

SUPPLEMENTARY APPENDIX

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to:

Characterization of *LcGAPC* and its transcriptional response to salt and alkali stress in two ecotypes of *Leymus chinensis* (Trin.) Tzvelev

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Supplemental data

Table S1 Sequences of the primers used for RACE.

Label	Primer sequence (5'-3')	Expected product (bp)	Tm °C
<i>LcGAPC</i> -F1	CAGGACTGGAGAGGTGG	376	55
<i>LcGAPC</i> -R	TTCACTCGTTGTCGTACC		
<i>LcGAPC</i> -F2	ACCACTAACTGCCTTGCTCCC	499	55
<i>18SrRNA</i> -F	AGTATGGTCGCAAGGCTGAA	574	53
<i>18SrRNA</i> -R	CATTCAATCGGTAGGAGCGA		
<i>LcGAPC</i> -3'GSP	GGCTATCAAGGCTTCATCTGAGGGA	430	54
<i>LcGAPC</i> -3'NGSP	ATGGGTTATGTTGAGGAAGATTGGTC		
<i>LcGAPC</i> -5'GSP	AACGGTCTTCTGTGTGGCAGTGATGGCA	766	58
<i>LcGAPC</i> -5'NGSP	GGTCATCAAACCCTCAATAATACCGAAG		
<i>LcGAPC</i> -ORF F	ATGGGCAAGATTAAGATCG	1014	55
<i>LcGAPC</i> -ORF R	CTGAGTCTTGCCATGTGG		
q <i>LcGAPC</i> -F	CAGGACTGGAGAGGTGG	87	48
q <i>LcGAPC</i> -R	TTCACTCGTTGTCGTACC		
<i>Lcactin</i> -F	GTGCTTCCCTCTATGCAAGTGGT	93	55
<i>Lcactin</i> -R	CTGTTCTTGGCAGTCTCCAGCTC		

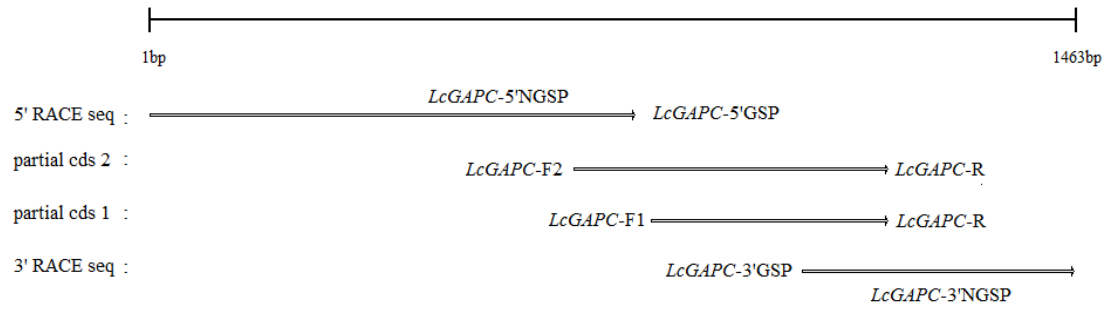


Figure S1 Schematic diagram representation of different primers for sequence amplification and fragment assembly.

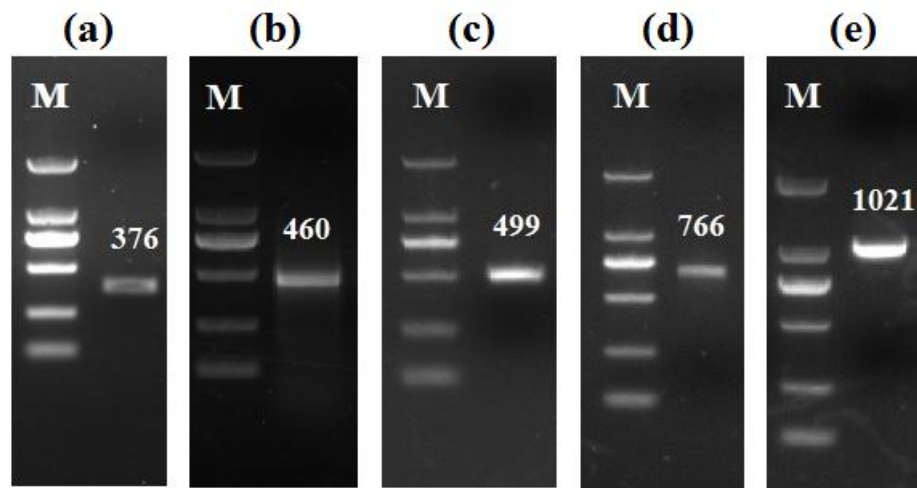


Figure S2 Isolation of *LcGAPC* fragments. M, DL2000 molecular size markers: 2000, 1000, 750, 500, 250 and 100bp DNA from top to bottom (TaKaRa, China). (a) The 376-bp conserved cDNA product for the *LcGAPC* gene; (b) The 499-bp 3'RACE products for the *LcGAPC* gene; (c) The 460-bp EST fragment combined with conserved cDNA; (d) The 766-bp 5'RACE products for the *LcGAPC* gene; (e) The 1021-bp full length cDNA products of the *LcGAPC* gene.

yellow-green	ATGGCAGATTAGATCGGAATCAACGGTGAATTCCTCTCTCTCTCGCGCGCGCTCTGATTTACCGGTGCTGCTGTTTTCTGTTGACCGT	100
gray-green	ATGGCAGATTAGATCGGAATCAACCGTGAATTCCTCTCTCTCTCGCGCGCGCTCTGATTTACCGGTGCTGCTGTTTTCTGTTGACCGT	100
yellow-green	GTGGGTGCTCTGTTTATTCGAGTGTCTGTTTCTCAGGTTTCGGAAGATCGGGAGCTCGTCGCCAGGTCGCCCTCCAGAGCAGCATCTCGA	200
gray-green	GTGGGTGCTCTGTTTATTCGAGTGTCTGTTTCTCAGGTTTCGGAAGATCGGGAGCTCGTCGCCAGGTCGCCCTCCAGAGCAGCATCTCGA	200
yellow-green	GCTGTCGCCCTCAACGACCCCTTCATCACCACCGTTACATGGTATACCTTCGATCTGCGATTTTACCCTGCTCCGATAGTAGATCTGGTGGCTAA	300
gray-green	GCTGTCGCCCTCAACGACCCCTTCATCACCACCGTTACATGGTATACCTTCGATCTGCGATTTTACCCTGCTCCGATAGTAGATCTGGTGGCTAA	300
yellow-green	AAGTTTTAGAGTGGTTTTACCTGTGATTTTCTGTTTACGATACCTGCTGTTGTTCTGTTTACGATACCTGCTGTTGTTCTGTTTACGATACCT	400
gray-green	AAGTTTTAGAGTGGTTTTACCTGTGATTTTCTGTTTACGATACCTGCTGTTGTTCTGTTTACGATACCTGCTGTTGTTCTGTTTACGATACCT	400
yellow-green	CATGCTTTGGTTACGGTCTGATAGTTCGCTGATTAGAGTCTGCTTTTGTCTCTGTAGTCTCTTTACTATTGGCAATAGTGTATTTTCTG	500
gray-green	CATGCTTTGGTTACGGTCTGATAGTTCGCTGATTAGAGTCTGCTTTTGTCTCTGTAGTCTCTTTACTATTGGCAATAGTGTATTTTCTG	500
yellow-green	TTTCTACGATCTGAGTGTGTGTCTGTTGGTGGCTGATTATACTACATATCATGATTCCTCTGTTGGTTTGTAAATGAGTGAATCTGTGGTT	600
gray-green	TTTCTACGATCTGAGTGTGTGTCTGTTGGTGGCTGATTATACTACATATCATGATTCCTCTGTTGGTTTGTAAATGAGTGAATCTGTGGTT	600
yellow-green	TGTGTATGACAGACTACATGTTCAAGTACGACACAGTTACGGCCACGGGAAGCACAGTGACATCAAGCTCAAGGACACAGACCTGCTTCGGG	700
gray-green	TGTGTATGACAGACTACATGTTCAAGTACGACACAGTTACGGCCACGGGAAGCACAGTGACATCAAGCTCAAGGACACAGACCTGCTTCGGG	700
yellow-green	AAGGCCAGTTATGTCCTCAGCGTCAGTATGTTGATTACGGACCTCTCGAAGGGTGTGTGGTTAAAGTTGTGAACATAATGTTGATGTTCT	800
gray-green	AAGGCCAGTTATGTCCTCAGCGTCAGTATGTTGATTACGGACCTCTCGAAGGGTGTGTGGTTAAAGTTGTGAACATAATGTTGATGTTCT	800
yellow-green	GTACTTGAAGGAACCGGAGGATCCCATGGCTGAGCTGTGCCGATATGTTGTGGAGTCTCCGGTCTTCACTGACAGGACAGGCGCT	900
gray-green	GTACTTGAAGGAACCGGAGGATCCCATGGCTGAGCTGTGCCGATATGTTGTGGAGTCTCCGGTCTTCACTGACAGGACAGGCGCT	900
yellow-green	GCTCAGTTGAAGGTATAATAATTGACCTCTGTTTTCGCTTACGATGATGATGATTAATTCGAGCTATAGCTCTTATGGTCTTGAATGAATAT	1000
gray-green	GCTCAGTTGAAGGTATAATAATTGACCTCTGTTTTCGCTTACGATGATGATGATTAATTCGAGCTATAGCTCTTATGGTCTTGAATGAATAT	1000
yellow-green	ATGAGGCTGAGAAAGAACTCTTTGGGTTTGTGAAGTTCATAGAAATGTTGCTGCTCCATATATATTTATTTCTTATAGGCTGAAAAGCAT	1100
gray-green	ATGAGGCTGAGAAAGAACTCTTTGGGTTTGTGAAGTTCATAGAAATGTTGCTGCTCCATATATATTTATTTCTTATAGGCTGAAAAGCAT	1100
yellow-green	GACAGAAACACACACACCTCAAGGTCGGAGAGTAAAGTAGAATCAATGTTGTTGCTTACGATGATGTTGGTGTTCAGGATAGC	1200
gray-green	GACAGAAACACACACACCTCAAGGTCGGAGAGTAAAGTAGAATCAATGTTGTTGCTTACGATGATGTTGGTGTTCAGGATAGC	1200
yellow-green	TATATAGACGACTGACTTCATCTAGTGTAGTCCACTGAGTCTGATTAACCATCAATTTATGGTTTACACAGATAGTGTCTTTAGCGGCTA	1300
gray-green	TATATAGACGACTGACTTCATCTAGTGTAGTCCACTGAGTCTGATTAACCATCAATTTATGGTTTACACAGATAGTGTCTTTAGCGGCTA	1300
yellow-green	TTTTAATTAATTTTCGATGCTAGCTGTATGCAAGTCCATTTATTTAGGGACTTAGCTTTTTCGATGATATCCAAACACTAGTAATGTGATGAAC	1400
gray-green	TTTTAATTAATTTTCGATGCTAGCTGTATGCAAGTCCATTTATTTAGGGACTTAGCTTTTTCGATGATATCCAAACACTAGTAATGTGATGAAC	1400
yellow-green	TACACTGTTCAATGAATATAGGTTGGTGGCAAGAGGTGGTCACTCTGCCCCCAAGCAAGAGTCCCTTAATGTTGGTGGTGGTCAATGAGGACAG	1500
gray-green	TACACTGTTCAATGAATATAGGTTGGTGGCAAGAGGTGGTCACTCTGCCCCCAAGCAAGAGTCCCTTAATGTTGGTGGTGGTCAATGAGGACAG	1500
yellow-green	TACACTGAGAGCTTAACATGTGCTCAATGTGACCTGACACCTAACCTGCTTGCCTTACCTTAAAGTATGTTCTGCTTCTGCTCATCTACCT	1600
gray-green	TACACTGAGAGCTTAACATGTGCTCAATGTGACCTGACACCTAACCTGCTTGCCTTACCTTAAAGTATGTTCTGCTTCTGCTCATCTACCT	1600
yellow-green	TGATGACTAGTGTGGGATGTGGTTCATTTGTATACCTGTAAATCATTCACAGGTCATTAATGACAACTTCGGTATATTAGGGGTTTGTAGAC	1700
gray-green	TGATGACTAGTGTGGGATGTGGTTCATTTGTATACCTGTAAATCATTCACAGGTCATTAATGACAACTTCGGTATATTAGGGGTTTGTAGAC	1700
yellow-green	CACGTGTCATGCTCAGTGGTAAAGTTTGTGGCAGTCTGGCAGTGTCTTAAATTAATCTGCTTTCGAACTTCGGTAAATGACATGGTGTACT	1800
gray-green	CACGTGTCATGCTCAGTGGTAAAGTTTGTGGCAGTCTGGCAGTGTCTTAAATTAATCTGCTTTCGAACTTCGGTAAATGACATGGTGTACT	1800
yellow-green	CCCTCAGCCACACAGAGACTGTTGATGCTCCCTCGAGCAGGACTGGAGAGTGGCAGAGTGGCAAGCTTTAATCATCAITCCGAGTACAGCATGGTGC	1900
gray-green	CCCTCAGCCACACAGAGACTGTTGATGCTCCCTCGAGCAGGACTGGAGAGTGGCAGAGTGGCAAGCTTTAATCATCAITCCGAGTACAGCATGGTGC	1900
yellow-green	TGCCAAGGTTGCCACGAACAGCAATGTGCAGTGTGTTTCAGACAGTGTGTTGAATGAATACCTGCTTTTCTCTATGGCTGTGTGTGATATGG	2000
gray-green	TGCCAAGGTTGCCACGAACAGCAATGTGCAGTGTGTTTCAGACAGTGTGTTGAATGAATACCTGCTTTTCTCTATGGCTGTGTGTGATATGG	2000
yellow-green	GTATATGTTTTCCATATGAGATAATCTGTGTTGTAAACATGTTTAAACAGCAAGCTTTGGCTCATGTTCAATATGTGCTGCTTATTTACAT	2100
gray-green	GTATATGTTTTCCATATGAGATAATCTGTGTTGTAAACATGTTTAAACAGCAAGCTTTGGCTCATGTTCAATATGTGCTGCTTATTTACAT	2100
yellow-green	TGTGAGCTTATGAGTGTCTGTTGATTTAAATGATTACAGCACTTCGCTTAATGATATAATGATTTTCAGGCTGTTGGTAAGGTTCTCTGAGCTGAA	2200
gray-green	TGTGAGCTTATGAGTGTCTGTTGATTTAAATGATTACAGCACTTCGCTTAATGATATAATGATTTTCAGGCTGTTGGTAAGGTTCTCTGAGCTGAA	2200
yellow-green	CACCAAGCTTACCGGTATGCTTCCGGGTTCCCTGTGGATGTGTGCTGTTGATCTCACTGTAGAACCGGAAGGCTGCATCATACAGGACATC	2300
gray-green	CACCAAGCTTACCGGTATGCTTCCGGGTTCCCTGTGGATGTGTGCTGTTGATCTCACTGTAGAACCGGAAGGCTGCATCATACAGGACATC	2300
yellow-green	AAGAGGCTAACGTAAGTGTGATCTTATGCACTCTCGAGACCTTGTGTGAGAAAGCTATTTGTCTGATACATGTTCTGCAATTTTGT	2400
gray-green	AAGAGGCTAACGTAAGTGTGATCTTATGCACTCTCGAGAGCTTGTGTGAGAAAGCTATTTGTCTGATACATGTTCTGCAATTTTGT	2400
yellow-green	GTCCGTGGGCTTCTCTTATTCCTGCTTACCTGCT	2500
gray-green	GTCCGTGGGCTTCTCTTATTCCTGCTTACCTGCT	2500
yellow-green	GAAACTCAAGGGTTCATGGGTTACGTTGAGGAAATTTGGTCTCCACGACTTATCGGTGACAGCAGGTATGCTGATGCTTATTTGCTGCTGCTGCTG	2600
gray-green	GAAACTCAAGGGTTCATGGGTTACGTTGAGGAAATTTGGTCTCCACGACTTATCGGTGACAGCAGGTATGCTGATGCTTATTTGCTGCTGCTGCTG	2600
yellow-green	AACTACAGTGTCTCAGATAGTAATCTGTTCCATGTTGATTTTCAATTAGGTCGAGCATCTTGATGCCAAGGCTGGAATGCTCTGAACGACC	2700
gray-green	AACTACAGTGTCTCAGATAGTAATCTGTTCCATGTTGATTTTCAATTAGGTCGAGCATCTTGATGCCAAGGCTGGAATGCTCTGAACGACC	2700
yellow-green	ATTTCGTCAAGCTTGTCTGGTGTATGACACAGTGGGTTACAGGTGAGTTGCCAAAGGATTTATTTCTACACATGCTATCCATGCTCTGCTT	2800
gray-green	ATTTCGTCAAGCTTGTCTGGTGTATGACACAGTGGGTTACAGGTGAGTTGCCAAAGGATTTATTTCTACACATGCTATCCATGCTCTGCTT	2800
yellow-green	CATAGCTCAGCCTGTAACACTCGGAATCTTCTCTGCGACACCGGCTTGTGACCTGATCCGCCACATGGCCAGAGCTCAGTA	2887
gray-green	CATAGCTCAGCCTGTAACACTCGGAATCTTCTCTGCGACACCGGCTTGTGACCTGATCCGCCACATGGCCAGAGCTCAGTA	2887

Figure S3 Complete nucleotide sequence of *GAPC* in two ecotypes of *Leymus chinensis*. The sequences were compared using DNAMAN software.

Note: Dark blue color denotes 100% similarity. Light blue color denotes non-identical nucleotides.



Figure S4 Structure analysis of *GAPC* gene in *Leymus chinensis*.

Note: The gene structure of *GAPC* was analyzed by GSDS (<http://gsds.cbi.pku.edu.cn/>). Exons are shown as yellow boxes and introns are shown as thin lines.

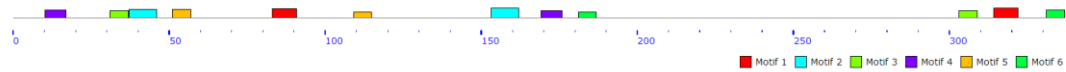
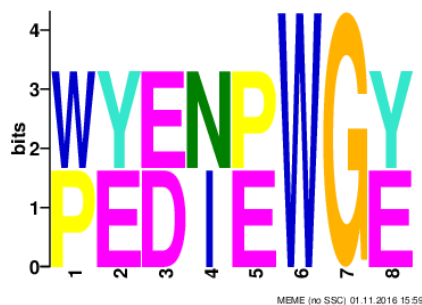
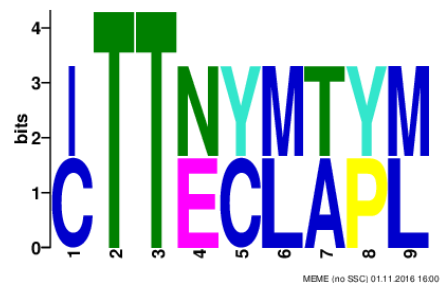


Figure S5 Predicted structure of *GAPC* proteins in *Leymus chinensis*.

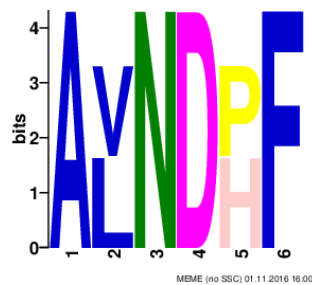
Note: The line represents the coding sequence and 6 motifs are shown. The amino acid sequences of *LcGAPC* were used as an input of MEME (<http://meme-suite.org/tools/meme>).



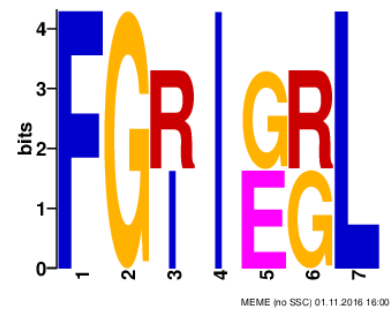
Motif 1



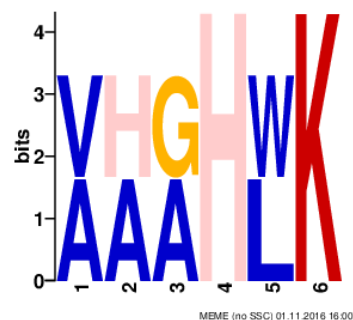
Motif 2



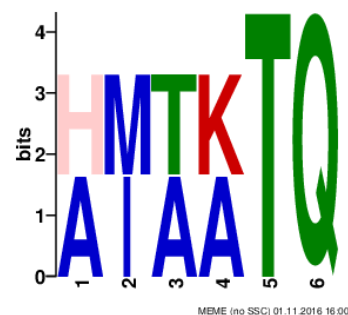
Motif 3



Motif 4



Motif 5



Motif 6

Figure S6 Motif LOGOs of *LcGAPC* generated by MEME.

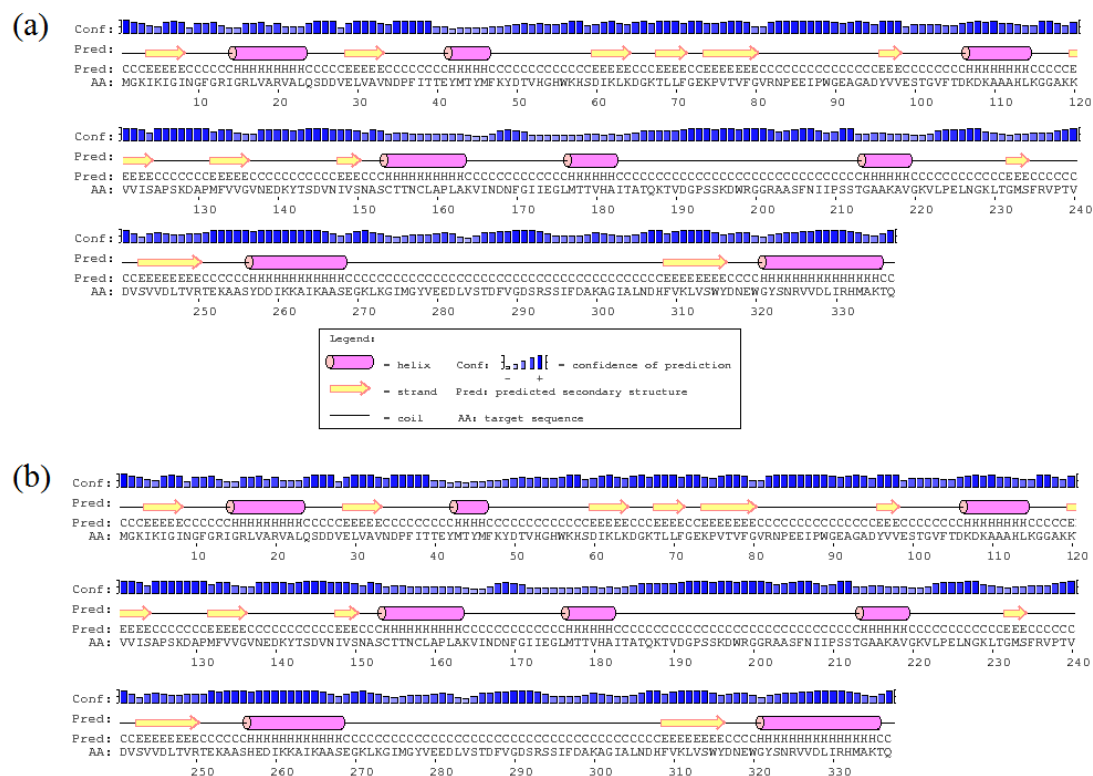


Figure S7 Predicted secondary structures of *LcGAPC* in the yellow-green type (a) and gray-green type (b) of *Leymus chinensis*.

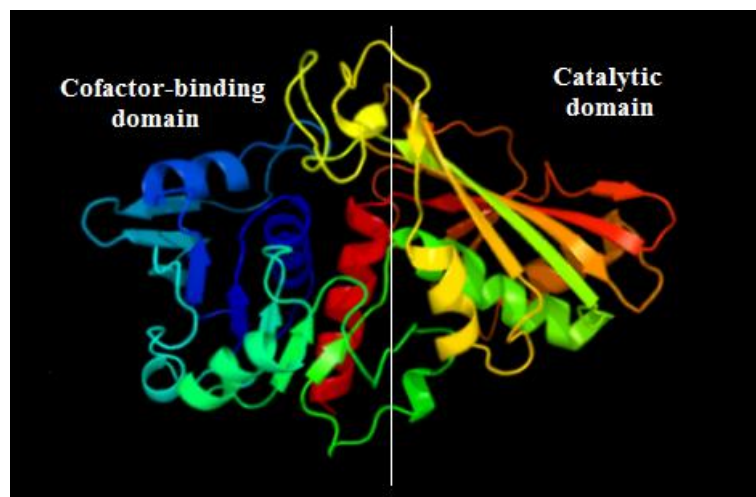


Figure S8 Predicted structure of a single *LcGAPC* subunit. The cofactor-binding domain is on left side and the catalytic domain is on the right side.

14 Nearest Neighbors					Names and origin	
id	site	distance	identity	comments		
G3PX_HORVU	cyto	5.8	99% [Uniprot]	SWISS-PROT45:Cytoplasmic.	Protein names	Recommended name: Glyceraldehyde-3-phosphate dehydrogenase 1, cytosolic EC=1.2.1.12
G3PD_MAIZE	cyto	25.4	95% [Uniprot]	SWISS-PROT45:Cytoplasmic.		
G3PC_MAIZE	cyto	29.6	95% [Uniprot]	SWISS-PROT45:Cytoplasmic.	Gene names	Name:GAPC
G3PC_PETHY	cyto	35.0	85% [Uniprot]	SWISS-PROT45:Cytoplasmic.	Organism	Hordeum vulgare (Barley)
G3PC_ANTMA	cyto	56.2	86% [Uniprot]	SWISS-PROT45:Cytoplasmic.	Taxonomic identifier	4513 [NCBI]
G3PC_PINSY	cyto	69.2	83% [Uniprot]	SWISS-PROT45:Cytoplasmic.		
G3PC_RANAC	cyto	72.5	86% [Uniprot]	SWISS-PROT45:Cytoplasmic.		
G3PC_CRAPL	cyto	73.0	83% [Uniprot]	SWISS-PROT45:Cytoplasmic.		
G3PC_PEA	cyto	75.3	83% [Uniprot]	SWISS-PROT45:Cytoplasmic.		
G3PC_PHYPA	cyto	78.5	79% [Uniprot]	SWISS-PROT45:Cytoplasmic.		
G3PC_MAGLI	cyto	81.4	87% [Uniprot]	SWISS-PROT45:Cytoplasmic.		
G3PC_ORYSA	cyto	82.6	91% [Uniprot]	SWISS-PROT45:Cytoplasmic.		
G3PC_TAXBA	cyto	94.5	83% [Uniprot]	SWISS-PROT45:Cytoplasmic.		
G3PC_CHLRE	cyto	104.7	67% [Uniprot]	SWISS-PROT45:Cytoplasmic.		
					Names and origin	
					Protein names	Recommended name: Glyceraldehyde-3-phosphate dehydrogenase 1, cytosolic EC=1.2.1.12
					Gene names	Name: GAPC1 Synonyms:GAPC, GPC1
					Organism	Zea mays (Maize)
					Taxonomic identifier	4577 [NCBI]

Figure S9 Prediction of LcGAPC protein localization sites in cells.

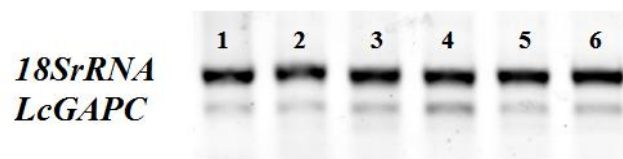


Figure S10 LcGAPC expression of yellow-green type and gray-green type of *Leymus chinensis* in different growth stage. Lanes 1, 3 and 5 represent the yellow-green type of LcGAPC expression in the turning-green stage, growth stage and heading stage; Lanes 2, 4 and 6 represent the gray-green type of LcGAPC expression in the turning-green stage, growth stage and heading stage.

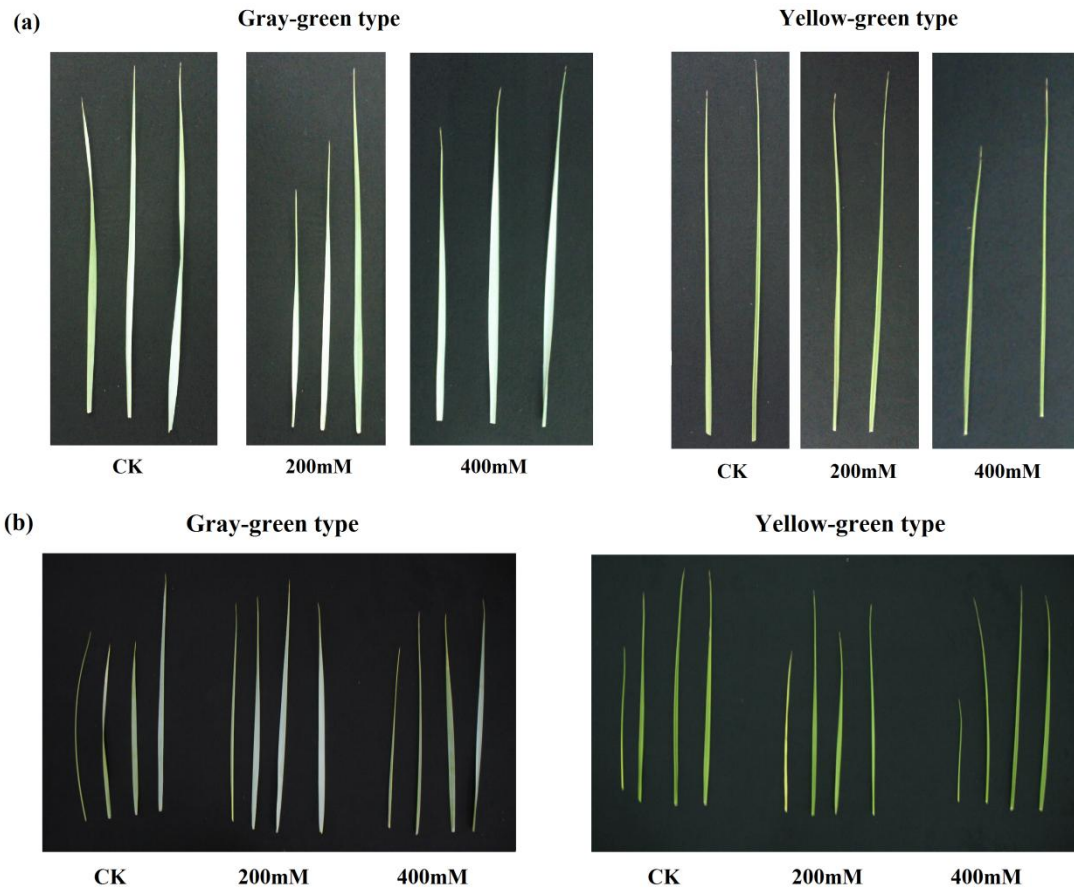


Figure S11 Phenotypes of *Leymus chinensis* subjected to different concentrations of NaCl for 24 hours (a) and 3 days (b).

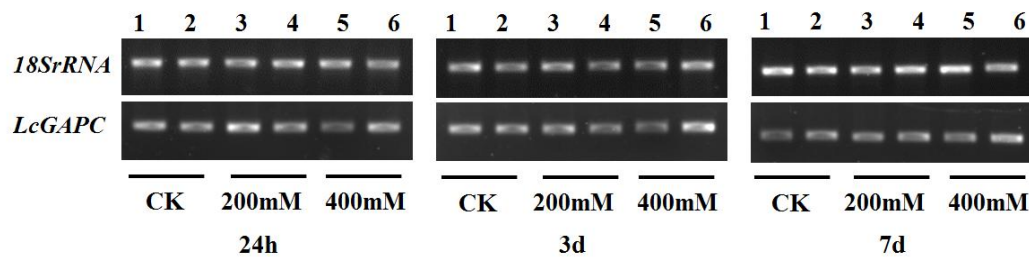


Figure S12 *LcGAPC* expression of *Leymus chinensis* in two ecotypes subjected to different concentrations of salt stress for 24 h, 3 d and 7 d. Lanes 1, 3 and 5 represent the yellow-green type of *Leymus chinensis*; Lanes 2, 4 and 6 represent the gray-green type of *Leymus chinensis*.