

Genotypic variation in agronomic traits and its assesment molecular markers among chinese Luobuma (*Apocynum* spp.) germplasm accessions

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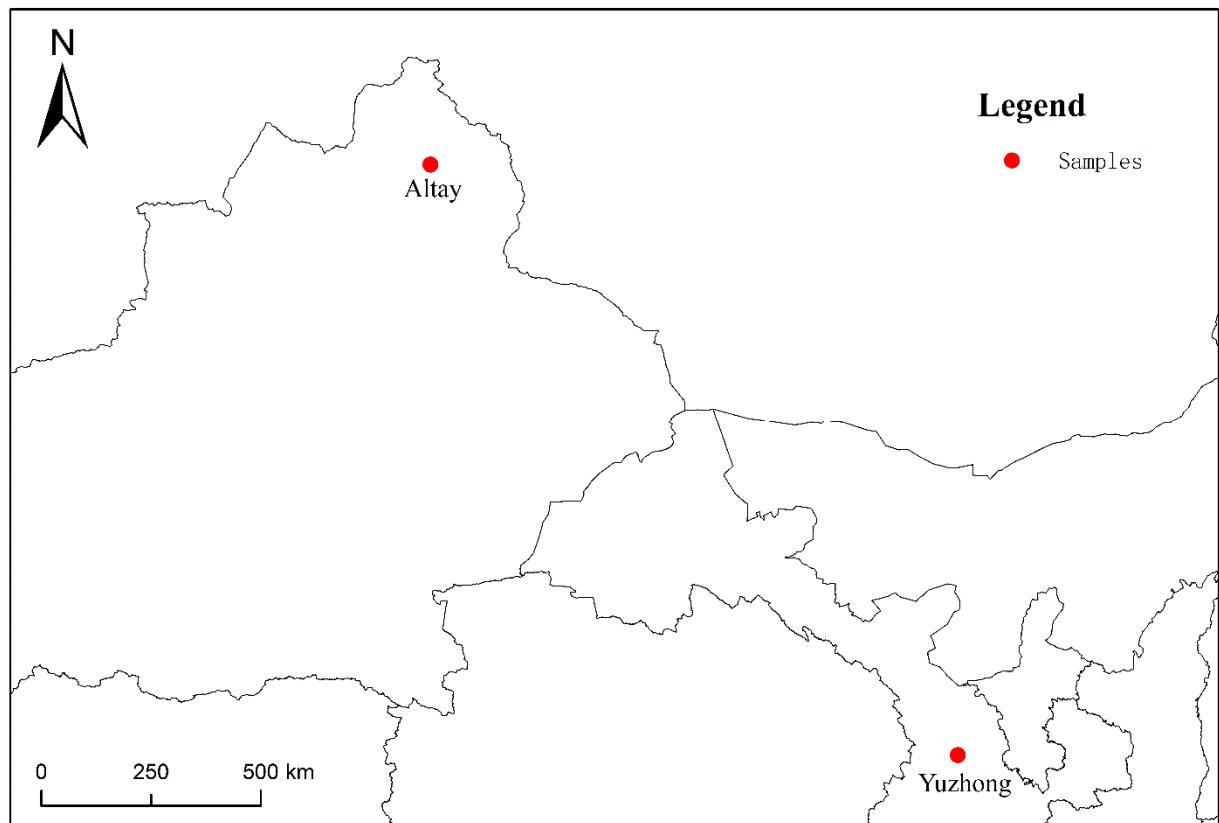


Figure S1. Distribution map of sampling locations.

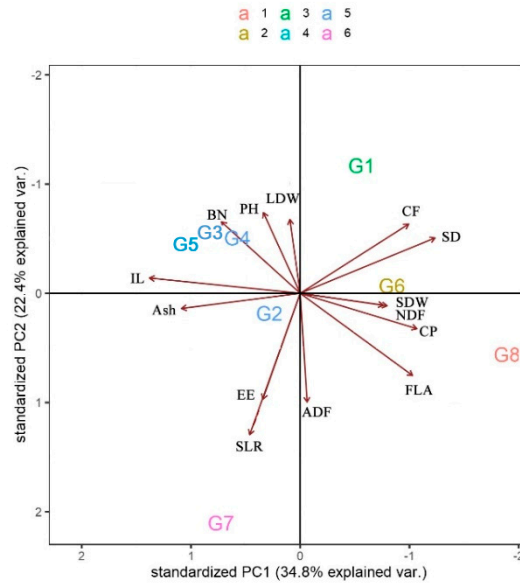


Figure S2. Principal component analysis of agronomic traits in different genotypes of *Apocynum* spp. and *Poacynum* spp. during early flowering stage from 2017-2019 at Altay.

Different color represents different group.

PH, plant height; SD, stem diameter; IL, internode length; BN, branch number; LDW, leaf dry weight; SDW, stem dry weight; SLR, stem to leaf ratio; ns, no significant; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fiber; EE, ether extract; CF, crude fiber; FLA, flavone.

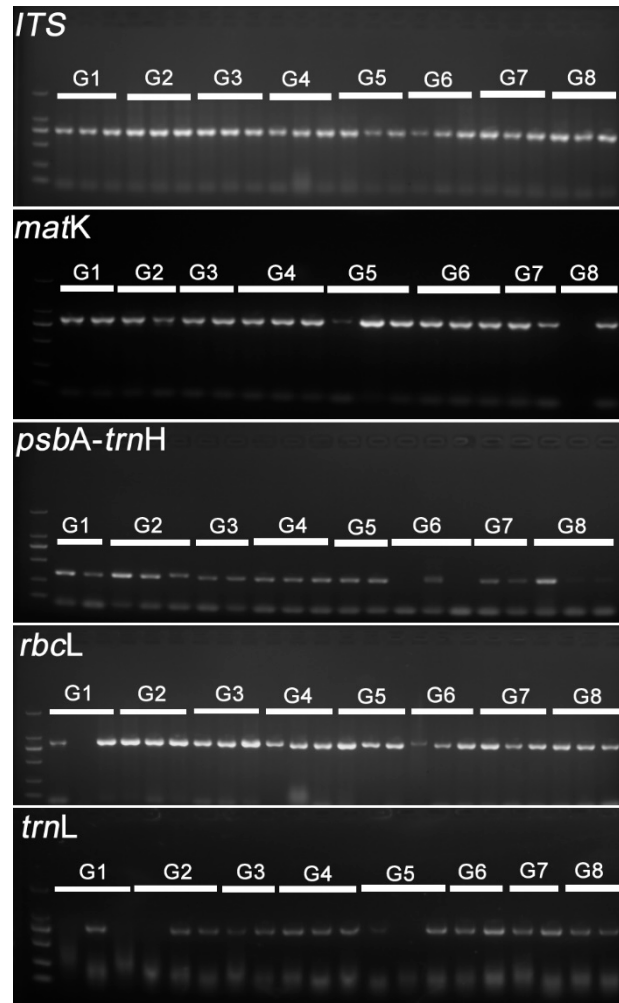


Figure S3. gel pictures of five molecular markers for 8 genotypes.

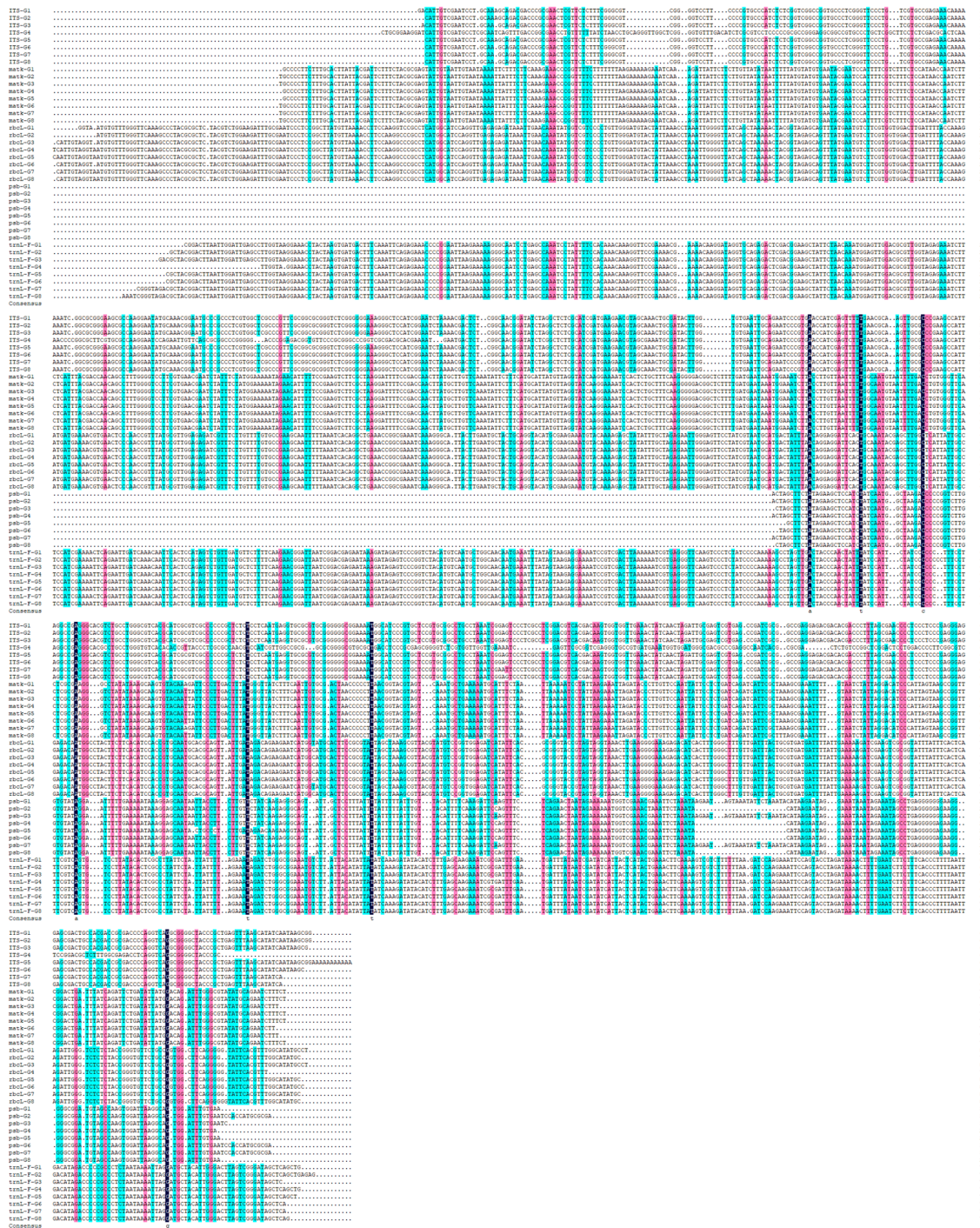


Figure S4. Comparison of sequences for eight genotypes ITS, matK, psbA-trnH, rbcL, trnL-F.

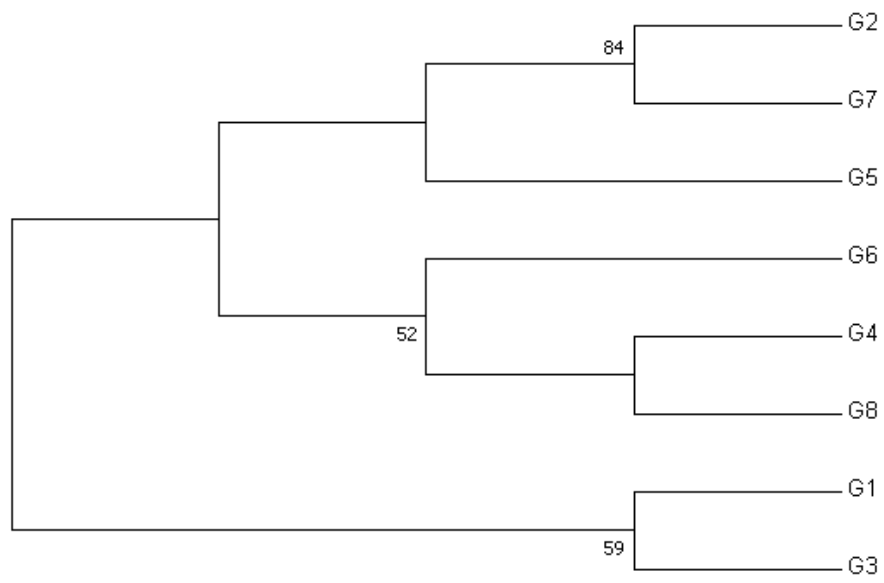


Figure S5. Neighbor-joining (NJ) tree for different phenotypes using matK + psbA-trnH + rbcL + trnL markers.

Table S1.

Trait mean, range, least significant differences ($LSD_{0.05}$), genotypic (σ^2_g), year (σ^2_y), accession-by-year interaction (σ^2_{gy}), and experimental error (σ^2_ϵ) variance components, and associated standard errors ($\pm SE$), estimated from 8 accessions, for yield related traits at the early flowering stage from 2017 to 2019 at Altay.

	PH (mm)	SD (mm)	IL (mm)	BN	LDW (g)	SDW (g)	SLR
Mean	991.0	21.45	60.5	5.52	26.41	25.35	0.94
Range	832.5-1116.1	18.3-24.6	50.3-65.4	3.86-7.33	22.08-33.83	21.18-27.81	0.85-1.20
$LSD_{0.05}$	59.78	3.05	4.19	12.64	35.07	16.63	0.72
σ^2_g	338.59 \pm 99.00	1.005 \pm 0.16	1.86 \pm 0.35	17.84 \pm 2.18	127.56 \pm 23.08	15.40 \pm 2.94	0.03 \pm 0.006
σ^2_y	ns	ns	ns	ns	ns	ns	0.004 \pm 0.002
σ^2_{gy}	161.08 \pm 58.45	0.20 \pm 0.05	0.46 \pm 0.13	1.02 \pm 0.40	21.71 \pm 7.76	15.40 \pm 2.94	0.03 \pm 0.006
σ^2_ϵ	1376.12 \pm 72.32	0.19 \pm 0.01	1.57 \pm 0.08	11.02 \pm 0.58	82.45 \pm 7.17	23.66 \pm 2.58	0.05 \pm 0.005

*PH, plant height; SD, stem diameter; IL, internode length; BN, branch number; LDW, leaf dry weight;

SDW, stem dry weight; SLR, stem to leaf ratio; ns, no significant.

Table S2

Trait average, range, least significant differences ($LSD_{0.05}$), genotypic (σ^2_g), year (σ^2_y), genotype \times year interaction (σ^2_{gy}), and experimental error (σ^2_ϵ) variance components, and associated standard errors ($\pm SE$), estimated from 8 genotypes, evaluated yield related traits at early flowering stage from 2018 to 2019 at Yuzhong.

	PH (mm)	SD (mm)	IL (mm)	BN	LDW (g)	SDW (g)	SLR
Mean	834.9	4.8	41.0	3.91	24.82	23.25	0.91
Range	798.9-891.8	4.5-5.3	37.8-46.3	3.27-4.43	22.55-26.45	20.81-25.47	0.86-0.95
$LSD_{0.05}$	62.95	2.50	2.42	4.16	17.70	18.61	0.24
σ^2_g	427.75 \pm 64.76	0.38 \pm 0.13	0.58 \pm 0.13	1.87 \pm 0.27	27.14 \pm 8.71	30.47 \pm 9.09	0.003 \pm 0.001
σ^2_y	ns	ns	ns	ns	ns	ns	ns
σ^2_{gy}	25.73 \pm 12.01	0.09 \pm 0.02	0.13 \pm 0.05	0.26 \pm 0.10	11.06 \pm 4.21	13.08 \pm 4.64	0.005 \pm 0.002
σ^2_ϵ	161.04 \pm 16.71	8.40 \pm 0.78	0.44 \pm 0.05	1.89 \pm 0.18	30.16 \pm 3.12	30.05 \pm 3.11	0.05 \pm 0.005

*PH, plant height; SD, stem diameter; IL, internode length; BN, branch number; LDW, leaf dry weight; SDW, stem dry weight; SLR, stem to leaf ratio; ns, no significant.

Table S3

Nutritional quality average, range, least significant differences ($LSD_{0.05}$), genotypic (σ^2_g), year (σ^2_y) and genotype with year (σ^2_{gy}), and experimental error (σ^2_ϵ) variance components, and associated standard errors ($\pm SE$), estimated from 8 genotypes, evaluated in early flowering stage at Altay.

	CP (%)	NDF (%)	ADF (%)	EE (%)	CF (%)	Ash (%)	FLA (mg/100g)
Mean	14.21	33.97	79.09	8.89	22.88	9.77	2.64
Range	12.97-16.88	27.63-36.52	71.89-84.81	6.67-15.17	18.63-26.12	9.1-10.85	2.37-2.99
$LSD_{0.05}$	3.42	5.22	30.94	17.56	20.12	3.23	0.69
σ^2_g	ns	ns	98.48 \pm 22.82	23.85 \pm 10.94	42.28 \pm 9.56	1.01 \pm 0.24	0.04 \pm 0.01
σ^2_y	ns	ns	ns	ns	ns	ns	ns
σ^2_{gy}	2.14 \pm 0.86	ns	ns	ns	ns	ns	ns
σ^2_ϵ	2.14 \pm 0.44	14.51 \pm 2.51	73.45 \pm 12.42	91.91 \pm 15.20	34.59 \pm 5.85	0.80 \pm 0.14	0.04 \pm 0.008

*CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; EE, ether extract; CF, crude fiber;

FLA, flavone; ns, no significant.

Table S4

Nutritional quality average, range, least significant differences ($LSD_{0.05}$), genotypic (σ^2_g), year (σ^2_y) and genotype with year (σ^2_{gy}), and experimental error (σ^2_ϵ) variance components, and associated standard errors ($\pm SE$), estimated from 8 genotypes, evaluated in full flowering stage at Altay.

	CP (%)	NDF (%)	ADF (%)	EE (%)	CF (%)	Ash (%)	FLA (mg/100g)
Mean	15.7	32.24	72.11	7.19	16.08	11.38	2.75
Range	13.07-18.26	24.67-35.90	68.97-78.74	6.63-7.92	11.23-17.71	8.02-17.62	2.49-3.35
$LSD_{0.05}$	3.6	16.04	14.31	2.86	11.6	16.41	1.6
σ^2_g	0.02 \pm 0.01	25.97 \pm 6.99	19.70 \pm 6.23	0.55 \pm 0.23	13.23 \pm 3.19	21.57 \pm 9.96	0.28 \pm 0.04
σ^2_y	ns	ns	ns	ns	ns	ns	ns
σ^2_{gy}	2.51 \pm 0.87	ns	ns	ns	ns	ns	0.04 \pm 0.01
σ^2_ϵ	1.72 \pm 0.35	29.75 \pm 5.04	31.78 \pm 5.38	1.01 \pm 0.21	11.24 \pm 1.98	76.58 \pm 12.95	0.01 \pm 0.004

*CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fiber; EE, ether extract; CF, crude fiber;

FLA, flavone; ns, no significant.

Table S5

Nutritional quality average, range, least significant differences ($LSD_{0.05}$), genotypic (σ^2_g), and experimental error (σ^2_ϵ) variance components, and associated standard errors ($\pm SE$), estimated from 8 genotypes, evaluated in early flowering stage at Yuzhong.

	CP (%)	NDF (%)	ADF (%)	EE (%)	CF (%)	Ash (%)	FLA (mg/100g)
Mean	18.87	22.79	63.98	5.11	24.52	14.8	1.78
Range	16.92-20.15	20.09-26.57	60.99-66.20	4.18-6.24	20.42-29.83	11.17-15.90	1.54-2.35
$LSD_{0.05}$	2.02	5.6	14.59	1.58	15.41	22.58	0.39
σ^2_g	ns	ns	16.64 \pm 7.05	0.19 \pm 0.08	ns	35.39 \pm 17.16	0.002 \pm 0.001
σ^2_ϵ	0.24 \pm 0.11	1.69 \pm 0.45	16.64 \pm 7.34	0.19 \pm 0.06	14.12 \pm 7.02	34.76 \pm 17.12	0.02 \pm 0.01

*CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fiber; EE, ether extract; CF, crude fiber;

FLA, flavone; ns, no significant.

Table S6

Correlation analysis of characters of *Apocynum* and *Poacynum* of different genotypes during early flowering stage from 2017 to 2018 at Yuzhong

Trait	Trait												
	SD	IL	BN	LDW	SDW	SLR	NDF	ADF	CP	CF	Ash	EE	FLA
PH	-0.21	0.21	0.16	0.41	0.61	0.84*	-0.56	-0.27	0.40	0.07	0.02	0.10	0.28
SD		-0.49	-0.51	0.10	0.04	0.09	0.18	-0.54	-0.53	0.12	0.21	-0.62	-0.06
IL			0.53	0.45	0.33	-0.22	0.51	-0.22	0.01*	0.64	-0.06	0.96**	0.02
BN				0.63*	0.63*	0.09	0.02	0.17	0.55	0.56	0.20	0.59	0.36
LDW					0.93**	0.43	0.10	-0.29	0.01**	0.75	0.52	0.40	0.28
SDW						0.66*	-0.13	-0.23	0.25	0.64	0.42	0.27	0.27
SLR							-0.66*	-0.14	0.41	-0.10	0.35	-0.30	0.19
NDF								-0.20	-0.47	0.45	0.18	0.49	-0.50
ADF									0.20	-0.42	0.10	0.06	-0.36
CP										-0.19	0.02	0.03	0.24
CF											0.00	0.57	0.27
Ash												-0.01	-0.32
EE													-0.09

*,** Significant at the 0.05 and 0.01 probability levels, respectively.

PH, plant height; SD, stem diameter; IL, internode length; BN, branch number; LDW, leaf dry weight; SDW, stem dry weight; SLR, stem to leaf ratio; ns, no significant; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fiber; EE, ether extract; CF, crude fiber; FLA, flavone.

Table S7. Primer information of different gene segments

Sequence name	Primer sequence (5'-3')	Annealing temperature (°C)	Length (bp)
<i>ITS</i>	F:GGAAGKARAAGTCGTAACAAGG	53	~640
	R:RGTTTCTTTTCCTCCGCTTA		
<i>psbA-trnH</i>	F:GTTATGCATGAACGTAATGCTC	60	~320
	R:CGCGCATGGTGGATTACAAATC		
<i>matK</i>	F:CCCRTYCATCTGGAAATCTTGGTTC	52	~710
	R:GCTRTRATAATGAGAAAGATTCTGC		
<i>trnL-F</i>	F:CGAAATCGGTAGACGCTACG	53	~640
	R:ATTTGAACTGGTGACACGAG		
<i>rbcL</i>	F:AGACCTWTTTGAAGAAGGTTTCWGT	53	~750
	R:TCGGTYAGAGCRGGCATRTGCCA		

Table S8. Sequences information of five sequences

sequence	Aligned length (bp)	Average of GC content (%)	Number of variable sites
<i>ITS</i>	683	61.05	8
<i>psbA-trnH</i>	314	35.68	37
<i>rbcL</i>	777	43.86	5
<i>trnL-F</i>	736	38.19	8
<i>matK</i>	728	34.78	7