

In Silico* Determination of Stress-Activated Metabolic Networks in *Medicago truncatula

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Distinguishing between metabolic networks that are involved in particular stress responses is a critical step in achieving a systems level understanding of the model legume *Medicago truncatula*. While some reactions within the network are invoked as part of a more general stress response, some are specifically activated by a certain elicitor, or class of elicitors. In this study, we compare the levels of activation within *a priori* determined metabolic networks for yeast elicited and methyl jasmonate elicited *Medicago truncatula* cell cultures. The level of activation for a given interaction is based upon the metabolite levels of its member nodes. The metabolite levels were derived from the polar components of GC-MS time course studies.

Our incomplete knowledge of *Medicago truncatula*'s metabolic networks combined with the presence of unknown molecules within the GC-MS spectra creates both challenges and opportunities. By using a variety of input networks, we are able to both distinguish between activated interactions of the treatments that occur within well studied regions of the metabolic networks as well as test predictions of interactions involving unknowns. Our initial source networks were derived from BNET and AraCyc. We used these source networks to begin distinguishing between the previously determined early and late stages of the two elicitors. A selected comparison of the early and late stages of yeast and methyl jasmonate elicited activated networks using BNET and AraCyc is shown in Table 1. We then created hypothetical network interactions reverse-engineered from the data. These networks were based on rank correlations with an alpha level of .00001 between given metabolites and upon significant mutual selection frequencies when GA-DFA is applied to the problem of distinguishing between early, late, and control samples for a given elicitor. By using these hypothetical networks, we are able to introduce the presence of unknowns into the set of possible interactions that can be activated as part of a given stress response. Table 2 shows a selected comparison of early and late stages of methyl jasmonate and yeast elicited *Medicago truncatula* samples using the aforementioned hypothetical networks.

Table1: Selected interactions derived from the BNET and AraCyc databases which are significantly activated in the presence of either the yeast or methyl jasmonate elicitor.

node1	node2	Methyl jasmonate early	Methyl jasmonate late	Yeast early	Yeast late
2-oxoglutarate	L-glutamate	1	0	0	0
2-oxoglutarate	succinate	0	0	0	1
4-aminobutanoate	L-glutamate	1	1	1	1
D-mannitol	D-fructose	1	1	0	1
D-mannitol	D-mannose	1	0	0	0
D-mannose	D-fructose	1	0	0	0
L-alanine	L-glutamate	0	1	0	1
L-alanine	L-serine	1	1	1	1

node1	node2	Methyl jasmonate early	Methyl jasmonate late	Yeast early	Yeast late	
L-alanine	beta-alanine	0	0	0	1	0
L-alanine	glycine	0	1	1	1	1
L-arginine	urea	1	1	1	0	1
L-asparagine	L-aspartate	1	1	1	1	1
L-aspartate	L-glutamate	1	0	1	1	1
L-aspartate	L-phenylalanine	1	1	1	1	0
L-aspartate	beta-alanine	1	1	1	1	1
L-aspartate	fumarate	1	1	1	1	0
L-glutamate	L-isoleucine	1	0	0	0	1
L-glutamate	L-phenylalanine	1	0	1	1	1
L-glutamate	L-valine	1	0	0	0	1
L-isoleucine	L-glutamate	1	0	0	0	1
L-leucine	L-glutamate	1	0	0	0	0
L-lysine	L-glutamate	1	0	0	0	1
L-phenylalanine	L-alanine	0	0	0	0	1
L-serine	glycine	1	1	1	1	1
L-threonine	glycine	1	0	0	0	1
L-valine	L-alanine	0	1	1	1	1
L-valine	L-glutamate	1	0	0	0	1
acetoacetate	succinate	0	1	1	0	0
allantoate	urea	1	1	1	0	1
alpha,alpha-trehalose	beta-D-glucose	1	1	1	1	0
beta-D-glucose	D-fructose	1	1	1	1	1
glycine	L-aspartate	1	0	0	0	0
glycine	L-glutamate	1	1	1	1	1
maleate	fumarate	1	1	1	1	1
maltose	beta-D-glucose	1	0	0	0	0
oxalate	succinate	0	0	0	1	1
succinate	fumarate	0	1	1	1	1
sucrose	myo-inositol	1	0	0	1	0

Table 2: Selected interactions derived from rank correlation and significant mutual selection by GA-DFA which are significantly activated in the presence of either the yeast or methyl jasmonate elicitor.

node1	node2	Methyl jasmonate early	Methyl jasmonate late	Yeast early	Yeast late
2-oxoglutarate	UNKNOWN-0021800-0174000	1	0	0	0
4-aminobutanoate	UNKNOWN-0013458-0142000	1	0	0	0
4-aminobutanoate	glycine	1	0	0	1
D-fructose	UNKNOWN-0029178-0217000	1	0	0	0
D-fructose	UNKNOWN-0034101-0204000	1	0	0	1
D-fructose	beta-D-glucose	1	0	0	1
HCO3-	UNKNOWN-0032718-0292000	0	0	1	1
L-arginine	UNKNOWN-0029670-0204000	1	0	0	0
L-arginine	allantoate	1	0	0	0
L-arginine	beta-alanine	0	0	1	0
L-asparagine	L-phenylalanine	1	0	0	0
L-asparagine	L-threonine	1	0	0	0
L-asparagine	UNKNOWN-0023305-0176000	1	0	0	1
L-asparagine	UNKNOWN-0032325-0204000	1	0	0	1
L-asparagine	allantoate	1	0	0	0
L-asparagine	beta-alanine	1	0	0	0
L-asparagine	urea	1	0	0	1

node1	node2	Methyl jasmonate early	Methyl jasmonate late	Yeast early	Yeast late	
L-glutamate	UNKNOWN-0025794-0213000	0	0	0	0	1
L-isoleucine	L-leucine	1	0	0	0	0
L-isoleucine	L-lysine	1	0	0	0	0
L-isoleucine	L-phenylalanine	1	0	0	0	0
L-isoleucine	L-threonine	1	0	0	0	0
L-isoleucine	L-valine	1	0	0	0	0
L-isoleucine	UNKNOWN-0023305-0176000	1	0	0	0	0
L-isoleucine	UNKNOWN-0032325-0204000	1	0	0	0	0
L-isoleucine	UNKNOWN-0047389-0204000	1	0	0	0	0
L-isoleucine	beta-alanine	1	0	0	0	0
L-leucine	L-phenylalanine	1	0	0	0	0
L-leucine	UNKNOWN-0023305-0176000	1	0	0	0	0
L-leucine	UNKNOWN-0036445-0389000	1	0	0	0	0
L-leucine	UNKNOWN-0047389-0204000	1	0	0	0	0
L-leucine	succinate	1	0	0	0	0
L-lysine	L-valine	1	0	0	0	0
L-lysine	UNKNOWN-0023305-0176000	1	0	0	0	0
L-lysine	succinate	1	0	0	0	0
L-phenylalanine	L-threonine	1	0	0	0	0
L-phenylalanine	L-valine	1	0	0	0	0
L-phenylalanine	UNKNOWN-0023305-0176000	1	0	0	0	0
L-phenylalanine	UNKNOWN-0029273-0299000	1	0	0	0	0
L-phenylalanine	UNKNOWN-0030528-0174000	1	0	0	0	0
L-phenylalanine	UNKNOWN-0032325-0204000	1	0	0	0	0
L-phenylalanine	UNKNOWN-0036445-0389000	1	0	0	0	0
L-phenylalanine	UNKNOWN-0047389-0204000	1	0	0	0	0
L-phenylalanine	beta-alanine	1	0	0	0	0
L-phenylalanine	succinate	1	0	0	0	0
L-threonate	UNKNOWN-0015748-0248000	1	0	0	0	0
L-threonate	UNKNOWN-0019609-0247000	1	0	0	0	1
L-threonine	L-valine	1	0	0	0	0
L-threonine	UNKNOWN-0023305-0176000	1	0	0	0	0
L-threonine	UNKNOWN-0029273-0299000	1	0	0	0	0
L-threonine	UNKNOWN-0030528-0174000	1	0	0	0	0
L-threonine	UNKNOWN-0031410-0406000	1	0	0	0	0
L-threonine	UNKNOWN-0032325-0204000	1	0	0	0	0
L-threonine	UNKNOWN-0036686-0204000	1	0	0	0	0
L-threonine	UNKNOWN-0047897-0204000	1	0	0	0	0
L-threonine	allantoate	1	0	0	0	0
L-threonine	beta-alanine	1	0	0	0	1
L-threonine	urea	1	0	0	0	0
L-valine	UNKNOWN-0023305-0176000	1	0	0	0	0