**Supplementary Tables**

**Supplementary Table 1. Dry-grind ethanol traits that were scored for a select maize inbred population.**

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| **Trait code** | **Trait Description** | **Trait type** |
| ASH\_P | Ash % (NIRS) Values between 1 - 10 |  |
| DEN\_N | Kernel Density |  |
| DG24P | Dry Grind Ethanol % after 24 Hrs Fermentation | Starch |
| DG48P | Dry Grind Ethanol % after 48 Hrs Fermentation | Starch |
| DG72P | Dry Grind Ethanol % after 72 Hrs Fermentation | Starch |
| GMADP | Grain Moisture (measured after Air Drying) | Moisture |
| OIL\_P | QM--Oil % | Oil |
| PRTNP | QM--Protein % | Protein |
| RFA\_P | QM--Crude Fiber | Fiber |
| STC\_P | QM--Grain Starch Content % | Starch |

**Supplementary Table 2. Top-ranked Three-enzyme solutions identified by interrogating a C4 carbon assimilation mathematical model.** The solutions are ranked by the level of predicted improvement. Each enzyme is colour coded to illustrate occurrence in multiple solutions.

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| --- | --- | --- | --- | --- | --- |
| **Number** | **Enzyme 1** | **Enzyme 2** | **Enzyme 3** | **Mean % increase in CO2 assimilation for enzyme levels in a 1.5-fold range around the optimal fold change levels** | **Optimal enzyme fold change levels achieving maximal CO2 assimilation** |
| 1 | Phosphoenolpyruvate carboxylase | Fructose-bisphosphatase | Phosphoribulokinase | 12.79 | [5.91,10,6.58] |
| 2 | Malate dehydrogenase (oxaloacetate-decarboxylating) (NADP+) | PGA-kinase-GAP-dehydrogenase complex in mesophyll | Fructose-bisphosphate aldolase | 12.78 | [10,2.92,10] |
| 3 | Malate dehydrogenase (oxaloacetate-decarboxylating) (NADP+) | Fructose-bisphosphate aldolase | 6-phosphofructo-2-kinase | 11.83 | [8.89,7.04,4.06] |
| 4 | Malate dehydrogenase (oxaloacetate-decarboxylating) (NADP+) | PGA-kinase-GAP-dehydrogenase complex in mesophyll | Fructose-bisphosphatase | 11.64 | [10,5.09,5.84] |
| 5 | Phosphoenolpyruvate carboxylase | Malate dehydrogenase (oxaloacetate-decarboxylating) (NADP+) | Fructose-bisphosphate aldolase | 11.44 | [3.55,10,10] |
| 6 | Malate dehydrogenase (NADP+) | Malate dehydrogenase (oxaloacetate-decarboxylating) (NADP+) | Fructose-bisphosphate aldolase | 11.39 | [6.8,10,9.8] |
| 7 | Malate dehydrogenase (oxaloacetate-decarboxylating) (NADP+) | Fructose-bisphosphate aldolase | Sedoheptulose-bisphosphatase | 11.39 | [10,8.79,3.73] |
| 8 | Malate dehydrogenase (oxaloacetate-decarboxylating) (NADP+) | Pyruvate, phosphate dikinase | Fructose-bisphosphate aldolase | 11.31 | [9.9,1.4,9.9] |
| 9 | Malate dehydrogenase (oxaloacetate-decarboxylating) (NADP+) | Phosphoglycerate kinase/ glyceraldehyde-3-phosphate dehydrogenase (NADP+) (phosphorylating) | Fructose-bisphosphate aldolase | 11.26 | [10,0.42,8.43] |

**Supplementary Table 3. Top-ranked Four-enzyme solutions identified by interrogating a C4 carbon assimilation mathematical model.** The solutions are ranked by the level of predicted improvement. Each enzyme is colour coded to illustrate occurrence in multiple solutions.

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| --- | --- | --- | --- | --- | --- | --- |
| **Number** | **Enzyme 1** | **Enzyme 2** | **Enzyme 3** | **Enzyme 4** | **Mean % increase in CO2 assimilation for enzyme levels in a 1.5-fold range around the optimal fold change levels** | **Optimal enzyme fold change levels achieving maximal CO2 assimilation** |
| 1 | Phosphoenolpyruvate carboxylase | Malate dehydrogenase (oxaloacetate-decarboxylating) (NADP+) | Phosphoglycerate kinase/ glyceraldehyde-3-phosphate dehydrogenase (NADP+) (phosphorylating) | Fructose-bisphosphate aldolase | 13.96 | [2.09,8.27,7.91,4.07] |
| 2 | Malate dehydrogenase (NADP+) | Malate dehydrogenase (oxaloacetate-decarboxylating) (NADP+) | Phosphoglycerate kinase/ glyceraldehyde-3-phosphate dehydrogenase (NADP+) (phosphorylating) | Fructose-bisphosphate aldolase | 13.86 | [10,10,10,10] |
| 3 | Malate dehydrogenase (oxaloacetate-decarboxylating) (NADP+) | Pyruvate, phosphate dikinase | Phosphoglycerate kinase/ glyceraldehyde-3-phosphate dehydrogenase (NADP+) (phosphorylating) | Fructose-bisphosphate aldolase | 13.78 | [10,6.35,9.73,9.15] |
| 4 | Malate dehydrogenase (oxaloacetate-decarboxylating) (NADP+) | Phosphoglycerate kinase/ glyceraldehyde-3-phosphate dehydrogenase (NADP+) (phosphorylating) | Fructose-bisphosphatase | Phosphoribulokinase | 13.62 | [8.45,2.59,3.07,3.7] |
| 5 | Phosphoenolpyruvate carboxylase | Malate dehydrogenase (oxaloacetate-decarboxylating) (NADP+) | Phosphoglycerate kinase/ glyceraldehyde-3-phosphate dehydrogenase (NADP+) (phosphorylating) | Fructose-bisphosphatase | 13.54 | [9.7,10,10,7.2] |
| 6 | Malate dehydrogenase (oxaloacetate-decarboxylating) (NADP+) | Phosphoglycerate kinase/ glyceraldehyde-3-phosphate dehydrogenase (NADP+) (phosphorylating) | Phosphoribulokinase | starch synthase (glycosyl-transferring) | 13.48 | [10,3.73,10,4.69] |
| 7 | Phosphoenolpyruvate carboxylase | Phosphoglycerate kinase/ glyceraldehyde-3-phosphate dehydrogenase (NADP+) (phosphorylating) | Phosphoribulokinase | starch synthase (glycosyl-transferring) | 13.47 | [8.33,10,10,8.01] |