

Table of Contents

Supplement 1: Orthogonal based ranking algorithm	2
Supplement 2: The model ODEs and rate laws of the sugarcane culm model.....	3
Supplement 3: Non-identifiability due to a functional relationship	5
Supplement 4: The rate laws and model ODEs of the gene regulatory network	7
Supplement 5: Gene Regulatory Network Model Results	10
Supplement 6: MATLAB model verification of the sugarcane culm model	11
Supplement 7: MATLAB model verification of the gene regulatory network.....	12

Supplement 1: Orthogonal based ranking algorithm

1. Calculate the sensitivity matrix Z .
2. Calculate the sum of squared values of each column of Z and choose the column with the highest value as the most estimable.
3. Add the chosen column to E_L where $L=1$ for the first iteration. With increasing number of iterations the size of E_L matrix grows as columns from Z are added to E_L .
4. Calculate an orthogonal projection $\hat{Z}_L$ for the column that exhibits the highest independence to the vector space spanned by E_L

$$\hat{Z}_L = E_L (E_L^T E_L)^{-1} E_L^T Z \quad (1)$$

5. As a measure of independence the residual matrix $Rs_L = Z - \hat{Z}_L$ is calculated.
6. The sum of squares value for each column of the Rs_L matrix is calculated, resulting in the vector Cs_L . The column corresponding to the largest sum of squares is chosen for the next estimable parameter.
7. Select the corresponding column in Z and augment it with the matrix E_L by marking the new column.
8. Iterate steps 4-7 until the cut-off value is reached or until all the parameters have been ranked and selected to be identifiable.

Supplement 2: The model ODEs and rate laws of the sugarcane culm model

The SBML model is available from the Biomodels database:

<http://www.ebi.ac.uk/biomodels-main/BIOMD0000000023>

The ODEs for the model are:

$$\frac{d[Glc]}{dt} = v_2 - v_3 + v_9$$

$$\frac{d[Fru]}{dt} = v_1 - v_4 - v_5 - v_8 + v_9$$

$$\frac{d[HexP]}{dt} = v_3 + v_4 + v_5 - 2v_6 - v_8 - v_{10}$$

$$\frac{d[Suc6P]}{dt} = v_6 - v_7$$

$$\frac{d[Suc]}{dt} = v_7 + v_8 - v_9 - v_{11}$$

Where the rate laws used in the Sugarcane culm model, as developed by Rohwer and Botha [40], are:

$$v_1 = V_{\max 1} \frac{[Fru_{ex}]}{k_{m1Fru,ex} \left(1 + \frac{[Fru]}{k_{i1Fru}} \right) + [Fru_{ex}]}$$

$$v_2 = V_{\max 2} \frac{[Glc_{ex}]}{k_{m2Glc,ex} \left(1 + \frac{[Glc]}{k_{i2Glc}} \right) + [Glc_{ex}]}$$

$$v_3 = V_{\max 3} \frac{\frac{[Glc]}{k_{m3Glc}} \frac{[ATP]}{k_{m3ATP}}}{\left(1 + \frac{[ATP]}{k_{m3ATP}} \right) \cdot \left(1 + \frac{[Glc]}{k_{m3Glc}} + \frac{[Fru]}{k_{m4Fru}} + \frac{0.113[HexP]}{k_{i3Glc6P}} + \frac{0.0575[HexP]}{k_{i4Fru6P}} \right)}$$

$$v_4 = V_{\max 4} \frac{\frac{[Fru]}{k_{m4Fru}} \frac{[ATP]}{k_{m4ATP}}}{\left(1 + \frac{[ATP]}{k_{m4ATP}} \right) \cdot \left(1 + \frac{[Glc]}{k_{m3Glc}} + \frac{[Fru]}{k_{m4Fru}} + \frac{0.113[HexP]}{k_{i3Glc6P}} + \frac{0.0575[HexP]}{k_{i4Fru6P}} \right)}$$

$$v_5 = V_{\max 5} \frac{\frac{[Fru]}{k_{m5Fru}} \frac{[ATP]}{k_{m5ATP}}}{\left(1 + \frac{[Fru]}{k_{i5Fru}} \right) \cdot \left(1 + \frac{[Fru]}{k_{m5Fru}} + \frac{[ATP]}{k_{m5ATP}} + \frac{[Fru][ATP]}{k_{m5Fru} \cdot k_{m5ATP}} + \frac{[ADP]}{k_{i5ADP}} \right)}$$

$$v_6 = V_6^f \left(\frac{(0.0575[HexP])(0.823 \mathbb{I}[HexP]) - \frac{[Suc][UDP]}{k_{eq6}}}{(0.0575[HexP])(0.823 \mathbb{I}[HexP]) \cdot \left(1 + \frac{[Suc6P]}{k_{i6Suc6P}}\right) + k_{m6Fru6P}(0.823 \mathbb{I}[HexP] + k_{i6UDPGlc}) \cdot \left(1 + \frac{[P]}{k_{i6P}}\right) + k_{m6UDPGlc}(0.0575[HexP]) \cdot \left(\frac{V_6^f}{V_6^r k_{eq6}}\right)} \right) \cdot \left(k_{m6UDP}[Suc6P] \cdot \left(1 + \frac{0.823 \mathbb{I}[HexP]}{k_{i6UDPGlc}}\right) + k_{m6Suc6P}[UDP] \cdot \left(1 + \frac{k_{m6UDPGlc}(0.0575[HexP])}{k_{i6UDPGlc} k_{m6Fru6P} \left(1 + \frac{[P]}{k_{i6P}}\right)}\right) + [Suc6P] \cdot \left(1 + \frac{0.0575[HexP]}{k_{i6Fru6P}}\right) \right)$$

$$v_7 = V_{\max 7} \frac{[Suc6P]}{k_{m7Suc6P} + [Suc6P]}$$

$$v_8 = -V_8^f \frac{[Suc][UDP] - \left(\frac{0.823 \mathbb{I}[HexP] \cdot [Fru]}{k_{eq8}}\right)}{[Suc][UDP] \left(1 + \frac{[Fru]}{k_{i8Fru}}\right) + k_{m8Suc}(k_{i8UDP} + [UDP]) + k_{m8UDP}[Suc] + k_{m8UDPGlc}[Fru] \cdot \left(1 + \frac{[UDP]}{k_{i8UDP}}\right) \cdot \left(\frac{V_8^f}{V_8^r k_{eq8}}\right) + k_{m8Fru}(0.823 \mathbb{I}[HexP]) \cdot \left(1 + \frac{k_{m8UDP} k_{m8Suc} [Suc]}{k_{i7UDP}}\right) + [Fru] \cdot \left(1 + \frac{[Suc]}{k_{i8Suc}}\right)}$$

$$v_9 = V_{\max 9} \frac{[Suc]}{\left(1 + \frac{[Glc]}{k_{i9Glc}}\right) \cdot \left(k_{m9Suc} \left(1 + \frac{[Fru]}{k_{i9Fru}}\right) + [Suc]\right)}$$

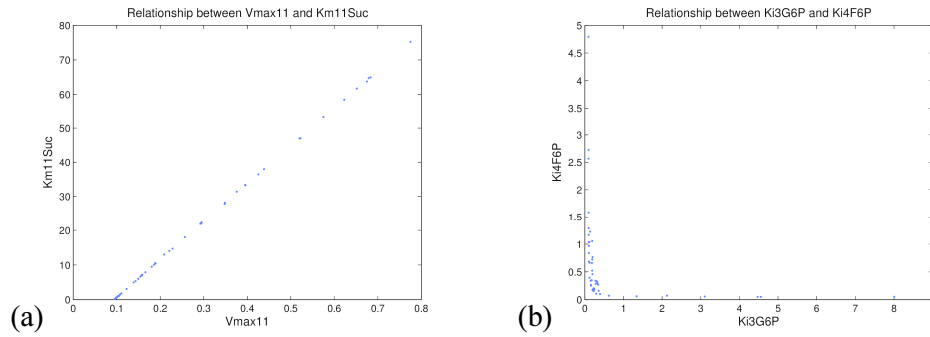
$$v_{10} = V_{\max 10} \frac{0.0575[HexP]}{k_{m10Fru6P} + 0.0575[HexP]}$$

$$v_{11} = V_{\max 11} \frac{[Suc]}{k_{m11Suc} + [Suc]}$$

Supplement 3: Non-identifiability due to a functional relationship

The orthogonal identifiability analysis of the ranking method determined that the three parameters, K_{i4F6P} , K_{i6F6P} and K_{m11Suc} , were non-identifiable. In order to determine if any functional relationships exist with the non-identifiable parameters an exhaustive functional analysis of these parameters was carried out. This analysis reveals a strong linear relationship between K_{m11Suc} and $V_{\max11}$ as shown in the Supplementary Figure 1a. A second nonlinear (hyperbolic) relationship was found between K_{i3G6P} and K_{i4F6P} , see Supplementary Figure 1b. No functional relationship was found between the last of the non-identifiable parameters, K_{i6F6P} , and any of the identifiable parameters. One method for dealing with non-identifiable parameters is to measure them directly. Although not typically possible in biological systems, the actual values are available for this model. Thus the estimation process was repeated using the actual values of the non-identifiable parameters while estimating the identifiable ones. The results of the estimation remain mixed, with roughly an equal number of parameters showing improvement and deterioration. Steady state analysis with the estimated parameters from Table 2 found the same stable steady state as was found with the actual parameter values (Supplementary Table 1). This confirms that although some of the parameters are non-identifiable the estimated parameter values remain valid for describing the actual dynamics of the system.

There is a clear indication that among the 9 identifiable parameters from the sensitivity method, some are still non-identifiable.



Supplementary Figure 1: Functional relationships between parameters. a) Linear; the relationship between K_{m11Suc} and $V_{\max11}$. b) near hyperbolic; the relationship between K_{i3G6P} and K_{i4F6P} .

Metabolite	Concentration	
	Actual Parameter	Estimated Parameter
Fru	1.00	1.00
Glc	1.00	1.00
HexP	0.10	0.67
HexP	10.00	0.63
Suc6P	0.07	0.45
Suc	1.40	0.32
Sucvac	0.20	0.34
Glycolysis	0.30	4.73
Phos	0.10	5.97
UDP	0.40	0.65
ADP	1.00	0.28
ATP	100.00	21.43
Glcex	100.00	21.43
Fruex	100.00	21.43

Supplementary Table 1: Steady state metabolite concentration as calculated using the actual parameter set from Rohwer and Botha [40] and with the estimated parameters.

Supplement 4: The rate laws and model ODEs of the gene regulatory network

The SBML model is available from the Sage Bionetworks' Synapse database:

<https://www.synapse.org/#!Synapse:syn2843038>

The model file is **model1.sbml** in the folder:

DREAM6_ParEst_Data_v4\Model1\Model Representations

The rate laws of the model are:

$$r_1 = \text{prol_strength}$$

$$r_2 = \text{pp1_mrna_degradation_rate} \times \text{pp1_mrna}$$

$$r_3 = \text{rbs1_strength} \times \text{pp1_mrna}$$

$$r_4 = \text{p1_degradation_rate} \times \text{p1}$$

$$r_5 = \text{pro2_strength} \times \text{as1} \times \text{rs1}$$

$$r_6 = \text{pp2_mrna_degradation_rate} \times \text{pp2_mrna}$$

$$r_7 = \text{rbs2_strength} \times \text{pp2_mrna}$$

$$r_8 = \text{p2_degradation_rate} \times \text{p2}$$

$$r_9 = \text{pro3_strength} \times \text{as3} \times \text{rs4}$$

$$r_{10} = \text{pp3_mrna_degradation_rate} \times \text{pp3_mrna}$$

$$r_{11} = \text{rbs3_strength} \times \text{pp3_mrna}$$

$$r_{12} = \text{p3_degradation_rate} \times \text{p3}$$

$$r_{13} = \text{pro4_strength} \times \text{as2} \times \text{rs2}$$

$$r_{14} = \text{pp4_mrna_degradation_rate} \times \text{pp4_mrna}$$

$$r_{15} = \text{rbs4_strength} \times \text{pp4_mrna}$$

$$r_{16} = \text{p4_degradation_rate} \times \text{p4}$$

$$r_{17} = \text{pro5_strength} \times \text{rs3}$$

$$r_{18} = \text{pp5_mrna_degradation_rate} \times \text{pp5_mrna}$$

$$r_{19} = \text{rbs5_strength} \times \text{pp5_mrna}$$

$$r_{20} = \text{p5_degradation_rate} \times \text{p5}$$

$$r_{21} = pro6_strength \times rs5$$

$$r_{22} = pp6_mrna_degradation_rate \times pp6_mrna$$

$$r_{23} = rbs6_strength \times pp6_mrna$$

$$r_{24} = p6_degradation_rate \times p6$$

$$as_1 = \frac{\left(\frac{p1}{v2_kd} \right)^{v2_h}}{\left(1 + \frac{p1}{v2_kd} \right)^{v2_h}}$$

$$as_2 = \frac{\left(\frac{p1}{v1_kd} \right)^{v1_h}}{\left(1 + \frac{p1}{v1_kd} \right)^{v1_h}}$$

$$as_3 = \frac{\left(\frac{p1}{v3_kd} \right)^{v3_h}}{\left(1 + \frac{p1}{v3_kd} \right)^{v3_h}}$$

$$rs_1 = \frac{1}{1 + \left(\frac{p6}{v5_kd} \right)^{v5_h}}$$

$$rs_2 = \frac{1}{1 + \left(\frac{p5}{v8_kd} \right)^{v8_h}}$$

$$rs_3 = \frac{1}{1 + \left(\frac{p4}{v6_kd} \right)^{v6_h}}$$

$$rs_4 = \frac{1}{1 + \left(\frac{p2}{v4_kd} \right)^{v4_h}}$$

$$rs_5 = \frac{1}{1 + \left(\frac{p4}{v7_kd} \right)^{v7_h}}$$

The naming of the variables are, *as* : activator binding site, *rs* : repression binding site, *rbs* : ribosomal binding site strength, *pro* : promoter strength, *p* : protein concentration, *pp* : protein production process.

The ODEs of the model are:

$$\frac{d(pp1_mrna)}{dt} = r_1 - r_2$$

$$\frac{d(p1)}{dt} = r_3 - r_4$$

$$\frac{d(pp2_mrna)}{dt} = r_5 - r_6$$

$$\frac{d(p2)}{dt} = r_7 - r_8$$

$$\frac{d(pp3_mrna)}{dt} = r_9 - r_{10}$$

$$\frac{d(p3)}{dt} = r_{11} - r_{12}$$

$$\frac{d(pp4_mrna)}{dt} = r_{13} - r_{14}$$

$$\frac{d(p4)}{dt} = r_{15} - r_{16}$$

$$\frac{d(pp5_mrna)}{dt} = r_{17} - r_{18}$$

$$\frac{d(p5)}{dt} = r_{19} - r_{20}$$

$$\frac{d(pp6_mrna)}{dt} = r_{21} - r_{22}$$

$$\frac{d(p6)}{dt} = r_{23} - r_{24}$$

Supplement 5: Gene Regulatory Network Model Results

Parameter Name	Ranking	Initialized Std. Dev.
rbs4_strength	1	0.001
pro6_strength	2	0.002
v8_h	3	0.003
pro1_strength	4	0.004
pro5_strength	5	0.005
pro3_strength	6	0.006
pro2_strength	7	0.007
pro4_strength	8	0.008
rbs6_strength	9	0.009
rbs5_strength	10	0.010
v5_h	11	0.011
v7_Kd	12	0.012
v6_Kd	13	0.013
p_degradation_rat	14	0.014
v4_h	15	0.015
v7_h	16	0.016
rbs1_strength	17	0.017
rbs3_strength	18	0.018
v6_h	19	0.019
v8_Kd	20	0.020
v3_h	21	0.021
rbs2_strength	22	0.022
v5_Kd	23	0.023
v2_h	24	0.024
v1_Kd	25	0.025
v2_Kd	26	0.026
v3_Kd	27	0.027
v1_h	28	0.028
v4_Kd	29	0.029

Supplementary Table 5: Ranking and standard deviation of parameters used in formulating the informed prior. The variances are used to initialize the diagonal of the state-estimation covariance matrix, P , and the process noise covariance matrix, Q .

Supplement 6: MATLAB model verification of the sugarcane culm model

The tables below shows the state values generated by the original SBML model in Copasi (Table 6a) and by the model implemented in MATLAB (Table 6b). In both cases the models are using the same set of initial parameter values.

# Time	Generated by Copasi				
	Fru	Glc	HexP	Suc6P	Suc
0	1	1	1	1	1
1	1.1721	1.1624	1.2339	0.5617	1.3941
2	1.3508	1.3310	1.4591	0.1824	1.7201
3	1.5320	1.5021	1.6742	0.0099	1.8363
4	1.7094	1.6705	1.8797	0.0025	1.7924
5	1.8816	1.8349	2.0761	0.0028	1.7475
6	2.0487	1.9958	2.2634	0.0032	1.7091
7	2.2112	2.1533	2.4422	0.0036	1.6770
8	2.3694	2.3078	2.6126	0.0039	1.6507
9	2.5234	2.4594	2.7751	0.0043	1.6300
10	2.6735	2.6085	2.9298	0.0046	1.6145

Supplementary Table 6a: Results from Copasi using the original SBML model of the sugarcane culm tissue and the default set of initial parameter values.

Time	Generated by MATLAB				
	Fru	Glc	HexP	Suc6P	Suc
0	1	1	1	1	1
1	1.1721	1.1624	1.2339	0.5617	1.3941
2	1.3508	1.3310	1.4591	0.1824	1.7201
3	1.5320	1.5021	1.6742	0.0099	1.8363
4	1.7094	1.6705	1.8797	0.0025	1.7924
5	1.8816	1.8349	2.0760	0.0028	1.7475
6	2.0487	1.9958	2.2634	0.0032	1.7091
7	2.2112	2.1533	2.4422	0.0036	1.6770
8	2.3694	2.3078	2.6126	0.0039	1.6507
9	2.5234	2.4594	2.7751	0.0043	1.6300
10	2.6735	2.6085	2.9298	0.0046	1.6145

Supplementary Table 6b: Results from Copasi using the original SBML model of the sugarcane culm tissue and the default set of initial parameter values.

Supplement 7: MATLAB model verification of the gene regulatory network

The tables below shows the state values generated by the original SBML model in Copasi (Table 7a) and by the model implemented in MATLAB (Table 7b). In both cases the models are using the same set of initial parameter values.

Generated by Copasi												
#Time	pp1_mrna	p1	pp2_mrna	p2	pp3_mrna	p3	pp4_mrna	p4	pp5_mrna	p5	pp6_mrna	p6
0	0	1	0	1	0	1	0	1	0	1	0	1
1	0.6321	0.6321	0.1580	0.4337	0.1622	0.4346	0.1580	0.4337	0.3950	0.5232	0.3950	0.5232
2	0.8647	0.7293	0.2271	0.2872	0.2518	0.2970	0.2271	0.2872	0.6204	0.5327	0.6204	0.5327
3	0.9502	0.8506	0.2605	0.2629	0.3145	0.2929	0.2605	0.2629	0.7266	0.6318	0.7266	0.6318
4	0.9817	0.9267	0.2739	0.2670	0.3523	0.3216	0.2739	0.2670	0.7671	0.7088	0.7671	0.7088
5	0.9933	0.9663	0.2784	0.2733	0.3722	0.3490	0.2784	0.2733	0.7796	0.7511	0.7796	0.7511
6	0.9975	0.9851	0.2797	0.2770	0.3820	0.3676	0.2797	0.2770	0.7823	0.7704	0.7823	0.7704
7	0.9991	0.9936	0.2801	0.2789	0.3866	0.3786	0.2801	0.2789	0.7823	0.7780	0.7823	0.7780
8	0.9997	0.9973	0.2803	0.2797	0.3888	0.3845	0.2803	0.2797	0.7819	0.7806	0.7819	0.7806
9	0.9999	0.9989	0.2804	0.2801	0.3897	0.3876	0.2804	0.2801	0.7815	0.7813	0.7815	0.7813
10	1.0000	0.9995	0.2805	0.2803	0.3902	0.3891	0.2805	0.2803	0.7812	0.7813	0.7812	0.7813

Supplementary Table 7a: Results from Copasi using the original SBML model of the gene regulatory network and the default set of initial parameter values.

Generated by MATLAB												
Time	pp1_mrna	p1	pp2_mrna	p2	pp3_mrna	p3	pp4_mrna	p4	pp5_mrna	p5	pp6_mrna	p6
0	0	1	0	1	0	1	0	1	0	1	0	1
1	0.6321	0.6321	0.1580	0.4337	0.1622	0.4346	0.1580	0.4337	0.3950	0.5232	0.3950	0.5232
2	0.8647	0.7293	0.2271	0.2872	0.2519	0.2970	0.2271	0.2872	0.6204	0.5326	0.6204	0.5326
3	0.9502	0.8507	0.2605	0.2629	0.3145	0.2929	0.2605	0.2629	0.7265	0.6318	0.7265	0.6318
4	0.9817	0.9268	0.2739	0.2670	0.3523	0.3216	0.2739	0.2670	0.7672	0.7088	0.7672	0.7088
5	0.9933	0.9663	0.2784	0.2733	0.3722	0.3490	0.2784	0.2733	0.7796	0.7511	0.7796	0.7511
6	0.9975	0.9851	0.2797	0.2771	0.3820	0.3676	0.2797	0.2771	0.7823	0.7704	0.7823	0.7704
7	0.9991	0.9936	0.2801	0.2789	0.3866	0.3786	0.2801	0.2789	0.7823	0.7780	0.7823	0.7780
8	0.9997	0.9973	0.2803	0.2797	0.3888	0.3845	0.2803	0.2797	0.7819	0.7806	0.7819	0.7806
9	0.9999	0.9989	0.2804	0.2801	0.3897	0.3876	0.2804	0.2801	0.7815	0.7813	0.7815	0.7813
10	1.0000	0.9996	0.2805	0.2804	0.3902	0.3891	0.2805	0.2804	0.7812	0.7813	0.7812	0.7813

Supplementary Table 7b: Results from Copasi using the original SBML model of the gene regulatory network and the default set of initial parameter values.