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Supplementary Material

Co-expression of xerophyte *Zygophyllum xanthoxylum ZxNHX* and *ZxVP1-1* enhances salt and drought tolerance in transgenic *Lotus corniculatus* by increasing cations accumulation

Ai-Ke Bao, Yan-Wen Wang, Jie-Jun Xi, Chen Liu, Jin-Lin Zhang and Suo-Min Wang

[A]State Key Laboratory of Grassland Agro-ecosystems, College of Pastoral Agriculture Science and Technology, Lanzhou University, Lanzhou 730 020, PR China.

[B]These authors have contributed equally to this work.

[C]Corresponding author. Email: smwang@lzu.edu.cn

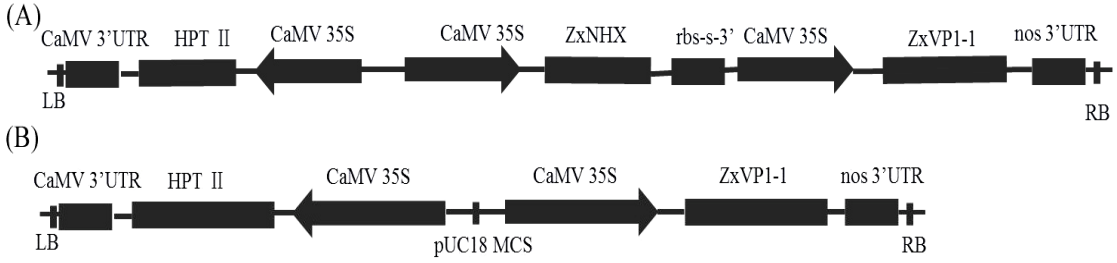

Fig. S1. Vector constructs used for *L. corniculatus*. (A) T-DNA region of the binary vector construct carried the *Z. xanthoxylum* tonoplast Na^+/H^+ antiporter gene *ZxNHX* and H^+-PPase gene *ZxVP1-1*. (B) The vector construct carried *ZxVP1-1*gene. LB, left border; RB, right border; nos, nopaline synthase terminator sequence; HPT, hygromycin phosphotransferase resistance gene; CaMV 35S, cauliflower mosaic virus 35S promoter sequence; rbs-s-3', ribosomal binding sites; pUC18 MCS, pUC18 plasmid multiple cloning site.

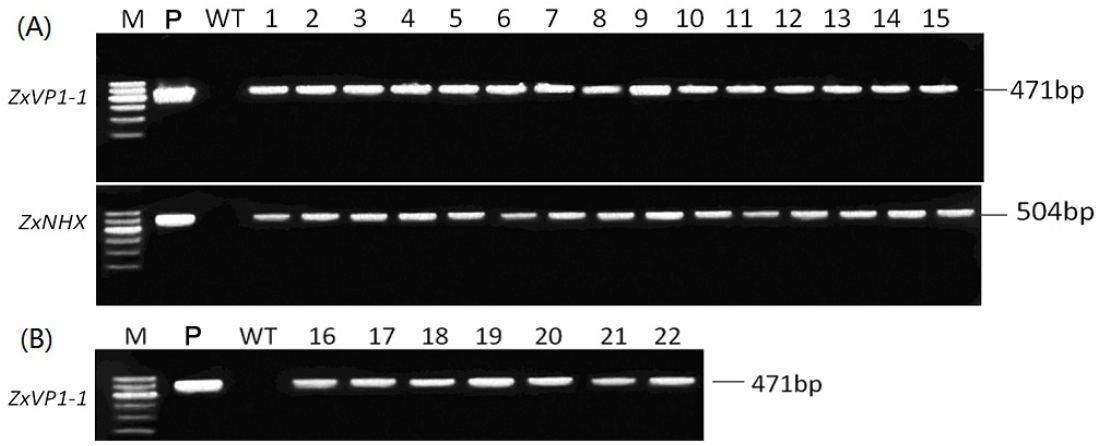

Fig. S2. PCR analysis of (A) *ZxNHX* and *ZxVP1-1*, and (B) *ZxVP1-1* in putative transgenic *L. corniculatus*. Specific PCR products (471 bp for *ZxVP1-1*, 504 bp for *ZxNHX*) were detected in putative transformants. M, DNA ladder; P, plasmids carrying *ZxNHX* and/or *ZxVP1-1*; WT, wild-type plants; 1-15, putative transgenic lines co-transferred *ZxNHX* and *ZxVP1-1*; 16-22, putative transgenic lines transferred *ZxVP1-1*.

Table S1. Transgenic *L. corniculatus* lines showed higher plant height and more shoot dry weight

Plants were watered every 2 days with 1/8 Hoagland nutrient solution for 4 weeks, then were treated with 200 mM NaCl for 10 d (Salt) and withheld water for 10 d (Drought), respectively. Data are means ± s.e. (n = 12). Different letters after data indicate significant difference at P<0.05 in same column (Duncan test). WT, wild-type plants; L1, 2, 3, 6, 8, 9, 10, 12, 13, 15, transgenic lines co-expression *ZxNHX* and *ZxVP1-1*; L 16, 18, 20, 21, 22, transgenic lines expressing *ZxVP1-1*

Lines	Control		Salt		Drought	
	Plant height (cm)	Shoot dry weight (g plant^{-1})	Plant height (cm)	Shoot dry weight (g plant^{-1})	Plant height (cm)	Shoot dry weight (g plant^{-1})
WT	35.3±0.7f	1.85±0.12c	23.8±1.1f	0.73±0.05h	30.8±1.2f	0.92±0.05g
L1	45.5±1.1a	2.41±0.10a	40.2±1.3a	1.86±0.05a	42.5±1.5a	1.83±0.04ab
L2	43.1±0.9b	2.32±0.09ab	39.1±0.7a	1.83±0.03a	40.2±1.6ab	1.88±0.05a
L3	43.4±2.1abc	2.25±0.07ab	38.4±0.6ab	1.82±0.04ab	36.8±0.8cd	1.72±0.08bcd
L6	40.6±2.3bcd	2.14±0.09b	36.6±1.4bcd	1.76±0.02bc	37.9±0.5c	1.60±0.07cd
L8	38.5±0.8d	1.92±0.06c	35.6±0.9d	1.63±0.05ef	36.1±1.0d	1.68±0.11cd
L9	39.8±1.6cd	2.03±0.11bc	36.2±0.5d	1.72±0.03cd	35.6±0.7d	1.61±0.05d
L10	40.5±1.8cd	2.19±0.12b	37.1±1.2bcd	1.75±0.04bcd	35.8±1.5cd	1.67±0.02d
L12	41.8±1.1bc	2.22±0.08b	37.9±0.4bc	1.69±0.03de	36.1±0.7d	1.73±0.08bcd
L13	43.9±2.1abc	2.16±0.13bc	39.2±1.8abc	1.79±0.07abc	37.5±1.3bcd	1.70±0.06cd
L15	39.5±0.6d	1.94±0.09c	36.2±1.3cd	1.62±0.03f	37.1±0.8cd	1.66±0.05cd
L16	36.2±1.0e	1.88±0.07c	30.4±0.9e	1.53±0.04g	33.4±1.1e	1.29±0.06f
L18	40.1±1.7cd	2.11±0.07b	36.6±0.9cd	1.71±0.06cd	35.9±0.9d	1.50±0.03e
L20	38.5±0.9d	1.91±0.05c	32.1±1.0e	1.61±0.04fg	32.5±0.3e	1.32±0.08f
L21	43.6±2.1ab	2.21±0.06b	39.1±0.8ab	1.73±0.04cd	36.9±1.3cd	1.68±0.05cd
L22	42.6±1.4bc	2.18±0.11b	37.2±1.2bcd	1.76±0.03c	37.6±0.8cd	1.74±0.04c