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Abstract: Effects of elevated CO2 (68 Pa versus ambient 38 Pa) on gene expression were studied in rice leaves grown in soil medium with three different nitrogen conditions (0, 0.6 and 1.2 g N per 8-L pot) in CO2 controlled chambers. Soluble protein contents were slightly decreased in leaves grown under elevated CO2 regardless of N supplies, whereas the polypeptide profiles of soluble protein analyzed by 2DE using the same amount of protein were totally unchanged between ambient and elevated CO2. In contrast, gene expressions examined by microarray analyses were significantly affected by elevated CO2. Forty-six up regulated genes (>1.5-fold) and thirty-five down regulated genes (<0.68-fold) were identified and these included many signal transduction and transcription regulation related genes. By contrast, the expressions of most of the genes for primary metabolism were not significantly altered. Although changes were small, the expressions of genes for enzymes involved in CO2 fixation (carbonic anhydrase, Rubisco, phosphoglycerate kinase and glyceraldehyde-3-phosphate dehydrogenase) were down regulated, whereas that of genes encoding enzymes for RuBP regeneration (fructose bisphosphate phosphatase, fructose

bisphosphate aldolase, sedoheptulose bisphosphate phosphatase and phosphoribulokinase) and starch synthesis (ADP-glucose pyrophosphorylase and starch synthase) were up regulated under elevated CO2. These results suggest that some sets of genes involved in primary metabolism pathway in the chloroplast are co-regulated by elevated CO2.

Rice plant response to long term CO_2 enrichment: Gene expression profiling

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Abbreviations: FACE, free air CO_2 enrichment ; 2DE, two dimensional gel electrophoresis; *RbcS*, Rubisco small subunit gene; Rubisco, ribulose-1,5-bisphosphate carboxylase/oxygenase; RuBP, ribulose-1,5-bisphosphate.

Key-words; elevated CO_2; photosynthesis; protein profiling; rice; transcript profiling.

ABSTRACT

Effects of elevated CO_2 (68 Pa versus ambient 38 Pa) on gene expression were studied in rice leaves grown in soil medium with three different nitrogen conditions (0, 0.6 and 1.2 g N per 8-L pot) in CO_2 controlled chambers. Soluble protein contents were slightly decreased in leaves grown under elevated CO_2 regardless of N supplies, whereas the polypeptide profiles of soluble protein analyzed by 2DE using the same amount of protein were totally unchanged between ambient and elevated CO_2. In contrast, gene expressions examined by microarray analyses were significantly affected by elevated CO_2. Forty-six up regulated genes (>1.5-fold) and thirty-five down regulated genes (<0.68-fold) were identified and these included many signal transduction and transcription regulation related genes. By contrast, the expressions of most of the genes for primary metabolism were not significantly altered. Although changes were small, the expressions of genes for enzymes involved in CO_2 fixation (carbonic anhydrase, Rubisco, phosphoglycerate kinase and glyceraldehyde-3-phosphate dehydrogenase) were down regulated, whereas that of genes encoding enzymes for RuBP regeneration (fructose bisphosphate phosphatase, fructose bisphosphate aldolase, sedoheptulose bisphosphate phosphatase and phosphoribulokinase) and starch synthesis (ADP-glucose pyrophosphorylase and starch synthase) were up regulated under elevated CO_2. These results suggest that some sets of genes involved in primary metabolism pathway in the chloroplast are co-regulated by elevated CO_2.

1. Introduction

Global atmospheric CO_2 assume increase to 55 Pa by the end of this century [1]. This could expect to significant beneficial effects on plants by the enhancement of CO_2 assimilation. For this reason, the physiological and morphological impacts of elevated CO_2 have been extensively studied in various plant species [2, 3]. Recently, FACE (free air CO_2 enrichment) facility has been developed all over the world and the effects of elevated CO_2 on plant growth are investigated at the managed field or natural ecosystem levels [2, 4].

Photosynthesis is generally enhanced by elevated CO_2 and this also stimulates the growth and productivity in most C_3 plants [5]. However, prolonged exposure of elevated CO_2 causes down regulation of photosynthesis typically showing reduction of Rubisco contents and light saturated CO_2 assimilation rate [5]. A high emphasis has been placed on the down regulation because this significantly decreases the stimulation of final plant productivity by elevated CO_2 [6]. In addition to this down regulation, plants also show some morphological changes under elevated CO_2. In general, plants became taller with larger stem diameter, increased branching and leaf number [5, 7]. In rice, significant increase in biomass of leaf sheath was observed under elevated CO_2 where transiently accumulate fixed carbon as starch [8]. This could be an acclimation response to elevated CO_2 by changing the morphological characteristics in plants.

The response of plants to elevated CO_2 can relate to various signaling factors. An increase in the levels of soluble sugars in the cell often observed in the leaves under elevated CO_2, can influence the expression of sugar responsive genes and hexokinase 1 is suggested to be a sensor of soluble sugars [9]. In addition, the redox state in chloroplast can be an another signal of sugar responsive genes[10]. Enhancement of photosynthesis under elevated CO_2 may led to the decrease in Pi level in leaves [11] and PHR1 and SIZ1 can take part in the regulation of expression of Pi responsive genes [12, 13]. Decrease in nitrogen content has been often observed in leaves under elevated CO_2 [5, 6]. Dof transcription factors can regulate the genes related to nitrogen assimilation [14] and it may play some role in this phenomenon. In this manner, many signal transduction and transcription regulation related factors were expected to involve in CO_2 response and in addition, it is possible that these could

interact with each other. However, the actual contributions of these signaling factors to CO_2 response in plants are still uncertain. In addition, these signaling factors including even most extensively characterized hexokinase have only reported in limited plant species [9-15]. These imply that there remain many questions left unanswered about the signaling mechanism of CO_2 response in plants.

In order to understand the molecular bases of CO_2 response, gene expression profiling using microarray were carried out in some plant species [16-20]. However, these profiles were diverse in response of genes related to metabolism and also selected signature genes. These suggest that response in gene expression to elevated CO_2 would significantly differ in plant species and seems difficult to find common features. In this study, we use rice, one of the most important crops for agriculture as a material and genome information and useful tools for molecular biology are well developed in this plant. Changes in gene expression due to exposure to elevated CO_2 were studied at protein level by two dimensional gel electrophoresis (2DE) and transcript level by microarray including 44,000 oligo DNA.

2. Materials and Methods

2.1. Plant materials and growth conditions

Rice (*Oryza sativa* L. spp. Japonica cv. Nipponbare) plants were grown in naturally illuminated semi-closed growth chambers [21] at two different CO_2 levels (38 and 68 Pa as ambient and elevated CO_2, respectively). Three seedlings were transplanted in 8-L plastic pots filled with inceptisols (this soil originally contained approximately 2 mg g^{-1} N) with three different nitrogen supplies (0, 0.6 and 1.2 g per pot as low, medium and high N, respectively) applied as coated urea (LP-70, Chisso Asahi Fertilizer Co. Ltd., Tokyo, Japan). P_2O_5 and K_2O were applied 1.0 g per pot as basal dressing. The CO_2 treatment was started just after transplanting. Four chambers were used for the study, two being assigned to elevated CO_2 and the other two assigned to ambient CO_2. In each chamber, we also rotated the pots once per week to minimize any effects of microclimatic variation within the chambers. We also rotated the pots among the four chambers and then re-established the treatment conditions every 3

weeks during the treatment period so as to minimize the impact of any variations among the chambers. All the chambers were maintained at 32/22°C (day/night) and 70% relative humidity. At 38 days after transplanting, the segments of about 2 cm for protein analysis and 20 cm for transcript analysis were harvested from the mid-section of the uppermost fully expanded 11th leaves (the flag leaf was 14th leaf in this study) at 11:00-12:00 and immediately frozen in liquid nitrogen. The samples were stored at -80°C until use.

2.2 Extraction of leaf soluble protein and 2DE

Total leaf soluble proteins were extracted as described previously [22]. Samples were homogenized using a chilled mortar and pestle in an extraction buffer containing 50 mM HEPES-KOH (pH 7.4), 10 mM $MgCl_2$, 1 mM EDTA, 5 mM DTT, 1 mM phenylmethylsulfonyl fluoride, 10 μM leupeptin, 5% polyvinylpolypyrrolidone and 10% (w/v) glycerol, with a small amount of sea sand. After total maceration, the homogenate was centrifuged at 15,000×g for 5 min and resultant supernatant was collected as a total leaf soluble protein extract. 2DE was carried out according to the method of Tsuchida et al. [23]. The total soluble proteins of 30 μg were mixed with 4% CHAPS, 7M urea, 2M thiourea, 50 mM dithiothreitol, 0.002% bromophenol blue and 2% IPG buffer (GE Healthcare, Piscataway, NJ, USA). The proteins were first separated by IEF using Immobiline DryStrip (pI 4-7) and Multiphor II Electrophoresis Units (GE Healthcare), then by 12% SDS-PAGE, and stained with silver.

The soluble protein concentration was measured according to Bradford [24] using bovine serum albumin as a standard.

2.3. RNA extraction and Microarray analysis

Total RNAs were isolated from leaves of four different plants grown in different pots for each treatment using the RNeasy plant mini kit (Qiagen, Carlsberg, CA, USA). Two sets of mixed total RNAs of two different plants (400 ng) were used for analysis. According to the manufacturer's

instruction, the RNAs were labeled by Cyanine-3 or Cyanine-5 dye with the Low RNA Input Linear-Amplification/Labeling Kit (Agilent Technologies, Santa Clara, CA, USA) and the labeled cRNAs were purified using the RNeasy plant mini kit. After purification, Cyanine-3 and Cyanine-5 labeled cRNAs were hybridized with 44k Rice Oligo Microarray slides (Agilent Technologies). After hybridization, the signals were detected by DNA microarray scanner (Agilent Technologies), and spot intensities were digitalized using Feature Extraction software (Agilent Technologies). Difference in the expression of transcripts between ambient and elevated CO_2 were compared at each N levels. To separate the effects of nitrogen supply to CO_2 partial pressure, the comparison of low and medium N was also carried out. In total, four comparative analyses were done with two biological replicates and two technical replicates by dye swap.

Statistical data mining was performed to identify genes differentially expressed between treatments. Variances were normalized between dye swap samples, and then genes with Cyanine-5 signal value greater than 100 were selected. The mean and standard deviation were calculated for each gene and the data were adopted for Z transformation to calculate Z scores to estimate significant difference (P<0.05) for each gene expression.

2.4. Semi-quantitative RT-PCR

RT-PCR was performed essentially as described by Fukayama et al. [25] using gene specific primers listed in Table 1. The 1st strand cDNA was synthesized from 5 μg of total RNA with oligo (dT)18 as the primer. AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster City, CA, USA) was first activated at 95 °C for 9 min, and PCR was carried out for 20–25 cycles of 15 sec at 95 °C, 30 sec at 60 °C and 60 sec at 72 °C, followed by a final extension step for 7 min at 72 °C. Expression of the rice actin gene (RAc1, **AB047313**) was examined as an internal control.

3. Results

3.1. Plant growth under elevated CO_2

The growth of rice under elevated CO_2 was quite similar to that under ambient CO_2 (Supporting Information Fig. S1). The leaves for analysis of 2DE and microarray were sampled at 38 days after transplanting. Until then, the plant length and leaf number of main stem had increased at almost same rate in ambient and elevated CO_2. Only the tiller number was enhanced by elevated CO_2 in medium and high N from 23 to 36 days after transplanting.

3.2. Analysis of protein profiles

Before analysis of 2DE and microarray, the content of total soluble protein was determined in different portions of the same leaves used for these analyses. Although the differences were not statistically significant, the soluble protein contents showed a tendency to decrease by elevated CO_2 and these were approximately 10% decline in all three N levels (Fig. 1). The CO_2 assimilation rate at same ambient CO_2 levels were also lower in plants grown at elevated CO_2 than that in control plants regardless of N supply levels (data not shown). These results confirm the occurrence of some physiological changes including the reduction of photosynthesis by elevated CO_2 as reported by Sakai et al. [21].

2DE was carried out to compare the expression pattern of soluble proteins and the typical results in leaves of plants grown with medium N were shown (Fig. 2). Most of major spots detected were considered to be the enzymes related to metabolism [23]. The intensities of representative spots related to the primary metabolism identified by previous study [23] as well as overall protein composition in elevated CO_2 were quite similar to that in ambient CO_2. Thus it was considered that the protein profiles were largely unchanged between control and elevated CO_2.

3.3. Transcript Profiles

Microarray analyses were performed to evaluate the difference in the expression of transcripts between ambient CO_2 and elevated CO_2. There were 115 differentially expressed genes ($P<0.05$) in all

three N levels by elevated CO_2 (Supporting Information Tables S1). Among them, the geometrical means calculated from low N, medium N and high N above 1.5-fold were listed as up-regulated genes in Table 1 and below 0.68-fold as down-regulated genes in Table 2. There were forty-six and thirty-five significantly up-regulated and down-regulated genes, respectively. Among up-regulated genes, the most abundant functional groups were genes related to signal transduction and transcriptional regulation and twenty-one genes were belonged to this category. It is noteworthy that seven protein kinase domain containing protein were observed in the up-regulated genes. Typical transcription factors such as MADS-box, WAKY, bHLH and Zn-finger, RING domain containing protein were also found. In the category of metabolism, two genes were participated in transport such as amino acid/polyamine and monosaccharide. Only one gene, phospho*enol*pyruvate carboxykinase were belonged to primary metabolism. Four genes including E-class P450 and Germin family protein were found in the category of stress related and two genes, senescence associated protein and legume lectin like domain containing protein were found in the category of cell growth and structural protein. In addition to these genes, twelve up-regulated genes were classified to unclear classification/unknown function and some of them were only similar to hypothetical proteins or no hit by BLAST.

In contrast to the up-regulated genes, relatively large number, twelve genes were classified as metabolism such as glycoside hydrolase, serine acetyltransferase, tyrosine decarboxylase and tryptophan synthase in the list of down-regulated genes (Table 3). Although only one protein kinase domain containing protein was found, there were many signal related genes including CBL-interacting protein kinase, synaptotagmin C and CONSTANS-like protein. In the category of stress related, there were six down-regulated genes and P450 family proteins were also found in the same as up-regulated genes. Two genes were classified as cell growth and structure and six genes were unclear classification or unknown function.

Difference in N supply significantly affected the transcript profile. The response of up-regulated genes such as protein kinase domain containing protein (**CB660294**) and Germin family protein were more enhanced in high N. In contrast, the expression of down-regulated genes such as Kinesin 4 and Viviparous-14 were more reduced under elevated CO_2 in low N.

In our results of microarray analysis, the number of genes its expressions were markedly affected by

elevated CO_2 were small in the category of primary metabolism. To investigate the effect of elevated CO_2 on the gene expression related to the primary metabolism, genes belonged to CO_2 fixation, RuBP regeneration, photorespiration, starch synthesis, sucrose synthesis, glycolysis, TCA cycle, N fixation and amino acid synthesis were selected and compared the expressions respond to elevated CO_2 (Supporting Information Table 2S). Although some of the genes, for instance, phosphoenolpyruvate carboxykinase and glutamine synthase were up-regulated, and pyruvate kinase and nitrate reductase were down regulated, the expressions of most of the genes related to photorespiration, sucrose synthesis, glycolysis, TCA cycle, N fixation and amino acid synthesis were mostly unaltered or differentially regulated among the pathway by elevated CO_2. Among the pathway of primary metabolism, the genes related to CO_2 fixation including chloroplastic carbonic anhydrase (CA), Rubisco small subunit (RbcS), phosphoglycerate kinase (PGK) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) tended to be down-regulated by elevated CO_2 (Fig. 3). However, the gene encoding Rubisco activase (Rca) was exceptionally up-regulated in this pathway. On the other hand, the genes related to RuBP regeneration pathway such as fructose bisphosphatase (FBPase), fructose bisphosphate aldolase (FBP aldolase), sedoheptulose bisphosphatase (SBPase) and phosphoribulokinase (PRK) were largely up-regulated by elevated CO_2. In starch synthesis pathway, the genes encoding ADP-glucose pyrophosphorylase (AGPaseS and L) and starch synthase (SS and GB-SS) also tended to be up-regulated by elevated CO_2. The response of some genes such as RbcS, GAPDH and FBPase were more marked in low N compared to high N. The results obtained by transcript profiling of primary metabolism suggest that the genes belonged to the same pathway of carbon metabolism in the chloroplast are co-regulated to elevated CO_2.and the responses of genes are partially enhanced by the limitation of N supply.

3.4. Validation of microarray data by RT-PCR

Semi-quantitative RT-PCR was conducted to verify microarray results (Fig. 4). In the category of signal transduction and transcriptional regulation, protein kinase domain containing protein and TPR-like domain containing protein as significantly up-regulated genes, and CBL-interacting protein

kinase 1 and synaptotagmin C as significantly down-regulated genes were selected for analysis. In addition to these genes, three genes, *Rbc*S, FBPase and AGPaseL from Fig. 3, participated in primary metabolism, were also analyzed. The band intensities of protein kinase domain containing protein, TPR-like domain containing protein, FBPase and AGPase were relatively higher in elevated CO_2 than that in ambient CO_2. In contrast, the band intensities of genes that showed down-regulation under elevated CO_2 by microarray were relatively lower in elevated CO_2 than that in ambient CO_2. These tendencies became obscure in high nitrogen treatment in some genes such as RbcS and AGPase. Although the differences in band intensities between ambient and elevated CO_2 were not always apparent, the results of RT-PCR were largely agreed with that of microarray analysis.

4. Discussion

Under elevated CO_2 condition, down-regulation of photosynthesis was observed in many plant species [5], whereas some plants such as radish did not show the down-regulation [26]. In rice, the reduction in photosynthetic rate under elevated CO_2 has been repeatedly reported using different facilities such as growth chamber [27] and FACE [28]. These imply that down-regulation of photosynthesis by elevated CO_2 reproducibly occurs in rice. In this study, a slight reduction in total soluble protein was observed in leaves of elevated CO_2 (Fig. 1). In general, the effect of CO_2 on physiology of plants was pronounced at later growth stage after heading in rice [21]. However, the response of transcript expression essentially preceded the changes in protein [29]. Although our results of total soluble protein did not show marked difference, it was considered to be a proper stage for analysis of both protein and transcript profiles.

Elevated CO_2 can influence the levels of some particular proteins, whereas overall protein profiles of soluble protein did not significantly alter by CO_2 treatment in this study (Fig. 2). Many studies have given much attention to the levels of Rubisco because the enzyme limits the photosynthetic CO_2 assimilation rate at current CO_2 level, but it considered to be excess under elevated CO_2. The reduction of Rubisco under elevated CO_2 was observed in many plant species including rice [2, 27, 30]. However, in rice, Rubisco contents were well correlated with total N contents regardless of CO_2

treatments [27]. In addition, both CO_2 limited and CO_2 saturated photosynthesis were decreased under elevated CO_2 in rice [28]. These findings suggest that the amounts of photosynthetic proteins other than Rubisco also decreased to similar extent at given total N under elevated CO_2. In contrast, the sucrose synthesis become an important factor determining the photosynthetic rate under elevated CO_2, and it was reported that the levels of a key enzyme of sucrose synthesis, sucrose phosphate synthase (SPS) increased under elevated CO_2 in rice [28, 31]. Considering these reports, it seems likely that changes in some protein levels could occur under elevated CO_2, whereas the expression levels relative to total soluble protein are not significantly affected in most of down-regulated proteins (as 2DE was carried out at total soluble protein base in this study) or the extent of change is small not to detect by 2DE in most of up-regulated proteins such as SPS. Also, it should be possible that detection of more low abundant proteins by improvement of detection sensitivity could identify differentially expressed protein such as signal transduction and transcription factors.

Although the protein composition largely unchanged, a large number of differentially expressed genes at transcript level were observed under elevated CO_2 (Table S2, 2 and 3). In the category of metabolism, phosphoenolpyruvate carboxykinase which related to glycolysis or glyconeogenesis was up-regulated by elevated CO_2. It was reported that elevated CO_2 increased the transcript levels of genes encoding enzymes of glycolysis and TCA cycle in soybean [16] and Arabidopsis [18]. Our results partly support these reports and suggested that the energy demand would increase to support enhanced photosynthesis and concomitant stimulation of growth under elevated CO_2. Large numbers of differentially expressed genes related to signal transduction/transcription regulation were found (Table 2 and 3), whereas the physiological role of these genes are mostly unknown. Among them, a CBL-interacting protein kinase was known to be regulated by metabolic sugar in Arabidopsis [32]. Protein kinase domain containing proteins are considered to be mediators of responses to diverse endogenous and environmental cues. In addition, two key enzymes of sucrose metabolism, sucrose phosphate synthase and sucrose synthase are regulated by phosphorylation [33, 34]. It is possible that these protein kinase domain containing proteins could function in the regulation of metabolism other than signal transduction. Elevated CO_2 can influence various signaling factors such as metabolic sugar, Pi and nitrogen status. However, genes already known to function in these signal transduction such as

hexokinases [9], PHR [13] and Dofs [14] did not significantly respond to elevated CO_2 in rice (Table S1, 2 and 3). It is likely that the expressions of many genes are regulated at the post-transcriptional levels. However, it is also possible that the genes regulated by transcriptional levels found in this study could more probably relate to signal transduction pathway in CO_2 response.

Although down-regulation of *Rbc*S by elevated CO_2 has been repeatedly reported [35-38], the information about the expression of other photosynthetic genes is still limited. In FACE, gene expression analyses using microarray have been reported [16-20], whereas the results obtained these studies were diverse and could not reach some definitive conclusions over the plants. These observations suggest that the response of gene expression differ with plant species and in FACE, various combined stress affect the results of gene expression. Actually, large changes in gene expression were observed in the comparison between growth chamber and field condition in *Arabidopsis* [19]. In addition, the developmental status significantly affects the profile of transcripts [17]. Particularly in tree species, it seems difficult to match the developmental status between treatments. Rice is easy to grow in uniform and the rates of leaf expansions are similar between ambient and elevated CO_2 (Fig. S1). Thus, the developmental status of leaves used in this study was quite similar between treatments. In this study, down-regulation of genes encoding primary CO_2 fixation and up-regulation of genes encoding RuBP regeneration and starch synthesis pathway were found (Fig. 3). Importantly, these results indicate that some sets of genes involved in these pathways respond similar way to elevated CO_2. These observations considered to be a reasonable response under elevated CO_2, because CO_2 fixation step such as Rubisco and carbonic anhydrase no longer restrict the photosynthetic rate under elevated CO_2, and RuBP regeneration capacity limits it. Increase in starch content is well characterized phenomena under elevated CO_2 [5], so it is also reasonable to increase gene expressions related to starch synthesis. These changes in gene expressions observed in the primary metabolism may reflect an acclimation response to utilize N more efficiently under elevated CO_2 where N availability in leaves is reduced. Interestingly, expression of gene for Rubisco activase shows opposite response to the other member of primary CO_2 fixation pathway. Antisense reduction of Rubisco activase led to increase in Rubisco contents [39, 40]. These reports suggest that some compensating regulations to photosynthesis are present between Rubisco and Rubisco activase.

Decrease in Rubisco activation by elevated CO_2 has been reported in many C_3 plants [30, 41, 42]. However, these decreases in Rubisco activation was not observed in rice [27]. Up-regulation of Rubisco activase might function in maintenance of the activation state of Rubisco under elevated CO_2 and it could be a response peculiar to rice.

The results in this study suggest that rice can response to acclimate to elevated CO_2 environment at the transcript levels, whereas these responses are largely not reflected to the protein levels. Exact mechanism of this inconsistency remained to be determined. Plants never speak, but the data of microarray analysis seem to tell us what rice wants to do under elevated CO_2. Information about gene expressions obtained in this study can provide a clue to improve photosynthesis and plant productivity in the future.

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Table 1. Primers used for RT-PCR.

Primer name	Primer sequence
PK-F	5’-TCACCGGGATGTGAAGACTA-3’
PK-R	5’-ATGAACGATGCACCATGGGC-3’
TPR-F	5’-CCGTTCATGGGGGCAGCTCA-3’
TPR-R	5’-GGCTTATTTATTTCACAAGCGT-3’
CBL-PK-F	5’-CCGAACCCGATGAAGAGGAT-3’
CBL-PK-R	5’-ATGATCCAGGGCGGCAATTC-3’
SynaC-F	5’-TGGACGTCGTGAACAACGGA-3’
SynaC-R	5’-GCGCCCAAAATGGATCACGA-3’
RbcS-F	5’-GCTTCGGCAACGTCAGCAAT-3’
RbcS-R	5’-CACACGAAACAAGGTGGGAG-3’
FBPase-F	5’-AGGACGTGTTACAGCCTGGA-3’
FBPase-R	5’-CACGCCTTGATGGTGCCAGA-3’
AGPase-F	5’-GTCCTGCATTTCTGAAGGCG-3’
AGPase-R	5’-CCGATGCCCCATCACATATT-3’
RAc1-F	5’-GCAACTGGGATGATATGGAGAA-3’
RAc1-R	5’-CCTCCAATCCAGACACTGTA-3’

Table 2. Significantly up-regulated genes under elevated CO_2.Rice plants were grown in low (LN), medium (MN) and high (HN) N supply with different CO_2 partial pressures (38 Pa or 68 Pa). Up-regulated genes by elevated CO_2 (68 Pa) in average of LN, MN and HN above 1.50 were indicated. Gene expression difference of MN relative to LN under ambient CO_2 (LN/MN) were also shown for comparison. Difference of gene expressions of LN, MN and HN in this list are all significant (P<0.05) by Z-Score method. [1*]Locus ID of Rice Annotation Project Database RAP-DB (http://rapdb.dna.affrc.go.jp/) was shown for genes without INSD accession number.

Description	Accession No.[1*]	Expression level (fold)				
		LN	MN	HN	LN/MN	Av.
Metabolism						
Amino acid/polyamine transporter II	AK102220	1.64	1.46	2.06	1.22	1.70
Thioredoxin family protein	AK069195	1.59	1.87	1.66	1.23	1.70
Phospholipid transfer protein	AF017359	1.66	1.57	1.85	0.98	1.69
Lipid transfer protein LPT II	AK058921	1.65	1.58	1.83	0.97	1.68
Phosphoenolpyruvate carboxykinase	AK102392	1.62	1.73	1.54	0.87	1.63
Monosaccharide transporter 1	AK108820	1.58	1.80	1.51	1.08	1.62
Cell growth/structure						
Senescence-associated protein 5	AK108529	2.09	1.76	2.34	0.79	2.05
Legume lectin, domain containing protein	AK108625	1.86	1.34	1.54	0.85	1.57
Signal transduction/Transcription						
Protein kinase domain containing protein	CB660294	3.64	4.69	7.98	1.26	5.14
Protein kinase domain containing protein	AK120189	2.56	2.99	8.04	0.87	3.95
Protein kinase domain containing protein	CI322564	1.87	2.24	3.68	1.14	2.49
MADS-box domain containing protein	AF058697	1.92	2.38	2.85	1.21	2.35
Cys-rich domain containing protein	Os03g0830200	2.26	1.68	2.55	0.91	2.13
LysM domain containing protein	Os06g0625300	1.64	1.95	2.48	1.03	2.00
Protein kinase domain containing protein	CI436252	1.69	1.64	2.09	1.04	1.80
Nf-Y-A subunit	AK106398	1.62	1.77	1.96	0.99	1.78
Protein kinase domain containing protein	AK111655	1.70	1.56	2.00	0.98	1.74
WRKY transcription factor 60	AU057193	1.46	1.65	1.93	1.40	1.67
bHLH domain containing protein	Os03g0728900	1.60	1.53	1.86	1.09	1.66
PEBP family protein	AB062676	1.56	1.69	1.64	1.13	1.63
bHLH domain containing protein	CI333262	1.40	1.73	1.72	0.87	1.61
Protein kinase domain containing protein	Os03g0678100	1.46	1.64	1.68	1.17	1.59
Zn-finger, RING domain containing protein	AK108566	1.59	1.38	1.67	1.08	1.54
Protein kinase domain containing protein	AK067467	1.50	1.46	1.66	1.20	1.54
No apical meristem protein	AK107090	1.38	1.50	1.74	1.21	1.53
Y/CCAAT-box binding factor A subunit	AK071595	1.42	1.46	1.72	1.00	1.53
TPR-like domain containing protein	AU071153	1.74	1.36	1.47	1.41	1.52
Protein phosphatase 2C-like protein	AK072292	1.45	1.45	1.66	1.33	1.52
Transcriptional activator HAP2	AK073742	1.43	1.48	1.63	1.06	1.51
Stress						
Germin family protein	AK059812	2.86	3.14	5.08	0.90	3.57
HcrVf2 protein	AK065753	1.36	1.96	2.68	1.08	1.93
E-class P450, group I family protein	CI560567	2.18	1.59	1.56	1.03	1.76
Heavy metal transport/detoxification protein	AJ308374	1.77	1.35	1.55	1.14	1.55
Unclear classification/unknown function						
No Hit	CI543502	2.37	2.13	4.16	1.14	2.76
Conserved hypothetical protein	AK111796	3.50	1.70	2.98	0.75	2.60
PGPS/D12	CI422497	2.76	1.84	2.85	0.86	2.44
Conserved hypothetical protein	AK063689	1.69	2.36	3.33	0.94	2.37
Hypothetical protein	AK069662	2.36	1.98	2.22	0.87	2.18
Unknown function 3588 containing protein	CI557027	1.82	1.53	1.89	1.13	1.74
Conserved hypothetical protein	AK064901	1.47	1.68	1.89	0.86	1.67
No Hit	CI441424	1.59	1.55	1.90	0.87	1.67
Conserved hypothetical protein	AK070327	1.48	1.89	1.58	0.79	1.64
DUF581 family protein	AK071528	1.64	1.53	1.73	0.86	1.63
Conserved hypothetical protein	AK060241	1.68	1.40	1.83	0.76	1.63
Hypothetical protein	AK071106	1.58	1.42	1.82	1.23	1.60
DUF966 family protein	AK119662	1.58	1.33	1.90	1.27	1.58

Table 3. Significantly down-regulated genes under elevated CO_2. Rice plants were grown in low (LN), medium (MN) and high (HN) N supply with different CO_2 partial pressures (38 Pa or 68 Pa). Down-regulated genes by elevated CO_2 (68 Pa) in average of LN, MN and HN below 0.68 were indicated. Gene expression difference of MN relative to LN under ambient CO_2 (LN/MN) were also shown for comparison. Difference of gene expressions of LN, MN and HN in this list are all significant (P<0.05) by Z-Score method. [1*]Locus ID of Rice Annotation Project Database RAP-DB (http://rapdb.dna.affrc.go.jp/) was shown for genes without INSD accession number.

Description	Accession No.	Expression difference (fold)				
		LN	MN	HN	LN/MN	Av.
Metabolism						
Glycoside hydrolase, family 18	AK065866	0.39	0.55	0.43	0.95	0.46
Serine acetyltransferase	AU166464	0.44	0.52	0.45	0.80	0.47
Serine acetyltransferase	Os03g0185000	0.48	0.56	0.50	0.80	0.51
Tyrosine decarboxylase 1	Os10g0400500	0.64	0.56	0.35	1.20	0.51
Sodium-dicarboxylate cotransporter-like	AK105756	0.60	0.50	0.46	0.72	0.52
FAD linked oxidase	AK103272	0.58	0.47	0.61	0.87	0.55
Hydrolase fold domain containing protein.	AK066814	0.48	0.60	0.59	1.32	0.55
Alpha-xylosidase	AK063966	0.46	0.63	0.65	0.96	0.58
Multicopper oxidase, type 1 family protein	CI452025	0.74	0.48	0.63	0.90	0.61
2OG-Fe(II) oxygenase like protein	AK067970	0.59	0.63	0.71	1.06	0.64
Tryptophan synthase, alpha chain	AK072595	0.66	0.72	0.61	0.86	0.66
Glycoside hydrolase, family 5 protein	AK063757	0.59	0.73	0.68	0.89	0.67
Cell growth/structure						
Kinesin 4	AK065848	0.40	0.62	0.61	0.98	0.54
Beta-tubulin	AK122099	0.46	0.67	0.51	0.81	0.55
Signal transduction/Transcription						
Viviparous-14	AK107649	0.35	0.44	0.58	1.30	0.45
Two-component response regulator ARR1	Os12g0586300	0.51	0.48	0.50	0.85	0.50
MAP65/ASE1 family protein	AK108923	0.47	0.36	0.68	0.92	0.51
Synaptotagmin C	AU057097	0.37	0.63	0.53	0.76	0.51
CBL-interacting protein kinase 1	AK061640	0.55	0.57	0.44	0.81	0.52
Protein kinase domain containing protein	AK107168	0.63	0.59	0.54	0.83	0.59
Myb, like domain containing protein	AK070421	0.63	0.59	0.68	0.74	0.63
EF-hand domain containing protein	AK066756	0.60	0.74	0.57	0.92	0.64
CONSTANS-like protein CO6	AK109630	0.72	0.67	0.58	0.84	0.65
Stress						
Cytochrome P450 family protein	AK064764	0.29	0.22	0.37	1.00	0.29
E-class P450, group I family protein	AK120757	0.42	0.45	0.42	0.78	0.43
Osmotin-like protein	AK060655	0.57	0.42	0.36	1.27	0.45
Bowman-Birk type trypsin inhibitor	AK120562	0.39	0.56	0.54	0.74	0.50
Heat shock protein DnaJ family protein	AK107961	0.54	0.66	0.59	1.04	0.59
Allergen V5/Tpx-1 related family protein	AK060409	0.55	0.65	0.66	1.35	0.62
Unclear classification/unknown function						
Conserved hypothetical protein	Os06g0554200	0.71	0.50	0.44	0.85	0.55
Hypothetical protein	AK108390	0.71	0.52	0.59	0.73	0.61
Conserved hypothetical protein.	AK101548	0.61	0.60	0.69	0.75	0.63
Conserved hypothetical protein	AK062656	0.73	0.56	0.62	1.01	0.64
Conserved hypothetical protein	AY224438	0.63	0.66	0.64	1.34	0.64
Conserved hypothetical protein	AK109132	0.69	0.62	0.66	0.78	0.66

Figure(s)

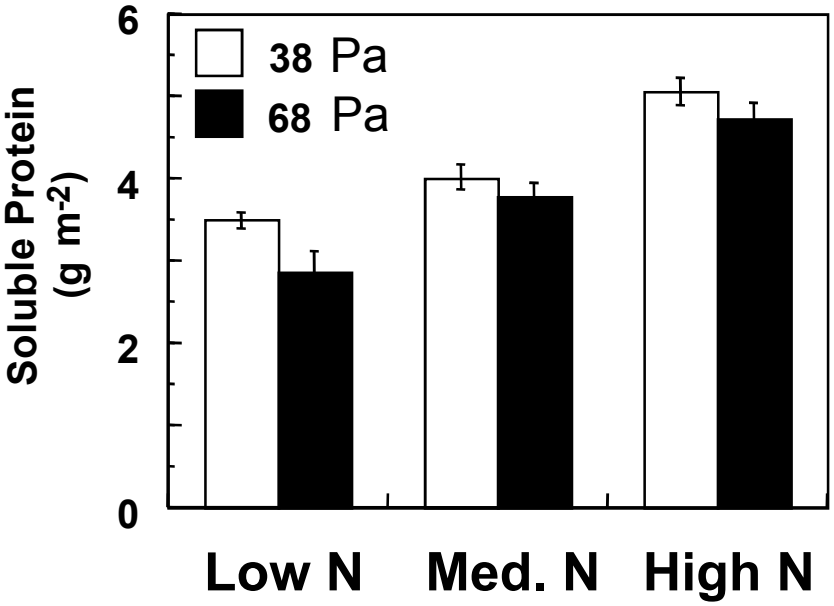

Fig. 1. Soluble protein content in the leaves. Rice plants were grown in low, middle and high N supply with ambient (38 Pa) and elevated (68 Pa) CO_2 partial pressures. The uppermost fully expanded 11th leaves were used. The levels of soluble protein are presented as mean ± SE obtained from five to eight independent measurements.

Figure(s)

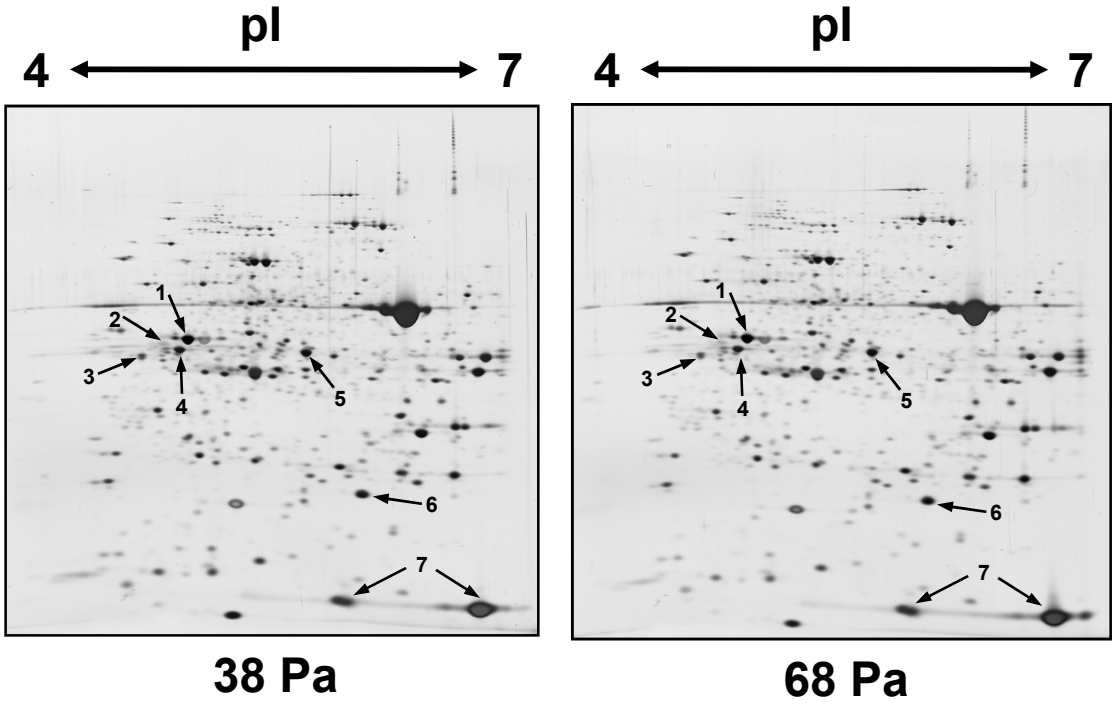

Fig. 2. Profiles of 2DE of soluble protein in leaves of rice grown under different CO_2 partial pressures. The representative data grown in ambient (38 Pa) or elevated (68 Pa) CO_2 with medium N supply are shown. Thirty μg protein was used for analysis. Proteins in the gels were stained with silver. Arrows indicate following representative proteins [23]: 1, phosphoglycerate kinase; 2, Rubisco activase small isoform; 3, sedoheptulose-1,7-bisphosphate phosphatase; 4, phosphoribulokinase; 5, glyceraldehyde 3-phosphate dehydrogenase; 6, carbonic anhydrase; 7, Rubisco small subunits.

Figure(s)

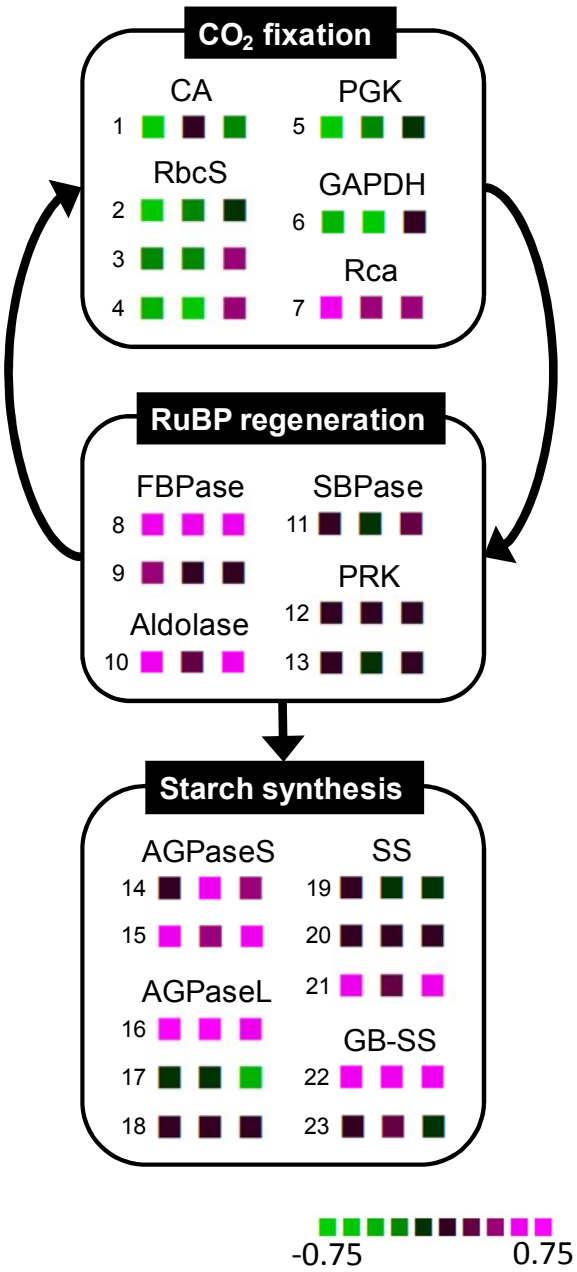

Fig. 3. Graphical representation of the expression of representative genes related to CO_2 fixation, RuBP regeneration and starch synthesis pathways in response to elevated CO_2. The color of dot indicates the degree of difference in gene expression at elevated CO_2 relative to ambient CO_2. Shades of pink and light green indicate up-regulation and down-regulation, respectively. The scale shows $\log_2$ at -0.75 to 0.75. Left, center and right dots indicate the results of low, medium and high N, respectively. Numbers in the panel indicate individual genes encoding following enzymes: 1-**AK060890**, carbonic anhydrase (CA); 2-**AK121444**, 3-**AK058875**, 4-**AK070257**, Rubisco small subunit (RbcS); 5-**AK062214**, phosphoglycerate kinase (PGK); 6-**AK067755**, glyceraldehyde-3-phosphate dehydrogenase (GAPDH); 7-**AK119513**, Rubisco activase (Rca); 8-**AK119536**, 9-**AK065201**, fructose bisphosphate phosphatase (FBPase); 10-**AF017362**, fructose bisphosphate aldolase (aldolase); 11-**AK062081**, sedoheptuloase bisphosphate phosphatase (SBPase); 12-**AK062081**, 13-**AK102236**, phosphoribulokinase (PRK); 14-**AK071826**, 15-**AK060270**, ADP-glucose pyrophosphorylase small subunit (AGPaseS); 16-**AK100910**, 17-**AK069296**, 18-**AK099643**, ADP-glucose pyrophosphorylase large subunit (AGPaseL); 19-**AF395537**, 20-**AK122098**, 21-**AY299404**, starch syntase (SS); 22-**AY069940**, 23-**AF383878**, granule-bound starch synthase (GB-SS). In the case of enzymes encoded by gene family, genes significantly expressed in the leaf blade analyzed by microarray (Miyao *et al*. unpublished data) were chosen.

Figure(s)

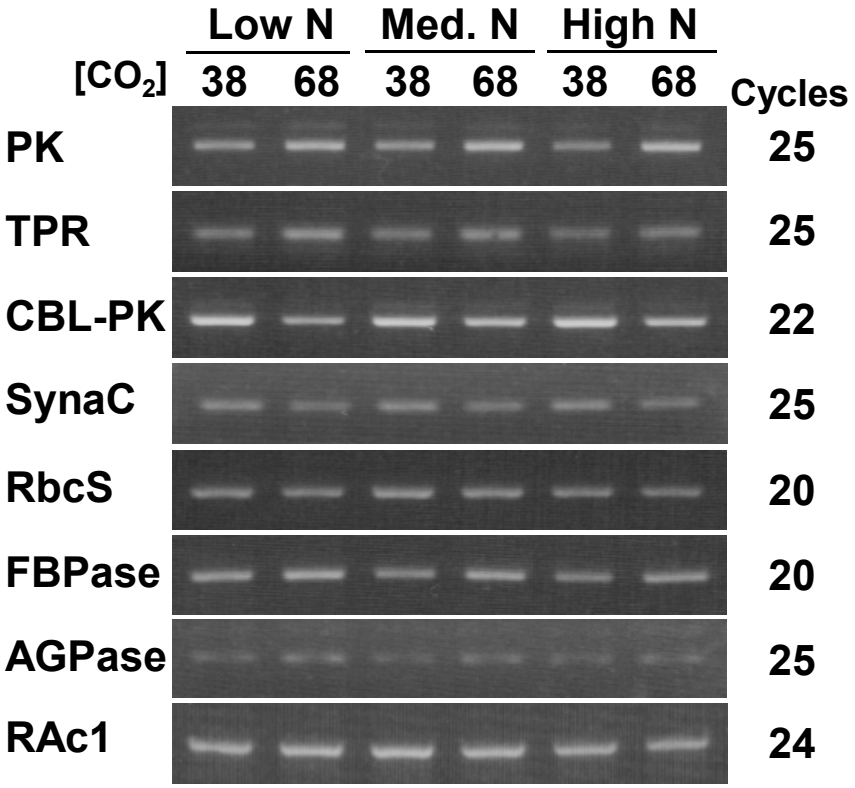

Fig. 4. Semi-quantitive RT-PCR analysis of expression of differentially expressed genes under elevated CO_2. Total RNA was subjected to RT-PCR using gene specific primer pairs (Table 3). Up-regulated genes in Table 1 (PK, TPR) and Fig. 3 (FBPase, AGPase), and down-regulated genes in Table 2 (CBL-PK, SynaC) and Fig. 3 (RbcS) were shown. Expression of the rice actin gene (RAc1, **AB047313**) was also examined as an internal control. PK; protein kinase domain containing protein (**AK120189**), TPR; TPR-like domain containing protein (**AU071153**), CBL; CBL-interacting protein kinase 1 (**AK061640**), SynaC; Synaptotagmin C (**AU057097**), RbcS; Rubisco small subunit (**AK121444**), FBPase; fructose-1,6-bisphosphate phosphatase (**AK119536**), AGPase, ADP-glucose pyrophosphorylase (**AK100910**).

Figure(s)

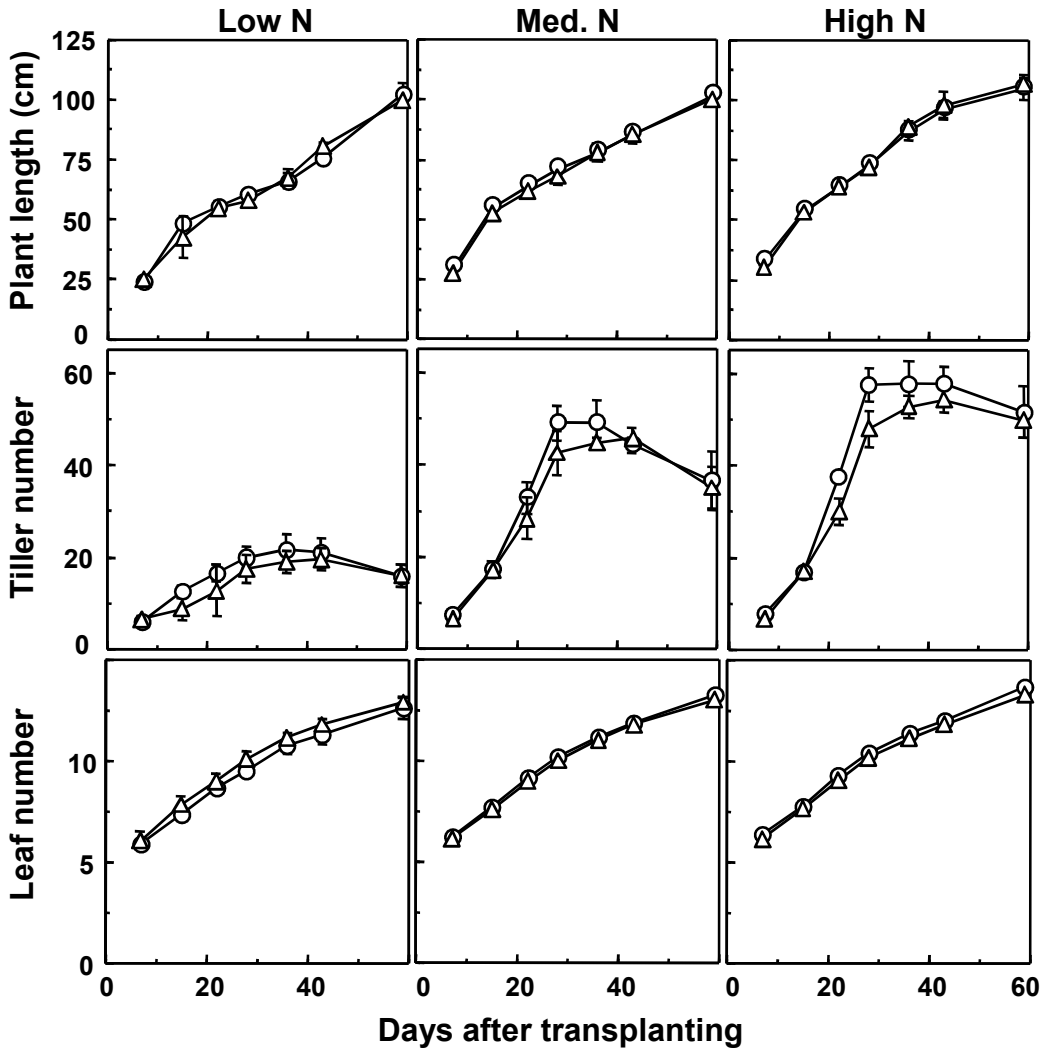

Fig.S1. Growth of rice plants under ambient or elevated CO_2. Rice plants were grown in low, middle and high N supply with ambient (38 Pa, open triangle) and elevated (68 Pa, open circle) CO_2 partial pressures. Plant length, tiller number and leaf number of the main stem were measured after transplanting (then, CO_2 treatment was started). The data were expressed as mean ±SE obtained from five to eight independent measurements.

Table(s)

Table S1. Differentially expressed genes under elevated CO_2. Rice plants were grown in low (LN), medium (MN) and high (HN) N supply with different CO_2 partial pressures (38 Pa or 68 Pa). Difference of gene expressions of LN, MN and HN in this list are all significant ($P<0.05$) by Z-Score method. Gene expression difference of MN relative to LN under ambient CO_2 (LN/MN) and the average of LN, MN and HN were also shown. [1*]Locus ID of Rice Annotation Project Database RAP-DB (http://rapdb.dna.affrc.go.jp/) was shown for genes without INSD accession number.

		Expression level (fold)				
Description	Accession No.[1*]	LN	MN	HN	LN/MN	Av.
Protein kinase domain containing protein	CB660294	3.64	4.69	7.98	1.26	5.14
Protein kinase domain containing protein	AK120189	2.56	2.99	8.04	0.87	3.95
Germin family protein	AK059812	2.86	3.14	5.08	0.90	3.57
No Hit	CI543502	2.37	2.13	4.16	1.14	2.76
Conserved hypothetical protein	AK111796	3.50	1.70	2.98	0.75	2.60
Protein kinase domain containing protein	CI322564	1.87	2.24	3.68	1.14	2.49
PGPS/D12	CI422497	2.76	1.84	2.85	0.86	2.44
Conserved hypothetical protein	AK063689	1.69	2.36	3.33	0.94	2.37
MADS-box domain containing protein	AF058697	1.92	2.38	2.85	1.21	2.35
Hypothetical protein	AK069662	2.36	1.98	2.22	0.87	2.18
Cys-rich domain containing protein	Os03g0830200	2.26	1.68	2.55	0.91	2.13
Senescence-associated protein 5	AK108529	2.09	1.76	2.34	0.79	2.05
LysM domain containing protein	Os06g0625300	1.64	1.95	2.48	1.03	2.00
HcrVf2 protein	AK065753	1.36	1.96	2.68	1.08	1.93
Protein kinase domain containing protein	CI436252	1.69	1.64	2.09	1.04	1.80
Nf-Y-A subunit.	AK106398	1.62	1.77	1.96	0.99	1.78
E-class P450, group I family protein	CI560567	2.18	1.59	1.56	1.03	1.76
Unknown function 3588 containing protein	CI557027	1.82	1.53	1.89	1.13	1.74
Protein kinase domain containing protein	AK111655	1.70	1.56	2.00	0.98	1.74
Amino acid/polyamine transporter II	AK102220	1.64	1.46	2.06	1.22	1.70
Thioredoxin family protein	AK069195	1.59	1.87	1.66	1.23	1.70
Phospholipid transfer protein	AF017359	1.66	1.57	1.85	0.98	1.69
Lipid transfer protein LPT II	AK058921	1.65	1.58	1.83	0.97	1.68
Conserved hypothetical protein.	AK064901	1.47	1.68	1.89	0.86	1.67
No Hit	CI441424	1.59	1.55	1.90	0.87	1.67
WRKY transcription factor 60	AU057193	1.46	1.65	1.93	1.40	1.67
bHLH domain containing protein	Os03g0728900	1.60	1.53	1.86	1.09	1.66
Conserved hypothetical protein	AK070327	1.48	1.89	1.58	0.79	1.64
DUF581 family protein	AK071528	1.64	1.53	1.73	0.86	1.63
PEBP family protein	AB062676	1.56	1.69	1.64	1.13	1.63
Phosphoenolpyruvate carboxykinase	AK102392	1.62	1.73	1.54	0.87	1.63
Conserved hypothetical protein	AK060241	1.68	1.40	1.83	0.76	1.63
Monosaccharide transporter 1	AK108820	1.58	1.80	1.51	1.08	1.62
bHLH domain containing protein	CI333262	1.40	1.73	1.72	0.87	1.61
Hypothetical protein	AK071106	1.58	1.42	1.82	1.23	1.60
Protein kinase domain containing protein	Os03g0678100	1.46	1.64	1.68	1.17	1.59
DUF966 family protein	AK119662	1.58	1.33	1.90	1.27	1.58
Legume lectin, domain containing protein	AK108625	1.86	1.34	1.54	0.85	1.57
Heavy metal transport/detoxification protein	AJ308374	1.77	1.35	1.55	1.14	1.55
Zn-finger, RING domain containing protein	AK108566	1.59	1.38	1.67	1.08	1.54
Protein kinase domain containing protein	AK067467	1.50	1.46	1.66	1.20	1.54
No apical meristem protein	AK107090	1.38	1.50	1.74	1.21	1.53
Y/CCAAT-box binding factor A subunit	AK071595	1.42	1.46	1.72	1.00	1.53
TPR-like domain containing protein	AU071153	1.74	1.36	1.47	1.41	1.52
Protein phosphatase 2C-like protein	AK072292	1.45	1.45	1.66	1.33	1.52
Transcriptional activator HAP2	AK073742	1.43	1.48	1.63	1.06	1.51

BTB/POZ domain containing protein	AK100472	1.53	1.40	1.55	1.33	1.49
Syntaxin	AK065950	1.43	1.43	1.61	1.09	1.49
Serine carboxypeptidase family protein	AK106800	1.49	1.42	1.51	1.06	1.47
DUF292 domain containing protein	AK099364	1.47	1.37	1.58	0.97	1.47
Hypothetical protein.	AK108125	1.41	1.41	1.58	1.02	1.47
Bacterial blight resistance protein.	Os11g0692300	1.46	1.38	1.53	1.09	1.45
Receptor-like protein kinase 6.	AK119388	1.41	1.32	1.64	0.97	1.45
Hypothetical protein.	AK058767	1.46	1.33	1.55	0.80	1.44
Photosystem II protein D1	Os04g0235200	1.37	1.50	1.46	0.74	1.44
Pectinesterase family protein	AK101494	1.54	1.33	1.44	1.02	1.43
Protein kinase domain containing protein	CI429782	1.37	1.37	1.53	1.12	1.42
Leucine-rich repeat containing protein.	AK120351	1.48	1.38	1.39	0.81	1.42
Conserved hypothetical protein	AK103995	1.36	1.41	1.46	0.79	1.41
Protein kinase domain containing protein	AK061220	1.48	1.36	1.37	0.90	1.40
Beta-ketoacyl-CoA-synthase	AK120567	1.44	1.33	1.44	0.91	1.40
Heat shock protein DnaJ family protein	AK068186	2.09	0.55	2.36	0.85	1.39
Tryptophan decarboxylase	CI191108	0.63	1.71	1.85	1.28	1.26
Hypothetical protein	AK110639	1.54	0.71	0.67	1.11	0.90
K+ potassium transporter family protein	Os01g0930400	1.36	0.65	0.54	0.85	0.78
Aldehyde oxidase	AK103597	1.46	0.71	0.43	0.73	0.77
MAP kinase-like protein	AK060552	0.74	0.74	0.71	0.88	0.73
Hypothetical protein	AK099797	0.70	0.75	0.71	0.84	0.72
Protein phosphatase type 2C	AK069274	0.74	0.67	0.72	1.17	0.71
Esterase/lipase/thioesterase like protein	AK120822	0.70	0.71	0.71	0.85	0.71
OsPK4	AK111746	0.74	0.68	0.70	0.81	0.70
Inositol 1, 3, 4-trisphosphate 56-kinase family	AK110903	0.64	0.74	0.72	1.12	0.70
Conserved hypothetical protein	AK067831	0.66	0.74	0.68	0.95	0.70
Protein phosphatase 2C-like protein	AU101828	0.69	0.75	0.65	0.99	0.69
Domain 01589 containing protein	AU085939	0.73	0.66	0.69	0.83	0.69
Conserved hypothetical protein	AK119672	0.49	0.38	1.66	1.15	0.68
Beta-amylase	AK067249	0.68	0.71	0.63	1.19	0.67
Tyrosine/nicotianamine aminotransferase	CI019806	0.70	0.60	0.72	0.86	0.67
Protein kinase family protein	CI339621	0.69	0.68	0.64	1.16	0.67
Glycoside hydrolase, family 5 protein	AK063757	0.59	0.73	0.68	0.89	0.67
Tryptophan synthase, alpha chain	AK072595	0.66	0.72	0.61	0.86	0.66
Conserved hypothetical protein	AK109132	0.69	0.62	0.66	0.78	0.66
CONSTANS-like protein CO6	AK109630	0.72	0.67	0.58	0.84	0.65
Conserved hypothetical protein	AY224438	0.63	0.66	0.64	1.34	0.64
2OG-Fe(II) oxygenase like protein	AK067970	0.59	0.63	0.71	1.06	0.64
Conserved hypothetical protein	AK062656	0.73	0.56	0.62	1.01	0.63
Myb, like domain containing protein	AK070421	0.63	0.59	0.68	0.74	0.63
EF-hand domain containing protein	AK066756	0.60	0.74	0.57	0.92	0.63
Conserved hypothetical protein	AK101548	0.61	0.60	0.69	0.75	0.63
Allergen V5/Tpx-1 related family protein	AK060409	0.55	0.65	0.66	1.35	0.62
Hypothetical protein	AK108390	0.71	0.52	0.59	0.73	0.61
Multicopper oxidase, type 1 family protein	CI452025	0.74	0.48	0.63	0.90	0.61
Heat shock protein DnaJ family protein	AK107961	0.54	0.66	0.59	1.04	0.59
Protein kinase domain containing protein	AK107168	0.63	0.59	0.54	0.83	0.59
Alpha-xylosidase	AK063966	0.46	0.63	0.65	0.96	0.58
Hydrolase fold domain containing protein	AK066814	0.48	0.60	0.59	1.32	0.55
Beta-tubulin	AK122099	0.46	0.67	0.51	0.81	0.55
Conserved hypothetical protein	Os06g0554200	0.71	0.50	0.44	0.85	0.55
FAD linked oxidase	AK103272	0.58	0.47	0.61	0.87	0.55
Chlorophyll a-b binding protein	AK103946	0.36	1.42	0.32	0.90	0.54

Kinesin 4	AK065848	0.40	0.62	0.61	0.98	0.54
Sodium-dicarboxylate cotransporter-like	AK105756	0.60	0.50	0.46	0.72	0.52
CBL-interacting protein kinase 1	AK061640	0.55	0.57	0.44	0.81	0.52
Serine acetyltransferase	Os03g0185000	0.48	0.56	0.50	0.80	0.51
Tyrosine decarboxylase 1	Os10g0400500	0.64	0.56	0.35	1.20	0.51
Synaptotagmin C	AU057097	0.37	0.63	0.53	0.76	0.51
MAP65/ASE1 family protein	AK108923	0.47	0.36	0.68	0.92	0.51
Bowman-Birk type trypsin inhibitor	AK120562	0.39	0.56	0.54	0.74	0.50
Two-component response regulator ARR1	Os12g0586300	0.51	0.48	0.50	0.85	0.50
Serine acetyltransferase	AU166464	0.44	0.52	0.45	0.80	0.47
Glycoside hydrolase, family 18	AK065866	0.39	0.55	0.43	0.95	0.46
Viviparous-14	AK107649	0.35	0.44	0.58	1.30	0.45
Osmotin-like protein precursor	AK060655	0.57	0.42	0.36	1.27	0.44
E-class P450, group I family protein	AK120757	0.42	0.45	0.42	0.78	0.43
Cytochrome P450 family protein	AK064764	0.29	0.22	0.37	1.00	0.29

Table(s)

Table S2. The expression of transcripts related to primary metabolism under elevated CO_2. Rice plants were grown in low (LN), medium (MN) and high (HN) N supply with different CO_2 concentrations (38 or 68 Pa). Av. indicates average of LN, MN and HN. Gene expression difference of MN relative to LN under ambient CO_2 (LN/MN) are also shown for comparison. [1*]Locus ID of Rice Annotation Project Database RAP-DB (http://rapdb.dna.affrc.go.jp/) was shown for genes without INSD accession number.

		Expression level (fold)				
Description	Accession No.[1*]	LN	MN	HN	LN/MN	Av.
CO_2 fixation						
Carbonic anhydrase	AK060890	0.80	1.00	0.93	0.70	0.91
Rubisco small subunit	AK059909	0.82	1.07	1.10	1.18	1.00
	AK121444	0.69	0.92	0.94	0.67	0.85
	AK058875	0.90	0.93	1.12	0.79	0.99
	AK070257	0.88	0.84	1.12	0.64	0.95
Phosphoglycerate kinase	AK062214	0.85	0.91	1.00	0.70	0.92
	CI054050	1.14	0.93	1.08	0.86	1.05
Glyceraldehyde-3-phosphate dehydrogenase	AK067755	0.88	0.82	1.01	0.77	0.90
	AF357884	1.03	1.09	1.11	1.01	1.08
Rubisco activase	AK119513	1.16	1.12	1.12	0.95	1.13
RuBP regeneration						
Transketolase	AK067452	0.98	0.95	1.15	0.71	1.03
Fructose-1,6-bisphosphatase	AK119536	1.33	1.36	1.24	1.06	1.31
	AK065201	1.12	1.03	1.01	0.97	1.05
Fructose-bisphosphate aldolase	AF017362	1.25	1.10	1.16	0.97	1.17
Sedoheptulose-1,7-bisphosphatase	AK062081	1.06	0.97	1.08	0.97	1.04
Ribulose phosphate epimerase	AK061772	1.06	1.00	0.99	0.97	1.02
Phosphoribulokinase	AK066164	1.05	1.05	1.05	0.99	1.05
	AK102236	1.06	0.99	1.03	0.85	1.03
	AK073304	0.95	1.00	0.95	0.94	0.97
	CI517473	1.03	1.06	0.99	0.94	1.03
Starch synthesis						
ADP-glucose pyrophosphorylase small subunit	AK071826	1.05	1.18	1.13	0.98	1.12
	AK060270	1.18	1.11	1.49	1.13	1.26
ADP-glucose pyrophosphorylase large subunit	AK100910	2.29	1.96	1.65	2.25	1.97
	AK069296	0.95	0.99	0.87	0.85	0.94
	AK099643	1.04	1.02	1.01	0.91	1.02
	AK071497	1.06	0.99	1.07	0.93	1.04
Starch synthase	AF395537	1.05	1.00	0.96	0.89	1.00
	AK122098	1.03	1.04	1.03	0.99	1.03
	AY299404	1.22	1.11	1.30	1.00	1.21
Granule-bound starch synthase	AY069940	1.36	1.27	1.37	1.13	1.33
	AF383878	1.06	1.10	1.00	1.02	1.05
	AF515481	1.06	0.97	1.04	0.99	1.02
Sucrose synthesis						
Triose phosphate/phosphate translocator	AK060343	1.15	1.08	1.13	0.89	1.12
	AK059788	0.97	0.98	0.91	0.90	0.95
Triosephosphate isomerase	AK060920	0.92	0.91	0.96	0.83	0.93
Fructose-1,6-bisphosphatase	AF378182	1.00	1.07	1.00	1.19	1.02
Phosphoglucomutase	AK062105	0.95	0.97	1.05	0.94	0.99
	AK068502	0.94	0.72	0.72	0.84	0.80
	AK069901	1.03	1.09	1.08	1.10	1.07
	AK064893	1.21	0.98	1.21	0.96	1.13
Sucrose-phosphate synthase	CI158157	1.16	1.00	0.96	0.96	1.04
	AK071732	0.96	0.89	0.95	0.80	0.93

	AK065273	1.03	1.09	1.06	0.91	1.06
	AK101676	0.92	1.09	0.93	1.00	0.98
	AF378184	1.49	1.21	1.10	0.96	1.27
Sucrose phosphate phosphatase	AK063330	0.98	0.96	1.03	0.88	0.99
	AK071525	0.98	0.96	0.92	0.88	0.95
Sucrose transporter	AK067030	1.04	1.01	1.12	1.06	1.06
	AF378185	1.27	1.12	1.13	0.89	1.17
	AK065430	0.97	1.01	1.00	1.05	0.99
Sucrose synthase	D21308	0.92	1.01	0.85	1.01	0.93
	AK102158	0.84	0.95	0.89	1.10	0.89
	AK061713	0.68	1.24	0.91	1.65	0.94
	AK061268	1.28	1.01	0.92	0.77	1.07
Glycolysis						
Phosphofructokinase	AK071065	1.04	1.03	1.09	1.19	1.05
	AK063963	0.91	0.91	0.99	0.96	0.94
	AK065052	0.84	0.85	0.79	0.66	0.83
	AK065250	0.93	1.00	1.00	1.21	0.98
PPi-phosphofructokinase	AK121093	0.93	0.92	0.91	1.09	0.92
	AK070279	0.86	0.89	0.98	0.76	0.91
	AK071798	0.86	0.91	1.08	1.20	0.95
	AK060464	0.85	0.96	0.96	0.99	0.93
	AK101073	0.87	1.02	1.02	0.95	0.97
Glyceraldehyde-3-phosphate dehydrogenase	AK062215	0.94	1.02	1.11	1.09	1.02
	AK064960	0.88	0.92	0.92	1.05	0.91
Phosphoglycerate kinase	AK070041	0.86	0.92	0.93	0.94	0.90
Phosphoglycerate mutase	AK068739	0.85	0.92	0.87	0.85	0.88
	AK068167	0.94	0.89	0.84	1.02	0.89
	CB645672	1.01	0.97	0.98	0.92	0.98
	AK065644	1.07	1.01	0.98	1.06	1.02
	CI319626	0.91	0.92	1.00	1.14	0.94
	AK058344	0.97	0.95	0.95	1.11	0.95
	CI008089	1.13	1.14	0.99	0.76	1.09
	Os11g0138600	1.12	0.99	0.90	0.92	1.00
	AK101802	0.99	0.97	0.98	1.01	0.98
Enolase	AK058348	0.99	0.93	0.99	0.96	0.97
	AK073662	0.86	1.01	1.05	1.03	0.97
	AK104904	0.97	0.92	0.92	0.86	0.94
	AK104877	0.86	0.94	0.96	1.06	0.92
Pyruvate kinase	AK061668	0.93	0.95	0.99	0.96	0.95
	AK100393	0.93	0.89	1.00	0.96	0.94
	AK100799	1.02	0.97	1.02	0.97	1.00
	AK071391	0.76	0.97	0.95	1.14	0.89
	AK101789	0.82	0.89	0.90	1.00	0.87
	AK103388	0.84	1.39	1.15	1.24	1.13
Phosphoenolpyruvate carboxylase	AK062201	1.03	1.02	1.01	1.07	1.02
	AY187619	0.94	1.05	0.95	0.98	0.98
	CI375311	0.95	0.94	0.95	1.01	0.95
	AK101274	1.03	1.21	1.10	1.08	1.11
	AK066635	0.94	0.97	1.01	0.89	0.97
	AK119573	0.98	1.03	1.03	1.01	1.01
Phosphoenolpyruvate carboxykinase	AK102392	1.62	1.73	1.54	0.87	1.63
TCA cycle						
Pyruvate dehydrogenase E1 alpha	AK098950	0.94	0.91	0.98	0.88	0.94
	AK069299	0.84	1.04	1.00	1.00	0.96

Pyruvate dehydrogenase E1 beta	AK069197	0.96	0.87	0.96	0.90	0.93
	AK069525	0.89	0.89	0.99	0.86	0.93
	AK071782	1.01	0.96	0.98	0.90	0.98
	AK063753	0.93	0.90	0.88	0.95	0.90
Citrate synthase	AK058734	1.03	1.05	0.94	1.02	1.01
	AK072950	0.97	0.95	1.02	0.88	0.98
	AF302906	0.96	0.89	0.95	0.87	0.94
NAD-dependent malic enzyme	AK070828	0.77	0.81	0.83	0.86	0.80
	AK061706	0.86	1.03	1.02	1.14	0.97
NADP-isocitrate dehydrogenase.	AK072762	0.97	1.01	1.00	1.04	0.99
	AF155333	0.87	0.99	0.99	1.25	0.95
NAD-isocitrate dehydrogenase	AK073096	1.07	0.98	0.97	1.00	1.01
	AK072240	1.00	0.98	1.05	0.92	1.01
Nitrogen fixation and amino acid synthesis						
Nitrate reductase	AK121810	0.74	0.57	0.80	1.07	0.70
Glutamine synthetase	X14244	1.05	0.99	0.86	0.70	0.97
	AK066867	0.89	0.95	0.93	0.98	0.92
	X14246	1.06	1.08	1.08	0.98	1.07
	AK109397	1.23	1.12	1.22	1.17	1.19
Fd-glutamate synthase	AK120896	1.02	1.04	1.01	0.89	1.02
NADH-glutamate synthase	AK105755	0.98	1.23	1.22	1.39	1.14
	AK070485	0.49	0.85	0.41	0.67	0.58
Nitrate transporter	AK061227	1.05	1.15	1.14	0.98	1.11
	AK104378	1.09	1.29	1.19	1.13	1.19
	AK068058	0.96	0.96	0.97	1.17	0.96
	AK065457	0.90	1.31	1.32	0.80	1.18
	AK105765	0.92	0.82	1.25	0.99	1.00
	AF140606	1.11	1.08	1.05	1.12	1.08