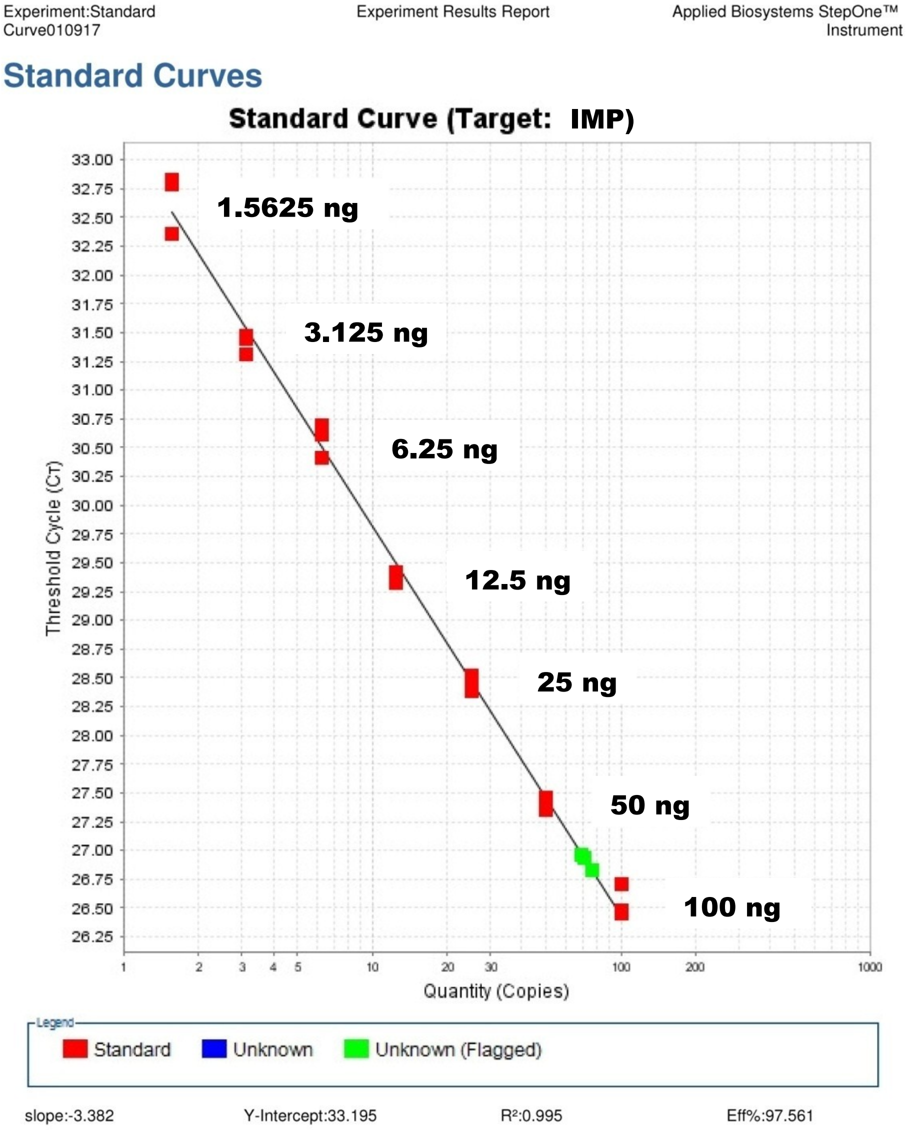


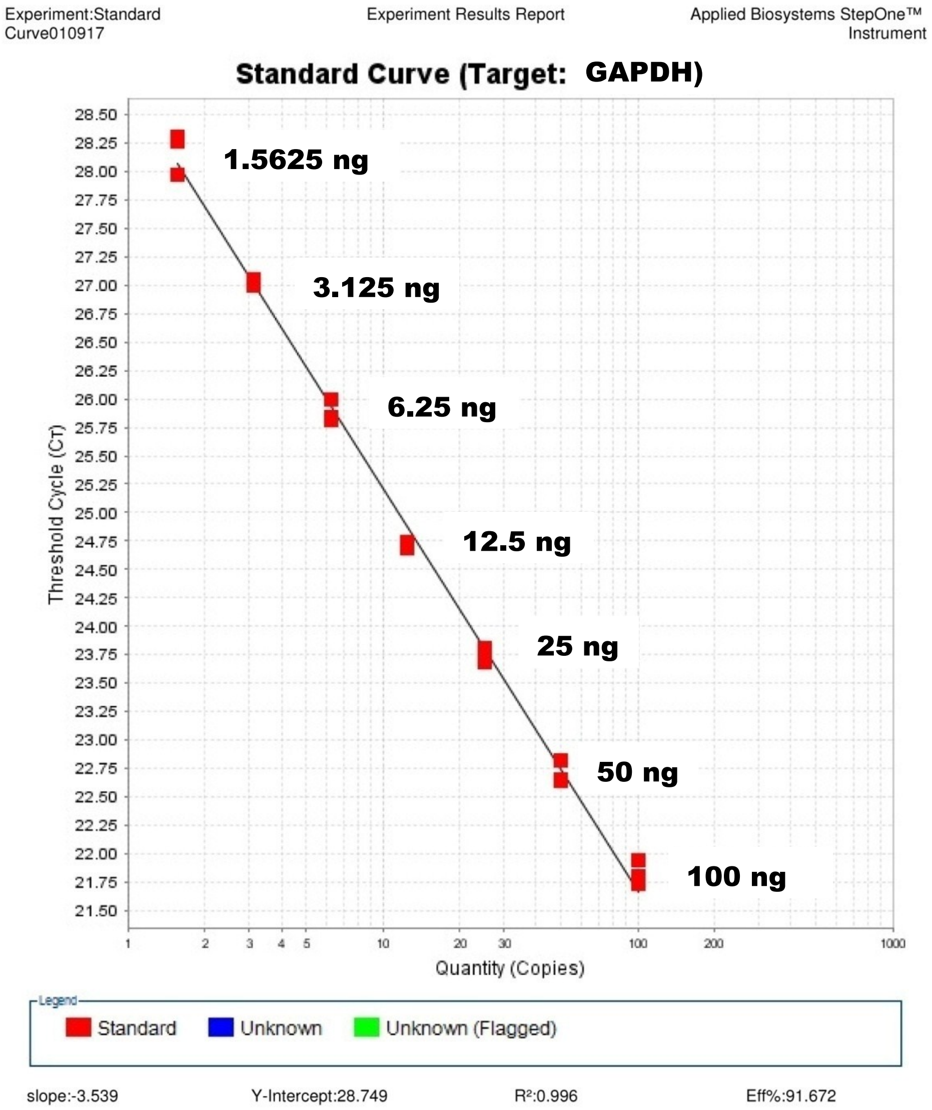
Experiment:Standard Curve010917 | Experiment Results Report | Applied Biosystems StepOne™ Instrument

Standard Curves

Standard Curve (Target: IMP)

slope:-3.382 Y-Intercept:33.195 R²:0.995 Eff%:97.561

Supplementary Fig. 1a. Standard curve of *IMP*. The detection limit of target gene (IMP) is 3.125 ng of RNA/ 10 µl reaction.



Supplementary Fig. 1b. Standard curve of *GAPDH*.

Supplementary Table 1. List of primers used in semi-quantitative RT-PCR.

Sl. No.	Target gene	Accession number	Primer sequence	Tm	Cycles for sqRT-PCR
Forward Library					
1.	*Alpha amylase*	JZ923597	Forward: 5’-GTGCCCAAGTGATCCAGAGT-3’ Reverse: 5’-TCGAAGAGGGTCATTTTTGG-3’	60.12^{0}C 60.04^{0}C	35
2.	*Inositol Monophosphatase3*	JZ923581	Forward: 5’-TTTGATTTGATGTCGCGAAG-3’ Reverse: 5’-CCGAGGTACAGGTGCAGAAT-3’	59.70^{0}C 60.13^{0}C	35
3.	*Tocopherol cyclase*	JZ923601	Forward: 5’-GCAACATGGCAGCACTAGAA- 3’ Reverse: 5’-TTGGGAGGAGGCTCTCTACA- 3’	60.02^{0}C 59.94^{0}C	35
4.	*Aspartic proteinase*	JZ923601	Forward: 5’-ATTGTGAAGCTGGAGCAAGG- 3’ Reverse: 5’- TGAAGACGTCCCCAAGAATC- 3’	60.40^{0}C 60.05^{0}C	35
5.	*NADP dependent malic enzyme*	JZ923606	Forward: 5’-GGCCGAGGTACAAATAACGA - 3’ Reverse: 5’- AACAAACCTGCAAGGACCAC- 3’	59.96^{0}C 60.01^{0}C	35
6.	*PEPCK*	JZ923598	Forward: 5’-GCGAGGTTGAGGTCTATGCT- 3’ Reverse: 5’-ACCACTCCCTCTTCATGCAC - 3’	59.46^{0}C 60.12^{0}C	35
7.	*Zm_FL51*	JZ923588	Forward: 5’- TGAAATGGTTTACCGTGCTG- 3’ Reverse: 5’-TGTTGTGTGGCACAAGTGAA - 3’	59.58^{0}C 59.75^{0}C	35
8.	*Zm_FL300*	JZ923602	Forward: 5’- TCTACTGAACAACAATCCTGCAA - 3’ Reverse: 5’- ATCTAGAAATAGCTGGGGATTGG - 3’	59.80^{0}C 59.59^{0}C	35
Reverse Library					
1.	*Metal transporter Nramp5*		Forward: 5’-TTCATCGTCGAGAGGGAAAC- 3’ Reverse: 5’-GTGGCTGCTTCTAGGCAAAC- 3’	60.20^{0}C 60.02^{0}C	35
2.	*Zm_RL303*		Forward: 5’- CGAGAATGTCGGCTTGAGTA - 3’ Reverse: 5’- CCTGATCTTAGGCCCTGACT - 3’	59.02^{0}C 58.35^{0}C	35
3.	*Zm_RL143*		Forward: 5’- GGCTCGAGGCATTTTCTCTAC-3’ Reverse: 5’- GGACGGAGGAGGCTAGGTTA-3’	60.35^{0}C 60.59^{0}C	35
4.	*Zm_RL365*		Forward: 5’-GTCCATCGACTACGCCTTTC- 3’ Reverse: 5’- CGAGAATGTCGGCTTGAGTA-3’	59.70^{0}C 59.02^{0}C	35

Supplementary Table 2. List of primers used in qRT-PCR experiments.

Sl. No.	Target gene	Accession No.	Primer sequence	size	Tm	L	From - To
1.	*Zea mays* NADP-dependent malic enzyme mRNA, complete cds	**AY864063.1**	F: 5'-AGGAGAAGAGGATGTTGCAG-3'	194	57.03	20	455-648
			R: 5'- TCGGAAGATTGACCCATACT-3'		57.05		
2.	*Zea mays* clone 263825 inositol monophosphatase 3 mRNA, complete cds	**EU963535.1**	F: 5'- TTTGTATGTGTCTCCGTTGG-3'	191	57.02	20	304-494
			R: 5'- CTATTGGTTCCAGCCTCTGT-3'		56.86		
3.	*Zea mays* thioredoxin h-like protein (LOC541940), mRNA	**NM_001111603.1**	F: 5'- CATTGCTCCTGTCTATGCTG-3'	100	57.02	20	180-279
			R: 5'- GTCCCATGAAGAGCTGAAGT-3'		56.86		
4.	*Zea mays* clone 337337 aspartic proteinase Asp1 precursor, mRNA, complete cds	**EU969925.1**	F: 5'-TAGCCTTGTTTCACAGCTCA-3'	103	57.22	20	585-687
			R: 5'-AACATCATCCCCAAAGAAGA-3'		57.02		
5.	*Zea mays* mRNA for phosphoenolpyruvate carboxykinase, complete cds	**AB018744.1**	F: 5'-GCAGACCATGTACCACTTCA-3'	169	57.07	20	1479-1647
			R: 5'-GCCGTACTTCTGCATCTTCT-3'		57.17		

L, length

Supplementary Table 3. Gene ontology term-based functional categorization of BABA-primed maize leaf (forward library) ESTs obtained by suppressive subtractive hybridization, based on biological process. Data distribution summary from BLAST2GO.

GO-term	**No. of sequences**
DNA repair	30
inositol phosphate dephosphorylation	12
phosphatidylinositol phosphorylation	12
response to cold	12
inositol biosynthetic process	12
L-ascorbic acid biosynthetic process	12
cell communication	12
signal transduction	12
single organism signaling	12
response to oxidative stress	11
response to salt stress	11
phloem sucrose loading	10
defense response	10
myo-inositol hexakisphosphate biosynthetic process	10
regulation of defense response	10
xanthophyll metabolic process	10
vitamin E biosynthetic process	10
fatty acid metabolic process	10
chlorophyll metabolic process	10

Supplementary Table 4. Gene ontology term-based functional categorization of control maize leaf ESTs, obtained by suppressive subtractive hybridization, based on biological process. Data distribution summary from BLAST2GO.

GO-term	**No. of sequences**
transport	113

Supplementary Table 5. Gene ontology term-based functional categorization of BABA-primed maize leaf (forward library) ESTs, obtained by suppressive subtractive hybridization, based on cellular component. Data distribution summary from BLAST2GO.

GO-term	No. of sequences
Nucleus	31
extracellular region	30
Mitochondrion	16
Cytosol	12
plasma membrane	12
chloroplast envelope	10
chloroplast thylakoid	10
Plastoglobule	10

Supplementary Table 6. Gene ontology term-based functional categorization of control maize leaf ESTs, obtained by suppressive subtractive hybridization, based on cellular component. Data distribution summary from BLAST2GO.

GO-term	No. of sequences
mitochondrion	144
integral component of membrane	113
chloroplast	31

Supplementary Table 7. Blast Top hit distribution of matched unigenes among different species generated from BLASTX in forward library. Data distribution summary from BLAST2GO.

Species	**#BLAST Top-Hits**
Zea mays	92
Lotus japonicas	16
Oryza sativa	2
Genlisea aurea	2
Sorghum bicolour	2
Heuchera sanguine	1
Setaria italic	1

Supplementary Table 8. Blast Top hit distribution of matched unigenes among different species generated from BLASTX in reverse library. Data distribution summary from BLAST2GO.

Species	**#BLAST Top-Hits**
Glycine max	121
Lotus japonicus	36
Vigna angularis var. angularis	34
Morus notabilis	5
Medicago truncatula	3
Cajanus cajan	3
Jatropha curcas	1

Supplementary Table 9. Gene ontology term-based functional categorization of BABA-primed maize leaf (forward library) ESTs, obtained by suppressive subtractive hybridization, based on molecular function. Data distribution summary from BLAST2GO.

GO-term	No. of sequences
zinc ion binding	46
nucleic acid binding	31
hydrolase activity, acting on acid anhydrides, in phosphorus-containing anhydrides	30
phosphoric diester hydrolase activity	30
alpha-amylase activity	30
calcium ion binding	30
inositol monophosphate 1-phosphatase activity	12
L-galactose-1-phosphate phosphatase activity	12
magnesium ion binding	12
tocopherol cyclase activity	10

Supplementary Table 10. Gene ontology term-based functional categorization of control maize leaf ESTs, obtained by suppressive subtractive hybridization, based on molecular function. Data distribution summary from BLAST2GO.

GO-term	**No. of sequences**
transporter activity	113
zinc ion binding	32

Supplementary Table 11. InterPro Scan (IPS) analysis showing the number of ESTs without IPS domains, with IPS domains, and GO domains in both libraries. Data distribution summary from BLAST2GO.

Type	Number of sequences	
	Forward Library	Reverse Library
without IPS	143	204
with IPS	49	0
with GOs	35	0

Supplementary Table 12. IPS domain analysis of functionally annotated ESTs from forward SSH library. Data distribution summary from BLAST2GO.

IPS Domain	No. of sequences
(IPR013780) Glycosyl hydrolase, all-beta	30
(IPR012850) Alpha-amylase, C-terminal beta-sheet	30
(IPR033121) Peptidase family A1 domain	2
(IPR021109) Aspartic peptidase domain	2
(IPR012301) Malic enzyme, N-terminal domain	1
(IPR016040) NAD(P)-binding domain	1
(IPR012302) Malic enzyme, NAD-binding	1
(IPR008210) Phosphoenolpyruvate carboxykinase, N-terminal	1

Supplementary Table 13. KEGG pathway analysis of functionally annotated ESTs from forward SSH library. Data distribution summary from BLAST2GO.

Pathway	**Pathway ID**	**Enzyme**	**No. of Seqs of Enzyme**
Starch and sucrose metabolism	map00500	ec:3.2.1.1 - glycogenase	30
Biosynthesis of antibiotics	map01130	ec:3.1.3.25 - phosphatase, ec:4.1.1.49 - carboxykinase (ATP)	13
Inositol phosphate metabolism	map00562	ec:3.1.3.25 - phosphatase	12
Phosphatidylinositol signaling system	map04070	ec:3.1.3.25 - phosphatase	12
Streptomycin biosynthesis	map00521	ec:3.1.3.25 - phosphatase	12
Aminobenzoate degradation	map00627	ec:3.1.3.41 - nitrophenyl phosphatase	12
Pyruvate metabolism	map00620	ec:1.1.1.39 - dehydrogenase (decarboxylating), ec:4.1.1.49 - carboxykinase (ATP), ec:1.1.1.38 - dehydrogenase (oxaloacetate-decarboxylating), ec:1.1.1.40 - dehydrogenase (oxaloacetate-decarboxylating) (NADP+), ec:4.1.1.3 - decarboxylase	3
Carbon fixation in photosynthetic organisms	map00710	ec:1.1.1.39 - dehydrogenase (decarboxylating), ec:4.1.1.49 - carboxykinase (ATP), ec:1.1.1.40 - dehydrogenase (oxaloacetate-decarboxylating) (NADP+)	3
Glycolysis / Gluconeogenesis	map00010	ec:4.1.1.49 - carboxykinase (ATP)	1
Citrate cycle (TCA cycle)	map00020	ec:4.1.1.49 - carboxykinase (ATP)	1

Supplementary Table 14. GO annotations of all the subtractive ESTs of forward library were performed using the Blast2GO program on the basis of biological process.

GO ID	GO Name	Parents (Name)	No. of Seqs
GO:0008152	metabolic process	biological_process	58
GO:0009987	cellular process	biological_process	56
GO:0006950	response to stress	response to stimulus	53
GO:0006281	DNA repair	single-organism metabolic process, DNA metabolic process, single-organism cellular process, cellular response to DNA damage stimulus	30
GO:0009058	biosynthetic process	metabolic process	24
GO:0009628	response to abiotic stimulus	response to stimulus	23
GO:0009266	response to temperature stimulus	response to abiotic stimulus	22
GO:0007165	signal transduction	single organism signaling, cellular response to stimulus, cell communication, regulation of cellular process	12
GO:0046855	inositol phosphate dephosphorylation	inositol phosphate catabolic process, phosphorylated carbohydrate dephosphorylation, single-organism carbohydrate metabolic process	12
GO:0009915	phloem sucrose loading	sucrose transport, phloem transport	10

Supplementary table 15. GO annotations of all the subtractive ESTs of forward library were performed using the Blast2GO program on the basis of molecular function

GO ID	GO Name	Parents (Name)	No. of Seqs
GO:0043169	cation binding	ion binding	59
GO:0016788	hydrolase activity, acting on ester bonds	hydrolase activity	42
GO:0003676	nucleic acid binding	heterocyclic compound binding, organic cyclic compound binding	31
GO:0016817	hydrolase activity, acting on acid anhydrides	hydrolase activity	30
GO:0016798	hydrolase activity, acting on glycosyl bonds	hydrolase activity	30
GO:0016160	amylase activity	hydrolase activity, hydrolyzing O-glycosyl compounds	30
GO:0016791	phosphatase activity	phosphoric ester hydrolase activity	12
GO:0009976	tocopherol cyclase activity	cyclase activity	10

Supplementary Table 16. GO annotations of all the subtractive ESTs of forward library were performed using the Blast2GO program on the basis of cellular component.

GO ID	GO Name	Parents (Name)	No. of Seqs
GO:0005634	nucleus	intracellular membrane-bounded organelle	31
GO:0005576	extracellular region	cellular_component	30
GO:0005739	mitochondrion	cytoplasmic part, intracellular membrane-bounded organelle	16
GO:0005886	plasma membrane	cell periphery, membrane, cell part	12
GO:0005829	cytosol	cytoplasmic part	12
GO:0010287	plastoglobule	chloroplast stroma, chloroplast part	10
GO:0009534	chloroplast thylakoid	plastid thylakoid, chloroplast, chloroplast part	10
GO:0009941	chloroplast envelope	plastid envelope, chloroplast part	10

Supplementary Table 17. GO annotations of all the subtractive ESTs of reverse library were performed using the Blast2GO program on the basis of biological process.

GO ID	GO Name	Parents (Name)	No. of Seqs
GO:0006810	transport	establishment of localization	113

Supplementary Table 18. GO annotations of all the subtractive ESTs of reverse library were performed using the Blast2GO program on the basis of molecular function.

GO ID	GO Name	Parents (Name)	No. of Seqs
GO:0005215	transporter activity	molecular_function	113
GO:0008270	zinc ion binding	transition metal ion binding	32

Supplementary Table 19. GO annotations of all the subtractive ESTs of reverse library were performed using the Blast2GO program on the basis of cellular component.

GO ID	GO Name	Parents (Name)	No. of Seqs
GO:0016021	integral component of membrane	intrinsic component of membrane	113
GO:0005739	mitochondrion	cytoplasmic part, intracellular membrane-bounded organelle	144
GO:0009507	chloroplast	plastid	31