2g28780	At3g01260	At3g30775	At4g03400
At1g20640	At1g53920	At1g74030	At2g28840	At3g01290	At3g45140	At4g04630
At1g21000	At1g54130	At1g74090	At2g28890	At3g02020	At3g45700	At4g04955
At1g21010	At1g55120	At1g74440	At2g29090	At3g02885	At3g45970	At4g05150
At1g21440	At1g55760	At1g74660	At2g29120	At3g02910	At3g46640	At4g05200
At1g21680	At1g55920	At1g74710	At2g29700	At3g03150	At3g47160	At4g05390
At1g21910	At1g56150	At1g75080	At2g29730	At3g03870	At3g47520	At4g08300
At1g22130	At1g56650	At1g75140	At2g29990	At3g05200	At3g47970	At4g08850
At1g22160	At1g58032	At1g76070	At2g30020	At3g05400	At3g47980	At4g08950
At1g22170	At1g58080	At1g76350	At2g30040	At3g06330	At3g48100	At4g09000
At1g22400	At1g58180	At1g77760	At2g30360	At3g06390	At3g48360	At4g09620
At1g22500	At1g59730	At1g77920	At2g30590	At3g07270	At3g48740	At4g10960
At1g22650	At1g59940	At1g77950	At2g30970	At3g07350	At3g48990	At4g11360
At1g22990	At1g59960	At1g77990	At2g31360	At3g07910	At3g49940	At4g11460
At1g23870	At1g59970	At1g78000	At2g31390	At3g09270	At3g50260	At4g12030
At1g24280	At1g60140	At1g78050	At2g31790	At3g09805	At3g50660	At4g12070
At1g25230	At1g60710	At1g78090	At2g31950	At3g10500	At3g50740	At4g12280
At1g25440	At1g61100	At1g78780	At2g32560	At3g10520	At3g50900	At4g13130
At1g25550	At1g61360	At1g79320	At2g32990	At3g10760	At3g50910	At4g13510

At4g13770	At4g25490	At4g36040	At5g04590	At5g16110	At5g38800	At5g50160
At4g14270	At4g25620	At4g36380	At5g04840	At5g17380	At5g39590	At5g50200
At4g14630	At4g25835	At4g36410	At5g04950	At5g18130	At5g40470	At5g50210
At4g15610	At4g25990	At4g36540	At5g05090	At5g18600	At5g40590	At5g50800
At4g15700	At4g26130	At4g36670	At5g05160	At5g18840	At5g40730	At5g50850
At4g17490	At4g26150	At4g37300	At5g06300	At5g19140	At5g40780	At5g51460
At4g17550	At4g26970	At4g37540	At5g06570	At5g19530	At5g40850	At5g51830
At4g17615	At4g28240	At4g37610	At5g06870	At5g19970	At5g41670	At5g52020
At4g17670	At4g29950	At4g38060	At5g07680	At5g20530	At5g41800	At5g53160
At4g17730	At4g30190	At4g38340	At5g07890	At5g20885	At5g41810	At5g53460
At4g17900	At4g30470	At4g38470	At5g08570	At5g22570	At5g42830	At5g54120
At4g17980	At4g31500	At4g38540	At5g09800	At5g23010	At5g42840	At5g54130
At4g18010	At4g31730	At4g38840	At5g10030	At5g23660	At5g42990	At5g54170
At4g18340	At4g31910	At4g39090	At5g10210	At5g24120	At5g43520	At5g54500
At4g18510	At4g32950	At4g39660	At5g10570	At5g24780	At5g43880	At5g54660
At4g19160	At4g33040	At4g39780	At5g10580	At5g24810	At5g44670	At5g54800
At4g19170	At4g33960	At4g39800	At5g10820	At5g24910	At5g44790	At5g55050
At4g19430	At4g34000	At4g40070	At5g12250	At5g25280	At5g45070	At5g55970
At4g20010	At4g34750	At5g01740	At5g12470	At5g25350	At5g45080	At5g56080
At4g21120	At4g34760	At5g01750	At5g12860	At5g27000	At5g45340	At5g56870
At4g21350	At4g34770	At5g02420	At5g12880	At5g27100	At5g46050	At5g57560
At4g21680	At4g34860	At5g02550	At5g13110	At5g27150	At5g47100	At5g57660
At4g22070	At4g34950	At5g02580	At5g13420	At5g27420	At5g47240	At5g58070
At4g22470	At4g35090	At5g03230	At5g13550	At5g35620	At5g47550	At5g58140
At4g23700	At4g35260	At5g03290	At5g13750	At5g35630	At5g47560	At5g58700
At4g23890	At4g35480	At5g03320	At5g14040	At5g36110	At5g48370	At5g58900
At4g24020	At4g35750	At5g03340	At5g14760	At5g36140	At5g49100	At5g59050
At4g24620	At4g36010	At5g03380	At5g15190	At5g36150	At5g49360	At5g59080
At4g24670	At4g36020	At5g04540	At5g15830	At5g37790	At5g49480	At5g59450
At5g59490	At5g61340	At5g62720	At5g64260	At5g65060	At5g66530	
At5g59930	At5g61420	At5g62900	At5g64410	At5g65210	At5g67390	
At5g60300	At5g62430	At5g64100	At5g64770	At5g65660	At5g67420	
At5g61010	At5g62480	At5g64120	At5g65010	At5g66170		

Table SII. Locus IDs of rice homologs of Arabidopsis Nitrate Responsive Genes used in the present study

LOC_Os01g04950	LOC_Os01g59150	LOC_Os03g09940	LOC_Os05g40420
LOC_Os01g07870	LOC_Os01g5965601	LOC_Os03g09970	LOC_Os05g41580
LOC_Os01g08040	LOC_Os01g5982657	LOC_Os03g09980	LOC_Os05g42350
LOC_Os01g10087624	LOC_Os01g60190	LOC_Os03g10640	LOC_Os05g44100
LOC_Os01g10099031	LOC_Os01g65410	LOC_Os03g12030	LOC_Os05g47540
LOC_Os01g1060	LOC_Os01g65460	LOC_Os03g12290	LOC_Os05g48200
LOC_Os01g11160	LOC_Os01g67580	LOC_Os03g12270 LOC_Os03g12360	LOC_Os05g49270
	_	_	_
LOC_Os01g14311779	LOC_Os01g71240	LOC_Os03g12660	LOC_Os05g49290
LOC_Os01g14326916	LOC_Os01g9688862	LOC_Os03g13240	LOC_Os05g49520
LOC_Os01g16960	LOC_Os01g9696575	LOC_Os03g15020	LOC_Os05g49770
LOC_Os01g17531283	LOC_Os02g01590	LOC_Os03g18130	LOC_Os05g49800
LOC_Os01g17540423	LOC_Os02g02170	LOC_Os03g18980	LOC_Os05g49900
LOC_Os01g17544603	LOC_Os02g02190	LOC_Os03g21260	LOC_Os05g50280
LOC_Os01g18050	LOC_Os02g02400	LOC_Os03g29950	LOC_Os05g50560
LOC_Os01g18572952	LOC_Os02g02780	LOC_Os03g42020	LOC_Os06g01630
LOC_Os01g18573668	LOC_Os02g03320	LOC_Os03g45920	LOC_Os06g02150
LOC_Os01g19264624	LOC_Os02g04170	LOC_Os03g48310	LOC_Os06g03860
LOC_Os01g19281692	LOC_Os02g04230	LOC_Os03g49380	LOC_Os06g04270
LOC_Os01g22134457	LOC_Os02g04710	LOC_Os03g51050	LOC_Os06g04370
LOC_Os01g22159901	LOC_Os02g07060	LOC_Os03g53230	LOC_Os06g05160
LOC_Os01g2290880	LOC_Os02g07630	LOC_Os03g56810	LOC_Os06g06440
LOC_Os01g2292845	LOC_Os02g08010	LOC_Os03g61290	LOC_Os06g08310
LOC_Os01g22427544	LOC_Os02g00010 LOC_Os02g10120	LOC_Os03g62370	LOC_Os06g09540
	LOC_Os02g10120 LOC_Os02g10290		LOC_Os06g15420
LOC_Os01g24484520		LOC_Os03g63330	
LOC_Os01g25129986	LOC_Os02g11070	LOC_Os04g13210	LOC_Os06g19960
LOC_Os01g25138002	LOC_Os02g12730	LOC_Os04g13220	LOC_Os06g26340
LOC_Os01g25380	LOC_Os02g14110	LOC_Os04g19740	LOC_Os06g35540
LOC_Os01g26097209	LOC_Os02g14770	LOC_Os04g23890	LOC_Os06g36650
LOC_Os01g26105560	LOC_Os02g15660	LOC_Os04g32460	LOC_Os06g37560
LOC_Os01g26363417	LOC_Os02g18670	LOC_Os04g35280	LOC_Os06g39750
LOC_Os01g26371840	LOC_Os02g33710	LOC_Os04g37430	LOC_Os06g45500
LOC_Os01g26485682	LOC_Os02g34560	LOC_Os04g43070	LOC_Os06g46000
LOC_Os01g26494207	LOC_Os02g35190	LOC_Os04g45290	LOC_Os06g48770
LOC_Os01g27141885	LOC_Os02g45520	LOC_Os04g46940	LOC_Os06g51150
LOC_Os01g27150951	LOC_Os02g50240	LOC_Os04g48390	LOC_Os07g01070
LOC_Os01g32080	LOC_Os02g51620	LOC_Os04g49890	LOC_Os07g07910
LOC_Os01g33790	LOC_Os02g53130	LOC_Os04g49900	LOC_Os07g09340
LOC_Os01g34920	LOC_Os02g54150	LOC_Os04g51610	LOC_Os07g22350
LOC_Os01g3780493	LOC_Os02g54820	LOC_Os04g52900	LOC_Os07g22720
LOC_Os01g3801474	LOC_Os02g55400	LOC_Os04g54810	LOC_Os07g23990
LOC_Os01g3888059	LOC_Os03g01120	LOC_Os04g55210	LOC_Os07g46460
LOC_Os01g3896887	LOC_Os03g01530	LOC_Os04g55230	LOC_Os07g47100
LOC_Os01g39380	LOC_Os03g03910	LOC_Os04g55410	LOC_Os08g01750
LOC_Os01g43020	LOC_Os03g04410	LOC_Os04g56160	LOC_Os08g03350
LOC_Os01g43910	LOC_Os03g04410 LOC_Os03g04920	LOC_Os04g58110	LOC_Os08g05570
_	LOC_Os03g05730	LOC_Os04g58620	
LOC_Os01g45990		_	LOC_Os08g07010
LOC_Os01g46380	LOC_Os03g06520	LOC_Os04g58710	LOC_Os08g09200
LOC_Os01g46570	LOC_Os03g06940	LOC_Os05g05590	LOC_Os08g14360
LOC_Os01g47530	LOC_Os03g07570	LOC_Os05g08430	LOC_Os08g20570
LOC_Os01g48960	LOC_Os03g07820	LOC_Os05g10730	LOC_Os08g25390
LOC_Os01g50030	LOC_Os03g08220	LOC_Os05g19500	LOC_Os08g27840
LOC_Os01g53000	LOC_Os03g08430	LOC_Os05g25550	LOC_Os08g31410
LOC_Os01g54560	LOC_Os03g08560	LOC_Os05g26840	LOC_Os08g31870
LOC_Os01g55350	LOC_Os03g09250	LOC_Os05g34170	LOC_Os08g31980
LOC_Os01g5898420	LOC_Os03g09930	LOC_Os05g35440	LOC_Os08g34580
LOC_Os08g35620	LOC_Os08g36480	LOC_Os08g36500	LOC_Os08g37380

LOC_Os08g39840	LOC_Os09g26160	LOC_Os11g01140	LOC_Os12g04220
LOC_Os08g39850	LOC_Os09g27050	LOC_Os11g04460	LOC_Os12g05110
LOC_Os08g40530	LOC_Os09g29070	LOC_Os11g05110	LOC_Os12g07190
LOC_Os08g44260	LOC_Os09g31130	LOC_Os11g05700	LOC_Os12g19300
LOC_Os09g12290	LOC_Os09g34990	LOC_Os11g07440	LOC_Os12g25200
LOC_Os09g14670	LOC_Os09g37230	LOC_Os11g10980	LOC_Os12g33080
LOC_Os09g20820	LOC_Os10g02970	LOC_Os11g17080	LOC_Os12g36100
LOC_Os09g20990	LOC_Os10g18400	LOC_Os11g18690	LOC_Os12g36950
LOC_Os09g23350	LOC_Os10g22450	LOC_Os11g18730	LOC_Os12g37260
LOC_Os09g24910	LOC_Os10g26570	LOC_Os11g19160	LOC_Os12g37580
LOC_Os09g25890	LOC_Os10g28240	LOC_Os11g19210	LOC_Os12g37840
LOC_Os09g25960	LOC_Os10g30580	LOC_Os11g29400	LOC_Os12g39660
LOC_Os09g25980	LOC_Os10g33040	LOC_Os11g42790	LOC_Os12g42200
LOC_Os09g25990	LOC_Os10g33210	LOC_Os11g44950	LOC_Os12g44150
LOC_Os09g26000	LOC_Os10g40600	LOC_Os12g01140	

<u>Table S III: Subsets of nitrate responsive genes in Arabidopsis (Locus IDs)</u>

A. List of 21 Early Responsive Genes in Arabidopsis thaliana

At1g24280	At5g53460	At5g13420	At4g05390	At2g33830	At2g15620	At1g43710
At5g66170	At5g41670	At4g36670	At3g47520	At2g27510	At1g77760	At1g12940
At5g61420	At5g40850	At4g31910	At2g38170	At2g16060	At1g55920	At1g12110
C	C	<u> </u>	C	C	C	C
	B. L	ist of 25 House I	Keeping Genes in	n Arabidopsis tha	liana	
				<u> </u>		
At1g13320	At1g62930	At3g01150	At4g05320	At4g34270	At5g15710	At5g25760
At1g13440	At2g07190	At3g18780	At4g26410	At4g38070	At5g46630	C
At1g47770	At2g28390	At3g32260	At4g27960	At5g08290	At5g55840	
At1g58050	At2g32170	At3g53090	At4g33380	At5g12240	At5g60390	
υ	υ	C	U	U	U	
	C. List	of Genes Expres	sed Only in Root	ts in <i>Arabidopsis</i>	thaliana	
		•				
At2g32990	At1g31230	At1g80460	At3g13560	At4g34760	At5g02550	At5g61420
At2g26650	At1g31180	At1g80380	At3g22890	At4g35090	At5g03230	At5g61340
At2g42060	At2g17060	At1g74440	At3g13730	At4g35480	At5g03320	At5g62480
At2g33710	At2g31790	At1g71970	At1g36640	At4g37300	At5g03340	At5g64120
At2g44380	At2g47160	At1g78090	At1g75140	At4g38060	At5g05090	At5g64260
At2g26820	At2g36090	At1g67870	At3g10960	At4g38470	At5g05160	At5g65280
At2g29990	At1g54130	At1g76350	At1g51800	At4g38540	At5g06300	At5g66170
At2g44940	At2g16005	At1g52200	At1g22170	At4g39090	At5g10030	At5g66530
At2g29700	At2g17500	At1g72220	At1g66930	At4g39660	At5g13750	At5g67390
At2g41310	At1g65690	At1g72160	At1g18590	At4g39800	At5g15190	At5g04840
At2g01530	At1g59730	At1g29390	At4g01440	At4g40070	At5g24120	At5g24810
At2g27830	At1g59940	At1g19020	At4g02810	At3g45700	At5g35630	At5g24910
At2g28780	At1g14780	At1g02310	At4g04955	At3g47160	At5g36110	At5g25350
At2g28840	At1g14870	At1g13420	At4g05200	At3g47970	At5g36140	At5g25280
At2g18690	At1g15290	At3g01260	At4g05150	At3g47980	At5g36150	At5g27000
At2g25680	At1g17060	At3g01290	At4g08300	At3g48740	At5g40590	At5g27100
At2g42350	At1g21680	At3g09270	At4g08850	At3g50260	At5g40780	At5g27420
At2g28250	At1g49500	At3g10500	At4g10960	At3g50740	At5g41800	At5g15830
At2g15830	At1g64170	At3g06390	At4g11360	At3g50660	At5g41810	At5g16110
At2g15970	At1g48320	At3g06330	At4g12070	At3g50900	At5g42840	At1g58180
At2g46620	At1g53920	At3g07270	At4g12030	At3g50910	At5g43520	At5g19530
At2g18280	At1g53885	At3g10760	At4g12280	At3g50970	At5g43880	At1g58080
At2g42890	At1g64590	At3g07910	At4g13130	At3g52950	At5g44790	At1g58032
At1g61660	At1g76070	At3g02885	At4g13510	At3g52960	At5g45070	At1g47128
At1g27170	At1g18470	At3g22840	At4g17900	At3g54900	At5g45080	At1g30260
At1g60710	At1g50110	At3g15650	At4g18340	At3g55740	At5g45340	At1g44160
At1g61360	At1g01190	At3g21510	At4g18510	At3g56620	At5g46050	At1g25230
At1g22990	At1g26665	At3g23570	At4g20010	At3g57630	At5g47100	At4g17550
At2g17710	At1g26670	At3g21710	At4g21120	At3g58990	At5g48370	At4g15610
At1g05300	At1g12820	At3g21700	At4g21350	At3g60300	At5g50800	At4g17615
At1g10120	At1g63010	At3g18820	At4g22070	At3g60330	At5g51830	At2g41660
At1g67110	At1g21440	At3g12670	At4g24620	At3g61460	At5g52020	At2g38640
At1g61740	At1g43650	At3g20340	At4g24670	At3g61820	At5g54500	At1g27100
At1g78780	At1g19440	At3g26210	At4g26130	At3g61830	At5g55970	
At1g79320	At1g19450	At3g25780	At4g28240	At3g63260	At5g58900	
At2g35930	At1g53180	At3g25790	At4g31730	At5g01740	At5g59450	
At2g24550	At2g43500	At3g19710	At4g31910	At5g01750	At5g59930	
At1g31200	At1g74090	At3g14205	At4g34000	At5g02420	At5g61010	
111551200	1115/40/0	110517200	1101557000	1113502720	11.5501010	

D. <u>List of Genes Expressed Only in Shoots in Arabidopsis thaliana</u>

At2g30590	At1g10470	At2g41730	At3g28740	At4g26150	At5g17380	At3g51860
At2g39920	At1g03080	At1g52870	At1g55120	At4g31500	At5g18600	At5g13550
At2g29090	At2g16060	At1g36370	At2g30360	At4g34770	At5g37790	At1g35140
At2g29120	At1g21010	At1g75080	At4g03400	At4g35750	At5g40730	At4g14270
At2g19810	At1g68600	At1g71030	At4g04630	At4g38840	At5g47550	At4g15700
At2g01140	At1g77990	At1g63090	At4g08950	At3g45140	At5g49360	At1g69040
At2g24290	At1g56150	At1g13440	At4g09000	At3g45970	At5g50160	_
At2g05540	At1g22400	At3g03870	At4g11460	At3g60160	At5g56870	
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At2g28550	At1g21910	At3g26280	At4g21680	At5g07890	At5g59080	
At2g21210	At1g32450	At3g13110	At4g22470	At5g08570	At5g60300	
At2g42600	At2g47190	At3g28910	At4g23890	At5g12250	At5g64770	
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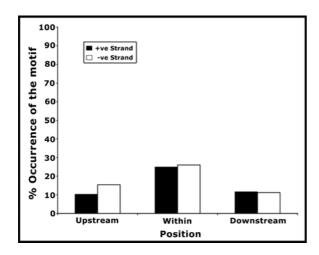
E. <u>List of Down-regulated genes in Arabidopsis thaliana</u>

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At2g33710	At1g54130	At1g64370	At4g08300	At3g55740	At5g45070	At5g24780
At2g33830	At2g16005	At1g71030	At4g08850	At3g56620	At5g45080	At1g58032
At2g44380	At2g17500	At1g20640	At4g10960	At3g57630	At5g46050	At1g47128
At2g38180	At1g65690	At1g19020	At4g11360	At3g60300	At5g47240	At1g44160
At2g32560	At1g14780	At1g02310	At4g12070	At3g60690	At5g48370	At1g28330
At2g40970	At1g14870	At1g02340	At4g12280	At3g61060	At5g49100	At1g25230
At2g39200	At1g21680	At3g03870	At4g13130	At3g61460	At5g49360	At4g14270
At2g39570	At1g64170	At3g01290	At4g13510	At3g61890	At5g50160	At4g14630
At2g29990	At1g48320	At3g09270	At4g13770	At3g62650	At5g53160	At4g17670
At2g44940	At1g53920	At3g10500	At4g17900	At5g01750	At5g54500	At4g15610
At2g19810	At1g53885	At3g06390	At4g19160	At5g02420	At5g55050	At4g17730
At2g07180	At1g74710	At3g06330	At4g19170	At5g02550	At5g55970	At4g17615
At2g43100	At1g56150	At3g03150	At4g19430	At5g02580	At5g56870	At1g67810
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At2g18690	At1g18470	At3g15630	At4g23700	At5g05160	At5g59050	
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At1g79320	At1g75080	At4g04630	At3g50260	At5g43520	At5g16110	
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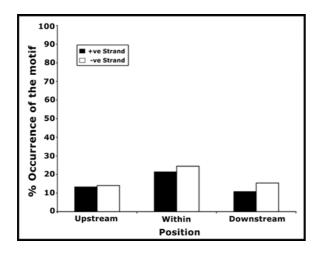
F. <u>List of Up-regulated Genes in Arabidopsis thaliana</u>

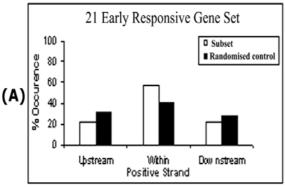
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At2g29120	At1g10470	At1g74440	At1g66200	At4g35260	At5g08570	At5g59930
At2g29730	At1g03080	At1g74660	At1g66140	At4g36040	At5g09800	At5g60300
At2g29700	At2g16060	At1g36370	At1g75140	At4g36020	At5g10030	At5g61420
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At2g43100	At1g59730	At1g67910	At1g22170	At4g37540	At5g10580	At5g62480
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At1g62660	At1g22500	At3g05400	At4g09620	At3g48360	At5g40730	At4g36540
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•	-	At3g17610	At4g21350	-	_	At1g44100
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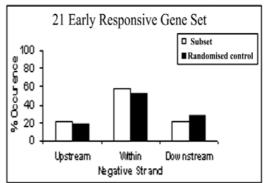
Supplementary Figure S1

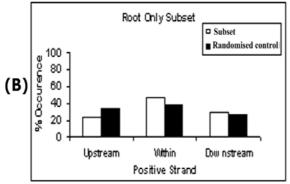


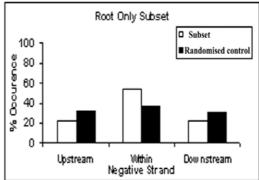
Supplementary Figure S2

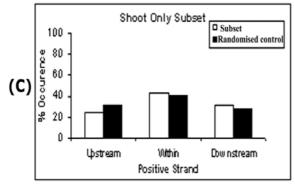


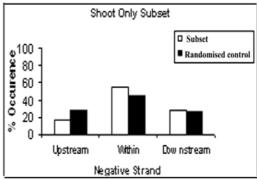


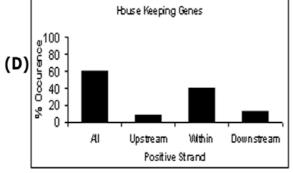


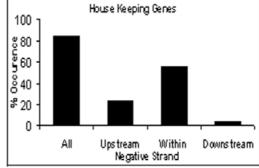




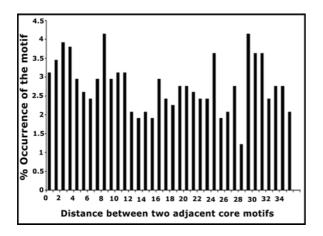




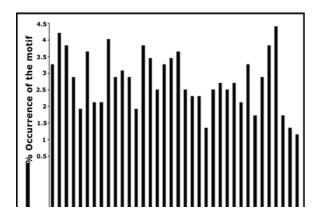




Supplementary Figure S4



Supplementary Figure S5



Supplementary Figure S6

