

Supplemental Material

Developmental and molecular physiological evidence for the role of phosphoenolpyruvate carboxylase in rapid cotton fibre elongation

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Ser

GhPEPC1 : MAAAGIRLSKKTTEMAGRKRVKMSISDAQRLRLVAFPKVSVDDDLVBYDAVLLDRFLDLDLQDLHGDDLRVTVQVQCYELSAFYEGKSTPKKRLBELGNVLTSLDPEGDSI VTRNS : 110
GhPEPC2 : -----MASFNNNNNNGKPEKLSASIDAQRLRLVBAKVSDDDLVBYDAVLLDRFLDLDLQDLHGDDLRVTVQVQCYELSAFYEGKSTPKKRLBELGNVLTSLDPEGDSI VTRNS : 103
AtPPC1 : -----MANRR-----LEKMASIDVHRLRLVBAKVSDDDLVBYDAVLLDRFLDLDLQDLHGDDLRVTVQVQCYELSAFYEGKSTPKKRLBELGNVLTSLDPEGDSI VTRNS : 97
AtPPC2 : -----MAARN-----LEKMASIDVHRLRLVBAKVSDDDLVBYDAVLLDRFLDLDLQDLHGDDLRVTVQVQCYELSAFYEGKSTPKKRLBELGNVLTSLDPEGDSI VTRNS : 97
LePPC1 : -----MTRRN-----LDKMASIDVHRLRLVBAKVSDDDLVBYDAVLLDRFLDLDLQDLHGDDLRVTVQVQCYELSAFYEGKSTPKKRLBELGNVLTSLDPEGDSI VTRNS : 97
LePPC2 : -----MTRRN-----LEKMASIDVHRLRLVBAKVSDDDLVBYDAVLLDRFLDLDLQDLHGDDLRVTVQVQCYELSAFYEGKSTPKKRLBELGNVLTSLDPEGDSI VTRNS : 97
ZmPEPC-C3 : -----MAALG----PKMERLSISDAQRLRLVBAKVSDDDLVBYDAVLLDRFLDLDLQDLHGDDLRVTVQVQCYELSAFYEGKSTPKKRLBELGNVLTSLDPEGDSI VTRNS : 99
ZmPEPC-C4 : -----MASTKAP---GPGSRHHSISDAQRLRLVBAKVSDDDLVBYDAVLLDRFLDLDLQDLHGDDLRVTVQVQCYELSAFYEGKSTPKKRLBELGNVLTSLDPEGDSI VTRNS : 101

GhPEPC1 : FSHMLNLGLAEEVQIAARRRRLKLRK-CDADENSATTESSDDEETKRLVLDLKKSPBEVFDALKNQTVDLVLTAAHTQSVRRSLLQKHGRIRNCLTQLYAKDITDDDKQ : 219
GhPEPC2 : FSHMLNLGLAEEVQIAARRRRLKLRK-CDADENSATTESSDDEETKRLVLDLKKSPBEVFDALKNQTVDLVLTAAHTQSVRRSLLQKHGRIRNCLTQLYAKDITDDDKQ : 212
AtPPC1 : FSHMLNLGLAEEVQIAARRRRLKLRK-CDADENSATTESSDDEETKRLVLDLKKSPBEVFDALKNQTVDLVLTAAHTQSVRRSLLQKHGRIRNCLTQLYAKDITDDDKQ : 207
AtPPC2 : FSHMLNLGLAEEVQIAARRRRLKLRK-CDADENSATTESSDDEETKRLVLDLKKSPBEVFDALKNQTVDLVLTAAHTQSVRRSLLQKHGRIRNCLTQLYAKDITDDDKQ : 206
LePPC1 : FSHMLNLGLAEEVQIAARRRRLKLRK-CDADENSATTESSDDEETKRLVLDLKKSPBEVFDALKNQTVDLVLTAAHTQSVRRSLLQKHGRIRNCLTQLYAKDITDDDKQ : 207
LePPC2 : FSHMLNLGLAEEVQIAARRRRLKLRK-CDADENSATTESSDDEETKRLVLDLKKSPBEVFDALKNQTVDLVLTAAHTQSVRRSLLQKHGRIRNCLTQLYAKDITDDDKQ : 206
ZmPEPC-C3 : FSHMLNLGLAEEVQIAARRRRLKLRK-CDADENSATTESSDDEETKRLVLDLKKSPBEVFDALKNQTVDLVLTAAHTQSVRRSLLQKHGRIRNCLTQLYAKDITDDDKQ : 208
ZmPEPC-C4 : ILSHMLNLGLAEEVQIAARRRRLKLRK-CDADENSATTESSDDEETKRLVLDLKKSPBEVFDALKNQTVDLVLTAAHTQSVRRSLLQKHGRIRNCLTQLYAKDITDDDKQ : 211

loopIII: regulatory function

conserved motif I for catalytic activity

1

GhPEPC1 : ELDEALQREIQAAFRTDEIRRTPTPQDEMAGMSYFHEHTVWKGVPKFLRRVDTALKNI GINERVYPYNAPLIQFSSWMGGDRDGNPRVTEVTRDVCLLARMMMAANLYYS : 329
GhPEPC2 : ELDEALQREIQAAFRTDEIRRTPTPQDEMAGMSYFHEHTVWKGVPKFLRRVDTALKNI GINERVYPYNAPLIQFSSWMGGDRDGNPRVTEVTRDVCLLARMMMAANLYYS : 322
AtPPC1 : ELDEALQREIQAAFRTDEIRRTPTPQDEMAGMSYFHEHTVWKGVPKFLRRVDTALKNI GINERVYPYNAPLIQFSSWMGGDRDGNPRVTEVTRDVCLLARMMMAANLYYS : 317
AtPPC2 : ELDEALQREIQAAFRTDEIRRTPTPQDEMAGMSYFHEHTVWKGVPKFLRRVDTALKNI GINERVYPYNAPLIQFSSWMGGDRDGNPRVTEVTRDVCLLARMMMAANLYYS : 316
LePPC1 : ELDEALQREIQAAFRTDEIRRTPTPQDEMAGMSYFHEHTVWKGVPKFLRRVDTALKNI GINERVYPYNAPLIQFSSWMGGDRDGNPRVTEVTRDVCLLARMMMAANLYYS : 317
LePPC2 : ELDEALQREIQAAFRTDEIRRTPTPQDEMAGMSYFHEHTVWKGVPKFLRRVDTALKNI GINERVYPYNAPLIQFSSWMGGDRDGNPRVTEVTRDVCLLARMMMAANLYYS : 316
ZmPEPC-C3 : ELDEALQREIQAAFRTDEIRRTPTPQDEMAGMSYFHEHTVWKGVPKFLRRVDTALKNI GINERVYPYNAPLIQFSSWMGGDRDGNPRVTEVTRDVCLLARMMMAANLYYS : 318
ZmPEPC-C4 : ELDEALQREIQAAFRTDEIRRTPTPQDEMAGMSYFHEHTVWKGVPKFLRRVDTALKNI GINERVYPYNAPLIQFSSWMGGDRDGNPRVTEVTRDVCLLARMMMAANLYYS : 321

2

Arg account for subunit interaction

3

4

5

GhPEPC1 : QIDELMFEISMWRQSDRLRRADELHRLR-SKK-DAKHYIFBWKQVPEPEYRVIILGDVRDKLYITREARRSLLANGSDDIPEEAATITVBOFLEPLELCYRSLQACGDREI : 438
GhPEPC2 : QIDELMFEISMWRQSDRLRRADELHRLR-SRR-DAKHYIFBWKQVPEPEYRVIILGDVRDKLYITREARRSLLANGSDDIPEEAATITVBOFLEPLELCYRSLQACGDREI : 431
AtPPC1 : QIDELMFEISMWRQSDRLRRADELHRLR-SRR-DAKHYIFBWKQVPEPEYRVIILGDVRDKLYITREARRSLLANGSDDIPEEAATITVBOFLEPLELCYRSLQACGDREI : 427
AtPPC2 : QIDELMFEISMWRQSDRLRRADELHRLR-SRR-DAKHYIFBWKQVPEPEYRVIILGDVRDKLYITREARRSLLANGSDDIPEEAATITVBOFLEPLELCYRSLQACGDREI : 424
LePPC1 : QIDELMFEISMWRQSDRLRRADELHRLR-SRR-DAKHYIFBWKQVPEPEYRVIILGDVRDKLYITREARRSLLANGSDDIPEEAATITVBOFLEPLELCYRSLQACGDREI : 425
LePPC2 : QIDELMFEISMWRQSDRLRRADELHRLR-SRR-DAKHYIFBWKQVPEPEYRVIILGDVRDKLYITREARRSLLANGSDDIPEEAATITVBOFLEPLELCYRSLQACGDREI : 425
ZmPEPC-C3 : QIDELMFEISMWRQSDRLRRADELHRLR-SRR-DAKHYIFBWKQVPEPEYRVIILGDVRDKLYITREARRSLLANGSDDIPEEAATITVBOFLEPLELCYRSLQACGDREI : 427
ZmPEPC-C4 : QIDELMFEISMWRQSDRLRRADELHRLR-SRR-DAKHYIFBWKQVPEPEYRVIILGDVRDKLYITREARRSLLANGSDDIPEEAATITVBOFLEPLELCYRSLQACGDREI : 431

GhPEPC1 : ADGSLLEDLRQVSTFGLSLVRLDIQBSDRHTDVLDAITTHLIGSGYREWTEERQEWLLSEISGRPLFCEDLPKTEBEADVLDLTFHVLAEPLSDPFGAYIISMATAPS : 548
GhPEPC2 : ADGSLLEDLRQVSTFGLSLVRLDIQBSDRHTDVLDAITTHLIGSGYREWTEERQEWLLSEISGRPLFCEDLPKTEBEADVLDLTFHVLAEPLSDPFGAYIISMATAPS : 541
AtPPC1 : ADGSLLEDLRQVSTFGLSLVRLDIQBSDRHTDVLDAITTHLIGSGYREWTEERQEWLLSEISGRPLFCEDLPKTEBEADVLDLTFHVLAEPLSDPFGAYIISMATAPS : 537
AtPPC2 : ADGSLLEDLRQVSTFGLSLVRLDIQBSDRHTDVLDAITTHLIGSGYREWTEERQEWLLSEISGRPLFCEDLPKTEBEADVLDLTFHVLAEPLSDPFGAYIISMATAPS : 534
LePPC1 : ADGSLLEDLRQVSTFGLSLVRLDIQBSDRHTDVLDAITTHLIGSGYREWTEERQEWLLSEISGRPLFCEDLPKTEBEADVLDLTFHVLAEPLSDPFGAYIISMATAPS : 535
LePPC2 : ADGSLLEDLRQVSTFGLSLVRLDIQBSDRHTDVLDAITTHLIGSGYREWTEERQEWLLSEISGRPLFCEDLPKTEBEADVLDLTFHVLAEPLSDPFGAYIISMATAPS : 535
ZmPEPC-C3 : ADGSLLEDLRQVSTFGLSLVRLDIQBSDRHTDVLDAITTHLIGSGYREWTEERQEWLLSEISGRPLFCEDLPKTEBEADVLDLTFHVLAEPLSDPFGAYIISMATAPS : 537
ZmPEPC-C4 : ADGSLLEDLRQVSTFGLSLVRLDIQBSDRHTDVLDAITTHLIGSGYREWTEERQEWLLSEISGRPLFCEDLPKTEBEADVLDLTFHVLAEPLSDPFGAYIISMATAPS : 541

GhPEPC1 : DVLAVELLQRECHVKQPLRVVPLEFKLADLDAAPAAVARLFSIDMYRNRINGKQVVMGYSDSGKDAGRLSAAWQLYKAOBELIKVAKQVGVKLLMFHGRGGTVGRGGGF : 658
GhPEPC2 : DVLAVELLQRECHVKQPLRVVPLEFKLADLDAAPAAVARLFSIDMYRNRINGKQVVMGYSDSGKDAGRLSAAWQLYKAOBELIKVAKQVGVKLLMFHGRGGTVGRGGGF : 651
AtPPC1 : DVLAVELLQRECHVKQPLRVVPLEFKLADLDAAPAAVARLFSIDMYRNRINGKQVVMGYSDSGKDAGRLSAAWQLYKAOBELIKVAKQVGVKLLMFHGRGGTVGRGGGF : 647
AtPPC2 : DVLAVELLQRECHVKQPLRVVPLEFKLADLDAAPAAVARLFSIDMYRNRINGKQVVMGYSDSGKDAGRLSAAWQLYKAOBELIKVAKQVGVKLLMFHGRGGTVGRGGGF : 644
LePPC1 : DVLAVELLQRECHVKQPLRVVPLEFKLADLDAAPAAVARLFSIDMYRNRINGKQVVMGYSDSGKDAGRLSAAWQLYKAOBELIKVAKQVGVKLLMFHGRGGTVGRGGGF : 645
LePPC2 : DVLAVELLQRECHVKQPLRVVPLEFKLADLDAAPAAVARLFSIDMYRNRINGKQVVMGYSDSGKDAGRLSAAWQLYKAOBELIKVAKQVGVKLLMFHGRGGTVGRGGGF : 645
ZmPEPC-C3 : DVLAVELLQRECHVKQPLRVVPLEFKLADLDAAPAAVARLFSIDMYRNRINGKQVVMGYSDSGKDAGRLSAAWQLYKAOBELIKVAKQVGVKLLMFHGRGGTVGRGGGF : 647
ZmPEPC-C4 : DVLAVELLQRECHVKQPLRVVPLEFKLADLDAAPAAVARLFSIDMYRNRINGKQVVMGYSDSGKDAGRLSAAWQLYKAOBELIKVAKQVGVKLLMFHGRGGTVGRGGGF : 651

loop I : PEP binding site

GhPEPC1 : THLAILSQPPDITIGSIRVTVQGEVIEQSGFEEHLCFRTLQRFATLLEHGMHPVPSKPEWRALDDEMAVVATKQYRSVVEQERFVEYFRNATPELEYGRMNI GSRPS : 768
GhPEPC2 : THLAILSQPPDITIGSIRVTVQGEVIEQSGFEEHLCFRTLQRFATLLEHGMHPVPSKPEWRALDDEMAVVATKQYRSVVEQERFVEYFRNATPELEYGRMNI GSRPS : 761
AtPPC1 : THLAILSQPPDITIGSIRVTVQGEVIEQSGFEEHLCFRTLQRFATLLEHGMHPVPSKPEWRALDDEMAVVATKQYRSVVEQERFVEYFRNATPELEYGRMNI GSRPS : 757
AtPPC2 : THLAILSQPPDITIGSIRVTVQGEVIEQSGFEEHLCFRTLQRFATLLEHGMHPVPSKPEWRALDDEMAVVATKQYRSVVEQERFVEYFRNATPELEYGRMNI GSRPS : 754
LePPC1 : THLAILSQPPDITIGSIRVTVQGEVIEQSGFEEHLCFRTLQRFATLLEHGMHPVPSKPEWRALDDEMAVVATKQYRSVVEQERFVEYFRNATPELEYGRMNI GSRPS : 755
LePPC2 : THLAILSQPPDITIGSIRVTVQGEVIEQSGFEEHLCFRTLQRFATLLEHGMHPVPSKPEWRALDDEMAVVATKQYRSVVEQERFVEYFRNATPELEYGRMNI GSRPS : 755
ZmPEPC-C3 : THLAILSQPPDITIGSIRVTVQGEVIEQSGFEEHLCFRTLQRFATLLEHGMHPVPSKPEWRALDDEMAVVATKQYRSVVEQERFVEYFRNATPELEYGRMNI GSRPS : 757
ZmPEPC-C4 : THLAILSQPPDITIGSIRVTVQGEVIEQSGFEEHLCFRTLQRFATLLEHGMHPVPSKPEWRALDDEMAVVATKQYRSVVEQERFVEYFRNATPELEYGRMNI GSRPS : 761

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GhPEPC1 : KRKPSGGIESLRAIPIPIAWTQRFHLPVWLGCGAARHVIVKQDIRNLHLOEMHNMWPFPRVTDLLEVMVFAKQDPIGIAALDKLLVSEDLWSEGLRRLRANPEETKRLS : 878
GhPEPC2 : KRKPSGGIESLRAIPIPIAWTQRFHLPVWLGCGAARHVIVKQDIRNLHLOEMHNMWPFPRVTDLLEVMVFAKQDPIGIAALDKLLVSEDLWSEGLRRLRANPEETKRLS : 871
AtPPC1 : KRKPSGGIESLRAIPIPIAWTQRFHLPVWLGCGAARHVIVKQDIRNLHLOEMHNMWPFPRVTDLLEVMVFAKQDPIGIAALDKLLVSEDLWSEGLRRLRANPEETKRLS : 867
AtPPC2 : KRKPSGGIESLRAIPIPIAWTQRFHLPVWLGCGAARHVIVKQDIRNLHLOEMHNMWPFPRVTDLLEVMVFAKQDPIGIAALDKLLVSEDLWSEGLRRLRANPEETKRLS : 864
LePPC1 : KRKPSGGIESLRAIPIPIAWTQRFHLPVWLGCGAARHVIVKQDIRNLHLOEMHNMWPFPRVTDLLEVMVFAKQDPIGIAALDKLLVSEDLWSEGLRRLRANPEETKRLS : 865
LePPC2 : KRKPSGGIESLRAIPIPIAWTQRFHLPVWLGCGAARHVIVKQDIRNLHLOEMHNMWPFPRVTDLLEVMVFAKQDPIGIAALDKLLVSEDLWSEGLRRLRANPEETKRLS : 865
ZmPEPC-C3 : KRKPSGGIESLRAIPIPIAWTQRFHLPVWLGCGAARHVIVKQDIRNLHLOEMHNMWPFPRVTDLLEVMVFAKQDPIGIAALDKLLVSEDLWSEGLRRLRANPEETKRLS : 867
ZmPEPC-C4 : KRKPSGGIESLRAIPIPIAWTQRFHLPVWLGCGAARHVIVKQDIRNLHLOEMHNMWPFPRVTDLLEVMVFAKQDPIGIAALDKLLVSEDLWSEGLRRLRANPEETKRLS : 871

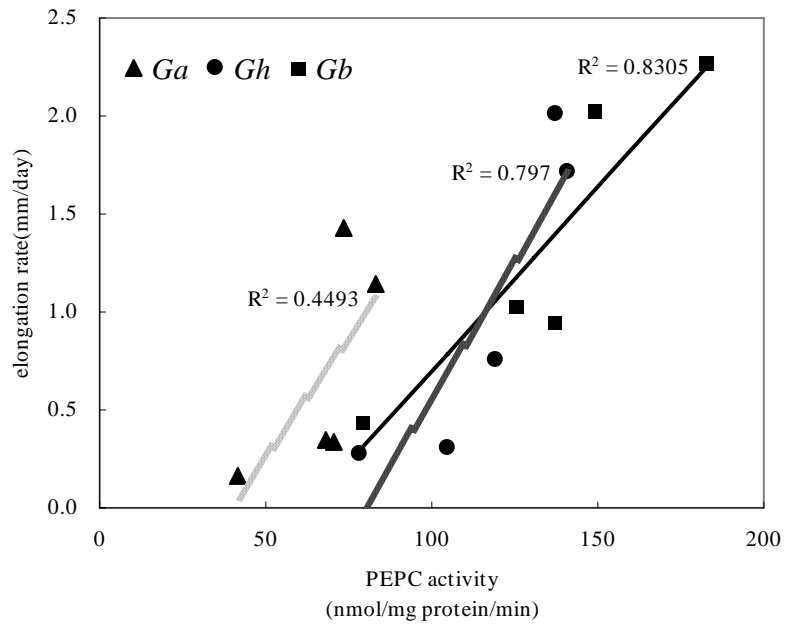
loop II: HCO₃ binding site

GhPEPC1 : FQVAGHRDLEEDPFLKQRLRLDAITTLNVQAYTLKRIREDPQVHVKVRPHLSREYMESSKAAAEVLVKNLFTSEYAPGLDITLTMKGIAAGHNTG : 978
GhPEPC2 : FQVAGHRDLEEDPFLKQRLRLDAITTLNVQAYTLKRIREDPQVHVKVRPHLSREYMESSKAAAEVLVKNLFTSEYAPGLDITLTMKGIAAGHNTG : 971
AtPPC1 : FQVAGHRDLEEDPFLKQRLRLDAITTLNVQAYTLKRIREDPQVHVKVRPHLSREYMESSKAAAEVLVKNLFTSEYAPGLDITLTMKGIAAGHNTG : 967
AtPPC2 : LQVAGHRDLEEDPFLKQRLRLDAITTLNVQAYTLKRIREDPQVHVKVRPHLSREYMESSKAAAEVLVKNLFTSEYAPGLDITLTMKGIAAGHNTG : 963
LePPC1 : LQVAGHRDLEEDPFLKQRLRLDAITTLNVQAYTLKRIREDPQVHVKVRPHLSREYMESSKAAAEVLVKNLFTSEYAPGLDITLTMKGIAAGHNTG : 964
LePPC2 : LQVAGHRDLEEDPFLKQRLRLDAITTLNVQAYTLKRIREDPQVHVKVRPHLSREYMESSKAAAEVLVKNLFTSEYAPGLDITLTMKGIAAGHNTG : 964
ZmPEPC-C3 : FQVAGHRDLEEDPFLKQRLRLDAITTLNVQAYTLKRIREDPQVHVKVRPHLSREYMESSKAAAEVLVKNLFTSEYAPGLDITLTMKGIAAGHNTG : 967
ZmPEPC-C4 : LQVAGHRDLEEDPFLKQRLRLDAITTLNVQAYTLKRIREDPQVHVKVRPHLSREYMESSKAAAEVLVKNLFTSEYAPGLDITLTMKGIAAGHNTG : 970

loopIV: regulatory function

conserved motif II for catalytic activity

Supplemental Fig. 1. Alignment of the deduced amino acid sequences of GhPEPC1 and GhPEPC2 with PEPCs from other plant species. Putative regulatory and catalytic domains are underlined. The arrow marks the phosphorylable Ser residue at the phosphorylation domain (underlined); *, the position of the C3 signature Ala; C (1-5), plant conserved Cys residues. The accession numbers of these known proteins in Genbank are as follows: GhPEPC1 (AF008939); GhPEPC2 (EU032328); AtPPC1 (AJ532901); AtPPC2 (AJ532902); LePPC1 (AJ243416); LePPC2 (AJ243417); ZmPEPC-C3 (X61489); ZmPEPC-C4 (NM_001111948).



Supplemental Fig. 2. Correlation analyses between PEPC activity and fiber elongation rate among *Ga*, *Gh* and *Gb*, (Data re-plotted from Figs 2A and B).