



Exploiting genome conservation between model and forage *Lotus* species: association mapping analyses to discover drought tolerance QTL in *Lotus tenuis*

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INTRODUCTION

Lotus tenuis is a diploid (2n=12), self-incompatible perennial forage species. Whereas, *L. japonicus* is a model species because of its diploid small genome, self-crossing, and short ontogenetic cycle. Due to its simpler genetics, *L. japonicus* has been the focus of legume genome and functional genomic programs allowing rapid generation of useful products for legume breeding (i.e. molecular makers, high-density linkage maps, genome database). Furthermore, comparative genetic studies showed genome-structure conservation among both species. An association mapping (LtAM) population including 100 *L. tenuis* genotypes was developed for identifying phenotypic traits and genomic region (QTL) controlling drought tolerance in *L. tenuis*.

MATERIAL AND METHODS

Plant phenotyping

- Plant spaced experiment
- 2 water environments (Irrigated vs Rainfed).
- 2 growing seasons.
- Alpha lattice experimental design, 5 replicates.
- Physiological and agronomical traits were measured.



Plant Genotyping

- Genomic DNA was extracted from an apical shoot (undeveloped leaves) with the DNeasy Plant Mini Kit (Qiagen).
- Eighty-eight SSR primers, previously developed in *L. japonicus*, were used for this assay.

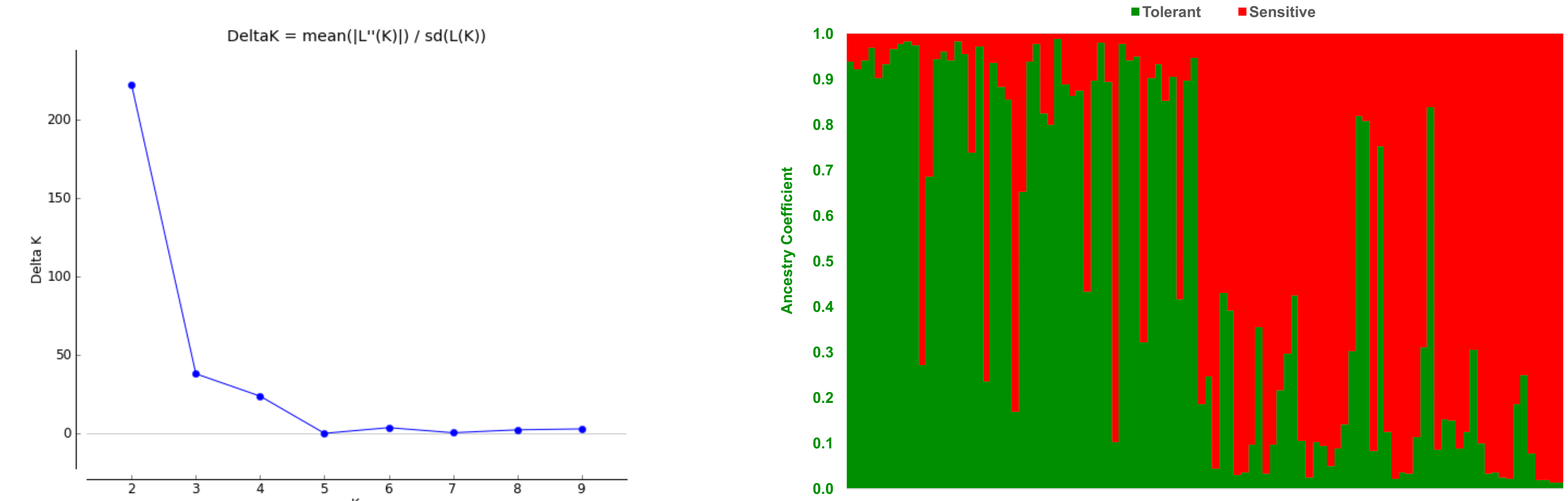
http://www.kazusa.or.jp/lotus/markerdb_index.html

- The PCR products were genotyped with an ABI 3130 xl automated sequencer and scored with GeneMapper® V4.0 (Applied Biosystems Inc.).

Data analyses

- ANOVA with mixed models in R (lme4)
- Population structure (STRUCTURE)
- Association Mapping (TASSEL)

Population structure analysis



Phenotypic characterization under field condition

(Inostroza et al., 2015. Crop and Pasture Science)

Table 1. Mean \pm standard error of the mean, range, and environment (E), genotype (G) and G \times E interaction effects of some physiological traits evaluated in 100 *Lotus tenuis* genotypes cultivated under irrigated and rainfed conditions during two seasons (summer-autumn 2011 and 2011-12 growing season)

In the 2011 season, the physiological traits were evaluated 60 days after planting (4 March); in the 2011-12 growing season, the physiological traits were evaluated on 21 February. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Physiological traits	Growing Seasons	Irrigated		Rainfed		ANOVA		
		Mean	Range	Mean	Range	E	G	G \times E
Xylem water potential (wps, MPa)	2011	-0.33 \pm 0.014	-0.7 to -0.11	-0.95 \pm 0.027	-1.57 to -0.34	***	**	**
	2011-12	-0.73 \pm 0.019	-1.24 to -0.33	-1.22 \pm 0.024	-1.84 to -0.61	***	n.s.	*
Osmotic potential (wpr, MPa)	2011	-1.37 \pm 0.009	-1.64 to -0.94	-1.61 \pm 0.009	-1.89 to -1.41	***	n.s.	n.s.
	2011-12	-1.38 \pm 0.008	-1.64 to -1.18	-1.35 \pm 0.012	-1.67 to -1.02	n.s.	*	n.s.
Pressure potential (wpp, MPa)	2011	1.03 \pm 0.016	0.64-1.39	0.77 \pm 0.026	0.24-1.49	***	***	**
	2011-12	0.65 \pm 0.020	0.16-1.11	0.24 \pm 0.019	0.01-0.86	***	n.s.	n.s.
Crop water stress index (CWSI)	2011	0.32 \pm 0.003	0.23-0.41	0.46 \pm 0.005	0.34-0.63	***	**	n.s.
	2011-12	0.34 \pm 0.010	0.21-0.91	0.55 \pm 0.008	0.36-1.06	***	**	n.s.
Specific leaf area (cm ² g ⁻¹)	2011	156.2 \pm 1.76	112.7-220.5	130.4 \pm 1.36	99.8-179.5	***	**	*
	2011-12	176.3 \pm 1.79	132.7-235.6	118.7 \pm 1.14	82.5-142.6	***	*	n.s.
Normalised difference vegetation index (NDVI)	2011	0.63 \pm 0.011	0.32-0.87	0.58 \pm 0.012	0.18-0.82	**	***	***
Relative stem elongation rate (cm cm ⁻¹ day ⁻¹)	2011	0.050 \pm 0.001	0.030-0.090	0.034 \pm 0.001	0.020-0.049	***	***	**
¹³ C isotopic discrimination (Δ ¹³ C _v ‰)	2011	-27.84 \pm 0.07	-29.44 to -25.98	-28.15 \pm 0.07	-29.85 to -26.83	**	**	n.s.

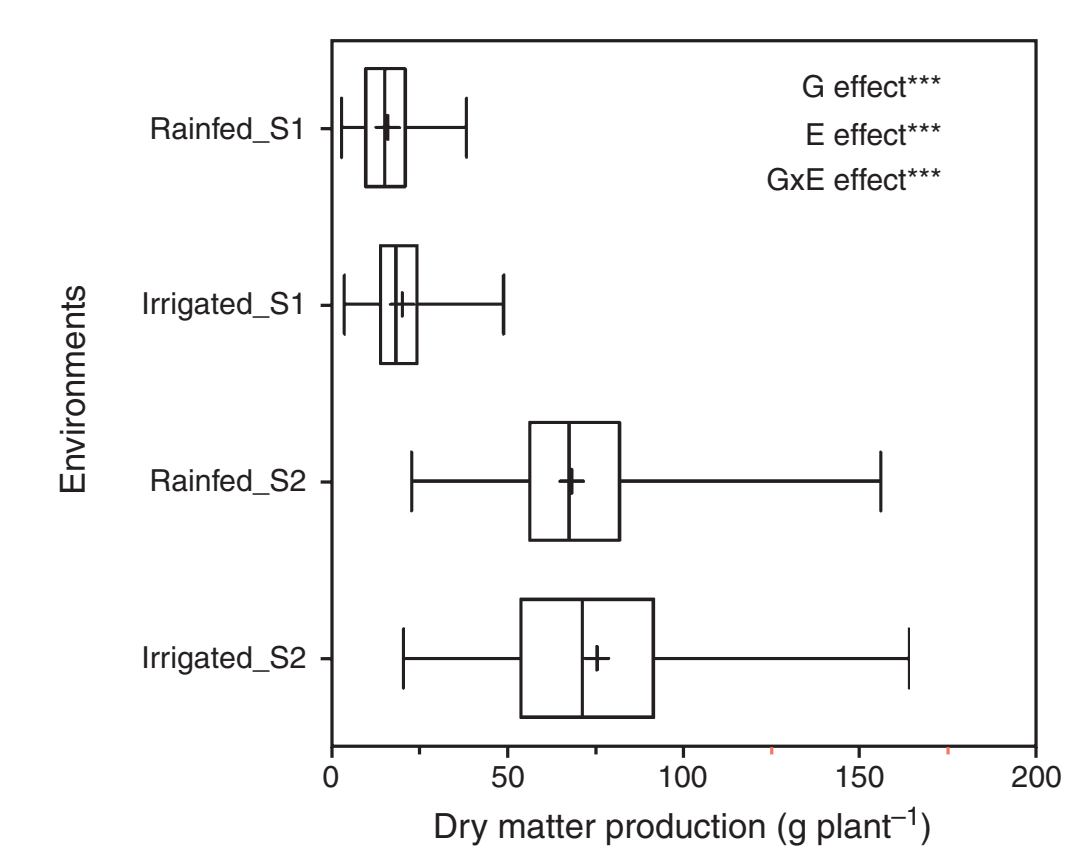


Fig. 3. Frequency distribution (box plot) and genotype (G), environment (E) and G \times E interaction effects for dry matter production of 100 *Lotus tenuis* genotypes evaluated during two seasons, summer-autumn 2011 (S1) and 2011-12 growing season (S2), and two water environments, irrigated and rainfed. The + symbol inside the box corresponds to the mean. ** $P < 0.01$; *** $P < 0.001$.

L. Tenuis drought tolerance QTL

Locus	Contig	Chr	cM	Ψ_x	Ψ_π	Ψ_p	SLA	CWSI	NDVI	RSER	BI	DM
TM0181	CM0181	1	0					*** (0.30)			** (0.12)	
TM0133	CM0133	1	12.1				** (0.11)				** (0.11)	
TM0117	CM0017	1	40.2							** (0.16)		
TM0847	LJT07F03	2	0.4					*** (0.34)				
TM0134	LJT34H20	2	3.2					** (0.12)				
TM2088	CM0120	2	36.5						*** (0.18)			
TM1805	CM0608	2	37.9									** (0.08)
TM0021	CM0021	2	60.9			** (0.15)						
TM0203	CM0152	3	64.8		** (0.15)							
TM0127	CM0127	3	82.4					*** (0.21)				
TM0256	CM0026	4	5.6		** (0.19)							
TM0212	CM0131	4	20.9			** (0.13)						
TM0030	CM0003	4	32.2					** (0.20)	** (0.19)			
TM0162	CM0046	4	53					** (0.10)				
TM0404	CM0046	4	53.8	** (0.15)								
TM0307	CM0307	4	56.2	** (0.14)								
TM0072	CM0072	5	7.6	** (0.12)								
TM0095	LJT02L13	5	37.6						** (0.13)			
BM2445	CM0148	5	44.1								** (0.12)	
TM1466	CM0200	5	47.3		** (0.18)		*** (0.23)					
TM0817	CM0738	6	13.6					*** (0.20)				** (0.05)
TM0632	CM0489	6	23.6	** (0.20)							** (0.20)	
TM1546	CM0013	6	37.7			** (0.17)						
TM0055	CM0055	6	61.5						** (0.05)			
TM1240	CM0314	6	66.6							** (0.07)		

***, ** for $P < 0.01$ and $P < 0.001$, respectively. Values in brackets are the variance proportion attributed to the marker effect.

Conclusion

Genomic knowledge in *Lotus* model species can be successfully transferred to agronomical important *Lotus* species. In this work, 25 genomic regions (QTL) were detected. These regions are controlling the expression of physiological traits involved in the *L. tenuis* drought tolerance.

Acknowledgements

This work was financial supported by FONDECYT 11100094

