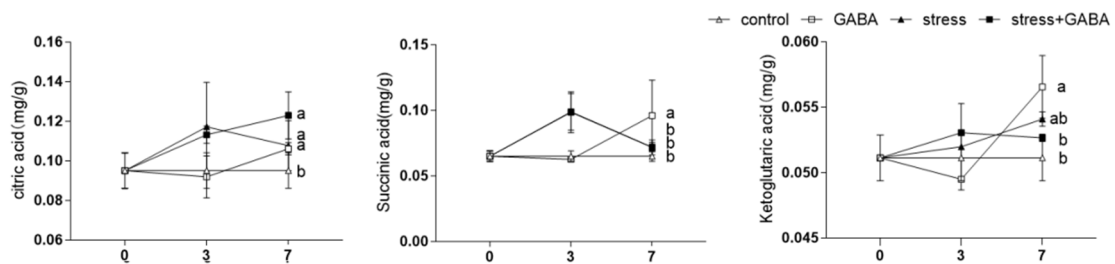
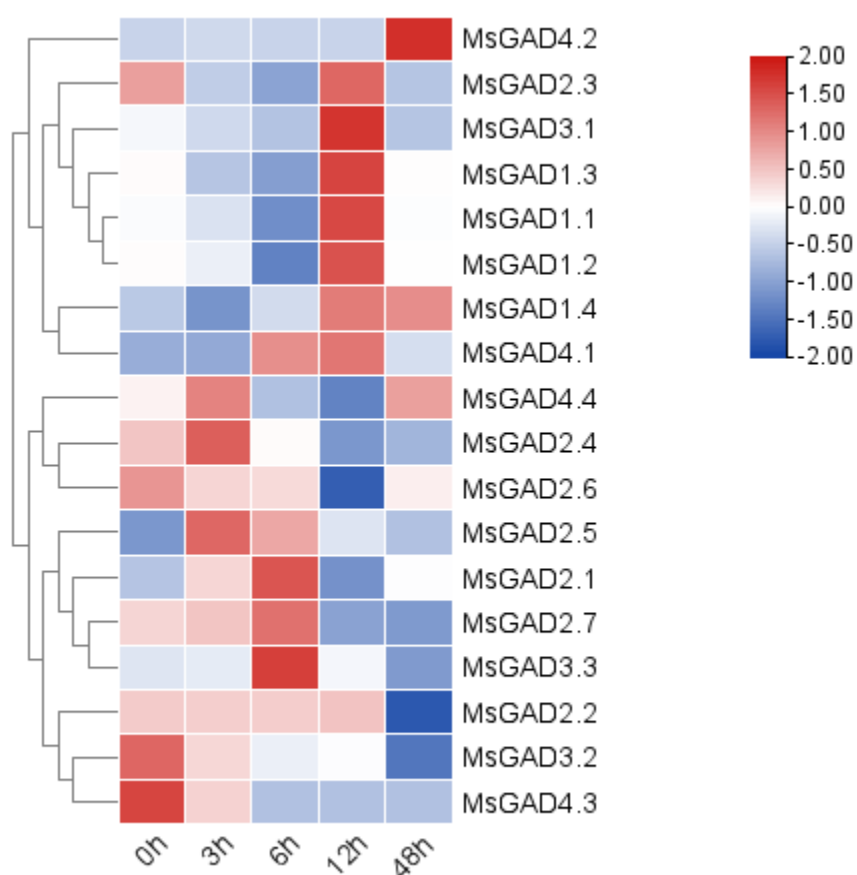


Supplementary Figure S1. Phenotypic chart of the effect of exogenous GABA on the alkali tolerance of Alfalfa Seedlings. The spraying concentrations of GABA were 0 mmol/L, 25 mmol/L, 50 mmol/L, 75 mmol/L and 100 mmol/L, observations on 10d and 21d.



Supplementary Figure S2. Succinic acid, citric acid and ketoglutaric acid in the leaves of alfalfa seedlings subjected to normal culture and alkali treatment with or without GABA. The seedlings were shown to one of the following four treatments: control, GABA (control + exogenous GABA), stress (alkali), and stress+GABA (alkali + exogenous GABA). Each value is the mean \pm SD of three independent experiments. (Duncan test: $P < 0.05$)



Supplementary Figure S3. Expression pattern analysis of predicted GAD genes in *M. sativa* leaves under different alkaline stress stages. Two-month-old *M. sativa* seedlings were precultured in a 1/2 Hoagland nutrient solution, then treated with 100 mmol/L NaHCO_3 (pH = 8.0) 0h, 3h, 6h, 12h and 48h. Immediately, the collected samples were frozen and stored at -80°C for RNA-seq. For all the above samples, three biological replicates were employed for each sample.

Gene name	Forward primer	Reverse primer
<i>MSGAD1</i>	CATTAGGGAGGATTCTCAAGGACT	CCAAAGCACTCTTTTAGCAACCAC
<i>MSGAD2</i>	ACAGTGTTCGAGATAGCAGACCA	TGTCCAAGAGATTCACTTTTTC
<i>MSGAD3</i>	ACCTGGAGGAGTTGAAGGGGAA	TAGTTTGAATCCTGGTAAGTGCTGC
<i>MSGAD4</i>	ACATCAAAGTGCTTCGTGCG	TGCAGTGTCAACCAAGGACTTCTC
<i>MSGABA-T</i>	CAGCGAGCTTACTGATTGTTGATGT	TCTTGGAACCTCGGAGCTATCTTGT
<i>MSGDH</i>	GACCGAGTTCCAAGGAGCTGATGC	CACAGTCACTCCACCAGCATTAGCA
<i>MSP5CS</i>	GCTAATTTGGAGATGGCTAAGCAG	CTTCCGACCGCAAATCGTCA
<i>MSPDH</i>	ACTCCTACCATCCTTCAGCCACCG	TTCCAACGTCCACGACAGGTCCTAT

Supplemental Table S1. Gene-specific primers designed for qRT-PCR.

M. sativa gene	Gene ID	M. truncatula orthologs	Sequence similarity
MsGAD1.1	MS.gene061565.t1	Medtr3g064740	98.425
MsGAD1.2	MS.gene05015.t1	Medtr3g064740	98.228
MsGAD1.3	MS.gene008330.t1	Medtr3g064740	98.228
MsGAD1.4	MS.gene049710.t1	Medtr3g064740	98.228
MsGAD2.1	MS.gene27393.t1	Medtr6g075210	98.798
MsGAD2.2	MS.gene065926.t1	Medtr6g075210	98.798
MsGAD2.3	MS.gene069682.t1	Medtr6g075210	98.597
MsGAD2.4	MS.gene041680.t1	Medtr6g075210	98.397
MsGAD2.5	MS.gene069681.t1	Medtr6g075210	98.397
MsGAD2.6	MS.gene069680.t1	Medtr6g075210	98.397
MsGAD2.7	MS.gene069679.t1	Medtr6g075210	98.196
MsGAD3.1	MS.gene008331.t1	Medtr3g064750	95
MsGAD3.2	MS.gene049711.t1	Medtr3g064750	92.537
MsGAD3.3	MS.gene061564.t1	Medtr3g064750	92
MsGAD4.1	MS.gene023240.t1	Medtr4g076070	67.647
MsGAD4.2	MS.gene028223.t1	Medtr4g076070	67.647
MsGAD4.3	MS.gene08383.t1	Medtr4g076070	64.706
MsGAD4.4	MS.gene34997.t1	Medtr4g076070	64.706

Supplemental Table S2. Naming of predicted GAD genes in M.sativa and their orthologs in M.truncatula. The characterized proteins having highest homology to the M.sativa proteins in alignment analyses are included for reference.