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Forage Seed Production in Northern Arid China

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Report Outline

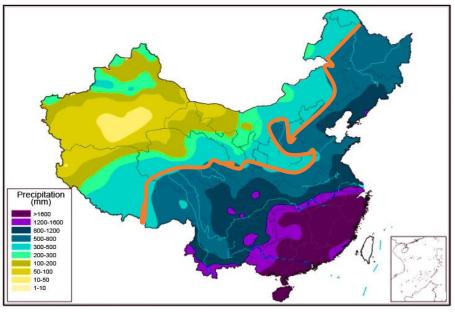
1. Background

- 2. Reproductive Characteristic
- **3. Variety Breeding**
- **4. Seed Production**
- **5. Application**

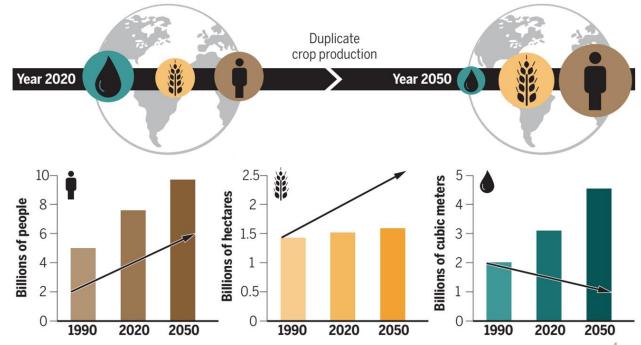
6. Acknowledgements

1. Background

- Climate change is leading us toward a hotter, more parched world. In the past decade, global losses in crop production due to drought totaled ~\$30 billion (Gupta *et al.*, 2020).
- China is a major grassland country, with a total grassland area of 4×10⁸ hm², accounting for 41.7% of the national territory (Fang et al., 2016).



我国干旱半干旱地区分布图 (Shan et al., 2010)



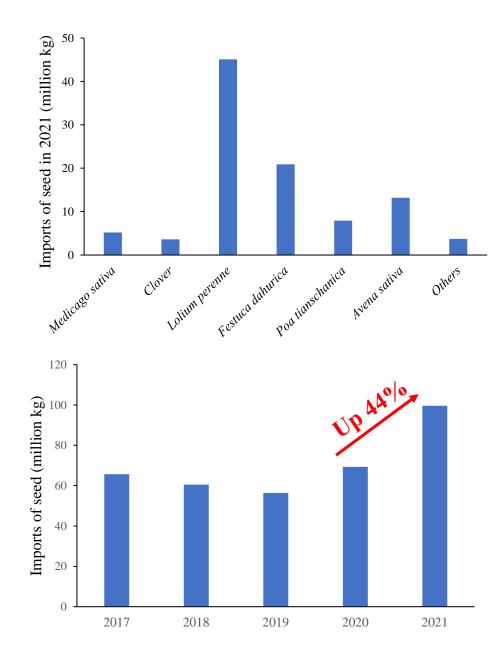
1. Background

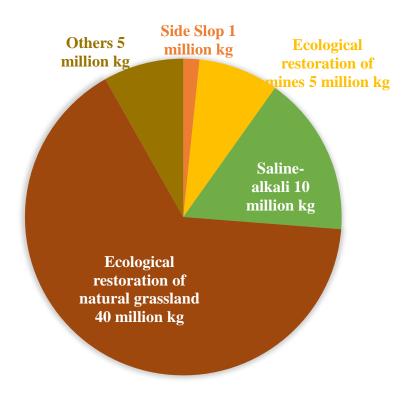
- Grassland degradation has become a serious issue, resulting in a decline of vegetation cover, the ecological service functions of grasslands have declined (Shen, 2016).
- Grass seeds are essential production resources for the improvement of degraded grasslands and soil and water conservation (Wang, 2013).
- Native grass play important role in the improvement of degraded grasslands and the management of desertification.











Prediction of Ecological Seed Demand in China in 2023

1. Background

- Cleistogenes songorica is an important perennial forage, and ecologically significant C₄ grass in desert areas where average annual rainfall is below 110 mm.
- Melilotus officinalis is an annual or biennial herb, which has adapted to extreme environmental conditions such as cold, drought and saline-alkali.
- > Lespedeza potaninii Vass is a perennial leguminous forage crop with drought, cold and barren tolerance.



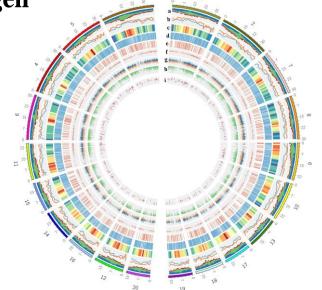
2. Reproductive Characteristic — *Cleistogenes songorica* as an example Study on the Divergence and Molecular Mechanisms of Dimorphic Floret in *C. songorica*

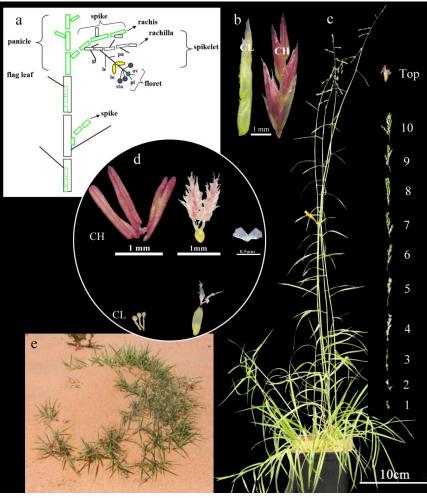
2. Reproductive Characteristic—*Cleistogenes songorica* as an example

- C. songorica develops two types of inflorescences in a single plant, enabling open pollination (chasmogamy, CH) on the top panicle, and self-pollination (cleistogamy, CL) on spike flowers embedded in the leaf sheath at each node.
- We sequenced and assembled a high-quality chromosome-level
 C. songorica genome (Zhang et al., 2021).
- > Domestication and variety breeding a new cultivar:

Cleistogenes songorica cv Tenggeli

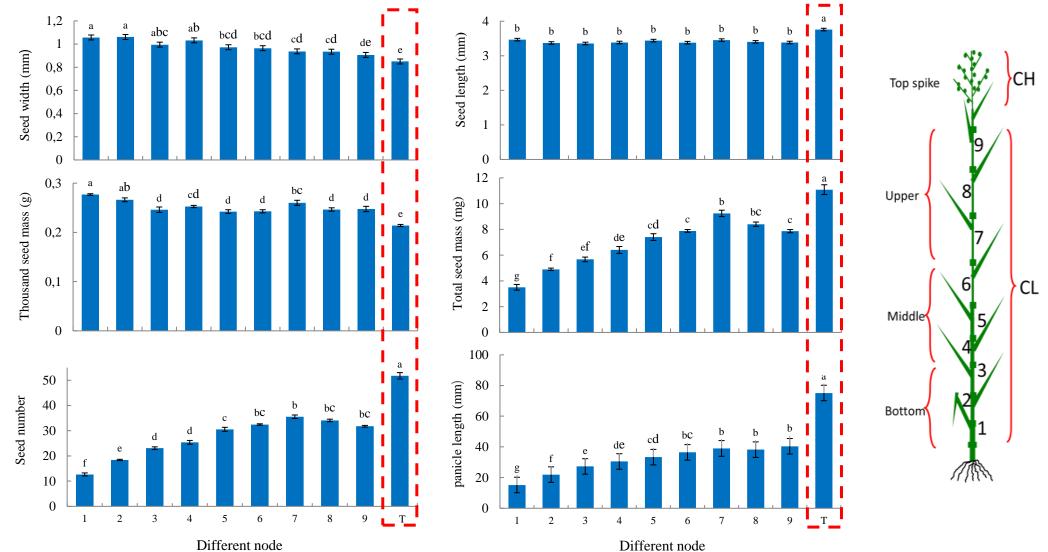
品种登记号:499	经第 七届全国草品种审
吅种豆尼与:499	经第 ~ 厢王国平吅种甲
品种名称:腾格里无芒隐子草	定委员会审定,该品种登记
申报单位: 兰州大学	为 ^{野生栽培} 品种, 经农业部
	公告,准予在适应区域正式
申报人: 王彦荣、张吉宇、	推广应用。
南志标、韩云华、李欣勇	全国草品种审定委员会
适应区域:	2016年7月21日
适宜我国西北干旱和半干旱荒漠: 区,年降雨量100~400mm的地区种植。	地





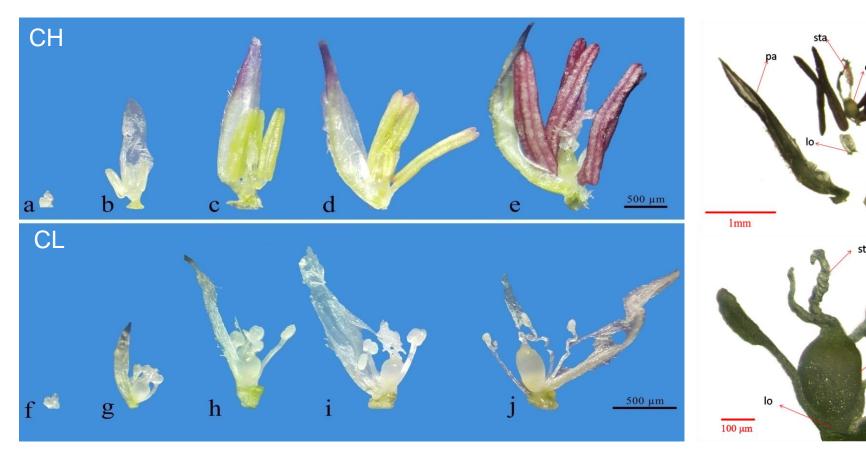
2.1 Seed divergence from different positions

There was progressive increasing in seed number, seed mass and length of spikelet from the bottom to the top



2.2 Different developmental stages of CH and CL flower

C. songorica could develop both CH and CL flowers, but there were some morphology difference between CH and CL floral organs



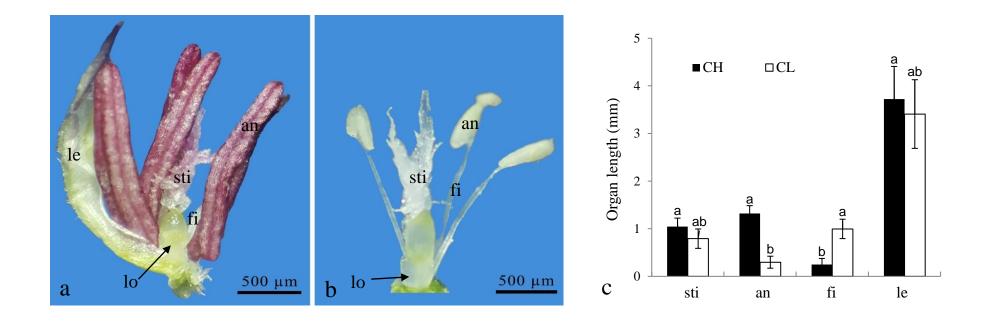
Different developmental stages of chasmogamy (CH) and cleistogamy (CL) flower

Floral organ structure of CH and CL

Note: lo, lodicule; ov, overy; le, lemma; pa, palea; sti, stigma; an, anthers; fi, filaments.

2.3 The morphology divergence of CH and CL flower

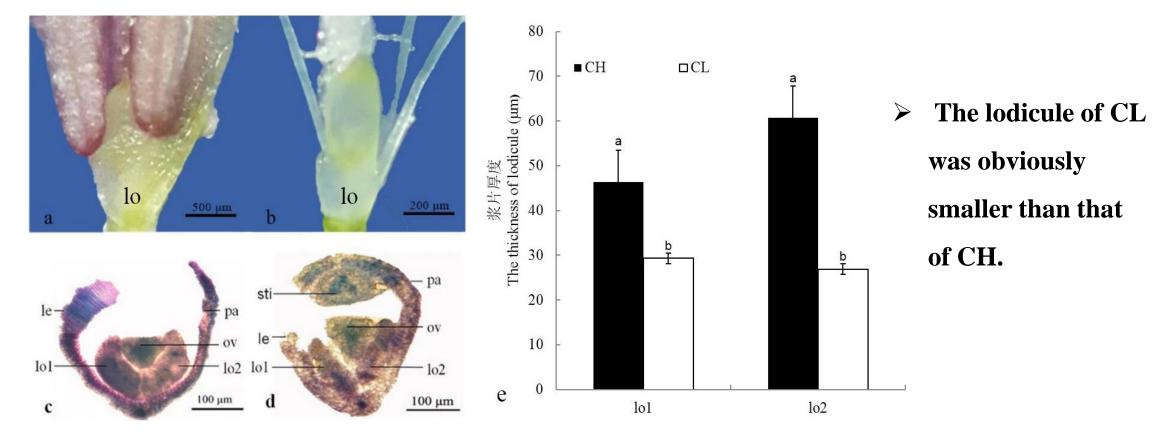
> The anther of CL was obviously smaller than that of CH, whereas the filaments were opposite.



Morphology of CH (a) and CL (b) flowers. c, Length of CH and CL floral organ

Note: lo, lodicule; ov, overy; le, lemma; pa, palea; sti, stigma; an, anthers; fi, filaments.

2.4 The lodicule divergence of CH and CL flower

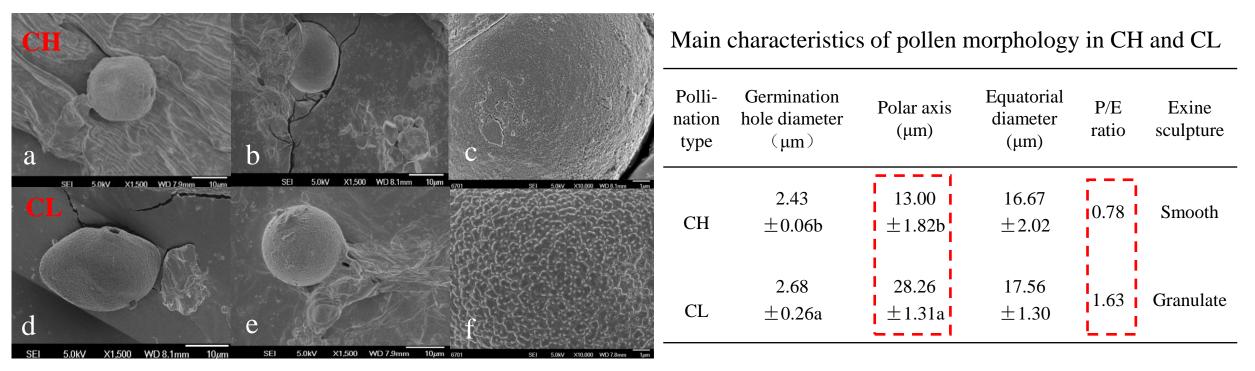


Microscopic structure of CH and CL lodicule

lo1, the first lodicule; lo2, the second lodicule; ov, overy; le, lemma; pa, palea; sti, stigma; an, anthers; fi, filaments.

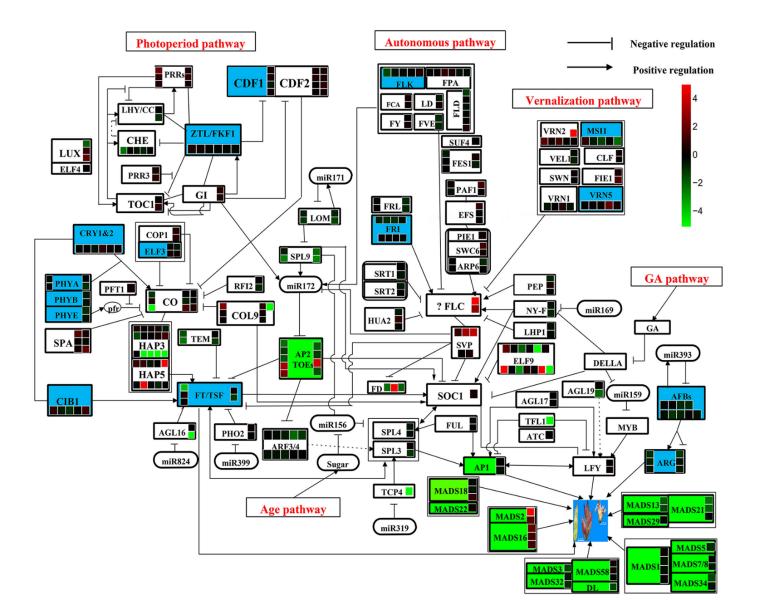
2.5 The pollen divergence of CH and CL flower

- Both CH and CL pollen grains have one germination aperture, and polar view of pollen grains are circular
- **Germination hole diameter of CL is larger than that of CH**

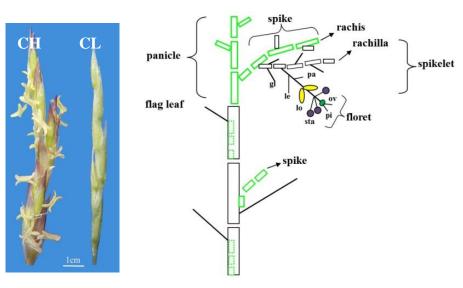


Micrographs of CH and CL pollen grain

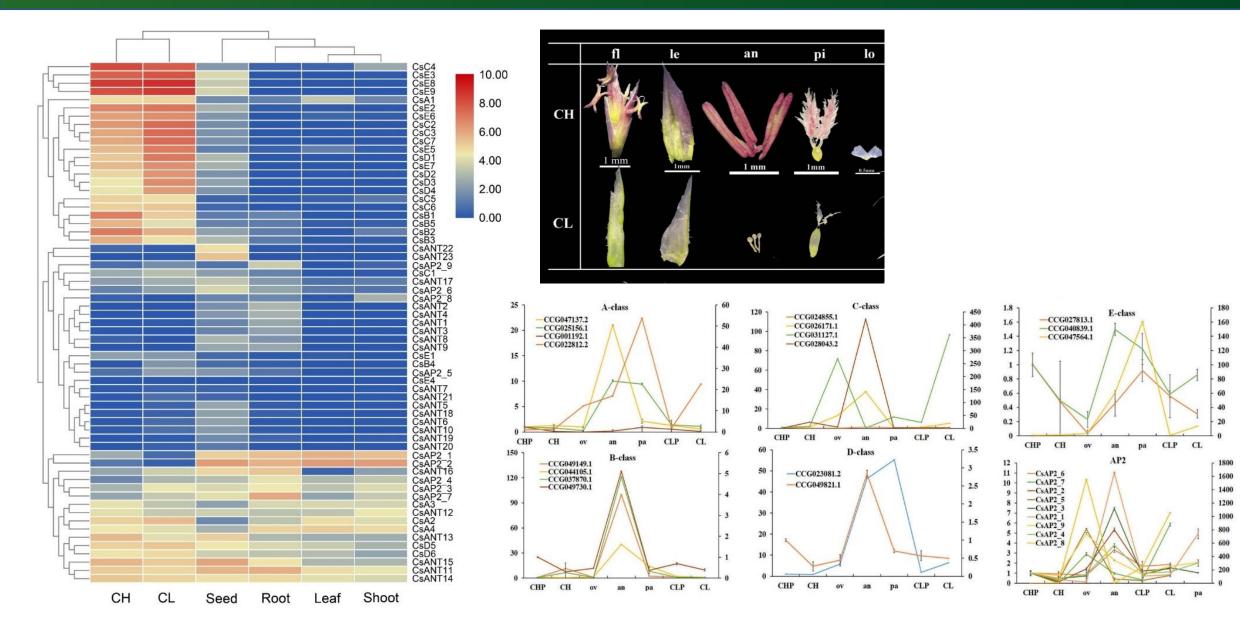
2.6 Constructed a flowering gene related network



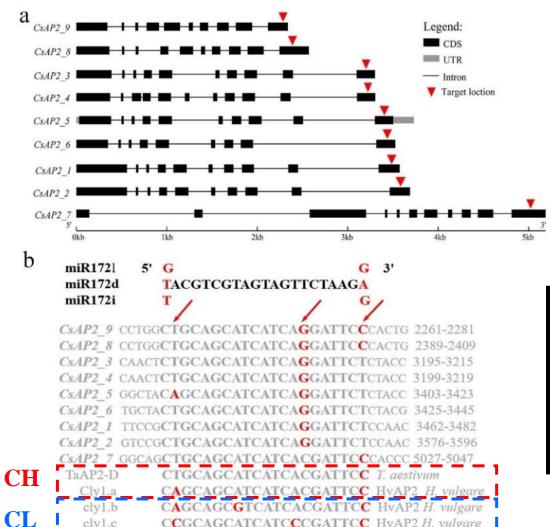
- Flowering gene network: 83 gene families including 302 genes;
- Expanded gene family, photoperiod pathway, may relate to regulated dimorphic flower development.



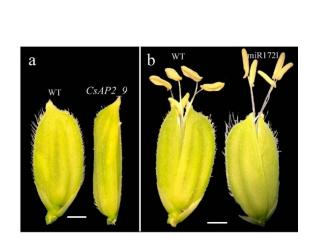
2.7 Identified the ABCDE model genes



2.8 miR172l and CsAP2_9 contribute to the regulation of cleistogamy



AGCATCATCAGGATTCT HvAP2L H. vulgare





CsAP2_9 transgenic
 lines showing abnormal
 palea, and smaller and
 thinner lodicules;

miR172l lines showing
 longer filaments, and
 reduced anther
 numbers.

3. Recurrent Selection Breeding — *Melilotus officinalis* as an example Recurrent selection of new breeding lines of *M. officinalis*

3. Variety Breeding—*Melilotus officinalis* as an example

- Sweet clover has been used as green manure and grazing leguminous forage in the southern US and later throughout the western region of the US and used to be addressed as the king of grazing pasture during first half of the 20th century (Clark, 2007).
- Yield and nitrogen-fixation of *M. officinalis* are considerably superior over two years old *Medicago* sativa (Mcewen and Johnston, 1985).
- > Breeding a M. officinalis new line: Melilotus officinalis cv Lanxi NO.1

Melilotus officinalis cv LanxiNO.1

		Hay yield of first year t ha ⁻¹	Total nitrogen of hay of kg N ha ⁻¹
	M. officinalis	9.4	143
ج	Medicago sativa	4.6	84

Global adaptation area of Melilotus

3.1 Genotypic variance components of *M. officinalis* HS families

➤ The genotypic variances were significant (P <0.05) among the 25 *M. officinalis* HS families for the agronomic and quality traits at two locations except SN, ADL and AIA.

Location		PH/cm	DB/ g plant ⁻¹	SD/mm	SN	LSR	Cou/%	CP/%	ADF/%	NDF/%	ADL/%	AIA/%
	Average	109.45	139.45	8.29	18.19	0.86	0.62	16.87	30.69	44.37	6.84	0.9
	Range	25.00-180.00	19.21-359.37	4.43-14.08	2-44	0.30-1.93	0.26-1.18	10.7-21.5	16.02-55.42	27.5-56.37	4.7-10.3	0.52-1.17
Yuzhong	σ^2_{g}	223.17±93.48	1327.33±420.75	0.58±0.29	n.s.	0.03±0.01	0.085 ± 0.04	0.91±0.51	3.10±1.73	2.89±1.67	n.s.	n.s.
	$\sigma^2_{\mathcal{E}}$	735.42±90.46	14451.44±156.54	2.79±0.31	65.49±6.8	0.10±0.01	0.31±0.36	3.46±0.48	19.81±2.49	18.29±2.34	0.37±0.05	0.01±0.001
	Average	128.51	122.89	7.5	14.3	0.49	0.39	11.89	33.01	47.48	7.89	0.81
T •	Range	43.01-192.00	17.7-384.98	3.40-11.50	2-38	0.23-1.14	0.32-0.82	7.97-15.46	18.8-48.2	37.81-57.11	5.64-10.51	0.10-1.60
Linze	σ_{g}^{2}	223.51±84.84	1327.33±420.73	0.31±0.18	n.s.	0.004 ± 0.002	0.088±0.035	0.34±0.14	2.36±1.28	3.58±1.48	n.s.	n.s.
	$\sigma^2_{\mathcal{E}}$	533.67±57.25	1451.44±156.54	2.36±0.25	52.66±5.6	0.018±0.002	0.01 ± 0.001	1.14±0.13	14.39±1.58	9.92±1.18	$0.84{\pm}1.04$	0.07 ± 0.01

				-

	PH/cm	DB/g plant ⁻¹	SD/mm	SN	LSR	Cou/%	CP/%	ADF/%	NDF/%	ADL/%	AIA/%
Average	118.84	131.21	7.89	16.24	0.67	0.51	14.48	31.83	45.87	7.44	0.86
Range	25.00-192.00	17.7-359	2.55-14.68	2-44	0.23-1.93	0.28-1.42	7.66-24.49	16.01-55.42	27.45-64.50	4.74-10.31	0.37-1.24
$\sigma^2_{\ gl}$	104.86±52.98	948.88±336.63	n.s.	n.s.	0.01 ± 0.005	n.s.	0.39±0.26	n.s.	n.s.	0.07 ± 0.04	0.002 ± 0.001
$\sigma^2_{\mathcal{E}}$	647.79±48.54	1975.83±164.82	3.09±0.23	21.52±1.77	0.05 ± 0.004	3.85±0.30	3.98±0.33	17.34±1.33	17.61±1.47	$0.54{\pm}0.04$	0.015±0.001
R	0.58	0.35	0.41	0.55	0.47	0.61	0.72	0.71	0.7	0.24	0.28

SD, stem diameter; SN, number of stems; PH, plant height; LSR, leaf/stem ratio; DB, dry biomass; Cou, coumarin, CP, crude protein; ADF, acid detergent fiber; NDF, neutral detergent fiber; ADL, acid detergent lignin; AIA, acid insoluble ash.

3.4 Comparative testing of different breeding lines of *M. officinalis*

Line3 had relatively low Cou, and high DB and PH which was an optimal line compared with the controls and other lines, and Line2 followed Line3.

Comparative test of different lines of *M. officinalis*.

	DB/(kg/ha)	PH/cm	LSR	CP/%	ADF/%	NDF/%	ADL/%	AIA/%	Cou/%
Line1	31.97% 14453.33±705.98a 40.37%	181.53±4.83a	0.66±0.01b	13.33±0.38a	39.55±0.83b	47.44±0.99b	7.17±0.09a	1.61±0.01b	-44.44% 0.40±0.00c -44.44%
Line2	40.37% 15373.33±600.22a 42.73%	182.07±4.11a	0.65±0.02b	13.45±0.62a	41.33±0.39ab	49.92±1.58a	7.27±0.14a	1.16±0.31c	0.40±0.00c -51.39%
Line3	15632.00±297.33a	183.30±4.00a	0.63±0.01b	13.02±0.15a	42.41±2.29a	49.76±2.05a	7.34±0.61a	1.53±0.13b	0.35±0.03d
CK- MoGon gnong	10538.67±140.58b	147.37±5.58b	0.72±0.04a	14.10±1.76a	35.48±2.14c	42.22±3.29c	6.18±0.18b	1.94±0.04a	0.96±0.05a
CK- Norgold	11365.33±230.82b	151.43±7.83b	0.75±0.01a	13.23±0.17a	39.45±0.81b	46.26±0.62b	6.32±0.51b	1.69±0.07b	0.48±0.03b

3.3 Predicted ΔG per selection cycle

- The genetic gains of all traits of the second recurrent selection cycle were lower than that of the first recurrent selection cycle in *M. officinalis*, except of PH and LSR.
- > The ΔG of all traits are positive, except for Cou.

Traits	Second recur	rent selection	First recurrent selection (Luo e al., 2016)				
	ΔG	%ΔG	ΔG	%ΔG			
DB/g	5.6	3.9	17.17	16.5			
PH/cm	4.9	4	4.08	2.6			
SD/mm	0.16	1.9	0.17	11.9			
SN	0.15	3.5	0.33	5.7			
LSR	0.03	4.5	0.02	2.4			
I Cou/%	-0.033	-6.66	-0.05	-11.8			
CP/%	0.006	0.04					
ADF/%	0.91	2.86					
NDF/%	0.86	1.88					
ADL/%	0.03	0.45					
AIA/%	0.006	0.66					

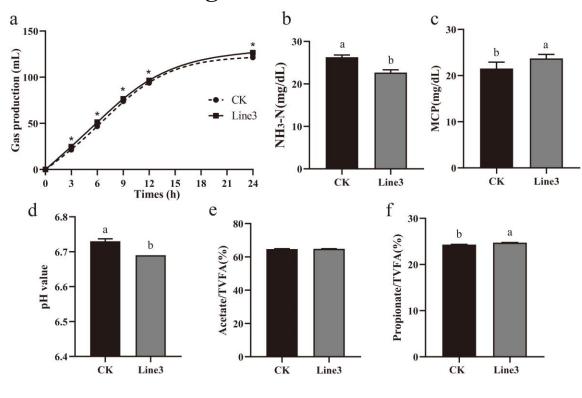
3.4 Comparative testing of different breeding lines of *M. officinalis*

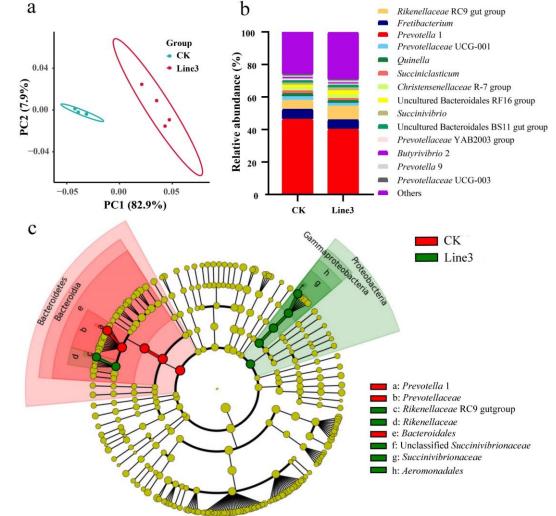


2020年黄花草木樨品系比较试验

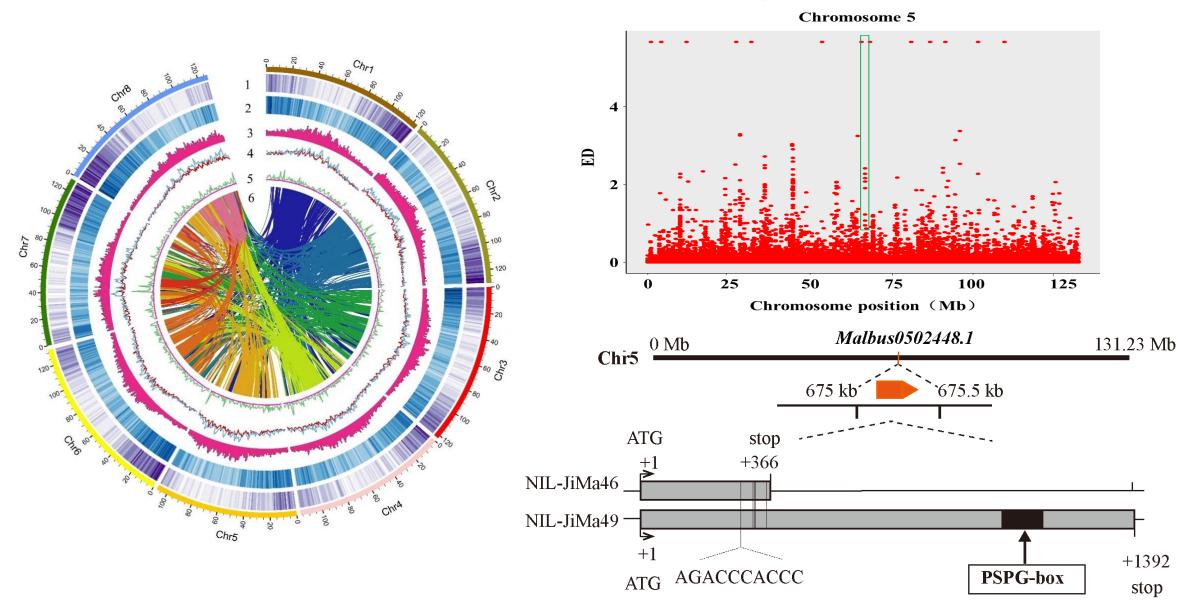
3.5 In vitro rumen fermentation profile

- The gas production of Line3 was significantly higher than that of the CK-MoGongnong, indicating that it might be due to differences in the contents of Cou.
- The abundance of *Prevotella* was higher in CK MoGongnong, suggesting that *Prevotella* in rumen is involve in the degradation of coumarin.

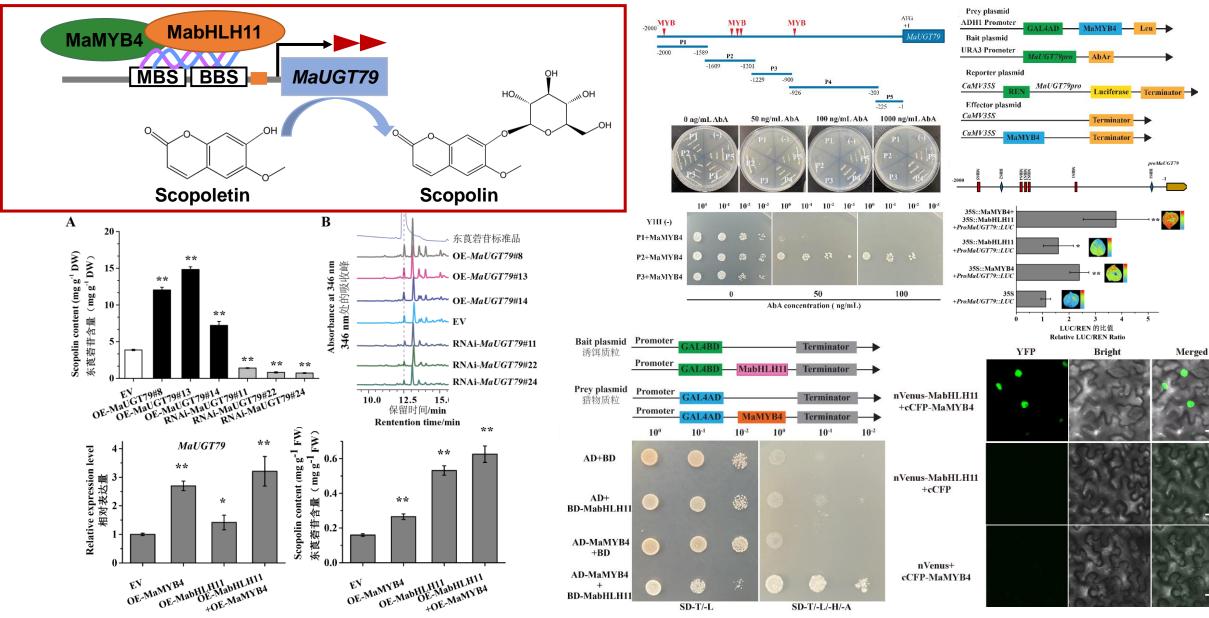




- The genome of *Melilotus albus* (2n = 2x = 16) size was 1.15 Gb.
- The MaUGT79 was identified based on BSA-seq.



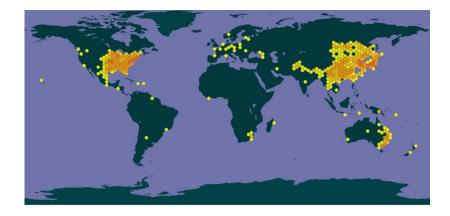
• The regulation model MaMYB4-MabHLH11-*MaUGT79* mediated scopolin biosynthesis in *M. albus*



4. Seed Production — Lespedeza potaninii vass as an example Development of Seed Production and Hardness Breaking Technique for L. potaninii vass

4. Seed Production Lespedeza potaninii vass as an example

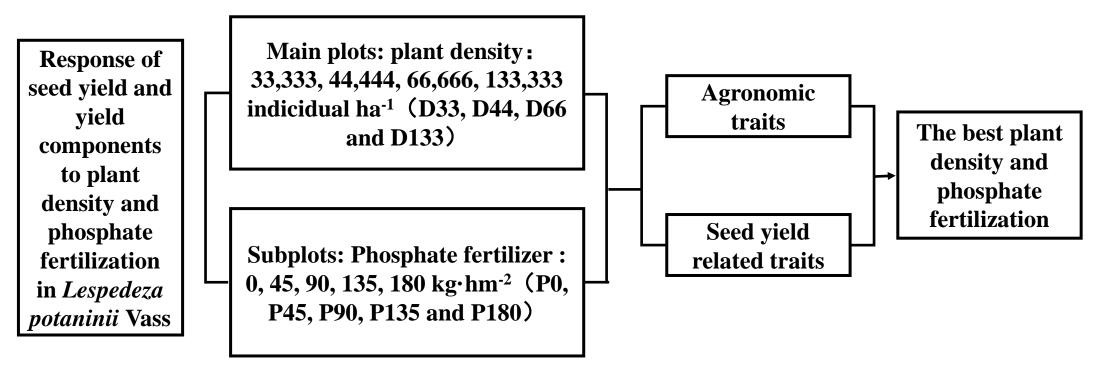
- L. potaninii plays an important role in livestock nutrition, grassland restoration and soil and water conservation in arid and semi-arid areas of China (Zhang et al., 2007).
- Under natural growing conditions, seed yield of *L. potaninii* is low.
- Breeding a L. potaninii new cultivar: L. potaninii cv Tenggeli (GS-CWV-2020-007)





4.1 Materials and methods

- > Materials: Lespedeza potaninii cv Tenggeli (ID: GS-CWV-2020-007)
- Location: Hexi Corridor in Zhangye City in Gansu Province, China (latitude: 39° 04' N, longitude: 100° 20' E, elevation: 1397 m), from 2019 to 2021 growing seasons.
- > Experimental design:



4.2 Variance analysis for Y, P and D on yield components

> Plant density, phosphate fertilizer and their interactions significantly affected seed yield.

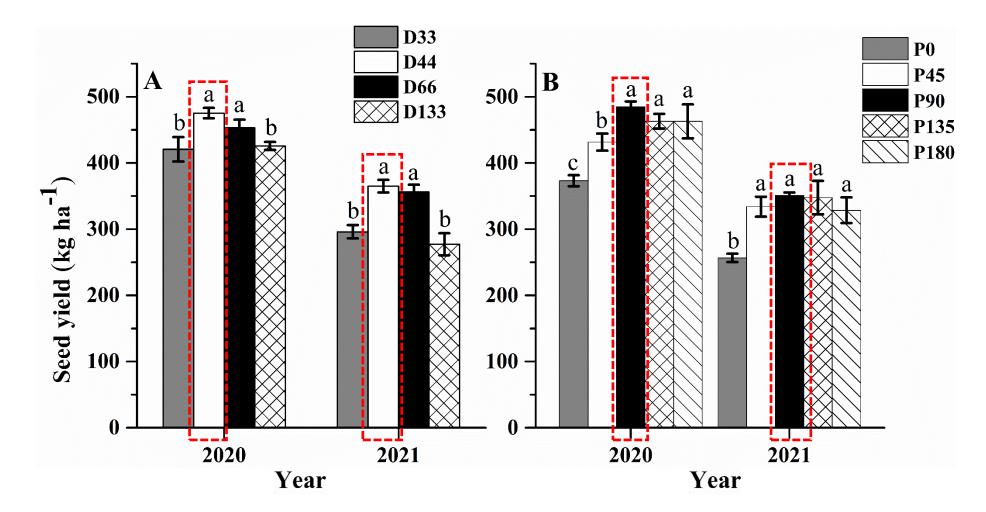
Source	Seed yield	Stems /m ⁻²	Racemes /Stem	Florets/ Raceme	Pods/ Raceme	1000 seeds weight	Seed yield per plant
Year (Y)	*	**	**	**	**	**	**
Plant density (D)	**	**	**	**	**	ns	**
Phosphate fertilizer (P)	**	**	**	ns	*	ns	**
D×P	**	**	**	ns	ns	*	**
D×Y	**	**	**	**	ns	ns	**
P×Y	ns	**	**	*	*	ns	ns
$\mathbf{D} \times \mathbf{P} \times \mathbf{Y}$	**	ns	ns	ns	ns	ns	ns

Analysis of variance for year (Y), plant density (D), phosphate fertilizer rate(P), and their interaction on seed yield and yield components

Effect of plant density and phosphate fertilization on seed yield in 2020 and 2021

Plant		Seed yiel	d in 2020	(kg·ha ⁻¹)			Seed yield	l in 2021	(kg·ha ⁻¹)			Mean s	eed yield (k	kg∙ha⁻¹)	
density	PO	P45	P90	P135	P180	P0	P45	P90	P135	P180	PO	P45	P90	P135	P180
D33	381.04	395.91	428.88	445.33	452.62	258.66	296.48	307.32	304.40	313.55	319.85b	346.19ab	368.10ab	374.87a	383.09a
D44	338.37	419.31	559.28	538.07	521.71	300.66	315.48	395.66	412.43	400.19	319.52c	367.40b	477.47a	475.25a	460.95a
D66	407.11	462.22	459.91	484.08	451.91	312.95	397.87	384.52	372.03	313.40	360.03b	430.04 a	422.21a	428.06a	382.65ab
D133	366.58	448.71	503.47	385.07	425.24	154.28	326.25	316.73	301.92	286.96	260.43d	387.48ab	410.10a	343.49c	356.10bc

4.4 Relationship between seed yield and D and P



Relationship between seed yield and plant density (A) and phosphate fertilizaer rate (B).

4.7 Correlation coefficients between yield components and seed yield

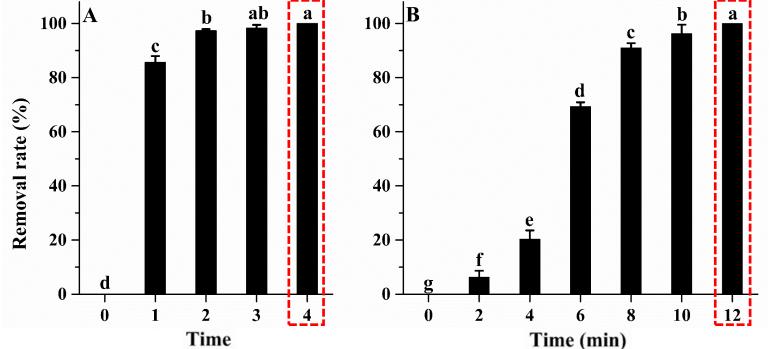
Correlation coefficients between contribution of yield components (stems per square meter, racemes per stem, florets per raceme, pods per raceme, 1000-seed weight and Seed yield per plant) and seed yield of *L. potaninii* Vass .

	Stems/m ⁻²	Racemes/Stem	Florets/Raceme	Pods/Raceme	1000-seed weight	Seed yield per plant	Correlation coefficients
Stems/m ⁻²	1	-0.836**	-0.833**	-0.832**	-0.335	-0.911**	-0.094
Racemes/Stem		1	0.791**	0.731**	0.241	0.897**	0.136
Florets/Raceme			1	0.858**	0.215	0.948**	0.454*
Pods/Raceme				1	0.209	0.862**	0.545*
1000-seed weight					1	0.305	0.013
Seed yield per plant						1	0.356

4.8 Persistent sepals of L. potaninii Vass cleaning

All the persistent sepals were removed when the seeds were treated four times with the seed coat breaking machine and 12 minutes with concentrated sulfuric acid.

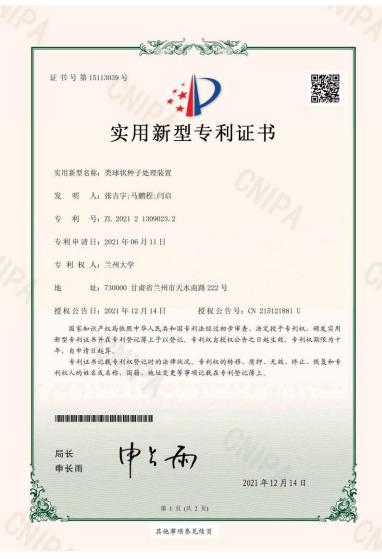




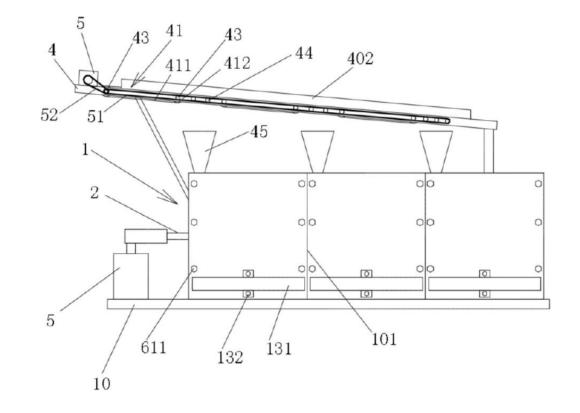
Effect of the seed coat breaking machine and concentrated sulfuric acid treatments on the persistent sepals of *L. potaninii* Vass

4.8 Persistent sepals breaking of L. potaninii Vass

Utility Model Patent



Sepals breaking machine model



Treatments	Percentage of damage (%)	Percentage of hard seeds (%)	Germination energy (%)	Germination rate (%)	Germination index	Vigor index
СК	0.00e	96.67a	2.00f	2.67f	0.72g	0.02f
1	0.67de	91.00a	8.33f	8.67f	4.56f	0.09ef
2	0.67de	80.33b	17.00e	18.00e	8.39e	0.16ef
3	1.00ced	73.67b	20.33e	23.67e	10.19e	0.21de
4	2.67bc	59.67c	34.00d	38.00d	17.01d	0.34cd
5	2.67bc	50.00d	43.33c	48.33c	21.38c	0.40c
6	2.33cd	46.67de	48.33bc	53.33bc	22.68c	0.39c
7	4.33b	38.67ef	56.00ab	60.00ab	28.36b	0.56b
8	7.00a	36.33f	57.33a	61.33a	31.68ab	0.87a
9	7.67a	33.00fg	61.67a	66.33a	33.14a	0.75a
10	8.00a	24.33g	60.00a	65.33a	31.59ab	0.50bc

Effects of sulfuric acid treatment on seed germination of L. potaninii Vass

Treatments	Percentage of hard seeds (%)	Germination energy (%)	Germination rate (%)	Germination index	Vigor index
СК	96.00a	4.00d	4.00e	1.93e	0.07d
5 min	84.67b	10.67d	12.66d	4.69e	0.17cd
10 min	51.33c	33.33c	44.00c	14.10d	0.45c
15 min	16.67d	67.33b	74.67b	26.94c	0.81b
20 min	14.67d	74.67ab	82.67a	35.8ab	1.13a
25 min	17.33d	72.67ab	81.33ab	34.12b	1.14 a
30 min	13.33d	80.00a	84.00 a	38.73 a	1.03 ab

Treatments	Percentage of hard seeds (%)	Germination energy (%)	Germination rate (%)	Germination index	Vigor index
СК	96.00a	4.00e	4.00g	1.93e	0.07e
80°C, 0.5 h	94.67a	5.33de	5.33fg	3.13e	0.14de
80°C, 1.0 h	92.67ab	7.33de	7.33efg	5.11de	0.19cde
80°C, 1.5 h	96.00a	6.67de	7.33efg	4.39de	0.15de
80°C, 2.0 h	92.00b	8.00de	8.00efg	4.67de	0.21bcde
90°C, 0.5 h	83.33ab	8.00de	9.33defg	4.87de	0.23bcd
90°C, 1.0 h	86.67ab	7.33de	10.67def	5.26de	0.21bcde
90°C, 1.5 h	87.33b	10.00d	12.00de	7.66d	0.40 a
90°C, 2.0 h	84.00b	10.67d	14.67d	7.95d	0.36ab
100°C, 0.5 h	74.00b	20.00c	21.33c	12.87 c	0.36ab
100°C, 1.0 h	61.00d	27.00b	30.00ab	16.92b	0.36ab
100°C, 1.5 h	53.33d	32.67 a	35.33a	21.75a	0.47a
100 °C, 2.0 h	36.00e	25.33b	28.00b	16.67b	0.35abc

Effects of high temperature treatment on seed germination of L. potaninii Vass

Treatments	Percentage of hard seeds (%)	Germination energy (%)	Germination rate (%)	Germination index	Vigor index
СК	96.00a	4.00c	4.00d	1.93d	0.07d
70° C	95.33a	4.00c	4.67d	1.70d	0.08cd
80°C	82.00b	10.00b	15.33c	4.66c	0.21b
90°C	60.00c	22.67a	38.00a	12.19a	0.42a
97°C	40.00d	12.00b	28.67b	7.52b	0.18bc

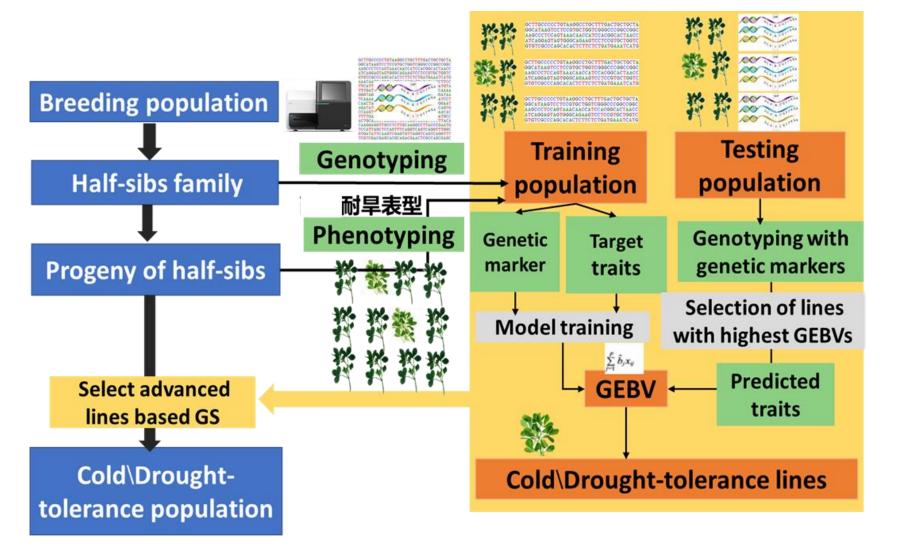
Effects of hot water treatment on seed germination of *L. potaninii* Vass

Effects of liquid nitrogen treatment on seed germination of L. potaninii Vass

Treatments	Percentage of	Germination	Germination	Germination	Vigor index
	hard seeds (%)	energy (%)	rate (%)	index	
СК	96.00a	4.00b	4.00b	1.93b	0.07 b
3 min	85.00b	8.00ab	14.00a	5.02ab	0.19a
5 min	84.66b	8.00 ab	13.33 a	5.05ab	0.16ab
<u>10 min</u>	81.33b	14.00a	16.67a	6.78a	0.17ab

5. Application of molecular breeding

Construction of Genomic Selection (GS) Model





Drought Tolerance and European Half-sib Families

Creation of Ms02Dr and Ms03Eu

Name	Abbreviation	Parental Source	Half-sib Family Progenies	Genotyping
Drought- Tolerance Half- sib family	Ms02Dr	23 drougt tolerant cultivars from China and other countries	199	Resequencing up to 35 X
European Half- sib family	Ms03Eu	10 cultivars from 7 European countries	136	GBS by CREA



Oct. 2020 in Yuzhong



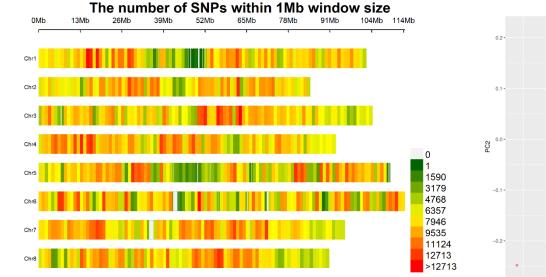
Apr. 2022 in Yuzhong

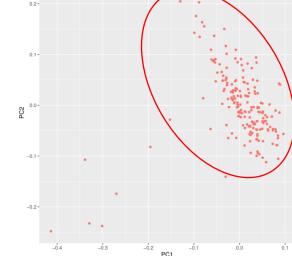
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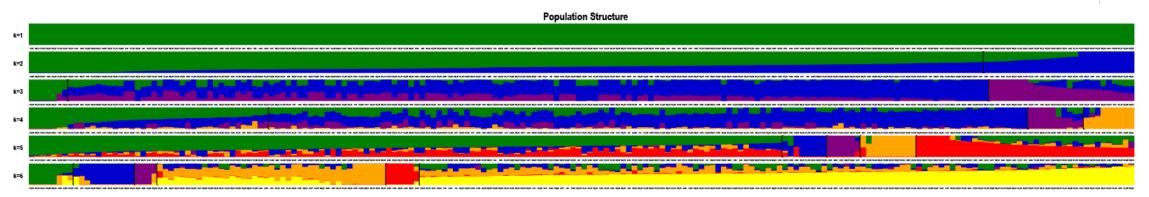


Resequencing of 199 Ms02Dr half-sib family

 10.37 million SNPs were obtained through 35X resequencing of 199 halfsib family. After filtering with LD=0.8, 6.21 million SNPs were obtained, distributed on 8 chromosomes.





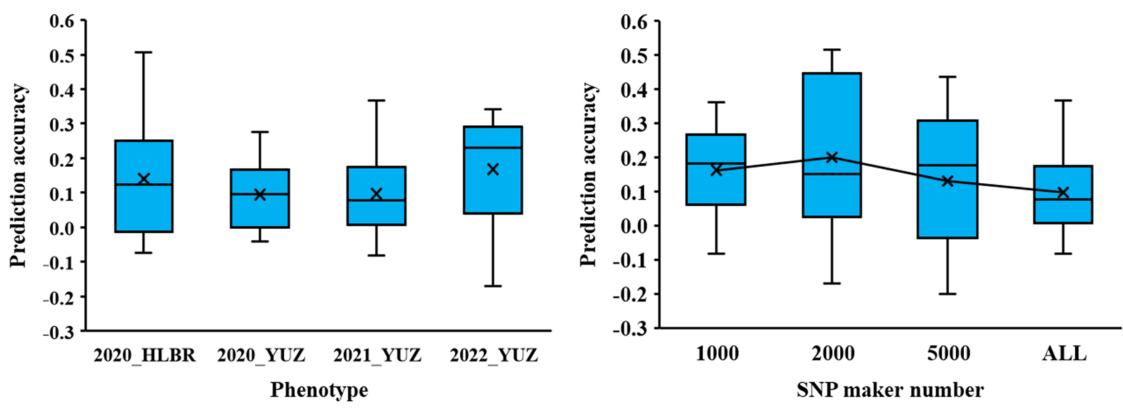


According to the PCA and population structure analysis of SNP, it conforms to the characteristics of its half-sib family (K=1).

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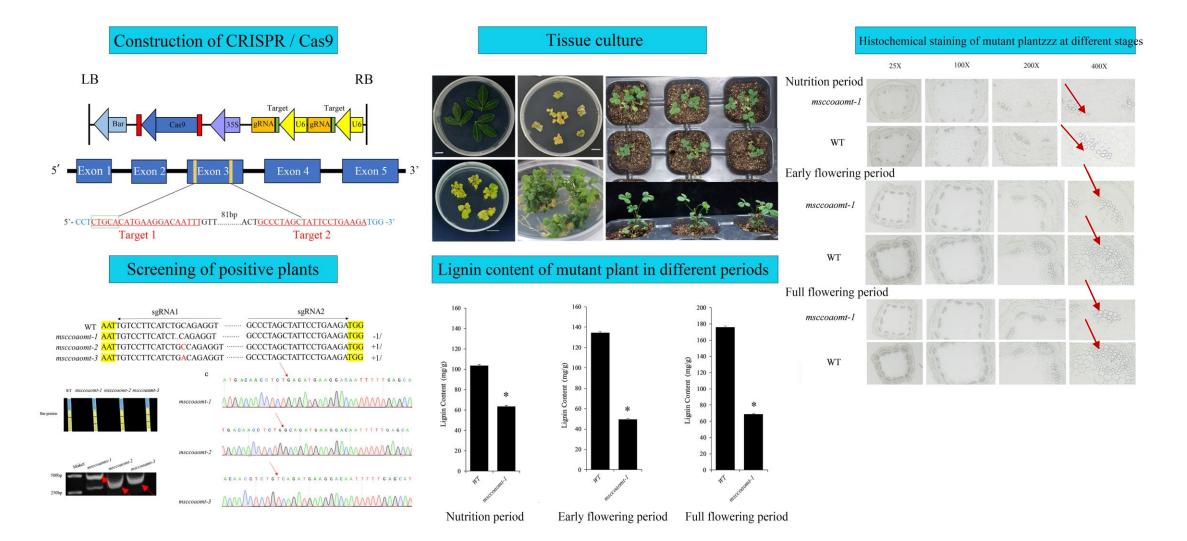


Construction of Genomic Selection (GS) Model Using gBLUP Method



The accuracy of predicting dry weight traits ranges from 9% to 15% with all SNPs participating in the prediction. Combining GWAS to select different numbers of topSNPs, the prediction accuracy can reach from 9% to 20%.

Breeding low lignin alfalfa lines by CRISPR/Cas9



5. Acknowledgements



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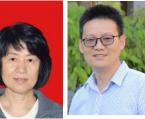


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