Expression of Photosynthesis Pathway Gene From Rice and Maize for Understanding Role in Plant Stress and Development Using Bioinformatics Approaches

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Research article

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Abstract

Background

Rice and maize go to family Poaceae contains many crops of agronomic trait and also represent two carbon metabolism systems, C3 and C4. Analysis of the maize sequence provides new insights into the employment of C3 genes to the C4 mechanism which allowed us to identify more orthologs in other crops. This investigation reports comparative account of genome wide in silico identification of C4 pathway related genes from Zea maize (Zm) and Oryza sativa (Os) from the available whole genome sequence information. The annotation of gene sequences, signature motif analysis, protein phosphorylation analysis, study of upstream cis-acting elements, phylogenetic tree construction, chromosomal locations, syntenic mapping and microarray expression analysis of C4 pathway related gene family from both the genomes have been attempted.

Results

A total of 30 and 37 C4-pathway genes have been predicted from rice and maize genome respectively. Multiple-sequence-alignment and signature motif analysis of these proteins of rice and maize revealed high conserveness. Phosphorylation analysis revealed that maize have high number than rice. The phylogenetic analysis of C4 related genes across both plant species clearly resulted in four sub-groups in both plants. In Rice, the 30 genes of C4 pathway related genes family are distributed on eleven out of twelve chromosomes, while in maize, they are randomly distributed on all the chromosomes. Most of the genes of Zm's chromosome 1 show syntenic relationship with chromosome 1. The cis-regulatory-elements of Zm and Os genes suggested its diverse functions associated with plant growth development, stress and hormone responsiveness as well as endosperm and meristem specific gene expression. This investigation of Zm and Os can now offer new insights into the role of different C4 pathway related genes and examine the comparative syntenic mapping between two monocot models and allows for better understanding about how genes evolve within monocots. Therefore, in silico investigation of C4-photosynthetic-pathway gene family needs to be supported by wet lab experimentation of the novel genes for elucidating their function in many biological courses.

Conclusion

Results revealed that photosynthetic pathway related gene play a potent role in stress response and plant growth and development.
pathway, on overproduction in C3 plants, it stimulated respiration in light and synthesis of amino and organic acids instead of increasing photosynthetic efficiency of rice plants the C4 pathway [24–25]. It has been observed that in the leaves of C3 plants, PEPC has an anaplerotic role replenishing the tricarboxylic acid cycle with intermediates, which are withdrawn for nitrate assimilation and the subsequent amino acid synthesis [26]. No prominent changes were observed in physiological and photosynthetic characteristics of transgenic rice in which C4 specific PPDK was introduced [27]. Although elevation above a threshold level generated a minor response. Overproduction of C4 specific NADP-ME led to increased photo-inhibition of photosynthesis, leaf chlorophyll bleaching and serious stunting because of increase in the NADPH/NADP ratio in the chloroplast [28].

In the present study, comparative genome wide in silico identification of C3/C4 gene to protein such as CA, PEPC, PECK, NADP-ME, NADP-MDH and PPDK involved in photosynthesis pathway of rice and maize. The member further were analyzed in detail in term as chromosomal location(s), gene structure, phylogenetic tree for evolutionary relationship construction and also analyzing the cis-regulatory elements associated with these genes in the promoter region. Further, comparative phylogeny and syntenic mapping and protein phosphorylation examine with putative protein signature sequences with their function. Further, we have attempted gene expression analysis development anatomical and stress condition during various stages of panicle and seed development implies their involvement in diverse developmental processes. rice and maize C3/C4 gene family have also been attempted.

**Materials And Methods**

Search of databases for the identification of photosynthetic genes/ proteins family members

In order to perform comparative analysis, the sequences of C3/C4 photosynthetic gene/ protein sequences such as CA, PEPC, PECK, NADP-ME, NADP-MDH and PPDK from rice (*O. sativa*) and Maize (*Z. mays*) were downloaded from different sources, TIGR (The Institute for Genomic Research; http://www.tigr.org/) and rice genome annotation project (http://rice.plantbiology.msu.edu/) for rice and NCBI The National Center for Biotechnology Information as primary database (http://www.ncbi.nlm.nih.gov/) for maize, respectively. And check all maize gene/ protein correspond to Maize genome database MaizeGDB http://www.maizegdb.org/ For the identification annotation of photosynthetic C3/C4 genes in rice and maize, the homology search of the C3/C4 genes photosynthetic genes proteins was performed by BLAST (http://www.ncbi.nlm.nih.gov/BLAST/) using blastp and tblastn algorithm [29–30]. Detailed analysis C3/C4 genes photosynthetic genes proteins in whole genomes of rice and sorghum, including their identification, classification, sequence analysis, domain signature analysis, chromosomal locations phylogenetic relationships and expression analysis were carried out as described below and gene list given in Table 1a and b.
(A) List of C3/C4 photosynthetic genes in Rice along with their chromosomal locations, alternative spliced forms, CDS and polypeptide length, localization and homologs.

| NAME               | Locus ID      | GENE DISCRIPTION                                                                 | Chr | CDS Coordinates (5'-3') | Nucleotide length | PPL  | PMW  | PpL | pfamhit     |
|--------------------|---------------|----------------------------------------------------------------------------------|-----|-------------------------|-------------------|------|------|-----|-------------|
| 1 PPDK             | LOC_Os05g33570| Orthophosphate dikinase precursor (EC 2.7.9.1)                                   | Chr5| 19737857–19718506       | 2844              | 948  | 10278| 6.3145 | PF01326.12  |
| 2 PPDK             | LOC_Os03g31750| Orthophosphate dikinase precursor (EC 2.7.9.1)                                   | Chr3| 18160127–18153143       | 2679              | 893  | 97212| 5.3604 | PF01326.12  |
| 3 NADP malic       | LOC_Os01g09320| NADP-dependent malic enzyme 3                                                    | Chr1| 4744591–4738905          | 1920              | 640  | 69865.8 | 7.1548 | PF00390.12  |
| 4 NADP malic       | LOC_Os01g52500| NADP-dependent malic enzyme 4                                                    | Chr1| 30166024–30171659        | 1932              | 644  | 70482.4 | 5.5251 | PF00390.12  |
| 5 NADP malic       | LOC_Os01g54030| NADP-dependent malic enzyme 5                                                    | Chr1| 31076571–31081377        | 1758              | 586  | 64269.8 | 6.9361 | PF00390.12  |
| 6 NADP malic       | LOC_Os02g44550| NADP-dependent malic enzyme 6                                                    | Chr2| 26993990–26988678        | 561               | 187  | 20398| 9.4713 | PF00390.12  |
| 7 NADP malic       | LOC_Os05g09440| NADP-dependent malic enzyme 7                                                    | Chr5| 5297419–5293555          | 1713              | 571  | 62934.9 | 5.5253 | PF00390.12  |
| 8 PEPCK            | LOC_Os03g15050| Phosphoenolpyruvate carboxykinase [ATP]                                          | Chr3| 8224217–8218791          | 2001              | 667  | 73771.8 | 7.5064 | PF01293.13  |
| 9 PEPCK            | LOC_Os04g50208| Phosphoenolpyruvate carboxykinase [ATP]                                          | Chr4| 29972696–29969234        | 1104              | 368  | 39279| 9.2502 | PF01293.13  |
| 10 PEPCK           | LOC_Os10g13700| Phosphoenolpyruvate carboxykinase [ATP]                                          | Chr10| 7444108–7438335          | 1605              | 535  | 59518.4 | 6.7889 | PF01293.13  |
| 11 PPC             | LOC_Os01g02050| PEPCase; Phosphoenolpyruvate carboxylase                                          | Chr1| 572138–562056             | 3108              | 1036 | 115840| 6.9464 | PF00311.10  |
| 12 PPC             | LOC_Os01g11054| PEPCase; Phosphoenolpyruvate carboxylase                                          | Chr1| 5899555–5909595          | 3045              | 1015 | 114197| 5.9411 | PF00311.10  |
| 13 PPC             | LOC_Os01g55350| PEPCase; Phosphoenolpyruvate carboxylase                                          | Chr1| 31865031–31859331        | 2775              | 925  | 105314| 6.594  | PF00311.10  |
| 14 PPC             | LOC_Os02g14770| PEPCase; Phosphoenolpyruvate carboxylase                                          | Chr2| 8184220–8172419          | 2907              | 969  | 109933| 6.1467 | PF00311.10  |
| 15 PPC             | LOC_Os08g27840| PEPCase; Phosphoenolpyruvate carboxylase                                          | Chr8| 16970571–16964694        | 2895              | 965  | 110056| 5.5479 | PF00311.10  |
| 16 PPC             | LOC_Os09g14670| PEPCase; Phosphoenolpyruvate carboxylase                                          | Chr9| 8697573–8692191          | 2928              | 976  | 110474| 6.1404 | PF00311.10  |
| 17 CA              | LOC_Os01g45274| Carbonic anhydrase                                                               | Chr1| 25696613–25705090        | 819               | 273  | 29117.4 | 8.2758 | PF00484.12  |
| 18 CA              | LOC_Os09g28910| Carbonic anhydrase                                                               | Chr9| 17572392–17567632        | 1002              | 334  | 36800.6 | 7.9867 | PF00484.12  |
| 19 CA              | LOC_Os11g05510| Carbonic anhydrase                                                               | Chr11| 2488205–2488622          | 342               | 114  | 11814.3| 7.4979 | PF00194.14  |
| 20 CA              | LOC_Os09g28130| Carbonic anhydrase                                                               | Chr9| 17075709–17075190        | 291               | 97   | 10802.2| 5.1666 | PF00194.14  |
| 21 NADP MDH        | LOC_Os08g33720| NAD malate dehydrogenase, chloroplastic                                         | Chr8| 21057561–21054659        | 1194              | 398  | 41538.7 | 7.5418 | PF00056.16  |
| 22 NADP MDH        | LOC_Os01g61380| malate dehydrogenase, chloroplastic                                             | Chr1| 35499017–35501765        | 1191              | 397  | 41787 | 7.8961 | PF00056.16  |
| 23 NADP MDH        | LOC_Os01g46070| malate dehydrogenase, mitochondrial                                           | Chr1| 26190752–26194517        | 1023              | 341  | 35460.9 | 8.8513 | PF00056.16  |
| 24 NADP MDH        | LOC_Os12g43630| malate dehydrogenase, glyoxysomal                                              | Chr12| 27099351–27094647        | 1071              | 357  | 37385.4 | 7.9869 | PF00056.16  |
| NAME  | Locus ID    | GENE DISCIPTION                      | Chr | CDS Coordinates (5'-3') | Nucleotide length | PPL  | PMW | Ppi | pfamhit     |
|-------|-------------|-------------------------------------|-----|------------------------|-------------------|------|-----|-----|-------------|
| 25    | NAD MDH LOC_Os10g33800 | malate dehydrogenase, cytoplasmic    | Chr10 | 17913818–17917850       | 999               | 333  | 35568.9 5.9726 PF00056.16 |
| 26    | NAD MDH LOC_Os08g44810 | malate dehydrogenase [NADP], chloroplastic | Chr8  | 28141042–28146270       | 1302              | 434  | 47008.9 7.3392 PF00056.16 |
| 27    | NAD MDH LOC_Os05g49880 | malate dehydrogenase, mitochondrial  | Chr5  | 28621585–28617595       | 1023              | 341  | 35435.9 8.2991 PF00056.16 |
| 28    | NAD MDH LOC_Os07g43700 | malate dehydrogenase, chloroplastic  | Chr7  | 26155933–26153825       | 1215              | 405  | 42221.7 9.0291 PF00056.16 |
| 29    | NAD MDH LOC_Os04g46560 | malate dehydrogenase, cytoplasmic    | Chr4  | 27605166–27608347       | 1059              | 353  | 38297.1 7.2212 PF00056.16 |
| 30    | NAD MDH LOC_Os03g56280 | malate dehydrogenase, glyoxysomal    | Chr3  | 32089685–32086001       | 1065              | 355  | 37023 8.0614 PF00056.16 |
Table 1

(B) List of C3/C4 photosynthetic genes in Maize along with their chromosomal locations, alternative spliced forms, CDS and polypeptide length, localizations and homologs.

| Gene symbol | Ch | Exon | Location | Protein ID | RNA ID | DOMAIN | Location |
|-------------|----|------|----------|------------|--------|---------|----------|
| umc2175     | 8  | 8    | NC_024466.1 (146068247..146092154) | NP_001168699.1 | NM_001175228.1 | cd00884 | CH beta CA CARBONIC ANHYDRAS |
| csu869(cah) | 3  | 7    | NC_024461.1 (215513277..215518550) | NP_001151431.1 | NM_001157959.1 | cd00884 | CH beta CA CARBONIC ANHYDRAS |
| LOC100280638| 4  | 5    | NC_024462.1 (56994835..56996762) | NP_001147028.1 | NM_001153556.1 | cd03124 | NF alpha CA CARBONIC ANHYDRAS |
| LOC100274185| 2  | 7    | NC_024460.1 (57684848..57687212) | NP_001142031.1 | NM_001148559.1 | cd03124 | NF alpha CA CARBONIC ANHYDRAS |
| LOC100283312| 7  | 10   | NC_024465.1 (127621625..127626283) | NP_001149686.1 | NM_001156214.1 | cd00884 | CH beta CA CARBONIC ANHYDRAS |
| LOC100279972| 9  | 4    | NC_024467.1 (109408583..109410241) | NP_001146392.1 | NM_001152920.1 | cl00012 | CH alpha CA CARBONIC ANHYDRAS |
| LOC100274597| 3  | 7    | NC_024461.1 (21550600..215509206) | NP_001152905.1 | NM_001159433.1 | cd00884 | CH beta CA CARBONIC ANHYDRAS |
| LOC100274349| 2  | 10   | NC_024460.1 (187773678..187778034) | NP_001140385.1 | NM_001146913.1 | cl00391 | CH beta CA CARBONIC ANHYDRAS |
| LOC100283752|    |      | 100283752 | NP_001150123.1 | NM_001156651.1 | NF beta CA CARBONIC ANHYDRAS |
| LOC100282652| 1  | 5    | NC_024459.1 (199145284..199147439) | NP_001149032.1 | NM_001155560.1 | cd03124 | CH alpha CA CARBONIC ANHYDRAS |
| LOC100275493| 3  | 14   | NC_024461.1 (215547921..215555236) | NP_001105539.1 | NM_001111891.1 | cl00391 | NF beta CA CARBONIC ANHYDRAS |
| pep1        | 9  | 10   | NC_024467.1 (62306266..62311652) | NP_001154820.1 | NM_001161348.1 | pfam00311 | PEPcase Phosphoenolpyruvate carboxylase |
| pep4        | 7  | 10   | NC_024465.1 (86459218..86464631) | NP_001105438.1 | NM_001111968.1 | pfam00311 | PEPcase Phosphoenolpyruvate carboxylase |
| pep7        | 5  | 10   | NC_024463.1 (144847532..144854986) | NP_001105503.1 | NM_001112033.1 | pfam00311 | PEPcase Phosphoenolpyruvate carboxylase |
| IDP1621     | 4  | 10   | NC_024462.1 (227810840..227818674) | NP_001130365.1 | NM_001136893.1 | cl21521 | pepcase Phosphoenolpyruvate carboxylase |
| LOC541622   | 1  | 11   | NC_024459.1 (35744408..35748808) | NP_001296837.1 | NM_001309908.1 | cd00484 | PEPCK Phosphoenolpyruvate carboxykinase |
| PCK2        | 9  | 11   | NC_024457.1 (142082093..142086899) | NP_001146178.1 | NM_001152706.1 | PEPCK Phosphoenolpyruvate carboxykinase |
| pdk1        | 6  | 19   | NC_024464.1 (146179394..146189973) | NP_001105738.2 | NM_001112268.2 | PRK09279 | CH PPDK Pyruvate kinase, pyruvate kinase |
| LOC100274067| 10 | 13   | NC_024468.1 (20391750..20418385) | NP_001141918.1 | NM_001148446.1 | pfam01326 | CH PPDK Pyruvate kinase, pyruvate kinase |
| LOC100381411| 33 | 33   | NC_024464.1 (110869370..110881829) | NP_001167723.1 | NM_001174252.1 | CH PPDK Pyruvate kinase, pyruvate kinase |
| me3         | 3  | 20   | NC_024461.1 (7276413..7281610) | NP_001105313.1 | NM_001111843.1 | PLN03129 | CH ME-NAD NAD-dependent enzyme |
| me2         | 6  | 23   | NC_024464.1 (139420838..139469949) | NP_001105382.2 | NM_001111913.2 | PLN03129 | CH ME-NAD NAD-dependent enzyme |
| me4         | 8  | 19   | NC_024466.1 (174612837..174617017) | NP_001105292.1 | NM_001111822.1 | PLN03129 | CH/CY ME-NAD NAD-dependent enzyme |
| LOC100284589| 6  | 9    | NC_024464.1 (130314932..130318270) | NP_001150965.1 | NM_001157493.1 | PLN03129 | CY/CY ME-NAD NAD-dependent enzyme |
| LOC100501486| 5  | 19   | NC_024463.1 (23916406..23923194) | NP_001183119.1 | NM_001196190.1 | PTZ00317 | MT ME-NAD NAD-dependent enzyme |
| LOC100286036| 3  | 20   | NC_024461.1 (201756875..201761835) | NP_001152396.1 | NM_001158924.1 | PLN03129 | CH ME-NAD NAD-dependent enzyme |
| mdh6        | 1  | 14   | NC_024459.1 (203210173..203213877) | NP_001105420.1 | NM_001111950.1 | PLN00112 | CH MDH NADP-Malate dehydrogenase |
| Gene symbol | Ch | Exon | Location          | Protein ID  | RNA ID           | DOMAIN | Location         |
|-------------|----|------|-------------------|-------------|------------------|--------|------------------|
| LOC100193663 | 4  | 4    | NC_024462.1       | NP_001132228.1 | NM_001138756.1 | PLN00106 | GL MDH NADP-Malat Dehydrogen |
|             |    |      | (83532085..83534409) |             |                  |        |                  |
| mdh5        | 1  | 7    | NC_024459.1       | NP_001105603.1 | NM_001112133.2 | PLN00135 | CY MDH NADP-Malat Dehydrogen |
| LOC100856934 | 7  | 1    | NC_024459.1       | NP_001241749.1 | NM_001254820.1 | PLN00106 | CH MDH NADP-Malat Dehydrogen |
| LOC100282134 | 8  | 1    | NC_024459.1       | NP_001148518.1 | NM_001155046.1 | PLN00106 | GL MDH NADP-Malat Dehydrogen |
| LOC100274264 | 7  | 6    | NC_024464.1       | NP_001142100.1 | NM_001148628.2 | PLN00106 | MT MDH NADP-Malat Dehydrogen |
| LOC100280767 | 2  |      |                   | NP_001147160.1 | NM_001153688.1 | PLN00135 | CY MDH NADP-Malat Dehydrogen |
| LOC100193743 | 2  | 1    | NC_024459.1       | NP_001132302.1 | NM_001138830.1 | PLN00106 | CH MDH NADP-Malat Dehydrogen |
| LOC100193491 | 10 |      |                   | NP_001132077.2 | NM_001138605.2 | PLN00106 | GL MDH NADP-Malat Dehydrogen |
| LOC100273428 | 9  |      | NW_007617880.1    | NP_001141337.1 | NM_001147865.1 | PLN00106 | MT MDH NADP-Malat Dehydrogen |
| LOC100273428 | 9  |      |                   |             |                  |        |                  |
Table 2

(A) cis-regulatory elements in the upstream region of C3 photosynthetic genes of rice. Elements responsive to plant growth and development, stress response and hormones were analyzed.

| GROUP     | GENE ID         | Plant growth and development | Biotic and abiotic stress response | Hormone response |
|-----------|-----------------|------------------------------|-----------------------------------|-------------------|
| PPDK      | Os05g0405000    |                              | ARE(1), MBS(1), TCA-rich repeats(1), | TCA element(2), ABRE(4), |
|           | Os03g0432100    | CAT box(1), circadian(1),    | MBS(2),                           | CGTCA-motif(1), TGACG-motif(1), ABRE(3) |
| NAD-      | Os01g0188400    | Skn1-motif(1), as-2-box(1),  | ARE(2), HSE(2),                   | TCA element(1), ABRE(1) |
| ME        | Os01g0723400    | CCGTCC-box(1),               | MBS(2), HSE(1), TCA-rich repeats(1), | CGTCA-motif(1), TGACG-motif(1), ABRE(1), TATC box(1) |
|           | Os01g0743500    | CCGTCC-box(1), O2 site(1), Skn1-motif(1), | MBS(1),                       | CGTCA-motif(1), TGACG-motif(1), ABRE(6) |
|           | Os02g0665000    | CCGTCC-box(1), Skn1-motif(1), | ARE(1), MBS(2), LTR(1),           | GARE-motif(1), TGA-element(1), |
|           | Os05g0186300    | CCGTCC-box(1),               | ARE(4), HSE(1), LTR(1),           | CGTCA-motif(2), TGACG-motif(2), ABRE(1), GARE-motif(1) |
| PEPCK     | Os03g0255500    | CCGTCC-box(1), circadian(1), | ARE(1), MBS(2), p-box(1), ERE(1)  |                  |
|           | Os04g0592500    | CAT box(1), CCGTCC-box(1), Skn1-motif(1), circadian(1), GCN4 motif(1), | ARE(1), box-w1(1), TCA-rich repeats(1), | TCA element(1), TGACG-motif(1), ABRE(3), TATC box(1), AuxRR-core(1) |
|           | Os10g0204400    | CAT box(1), CCGTCC-box(1), Skn1-motif(3), | ARE(1), MBS(3), HSE(2), TCA-rich repeats(1), | TGACG-motif(1), ABRE(5) |
| PEPC      | Os01g0110700    | CAT box(1), O2 site(1), circadian(1) | ARE(1), HSE(1),                   | TCA element(1), TGACG-motif(1), ABRE(1) |
|           | Os01g0208700    | CAT box(1), Skn1-motif(2),   | ARE(1), MBS(1), TCA-rich repeats(2), LTR(1), | CGTCA-motif(2), TCA element(1), TGACG-motif(2), TATC box(1), |
|           | Os01g0758300    | Skn1-motif(3), circadian(2), | MBS(1),                           | CGTCA-motif(1), TGACG-motif(1), GARE-motif(1) |
|           | Os02g0244700    | CCGTCC-box(1), O2 site(2), circadian(2), | ARE(1), MBS(2), box-w1(1), TCA-rich repeats(1), | CGTCA-motif(2), TGACG-motif(2) |
|           | Os08g0366000    | circadian(1)                  | ARE(1), HSE(1), TCA-rich repeats(1), WUN-motif(1), | GARE-motif(1) |
|           | Os09g0315700    | CCGTCC-box(2), Skn1-motif(1), circadian(1), | ARE(3), HSE(3), TCA-rich repeats(1), WUN-motif(1), | TCA element(1) |
| BETA-     | Os01g0639900    | CAT box(1), MSA-like(1), Skn1-motif(1), circadian(1), GCN4 motif(1), | ARE(1),                  | CGTCA-motif(2), TGACG-motif(2), ABRE(5) |
| CA        | Os09g0464000    | CCGTCC-box(1), MSA-like(1), Skn1-motif(1), as-2-box(1), GCN4 motif(1) | ARE(1), MBS(1) | ABRE(1) |
| ALPH-     | Os11g0153200    | CCGTCC-box(1), O2 site(1), circadian(1), as-2-box(2) | ARE(2), HSE(6),                  | ERE(1) |
|          | Os09g0454400    |                              |                                  |                  |
| NAD-      | Os08g0434300    | CAT box(1),                   | ARE(3), MBS(2), TCA-rich repeats(2), WUN-motif(1), | TGACG-motif(1), GARE-motif(1), p-box(1), ERE(1) |
| MDH       | Os01g0829800    | CAT box(1)                    | MBS(2),                           | CGTCA-motif(2), TGACG-motif(2), ABRE(1), CE3(1) |
|           | Os01g0649100    | CCGTCC-box(1), Skn1-motif(4), circadian(2), GCN4 motif(1) | ARE(2), MBS(3), TCA-rich repeats(1) | CGTCA-motif(1), TGACG-motif(1), GARE-motif(1), p-box(1) |
|           | Os12g0632700    | Skn1-motif(3),               | TCA-rich repeats(1)               | TCA element(1), TGACG-motif(2), ABRE(2), GARE-motif(1), p-box(1), TATC box(2) |
|           | Os10g0478200    | CAT box(1), O2 site(2), Skn1-motif(2), | ARE(1), MBS(1), HSE(1), TCA-rich repeats(1), LTR(2), | CGTCA-motif(2), TGACG-motif(2), ABRE(2), |
|           | Os08g0562100    | O2 site(2), Skn1-motif(1),   | HSE(1)                           | CGTCA-motif(1), TCA element(1), TGACG-motif(1), ABRE(1), GARE-motif(1), p-box(1), TATC box(1), |
|           | Os05g0574400    | CAT box(1), Skn1-motif(1), circadian(1), GCN4 motif(1), | MBS(2), WUN-motif(1), LTR(2), ERE(1), | CGTCA-motif(1), TCA element(1), TGACG-motif(2), ABRE(1), GARE-motif(1), motif lib(2) |
|           | Os07g0630800    | Skn1-motif(1)                 | MBS(2), box-w1(1), TCA-rich repeats(1), | CGTCA-motif(1), TCA element(3), TGACG-motif(1), ABRE(2), ERE(1), AuxRR-core(1) |
|           | Os04g0551200    | circadian(1)                 |                                  | TCA element(1) |
|           | Os03g0773800    | CCGTCC-box(1) O2 site(1), Skn1-motif(1) | ARE(1), MBS(1),                  | CGTCA-motif(3), ABRE(4), TGACG-motif(3) |
Table 2(B) Cis-regulatory elements in the upstream region of C4 photosynthetic genes of maize. Elements responsive to plant growth and development, stress response and hormones were analyzed.

| Gene ID      | Gene       | Growth and Development | Biotic/Abiotic Stress | Hormonal                          |
|--------------|------------|------------------------|-----------------------|-----------------------------------|
| GRMZM2G097457 | ZmPPDK    | O2-site (1), Skn-1_motif (2) | ARE (4), MBS (1)     |                                   |
| GRMZM2G306345 |           | circadian (1), Skn-1_motif (2) | TC-rich repeats (1), ARE (1), HSE (1), MBS (2) | TGACG-motif (1), CGTCA-motif (1) |
| GRMZM2G122479 | ZmNADP-ME | O2-site (2), GCN4_motif (2), Skn-1_motif (3) | MBS (2), ARE (1), Box-W1 (1), TC-rich repeats | ABRE (4), CGTCA-motif (1), TGACG-motif (1) |
| GRMZM2G159724 |           | Skn-1_motif (2), circadian (1) | MBS (1), LTR (1), HSE (1), Box-W1 (1), ARE (1), MNF (1) | TGACG-motif (1), TGA-box (1), TATC-box (1) |
| GRMZM2G404237 |           | Skn-1_motif (1), circadian (1) | ARE (1), TC-rich repeats (1), LTR (1) | CGTCA-motif (3), TCA-element (1), TGACG-motif (3), TATC-box (1) |
| GRMZM5G886257 |           | circadian (1), Skn-1_motif (1) | MNF (1), ARE (1), LTR (1) | CGTCA-motif (1), TGACG-motif (1), ABRE (5) |
| GRMZM2G118770 |           | Skn-1_motif (2) | MBS (1), HSE (2) | ABRE (1), AuxRR-core (1), TCA-element (1) |
| GRMZM2G001696 | ZmPEPCK   | Skn-1_motif (1) | ARE (2), MBS (3) |                                   |
| GRMZM5G870932 |           | Skn-1_motif (1), O2-site (1), circadian (3) | ARE (3), 5UTR Py-rich stretch (2), MBS (1), TC-rich repeats (1) | ABRE (1), CGTCA-motif (1), ERE (1), TGA-element (1), TGACG-motif (1), TCA-element (1) |
| GRMZM2G082780 | ZmPEPC    | circadian (2), Skn-1_motif (2), GCN4_motif (1) | ARE (1) | TGA-element (1) |
| GRMZM2G069542 |           | Skn-1_motif (1), GCN4_motif (1) | MBS (2), ARE (1) | TGACG-motif (1), TCA-element (1), ABRE (1) |
| GRMZM2G074122 |           | circadian (1), O2-site (1) | TC-rich repeats (2), MBS (1) |                                   |
| Gene ID | Description | Promoter Features |
|---------|-------------|-------------------|
| GRMZM2G083841 | circadian (2) | Box-W1(1) MBS (1) 5UTR Py-rich stretch(1) |
| GRMZM2G10714 | O2-site(1) | ARE (1) Box-W1 (1) |
| GRMZM2G473001 | circadian (2) | ARE(1) |
| GRMZM2G121878 | ZMCA | 5UTR Py-rich stretch(2) ABRE(1) |
| GRMZM2G348512 | circadian(1) | TC-rich repeats(1) EIRE(1),MNF(2) |
| GRMZM2G145101 | circadian(1) | ARE(3) |
| GRMZM2G414528 | Skn-1_motif(3) | 5UTR Py-rich stretch(2) ABRE(7) |
| GRMZM2G068455 | ZmNADP-MDH | circadian(1), CCGTCC-box(1) ARE(1) Box-W1(1) LTR(8) |
| GRMZM2G141289 | CCGTCC-box(1) | ARE(1) |
| GRMZM2G161245 | Skn-1_motif(1) | MBS (1) Box-W1 (1) |
| GRMZM2G154595 | circadian(3) | TC-rich repeats(3) motif ib(1) |
| Gene Name | Motifs and Elements |
|-----------|---------------------|
| GRMZM2G466833 | Skn-1_motif(3)  
GCN4_motif(2)  
MBS(2)  
Box-W1(1)  
ARE(1)  |
|          | TGACG-motif(1)  
TCA-element(1)  
TATC-box(1)  
GARE-motif(1)  
ABRE(1)  
CGTCA-motif(1)  |
| GRMZM2G072744 | Skn-1_motif(1)  
O2-site(1)  
ARE(1)  |
|          | TGACG-motif(1)  
TCA-element(1)  |
| GRMZM2G415359 | circadian(3)  
Skn-1_motif(5)  
TC-rich repeats(2)  
MBS(1)  
L-box(1)  
EIRE(1)  |
|          | CGTCA-motif(4)  |
| GRMZM2G035767 | O2-site(1)  
Skn-1_motif(2)  
MBS(1)  |
|          | TGACG-motif(2)  
CGTCA-motif(2)  
GARE-motif(1)  |
| GRMZM2G101290 | Skn-1_motif(3)  
CCGTCC-box(1)  
ARE(1)  
MBS(1)  |
|          | CGTCA-motif(1)  
TCA-element(1)  
TGACG-motif(1)  
TGA_element(2)  |
| PROTEIN ID   | SIGNATURE SEQ 1                | SIGNATURE SEQ 2                                      |
|-------------|--------------------------------|------------------------------------------------------|
| Os05g0405000 | EFFSFGTNDLTQMTFGYSR            | GGMHAAVGLTARGGTMTHAANVAR                             |
|             | PEP-utilizing enzymes signature 2. | PEP-utilizing enzymes phosphorylation site signature. |
| Os03g0432100 | EFFSFGTNDLTQMTFGYSR            | GGMNAAGLTTARGGTMTHAANVAR                             |
|             | PEP-utilizing enzymes signature 2. | PEP-utilizing enzymes phosphorylation site signature. |
| Os01g0188400 | FNDDIQGTASVVLAGLL              | KERDAHY                                             |
|             | Malic enzymes signature.        | TYSR_PHOSPHO_SITE Tyrosine kinase phosphorylation site. |
| Os01g0723400 | FNDDIQGTASVVLAGSGLV            | TWNSKGRAVFA                                        |
|             | Malic enzymes signature.        | Aminotransferases class-II pyridoxal-phosphate attachment site. |
| Os01g0743500 | FNDDIQGTAAVVLAGLI              | KERDAHY                                             |
|             | Malic enzymes signature.        | Tyrosine kinase phosphorylation site.                |
| Os02g0665000 | NOT FOUND                      | NOT FOUND                                           |
| Os05g0186300 | FNDDIQGTASVVLAGLI              | RPDDLVKY                                            |
|             | Malic enzymes signature.        | Tyrosine kinase phosphorylation site.                |
| Os03g0255500 | LIGDDEHCWSDTGVSN               | Phosphoenolpyruvate carboxykinase (ATP) signature.  |
| Os04g0592500 | RDQEVSY                        | Tyrosine kinase phosphorylation site.                |
| Os10g0204400 | LIGDDEHCWSDNGISN               | Phosphoenolpyruvate carboxykinase (ATP) signature.  |
| Os01g0110700 | VTLAHPTQINRR                   | VMGVYSDSGKDAG                                       |
|             | Phosphoenolpyruvate carboxylase active site 1. | Phosphoenolpyruvate carboxylase active site 2.       |
| Os01g0208700 | VTLAHPTQSVRR                   | IMIGYSDSGKDAG                                       |
|             | Phosphoenolpyruvate carboxylase active site 1. | Phosphoenolpyruvate carboxylase active site 2.       |
| Os01g0758300 | VTLAHPTQSVRR                   | VMIGYSDSGKDAG                                       |
|             | Phosphoenolpyruvate carboxylase active site 1. | Phosphoenolpyruvate carboxylase active site 2.       |
| Os02g0244700 | VFTAHPTQSVRR                   | VMIGYSDSGKDAG                                       |
|             | Phosphoenolpyruvate carboxylase active site 1. | Phosphoenolpyruvate carboxylase active site 2.       |
| Os08g0366000 | VTLAHPTQSVRR                   | VMIGYSDSGKDAG                                       |
|             | Phosphoenolpyruvate carboxylase active site 1. | Phosphoenolpyrivate carboxylase active site 2.       |
| Os09g0315700 | VTLAHPTQSVRR                   | VMIGYSDSGKDAG                                       |
|             | Phosphoenolpyruvate carboxylase active site 1. | Phosphoenolpyruvate carboxylase active site 2.       |
| Os01g0639900 | CADSRVCP                       | EYAVCALKVELIVVGHRSRCG                               |
|             | Prokaryotic-type carbonic anhydrases signature 1. | Prokaryotic-type carbonic anhydrases signature 2.   |
| Os09g0464000 | CADSRVCP                       | EFAVNTLEVENLVVGHRSRCG                               |
|             | Prokaryotic-type carbonic anhydrases signature 1. | Prokaryotic-type carbonic anhydrases signature 2.   |
| Os11g0153200 | NOT FOUND                      | NOT FOUND                                           |
| Os09g0454400 | SEHTINGTRFAEMHMV               | Alpha-carbonic anhydrases signature.                |
| Os08g0434300 | VTTLDVVRANTTFV                 | Malate dehydrogenase active site signature.         |
| Os01g0829800 | VTTLDVVRANTFI                  | RNCDITSY                                            |
|             | Malate dehydrogenase active site signature. | Tyrosine kinase phosphorylation site.                |
| Os01g0649100 | VTTLDVVRAKTFY                  |                                                     |
Malate dehydrogenase active site signature

| Gene ID      | Signature | Description                                      |
|--------------|-----------|--------------------------------------------------|
| Os12g0632700 | VTTLDVVRANTFV | Malate dehydrogenase active site signature.       |
| Os10g0478200 | LTRLDHNRALQI  | Malate dehydrogenase active site signature.       |
| Os08g0562100 | LTRLDENRAKCQL | Malate dehydrogenase active site signature.       |
| Os05g0574400 | VTTLDVVRKTFY  | Tyrosine kinase phosphorylation site.              |
| Os07g0630800 | VTTLDVVRANTFV | Malate dehydrogenase active site signature.       |
| Os04g0551200 | LTRLDHNRALQV  | Malate dehydrogenase active site signature.       |
| Os03g0773800 | VTTLDVARANTFV | Malate dehydrogenase active site signature.       |
| Protein ID | Signature seq 1                      | Signature seq 2                      |
|------------|-------------------------------------|-------------------------------------|
| NP_001168699.1 | CSDSRVCP carbonic anhydrases signature 1. | EYAVCALKEVLEVIGHSCCG Prokaryotic-type carbonic anhydrases signature 2. |
| NP_0011151431.1 | CSDSRVC Prokaryotic-type carbonic anhydrases signature 1. | EYAVCALKEVLEVIGHSCCG Prokaryotic-type carbonic anhydrases signature 2. |
| NP_001147028.1 | Not found Not found | |
| NP_001142031.1 | SEHTVDGRRFVPVELHMV Alpha-carbonic anhydrases signature. | |
| NP_001152905.1 | CSDSRVCP Prokaryotic-type carbonic anhydrases signature 1. | EYAVCALKEVIVVIGHSCG |
| NP_001140385.1 | CADSRVCP Prokaryotic-type carbonic anhydrases signature 1. | EFAVNTLQVENLVIGHSRCG Prokaryotic-type carbonic anhydrases signature 2. |
| NP_001149032.1 | SEHTVDGRRYAMELHMV Alpha-carbonic anhydrases signature. | |
| NP_001105359.1 | CSDSRVCP Prokaryotic-type carbonic anhydrases signature 1. | EYAVCALKVQIVVIGHSCG Prokaryotic-type carbonic anhydrases signature 2. |
| NP_001154820.1 | VFEALKNQTVDLVFQTAHPTQSARR Phosphoenolpyruvate carboxylase active site 1. | VMVGYSDSGKDAG |
| NP_001105438.1 | VLTAHPTQSVRR Phosphoenolpyruvate carboxylase active site 1. | VMIGYSDSGKDAG |
| NP_001105503.1 | VFTAHPTQSVRR Phosphoenolpyruvate carboxylase active site 1. | VMIGYSDSGKDAG |
| NP_001105808.1 | IGRGRGFVVRCCYAAATGEPAVK Protein kinases ATP-binding region signature. | VAHRDVFDPNLI |
| NP_001105774.1 | IGRGRGFGVVRCCSRATGDAFAVK Protein kinases ATP-binding region signature. | VVHRDVFDPNVL |
| NP_001105773.1 | IGRGRGFGVVRCCSRATGDAFAVK Protein kinases ATP-binding region signature. | VAHRDVFDPNLI |
| NP_001105772.1 | IGRGRGFGVVRCCSRATGDAFAVK Protein kinases ATP-binding region signature. | VAHRDVFDPNLI |
| NP_001130365.1 | KDVLEGDPY Tyrosine kinase phosphorylation site. | |
| NP_001296837.1 | LIGDDEHCWSDNVSN Phosphoenolpyruvate carboxykinase (ATP) signature. | |
| NP_001296837.1 | LIGDDEHCWSDNVSN Phosphoenolpyruvate carboxykinase (ATP) signature. | |
| NP_001146178.1 | LIGDDEHCWSENVSN | |
Phosphoenolpyruvate carboxykinase (ATP) signature.

| Accession | Sequence | Description |
|-----------|----------|-------------|
| NP_001105738.2 | EFFSFGTNDLTMGFYSR | PEP-utilizing enzymes signature 2. |
| NP_0011141918.1 | Not found | Not found |
| NP_001167723.1 | KWINERAY | Tyrosine kinase phosphorylation site. |
| NP_001105313.1 | FNDDIQGTAVLAGLL | Malic enzymes signature. |
| NP_001105383.2 | FNDDIQGTAVLAGLL | Malic enzymes signature. |
| NP_001105292.1 | FNDDIQGTAVLAGL | Malic enzymes signature. |
| NP_001150965.1 | FNDDVQGTAVLAGL | Malic enzymes signature. |
| NP_001152396.1 | FNDDIQGTAVLAGLI | Malic enzymes signature. |
| NP_001147966.1 | KITGEFGY | Tyrosine kinase phosphorylation site. |
| NP_001105420.1 | LTRLDENRAKCQL | MDHactive site signature. |
| NP_001132228.1 | VTTLDVVRANTFV | MDHactive site signature. |
| NP_001105603.1 | LTRLHNRALGQI | MDHactive site signature |
| NP_001241749.1 | VTTLDVVRANTFV | MDHactive site signature. |
| NP_001148518.1 | VTTLDVARANTFV | MDHactive site signature. |
| NP_001142100.1 | VTTLDVRAKTFYA | MDHactive site signature. |
| NP_001147160.1 | LTRLHNRALGQI | MDHactive site signature |
| NP_001132302.1 | VTTLDVVRANTFV | MDHactive site signature. |
| NP_001132077.2 | VTTLDVVRANTFV | MDHactive site signature. |
| NP_00114337.1 | VTTLDVRAKTFYA | NADP-MDHactive site signature. |
| NP_001307728.1 | Not found | |

Chromosomal distribution/ Synteny between rice and maize
The maize genome size is greater than rice genome but has two fewer chromosomes relative to rice. Chromosomal map for rice genes was constructed by using chromosome map tool available in Oryza base Integrated Science Database (http://viewer.shigen.info/oryzavw/maptool/MapTool.do) and mapviewer http://www.ncbi.nlm.nih.gov/projects/mapview/map_search.cgi?taxid=4577&build=100.0 and maize bin viewer of MaizeGDB for maize.

Phylogenetic analysis

Amino acid sequences of all the identified C3/C4 photosynthetic genes from rice and maize were aligned separately using ClustalW and the phylogenetic tree was constructed using NJ method of MEGA version 4.0.02 [31–32]. Each node was tested using the bootstrap approach by taking 5000 replications to ascertain the reliability of nodes. The number indicated percentages against each node.

Analysis of Signature and Phosphorylation site prediction

To identify the signature sequences within the protein sequences of C3/C4 photosynthetic genes, the deduced protein sequences of all the C3/C4 photosynthetic genes in rice and maize were analyzed using online SMART motifscan (http://myhits.isb-sib.ch/cgi-bin/motif_scan) and fingerPRINTscan (http://www.ebi.ac.uk/Tools/pfa/fingerprintsscan/). Prediction of Serine, threonine and tyrosine specific Phosphorylation site prediction of C3/C4 protein sequences (Supp Table 1) is done by employing tools NetPhos 2.0 server [33].

Cis-acting regulatory elements/promoter analysis

For promoter analysis, sequences 1,000 bp upstream of the initiation codon of the putative all C3/C4 genes such as CA, PEPC, PEPCX, NADP-ME, NADP-MDH and PPDK were retrieved and subjected to search using CARE program (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) of Plant CARE database to identify cis-regulatory elements [34].

Expression Analysis using publically available Microarray data

Data expression data of rice C3/C4 genes were retrieved from project Web site (http://www.ricearray.org/expression/expression.php) and Gene expression omnibus (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE6901) using chromosome map tool available in Oryza base Integrated Science Database (http://viewer.shigen.info/oryzavw/maptool/MapTool.do) and mapviewer http://www.ebi.ac.uk/Tools/pfa/ngerprintscan/). Data expression data of rice C3/C4 genes were retrieved from project Web site (http://www.ricearray.org/expression/expression.php) and Gene expression omnibus (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE6901) using chromosome map tool available in Oryza base Integrated Science Database (http://viewer.shigen.info/oryzavw/maptool/MapTool.do) and mapviewer http://www.ebi.ac.uk/Tools/pfa/ngerprintscan/). For promoter analysis, sequences 1,000 bp upstream of the initiation codon of the putative all C3/C4 genes such as CA, PEPC, PEPCX, NADP-ME, NADP-MDH and PPDK were retrieved and subjected to search using CARE program (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) of Plant CARE database to identify cis-regulatory elements [34].

Result And Discussion

Chromosomal Mapping of Maize photosynthetic pathway genes

All of the 6 genes [37 isoforms viz CA-11, PEPC-4, PEPCX-2, PPDKX-3, NADP-ME-6 and NADP-MDH-11 isoforms] of C4 photosynthetic pathway were mapped across all ten chromosomes of Zea mays. While for one gene of PEPCX [NM_001309908.1] no matches were found, out of 11 genes of NADP-MDH two genes [NM_001309908.1 and NM_001147865.1] were not placed while for one gene [NM_001302799.1] no matches were found. On further analysis it was found that only a single gene of beta Carbonic Anhydrase [NM_001111889.1] was present as two copies at a position on larger arm of chromosome 3 and 8 shown connected by a blue line, while rest of them were present as single copies. Genes of carbonic anhydrase were present in 4 alpha and 7 beta form. Alpha forms were concentrated on Chromosome 1, 2, 4 and 9 while beta forms were concentrated on 2, 3, 7 and 8. Most of the genes were scattered across 10 chromosomes except Malate dehydrogenase, 5 isoforms of which were present on Chromosome 1 and rest were distributed across chromosome 1, 3, 4 and 6, while out of 2 genes of PEPCX only one [NM_001152706.1] is found on larger arm of Chromosome 9. Out of three genes of PPDK two were present on chromosome 6 while Chromosome 10 had only single gene. 6 isoforms of NADP-ME were distributed across Chromosome 3, 5, 6 and 8. All four isoforms of PEPC genes were scattered respectively on different chromosome namely 4, 5, 7 and 9.

Chromosomal Mapping of Rice photosynthetic pathway genes

All of 6 genes [30 isoforms viz CA-4, PEPC-6, PEPCX-3, PPDKX-2, NADP-ME-5 and NADP-MDH-10 isoforms] of C3 photosynthetic pathway were mapped across all twelve chromosomes of Oryza sativa and it was found that all genes existed as single copy. All genes were scattered but most of isoforms were concentrated on Chromosome 1 and no gene was present on Chromosome 6, while single isoforms were concentrated on Chromosome 7, 11 and 12. Out of four isoforms of Carbonic anhydrase two alpha forms were present on Chromosome 9 and 11 while two beta forms were present on Chromosome 1 and 9. Two isoforms of gene PPDK were present on Chromosome 3 and 4. Out of four isoforms of gene NADP-ME, three were present on Chromosome 1 and one was present on Chromosome 2. Isoforms of PEPC were present on Chromosome 1, 2, 8 and 9. Isoforms of NADP-MDH were present on 1, 3, 4, 5, 7, 8, 10 and 12. No gene was present on Chromosome 6.

Chromosomal distribution and the synteny between rice and maize

There is some co-linearity between rice and maize at whole genome level. The orthologs from photosynthetic gene families in both rice and maize were mapped and their corresponding chromosome locations are summarized (Fig. 1A & B). Expansion and inversion of some chromosome region were also revealed during comparative synteny in rice. Most of the genes present on Chromosome 1 of rice showed synteny with genes present on Chromosome 1, 3, 6, 8
and 9. Distribution of photosynthetic genes across chromosome of rice and maize is not uniform but phylogenetic analysis has revealed macro colinearity in between the isoforms of gene to a great degree. Isoforms of genes present on Chromosome 1 of rice showed a higher synteny with genes of maize with beta form of Carbonic Anhydrase [Os01g0639900] of rice present on chromosome 1 depicting synteny with 4 other beta isoforms(NM_001175228.1) present on Chromosome 8 and (NM_001156555.1, NM_001159433.1 and NM_001157959.1) present on Chromosome 3 respectively. 2 isoforms of NADP-ME (Os01g0723400 and Os01g0743500) on rice showed synteny with NADP-ME (NM_001158924.1) on Chromosome 3 of maize. Alpha form of Carbonic Anhydrase of rice (Os11g0153200) on Chromosome 11 showed synteny with beta form of Carbonic anhydrase (NM_001155560.1 and NM_001148559.1) on Chromosome 2 of maize. Alpha form of Carbonic anhydrase (Os09g0454400) present on Chromosome 9 showed synteny with alpha Carbonic anhydrase (NM_001155560.1 and NM_001156214.1) present on Chromosome 1 and Chromosome 2. Two genes of PPDK present on Chromosome 3 and 5 (Os03g0432100 and Os05g0405000) of rice showed synteny with PPDK Gene (NM_001112268.2) of maize on Chromosome 6. Also PPDK gene of maize (NM_001174252.1) on Chromosome 6 showed synteny with PPDK gene (NM_001148446.1) on Chromosome 10 of maize.

Comparative phylogenetic analysis between rice and maize individual C3/C4 genes

Phylogenetic analysis of photosynthetic genes of C4 and C3 plants [Zea mays and Oryza sativa] was done and evolutionary trees were constructed using MEGA 4. These evolutionary trees were used to develop relationship among isoforms of same gene among C4 and C3 plants [Zea mays and Oryza sativa] depicted through chromosomal map. Amino acid sequences of C3 and C4 photosynthetic genes of rice and maize were aligned using Clustal W to study their phylogenetic relationship by constructing rooted (Fig. 2a,b,c,d,e & f) tree using MEGA4. Rooted trees for 6 genes were constructed and analysed for synteny. Phylogenetic trees of 6 genes showed variance to a great degree. In carbonic anhydrase, Phylogenetic analysis revealed tree to be constructed of two big clusters A or beta form and B (only alpha form), where cluster A was subdivided into two small clusters a and a2. a1 was further dividing into x and y, while a2 into m and n clusters. Cluster B was dividing into two small cluster b1 and b2. In cluster a1 Beta isoform of CA (Os01g0639900) of rice present on chromosome 1 showed higher degree of synteny with 4 other beta isoforms (NM_001175228.1) present on Chromosome 8 and (NM_001156555.1, NM_001159433.1 and NM_001157959.1) present on Chromosome 3 respectively and NM_001156651.1, NM_001159433.1 also showed synteny with each other(100%). In cluster b2 beta form of CA (Os09g0464000) present on chromosome 9 showed synteny with CA of maize NM_001146913.1 and NM_001156214.1 present on chromosome 2 and 7 respectively. In cluster b alpha form of CA of rice (Os09g0454400 and Os11g0153200) present on Chromosome 9 and 11 showed synteny of CA with maize (NM_001148559.1 and NM_001155560.1) present on Chromosome 2 and 1 respectively. In PPDK, phylogenetic analysis of PPDK gene showed tree to be dividing into 2 clusters A and B. In cluster A gene of PPDK present on rice (Os03g0432100 and Os05g0405000) present on Chromosome 3 and 5 showed synteny with PPDK and PEPCK gene of maize (NM_00112268.2) present on Chromosome 6. Cluster B showed relationship between PPDK gene of maize NM_001174252.1 and NM_001148446.1 present on Chromosome 6 and 10 respectively. In PEPCK, Phylogenetic analysis revealed tree to be dividing into two clusters A and B respectively. In Cluster A gene of PEPCK of rice (Os03g0255000, Os10g0204400) present on Chromosome 3 and 10 respectively showed synteny with PEPCK gene of maize (NM_001152706.1) present on Chromosome 9 and (NM_001309908.1) for which no matches were found. Cluster B showed a single gene of PEPCK (Os04g0592500) present on Chromosome 4 in relation with Cluster A. In PEPCK, Phylogenetic analysis revealed tree to be dividing into two big clusters A and B respectively. Cluster A was further divided into two cluster big a and small b, where cluster a was again divided into one big a1 and one small cluster a2. a1 was further divided into x1(x1 and x2) and y clusters. Cluster y revealed PEP gene of rice (Os02g0244700) present on chromosome 2 in synteny with two isoforms of PEPCK gene of maize (NM_001136893.1, NM_001112033.1) present on Chromosome 4 and 5. Cluster x revealed PEPC gene of rice (Os09g0315700) present on Chromosome 9 in synteny with PEPC gene of maize (NM_001111968.1) present on Chromosome 7. In NADP-ME, phylogenetic analysis revealed tree to be dividing into two clusters big A and small B respectively. Cluster A was further divided into one big cluster a1 and one small cluster a2. a1 into x and y and x into x1 and x2.x1 was further divided into m and n. Cluster x2 revealed relationship between isoform of NADP-ME gene of maize (Os01g0743500) present on Chromosome 1 with isoform of NADP-ME gene of maize (NM_00111822.1) present on Chromosome 8. Cluster x1 revealed 3 relationships involving cluster m1, m2 and n. Cluster m1 revealed relationship between isoform of NADP-ME gene of rice (Os01g0723400) present on Chromosome 1 with isoform of NADP-ME gene of maize (NM_001158924.1) present on Chromosome 3. Cluster m2 revealed relationship between isoform of NADP-ME gene of rice (Os05g0186300) present on Chromosome 5 with isoform of NADP-ME gene of maize (NM_001157959.1) present on Chromosome 6. Cluster n revealed relationship between isoform of NADP-ME gene of rice (Os01g0188400) present on Chromosome 1 with two isoforms of NADP-ME gene of maize (NM_001111963.1, NM_001111913.2) present on Chromosome 3 and 6 respectively. In NADP-MDH, phylogenetic analysis revealed tree to be dividing into two big clusters A and B respectively. Cluster A was further divided into 4 clusters a, b, c and d and cluster B into two x and y respectively. Cluster a1 was further divided into x and y and x into x1 and x2.x1 was further divided into m and n. Cluster a1 revealed relationship between isoform of NADP-ME gene of rice (Os01g0649100, Os05g0574400) present on Chromosome 1 and 5 with 5 isoforms of NADP-ME gene of maize (NM_001148628.2) present on Chromosome 6 and NM_001147865.1 which was not placed. Cluster b1 revealed relationship between isoform of NADP-ME gene of rice (Os12g0632700) present on Chromosome 12 with isoform of NADP-ME gene of maize (NM_001133605.2) present on Chromosome 3. Cluster b2 revealed relationship between isoform of NADP-ME gene of rice (Os03g0773800) present on Chromosome 3 with isoform of NADP-ME gene of maize (NM_001155046.1) present on Chromosome 1. Cluster c revealed relationship between isoform of NADP-ME gene of rice (Os07g0630800) present on Chromosome 7 with isoform of NADP-ME gene of maize (NM_001320709.1) for which no matches were found. Cluster d consisting of d1 and d2 revealed relationship between two isoforms of NADP-ME gene of rice (Os01g0829800, Os08g0434400) present on Chromosome 1 and 8 with three isoforms of NADP-ME gene of maize (NM_001138303.1, NM_001254820.1 and NM_00128756.1) on Chromosome 1,1 and 4 respectively. Cluster x revealed relationship between isoform of NADP-ME gene of rice (Os08g0562100) present on Chromosome 8 with isoform of NADP-ME gene of maize (NM_00111950.1) present on Chromosome 1. Cluster y revealed relationship between isoform of NADP-ME gene of maize (Os08g0562100) present on Chromosome 3 with isoform of NADP-ME gene of maize (NM_00111950.1) present on Chromosome 1. Cluster y2 revealed relationship between isoform of NADP-ME gene of rice (Os10g0478200) present on Chromosome 10 with isoform of NADP-ME gene of maize (NM_00111233.2) present on Chromosome 1 and NM_001153688.1 which was not placed.

Evolutionary relationship of C3/C4 photosynthetic pathway genes in rice and maize
A rooted tree was constructed to examine evolutionary relationship among C3 and C4 photosynthetic genes of rice and maize by aligning their amino acids using Clustal-W method and tree was constructed using MEGA 4.0 Minimum Evolution method (Fig. 3A). Evolutionary phylogenetic tree analysis revealed tree to be constructed of two big clusters:- A] Cluster A consists of gene of NADP-MDH, Carbonic Anhydrase and PPDK. B] Cluster B consists of gene of NADP-ME, PEPC and PEPPCK. Cluster A consist of 4 small clusters. Cluster a reveal a relationship among NADP-MDH gene of rice and maize. Evolutionary analysis shows NADP-MDH gene of glyoxysomal and mitochondrial origin of rice and maize to have evolved together and also revealing a relation between glyoxysomal and chloroplastic NADP-MDH gene of rice and maize respectively. Cluster b also revealed relation between cytosolic and chloroplastic genes of NADP-MDH of rice and maize. Cluster c revealed a relation between chloroplastic origin of beta CA of rice and maize. Cluster d revealed a unique relationship between single isoform of PEPPCK gene of rice (Os04g0592500) and two isoforms of PPDK gene of maize (NP_001141918.1, NP_001167723.1). Cluster B consist of three clusters. Cluster e revealed relation between alpha CA of rice and maize and that of NADP-ME of maize with PEPC gene of rice. Cluster f and g revealed relation between PEPC and NADP-ME of rice and maize respectively Also individual analysis of rice and maize genes was also done by constructing tree by respectively aligning their amino acids through Clustal-W method showed in (Fig. 3B & 3C). Figure 3B depicts evolutionary tree of photosynthetic genes of rice and depict common origin of all photosynthetic genes respectively except that of PEPPCK gene (Os04g0592500). Figure 3C depicts evolutionary tree of photosynthetic genes of maize and depict common origin of all photosynthetic genes respectively except that of PPDK gene (NP_001141918.1).

Cis-Regulatory Elements Analysis

A total of 25 CARE [14 in rice and 11 in maize] viz ARE, MBS, TC-rich repeats, 5 UTR Py-rich Stretches, GC-motif, HSE, LTR, Box-W1, Box-W2, GCC-Box, Pc-CMA2a, WUN motif, MNF, EIRE and ABRE were observed in rice (-1 kb) and maize (-5 kb). Upstream regions were analyzed using Plant CARE to study correlation of transcriptional regulation of C3 and C4 photosynthetic genes among rice and maize. These elements were mainly associated with plant growth and development, stress response and hormone responsiveness, meristem-specific expression but here we focus on stress responsiveness element which frequency distribution both rice and maize were shown in Fig. 4 & listed in supplementary Table 1. A total number of 25 cis-regulatory elements (CREs) [14 in rice and 11 in maize] which had stress responsive role were identified namely anoxia-response element (ARE), MYB-binding site (MBS), defense and stress responsive elements (TC-rich repeats), wound-responsive element (WUN-motif), heat shock element (HSE), ABA-response element (ABRE), 5 UTR Py-rich Stretches, LTR, Box-W1, GCC-Box, Pc-CMA2a, WUN-motif, MNF, EIRE and ABRE. Name and frequencies of identified CARE with respect to C4 photosynthetic genes of rice and maize is listed in respectively Table 2A and 2B. Presence of the stress related motifs showed altered gene expression of photosynthetic genes under stress. In Rice, Anoxia-response elements (ARE) involved in anaerobic induction was most commonly observed in almost all genes in moderate frequency with higher expression in NADP-MDH gene Os08g0434300. MYB-binding site (MBS) was also present in moderate frequency in almost all genes with higher expression in PEPPCK gene (Os10g0204400) and NADP-MDH gene (Os01g0649100). TC-rich repeats present in moderate frequency in almost all genes. 5 UTR Py-rich Stretches were observed in moderate frequency in almost all genes with most expression in NADP-MDH gene (Os03g0773800). GC-motif present in moderate quantity in few genes with most expression in PPDK gene (Os05g0405000) and in Carbonic Anhydrase (Os01g0639900). HSE heat shock element factor involved in heat stress response were present in moderate frequency in few genes with most expression observed in Carbonic Anhydrase (Os11g0153200). LTR and L-box were present in low frequency in only 4 different genes. Box-W1- fungal elicitor responsive element (TTGACC) were present in low frequency in only 3 genes. GCC-Box, WUN-motif and Pc-CMA2a were present only in 3, 3 and 2 genes with low frequency. MNF and EIRE were present in low frequency in only single genes. In Maize, Anoxia-response elements (ARE) involved in anaerobic induction was most commonly observed in almost all genes in moderate frequency with higher expression in PPDK gene (ZM2G097457). MYB-binding site (MBS) were also present in moderate frequency in almost all genes with higher expression in PEPPCK gene (ZM2G001696). TC-rich repeats present in moderate frequency in few genes with most expression in NADP-MDH gene (Zm2G1545595). 5 UTR Py-rich Stretches were observed in moderate frequency in 4 genes. HSE involved in heat stress response were present in low frequency in only 3 genes. LTR were present in low frequency with exception of NADP-MDH gene (Zm2G068455) with higher expression. L-box was present in low frequency in single gene. Box-W1- fungal elicitor responsive element (TTGACC) was present in low frequency in only 7 genes. EIRE was present in low frequency in only single genes. MNF was present in low frequency in only 5 genes. ABRE present in higher frequency in only single gene of Carbonic Anhydrase (Zm2G6414528).

Post translational modification (phosphorylation) analysis of C3/ C4 protein sequences of rice and maize

Graphs of phosphorylation site of 6 photosynthetic genes of C3 and C4 plants were examined and data was analysed and summarized in (Supp Table 2A & 5A & 5B). In Os-CA Serine specific phosphorylation sites were present in abundance in all isoforms of genes of rice while that of Threonine and Tyrosine varied in all isoforms with most Threonine specific sites were present in Os09g0454400 and most Tyrosine specific sites were present on Os01g0639900. In general more phosphorylation sites were present on beta CA than alpha CA in rice. 2] Zm-CA In maize Serine specific phosphorylation sites were present in abundance in all isoforms of genes with highest being in NP_001105359.1. In general Threonine specific phosphorylation sites were less present with their absence in NP_001151431.1. Tyrosine specific phosphorylation sites were also present in moderate quantity in all isoforms of genes of CA. In general NP_001105359.1 isoform of beta CA gene of maize showed higher number of phosphorylation sites. Here clearly revealed that in comparison of both maize and rice more phosphorylation active sites were present on beta CA isoforms of gene and more present on maize especially in NP_001105359.1. 3] Os-PEPC Almost equivalent number of Serine, Threonine, and Tyrosine specific phosphorylation sites present in all isoforms of PEPC genes. 4] Zm-PEPC, Almost equivalent number of Serine, Threonine, and Tyrosine specific phosphorylation sites present in all isoforms of PEPPCK genes except NP_001130365.1 where less sites are present. Note that, In comparison of both maize and rice more phosphorylation active sites were present on rice. 5] Os-PEPPCK Almost equivalent number of Serine and Threonine specific sites were present on all isoforms of PEPPCK genes of rice. Tyrosine specific sites were present in moderate number in all isoforms except Os04g0592500 where number was less. 6] Zm-PEPPCK, Almost equivalent number of Serine, Threonine, and Tyrosine specific phosphorylation sites present in all isoforms of PEPPCK gene of maize. It to be note that in comparison of both maize and rice more phosphorylation active sites were present on rice especially Os03g0255500. 7] Os-NADP-MDH Only equivalent number of low Tyrosine specific phosphorylation active sites was present on all isoforms of genes while that of Serine and Threonine specific sites number varied. Serine specific sites were present more in Os01g0649100 of glyoxysomal origin
while Threonine specific sites were present in higher number in Os03g0773800 of rice. 8] Zm-NADP-MDH, Number of sites for Serine, Threonine and Tyrosine specific phosphorylation sites varied in number for all isoforms of genes. In general number of Threonine specific sites was present in higher quantities in Zm-NADP-MDH compared to all other genes in maize and rice. More number of phosphorylation sites was present on NP_001132228.1 of glyoxysomal origin. Here clearly revealed that In comparison of both maize and rice more phosphorylation active sites were present on maize especially NP_001132228.1. 9] Os-NADP-ME Moderate number of Serine, Threonine and Tyrosine specific sites were present on all isoforms while more number of sites was present on Os05g0186300. 10] Zm-NADP-ME Almost equivalent number of Serine, Threonine, and Tyrosine specific phosphorylation sites present in all isoforms of NADP-ME gene of maize with most expression in NP_001105313.1. In comparison of both maize and rice more phosphorylation active sites were present on maize especially NP_001105313.1. 11] Os-PPDK Almost equivalent number of Serine, Threonine, and Tyrosine specific phosphorylation sites present in all isoforms of PPDK gene of rice. 12] Zm-PPDK, Number of Serine, Threonine, and Tyrosine specific phosphorylation sites varied with most number of sites present in NP_001105738.2. In comparison of both maize and rice more phosphorylation active sites were present on maize especially NP_001105738.2.

Identification of protein signature

A table of signature sequences of rice and maize were listed and following observations were made and showed in (Table 3A & 3B), isoforms of PPDK gene revealed two conserved signature sequences. Isoforms of NADP-ME gene revealed two sequences - one conserved and one variable among all isoforms. Two isoforms of PEPCK gene showed common signature [ATP signature] while one Os04g0592500 showed varied signature [Tyrosine kinase phosphorylation site]. Six isoforms of PPCK gene showed two common signature sequences. Beta Carbonic anhydrase isoforms showed two common signature sequences while only one alpha Carbonic Anhydrase showed one common signature while no signature sequences were found in Alpha CA (Os11g0153200). NADP-MDH isoforms showed one common signature sequences to all while only a second tyrosine kinase phosphorylation site was present in (Os01g0829800 and Os08g0562100). While maize, All alpha and beta isoforms of Carbonic Anhydrase had different conserved sequences while no sequences were found for alpha CA (NP_001146392.1). All PEPCK isoforms have two signature sequences varying for each gene. All NADP-MDH isoforms have almost single conserved sequence with no signature sequences found for NP_001307728.1. All NADP-ME isoforms have almost two conserved signature sequences with single sequence present in NP_001147966.1. All 9 PEPCK isoforms of gene have almost same signature sequences. All two PECK isoforms of gene have same signature sequences.

Expression profiling during plant anatomical stage and across different plant tissues shows differential transcriptional regulation in rice and maize.

In rice, we observed a much diverse pattern of expression of C4 genes during anatomical stage (Fig. 6A). The genes which showed very low expression throughout the anatomical stage were OsPEPCK-2, OsPEPCK-3, OsPEPC-1 and OsPEPC-2. It was further revealed that while several C4 genes show near constant expression pattern (at a either down regulated or up regulated, a few members showed tissue specific expression and few are ubiquitously expressed across all plant tissues. OsPEPC-3 showed down regulated expression and moderate up regulated expressions were observed at Suspension, stroma, ovary, developing anther, mature anther, embryo sac and embryo stage. Cytosolic enzyme, OsNADP-MDH-5, uniquely showed predominantly high up regulated expression at all stages. Further observations revealed that chloroplastic OsNADP-ME-1, OsPEPC-4, chloroplastic OsCAAlfa-1, glyoxysomal OsNADP-MDH-3 and glyoxysomal OsNADP-MDH-7 were found to be fairly consisten up regulated. OsPPDK-1 was found to be high up regulated at stages which include internode pith parenchyma, root tip, spikelet, embryo and endosperm, which highlights its significant role in plant nutrition and protection and down regulated at stoma and ovary stage. Chloroplastic OsPPDK-2 was found to give active up regulated expression at callus, suspension cell and dry seed stage. Chloroplastic, NADPME-2 showed overall moderate up regulated expression. Cytoplasmic OsNADPME-3, Chloroplastic OsNADPME-4 and OsNADPME-5 showed overall down regulated expression. Interestingly, OsNADPME-3 exhibit up regulated expression specifically at dry seed, embryo-sac and endosperm stage. OsPEPCK-1 showd tissue specific function and was high up regulated for leaf, root, leaf and endosperm. OsPEPC-5 was not expressed and show down regulated expression for coleoptiles and germination seed. Chloroplastic OsCAAlfa-1 showed varied expression from down regulated to moderate up regulated expression. OsCA Beta-2, a chloroplastic enzyme, exhibit down regulated expression for developing anther which is crucial reproductive structure. For all other stages the expressions were up regulated. OsCA Alfa-2 exhibit down regulated expression at all anatomical stage. Both glyoxysomal OsNADP MDH-1 and OsNADPMDH-2 showed moderate up regulated expression except for down regulated expression for dry leaf and flaq leaf stage respectively. Glyoxysomal OsNADP MDH-4 expressed fairly up regulated but expressed down regulated at whole plant stage. Further study revealed that chloroplastic OsNADP-MDH-6 was down regulated at dry seed, coleoptiles and root tip stage where as it showed high up regulated expression for all other specific tissues. Glyoxysomal OsNADP MDH-8 and cytosolic OsNADP MDH-9 showed no significant role across all anatomical stage. Glyoxysomal OsNADP MDH-10 was found to be differentially expressed across the various anatomical stage. It showed high expression for specific tissues including whole plant, seedling shoot, leaf, flaqleaf, spikelet and down regulated expression for suspension cell, root tip, SAM and developing anther. It showed moderate up regulated expression for other specific tissues.

To know some facts, functions and diverse expressions of C4 genes in *Zea mays*, we analyzed the micro-array based expression pattern at different anatomical stage (Fig. 6B)
regulated expression for root, root tips and ear inter-node. ZmPEP-2 exhibit varied expression. It showed up regulated expression at adult, leaf, blade (lamina), juvenile leaf, foliar life, shoot, leaf and seedling stage whereas it is fairly down regulated at shoot apex, ear internode, internode, culm (main stem) and pollen stage. Further observations revealed that cytosolic enzyme Zm NADP-2 uniquely up regulated expressed across all stages and highly up regulated at ear internode, pulvinus and glume stage. Notably, Chloroplastic ZmNADPME-3 showed up regulated expression at embryo and endosperm stage. It confirms that this enzyme played a vital role in fertilization and providing nutrition. ZmPEPCK-1 showed up regulated expression for adult leaf and blade (lamina) and fairly upregulated at juvenile stage, foliar leaf, shoot, leaf and seedling stage and for rest of the stage it showed either no expression or negligible expression.

ZmPDK-1, a chloroplast localised enzyme expressed high up regulated at endosperm stage which clearly shows that it holds nutritive function in plants and its overall expression was down regulated. Mitochondrial ZmMDH-3 showed overall fairly up regulated expression except at root stage where it showed up regulated expression. Zm MDH-4, a cytosolic enzyme showed upregulated expression except at pollen, carpoply, shoot apex and lateral root stage.

Mitochondrial ZmNADPME-4 showed high up regulated expression for pollen and gave no expression that is, down regulated at endosperm and caropsis stage. Glyoxysomal ZmMDH-5 showed up regulated expression for root tip and fairly up regulated for endosperm and embryo stage. Chloroplastic enzymes ZmCA Beta-4 and ZmCA Beta-5 all down regulated expression across all stages except at pollen stage where ZmCA Beta-4 expressed and predominantly up regulated. Glyoxysomal ZmMDH-6, ZmPEP-3 and chloroplastic ZmPDK-2 were expressed at down regulation in most of the stages, with moderate and up regulated expression in some specific stage that indicate their stage specific role.

Expression profiling during plant development and across different plant tissues shows differential transcriptional regulation in rice and maize.

To gain some insights into the possible function of C4 genes in rice, we analyzed the micro-array based expression pattern at different developmental stage (see method). During analysis, we observed a much diverse pattern of expression of C4 genes during all development stages. (Fig. 7A). The enzymes which showed overall down regulated expression throughout the developmental stage studies were OsPEPCK-2, OsPEPCK-3, OsPEP-1, OsPEPCK-2 and OsPEPC3. Interestingly, OsPEPCK-3 showed major involvement only at Panicle 6 and had up regulated expression. OsPEPCK-1, OsPEPCK-2 and OsPEPC3 genes were expressed in most of the stages with moderate up regulation expression in some specific stage. Amongst the glyoxyosomal enzymes, OsNADP MDH-1, OsNADPMDH-2, OsNADPMDH-3 & OsNADPMDH-4, OsNADPMDH-3 was highly up regulated. OsNADP MDH-1 was moderately down regulated at pre-germination stage while OsNADP MDH-5, OsNADPMDH-2 and OsNADP MDH-4 were found to be moderate up regulated for P1, P2, P3 and P4 stage. OsNADPMDH-5, a cytosolic enzyme showed most predominately up regulated expression across all the stages. The expression for OsNADP MDH-6, which is localised in chloroplast, was found to be down regulated at pre-germination stage and moderate down regulated at S5 stage. Other gene enzymes were found to be differentially expressed across the various developmental stages. Among glyoxyosomal OsNADP MDH-7 & OsNADP MDH-1, OsNADP MDH-7 showed up regulated expression at every stage except for S4 and S5 where moderate up regulated expression was found while OsNADP MDH-10 showed moderate down regulated expression for P1, P2, P3 stage and moderated up regulated expression for callus suspension and germination seedling stage which is crucial for initiating development of plant. In Glyoxyosomal OsNADP MDH-8 and Cytosolic OsNADP MDH-9, overall expression at all stages is down regulated. OsPEPC-4, OsPEPC-5 showed up regulated expression except for down regulated expression at maturing seedling stage in OsPEP-5. Chloroplastic OsCA Beta-2 and OsCA Alfa-1 expressions varied from moderate up regulated to high upregulated. P1, P2 and P3 stage was found to be down regulated in OsCA Alfa-1. Further studies revealed that, OsCABeta-1 and OsCA Alfa-2 showed gross down regulated expression. Chloroplastic OsCABeta-1 showed up regulated expression for stages including pre-germination, tillering stage, S1 and S5. It was further observed that, among chloroplast localised enzyme, OsPDK1 and OsPDK2, the later showed down regulated expression for 1st leaf, 2nd leaf, 3rd leaf, tiller initiation, tillering stage, P1, P2, P3,P4, P5 stages whereas OsPDK1 showed fairly high upregulated expression for the same stages. Chloroplastic enzymes, OsNADP ME-1 & OsNADP ME-2, were found to be consistently upregulated except for the down regulated expression at pregermination stage in OsNADP-ME-2. Further expressions revealed that, cytosolic OsNADP-ME-3 and chloroplast localised OsNADP-ME-4 and OsNADP-ME-5 showed overall expression of down regulated. At Pregeneration, S4 and S5 stage upregulated expression was observed in OsNADP- ME-3. OsPEPCK-1 has shown varied expressions. At 2nd leaf, S4 & S5 stage, expressions were observed most up regulated while at callus suspension, pregermination, 3rd leaf, tiller initiation, tillering stage, P5, P6, S1, S2 and S3 moderate up regulated expressions were observed.

To gain insights into potential physiological function in Z. mays, we have studied their expression at different stages of plant development and across various plant tissues showed in (Fig. 7B). The enzymes that showed the down regulated expression throughout were chloroplastic enzymes, ZmCA Alfa-2, ZmCA Beta-4, ZmCA Beta-5 and ZmPDK-2. ZmMDH-4 which is localised in cytosol uniquely showed predominately up regulated at all developmental stage except for dough stage which is down regulated. ZmCABeta-1 and chloroplastic enzymes ZmCA Beta-2 and ZmCA Beta-3 showed moderate up regulated expression for seedling stage which is important to understand the cotyledon patterns. ZmCABeta-2 exhibit fairly up regulated expression at anthesis and inflorescence formation. ZmCA Alfa-1, a chloroplastic enzyme exhibit overall down regulated expression at all developmental stage. Further, chloroplastic ZmMDH-1 was found to exhibit down regulated expression at dough stage, fruit formation and germination stage while up regulated expressions were observed for inflorescence formation and seedling stage, which holds many important functions during reproduction and provide nutrients to fruits and flowers. Further studies revealed that, ZmNADP-ME-1 showed overall down regulated expression except for seedling stage, for which moderate up regulated expression was observed. ZmPEP-1 and glyoxyosomal ZmMDH-2 showed overall moderate down regulated expression at all stages. ZmPEP-2 expressed up regulated only for seedling stage and its overall expression is down regulated. Further it was observed that ZmNADP ME-2, localised in cytosol, showed varied expressions from down regulated to high regulated. For dough stage and formation, down regulated expression was observed whereas fairly up regulated expression was observed for anthesis and seedling stage. The enzyme expressed most up regulated for inflorescence and stem elongation stage. ZmNADPME-3, a chloroplastic enzyme, exhibits high expression for dough stage and fruit formation and was up regulated. ZmPEPCK, glyoxyosomal ZmMDH-6 and ZmPEPC-3, showed overall down regulated expression except for seedling stage, dough stage and germination stage, where respectively these enzymes expressed up regulated. The expression exhibit by chloroplastic ZmPPDK-1 was up regulated for dough stage and fruit formation whereas at rest of the stages, its expression was down regulated. The expression of mitochondrial ZmMDH-3, was overall fairly up regulated except for germination stage, where its expression was up regulated the most. Mitochondrial ZmNADP ME-4 was found to be differentially expressed across the various developmental stages. The enzyme did not expressed for dough stage and fruit formation stage and fairly up regulated at stem elongation, seedling stage and germination stage. Notably, it was
highly expressed for anthesis and inflorescence formation and thus expressed up regulated. ZmMDH-5 which is localised in glyoxysomes consistently expressed down regulated across all developmental stage.

Regulation of Transcription of photosynthetic genes in Rice and Maize under Stress

Analysis of expression of C3 and C4 photosynthetic genes under stress conditions was done using publically available microarray databases result of which is showed in Fig. 8A & 8B.

Expression of rice genes for stress (Seedling, Drought, Salt and Cold)

NADP-MDH gene isoforms (6 and 12) were down regulated, NADP-MDH (1, 2, 3, 4, 5, 8, 9 and 11) was upregulated while moderate expression was observed in NADP-MDH (7 and 10). PEPC gene isoforms (1, 3, and 6) were upregulated while PEPC isoforms (2, 4 and 5) were downregulated. PECK gene isoforms (2 and 3) were downregulated while isoform (1) was upregulated. PPDK gene isoform (2) was upregulated and isoform (1) was downregulated. NADP-ME gene isoform (1 and 3) was upregulated while (2, 4 and 5) was downregulated. Carbonic Anhydrase gene isoform alpha (1 and 2) was downregulated while beta (1 and 2) was upregulated.

Expression of maize genes for stress (Water and drought)

All 6 isoforms of NADP-MDH gene were downregulated as well as moderate for almost all stress factors. All three isoforms of PEPC gene isoforms were generally downregulated for almost all stress factors with exception for Water (6, 7, 13 and 14) respectively. Single PECK gene isoform was downregulated for stress factor (Water1-7) moderate for stress factor (Water 8, 9, 10, 11 and 12) while upregulated for stress factor (Water 13 and 14). Two isoforms of PPDK gene showed moderate and downregulation for various stress factors. All 4 isoforms of NADP-ME gene were downregulated as well as moderate for almost all stress factors. All five isoforms of beta Carbonic Anhydrase were downregulated for almost all stress factors except Water (13 and 14) while all both isoforms of alpha Carbonic Anhydrase were also down regulated for all stress factors. Comparative analysis of regulation of photosynthetic genes of rice and maize under stress reveals a total of down regulation of genes of maize in response to stress factors while that of rice was generally upregulated and moderate. This result is corresponding to CARE analysis given in Fig. 4A and B which showed number and frequency of cis acting regulatory elements high in rice (14 in rice and 11 in maize) CARE such as ARE, MBS, TC-rich repeats, GC motif, HSE were present in higher frequency in rice thus corresponding to the fact of upregulation and moderate expression of C3 photosynthetic genes of rice under stress.

Conclusion

In this investigation, we conducted a comprehensive insilico investigation and identification C3/C4 photosynthesis gene/protein sequences in rice and maize. A complete overview of CA, PEPC, PECK, NADPH-ME, NADP-ME and PPDK genes/ protein sequences in rice and maize is presented, including the chromosomal mapping, evolutionary phylogeny relationship, serine, threonine and tyrosine specific phosphorylation site and protein signature for assessment of posttranslational modification and their cis acting regulatory elements analysis. Analysis of protein signature revealed some conserve protein motif present in each protein sequences between rice and maize. Protein phosphorylation analysis clearly revealed role in posttranslational modification in C3/C4 genes in both crops but maximum present in maize. The cis-regulatory element analysis of the C3/C4 photosynthesis gene revealed the major putative functions as regulation of genes associated with plant growth development, abiotic and biotic stress, growth hormone and light response. Presence of high number of stress responsive cis acting element in upstream suggests that these proteins might be unregulated in plant stress tolerance. The expression data needs to be correlated in revealing the function of these protein and role in plant stress. A comprehensive analysis on gene expression may provide a better assessment of the prospective genes/protein for crop improvement. Genome editing based cisgenic approach may be utilized to validate its potential candidature for crop improvement.

Declarations

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Authors’ contributions

SP conceived the idea and design the all experiment, SP and VR performed experiments and wrote the manuscript critically evaluated the manuscript and approved the final version of the manuscript.

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Availability of data and materials

Most data supporting the results are included in the article. The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate
The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Competing interests

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**Figures**

**Figure 1**

Genomic distribution and syntenic mapping of C3/C4 photosynthetic genes from rice and maize
Figure 2
Phylogenetic tree of all CA, PEPC, NADP-MDH, NADP-ME, PECK, PPDK protein sequences of rice and maize. All genes showed individual tree Fig 1. [A] for CA, [B] for PEPC, [C] NADP-MDH, [D] for NADP-ME, [E] for PECK, & [F] for PPDK. The phylogenetic tree was constructed by N-J method using MEGA version 4.0.02.
Figure 3

Evolutionary phylogenetic tree analysis of whole CA, PEPC, NADP-MDH, NADP-ME, PEPCK, PPDK protein sequences between rice and maize. Construction of the tree was based on minimum evolution method of MEGA version 4.0.02 using 5000 bootstrap replicates.
Figure 4

Prediction and frequency distribution of Cis-regulatory elements in the upstream region of CA, PEPC, NADP-MDH, NADP-ME, PEPCK, PPDK genes rice and maize
Figure 5

Protein phosphorylation frequency prediction (Serine, threonine & tyrosine specific) using Netphos (CBS Server)
Figure 6

Expression profiling of C3/C4 photosynthesis genes of Rice and Maize at different developmental stages using microarray data. Heat map and hierarchical cluster display differential expression profile for above genes. Various stages are listed in the temporal order of development. The colour bar on top represents relative expression values.
Figure 7

Expression profiling of C3/C4 photosynthesis genes of Rice and Maize at different Anatomical (tissue specific) using microarray data. Heat map and hierarchical cluster display differential expression profile for above genes. Different tissue are listed in the temporal order of development. The colour bar on top represents relative expression values.
Figure 8

Expression profile of C3/C4 photosynthesis genes under abiotic stress conditions. Heat maps show the microarray-based expression pattern of C3/C4 genes from Rice (A) and Maize (B) under conditions of seedling, salt, drought and cold as indicated at the bottom of the heat map. Color bars at the bottom of each of the heat maps show the corresponding scale for log2 fold change in expression. Heat maps were generated using hierarchical clustering for which weighted average linkage method and Pearson correlation distance metric were used.

Supplementary Files

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