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RESEARCH ARTICLE

Analysis of genetic diversity of Ruthenia Medic (*Medicago ruthenica* (L.) Trautv.) in Inner Mongolia using ISSR and SSR markers

Hong-yan Li · Zhi-yong Li · Li-yan Cai · Wen-gui Shi · Fu-gui Mi · Feng-ling Shi

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Abstract Ruthenia Medic is tolerant to drought, cold, high salinity, resistance to trampling and high quality features. Inter-simple sequence repeat (ISSR) and simple sequence repeat (SSR) molecular markers were employed for the first time to access the genetic diversity and relationships of 30 wild Ruthenia Medic accessions obtained from Inner Mongolia in the present study. A total of 94 bands were amplified by ten ISSR primers, of which 83 (88.5 %) were polymorphic, and 57 polymorphic bands (80.4 %) were observed in 69 bands amplified by ten SSR primers. Shannon's information index (I = 0.487), and average expected heterozygosis (He = 0.329) generated by ISSR primer were higher than that of SSR analysis (I = 0.372, He = 0.231). The study indicated that ISSR were more effective than SSR markers for assessing the degree of genetic variation of Ruthenia Medic. UPGMA cluster analysis revealed inconsistencies in the clustering patterns, as the Mantel's test between the dendrograms for ISSR and SSR data indicated a poor fit for the ISSR and SSR data types (r = 0.0970). Whereas the pattern of clustering of the genotypes remained relatively the same in ISSR and combined data of ISSR and SSR. The results of principal components analysis also supports their UPGMA clustering. These results have an important implication for Ruthenia Medic germplasm characterization, improvement, and conservation.

Keywords Genetic diversity · ISSR markers · *Medicago ruthenica* (L.) Trautv. · SSR markers

H. Li · Z. Li (✉) · L. Cai · W. Shi
The Institute of Grassland Research (CAAS)/ SharaQin Key Wild Scientific Monitoring Station for Forage Resources of Ministry of Agriculture, Inner Mongolia, Hohhot 010010, China
e-mail: zhiyongli1216@126.com

F. Mi · F. Shi
Inner Mongolia Agricultural University, Hohhot 010010, China

Introduction

Medicago ruthenica (L.) Trautv. is an diploid ($2n = 2x = 16$) perennial legume species widely distributed in China as including 10 provinces of northern China, especially in Helongjiang grasslands, Inner Mongolia dry grasslands, Xinjiang hill grasslands, and the Qinghai-Tibet plateau grasslands, which commonly grows on sunny hillsides, dry stony slopes, embankments, and in mixed grass steppes and moist meadows, at times in large numbers. (Small and Jomphe 1989; Gen et al. 1995; Hao and Shi 2006). This species has been collected mostly from the Transbaikal region to the Pacific Ocean, and south to about 34°N latitude (Small 2011). It is tolerant to drought, high salinity, and alkaline environments. Therefore, it is regarded as a promising legume for forage in arid and

semi-arid areas. It held that Ruthenia Medic seen far more capable of existing on poor stony soils under conditions of the continental North than *Medicago sativa* L., and so has potential application in low-input agricultural systems (Small 2011).

Ruthenia Medic is expected to become a high quality raw materials resources for improved alfalfa resistance genes (Campbell et al. 1997, 1999; Li et al. 2012). Evaluation of genetic diversity and relationships among various accessions is fundamental importance for plant breeding programs. This information can provide predictive estimates of genetic variation within a species, thus facilitating breeding material selection (Qi et al. 2008). Molecular markers have been shown to be a very powerful tool for genotype characterization and estimation of genetic diversity. In recent years, various molecular markers, including random amplified polymorphic (RAPD) (Deshwall et al. 2005; Teklewold and Becker 2006), amplified fragment length polymorphisms (AFLP) (Laurentin and Karlovsky 2006; Wang et al. 2010a), and simple sequence repeats (SSRs or microsatellites) (Beyene et al. 2006; Wang et al. 2010b) have been used to detect genetic diversity in different plant. The ISSR and SSR marker systems are widely used to evaluate genetic diversity (Ariss and Vandemark 2007; Uysal et al. 2010; Yang et al. 2010a; Iwata et al. 2005; Ofori et al. 2008; Panguluri 2007). These studies have given important clues in understanding species relationship, which may further assist in developing and planning breeding strategies. However, reports on genetic diversity using molecular markers are rarely available in the Ruthenia Medic germplasm (Li et al. 2008a, b; Li et al. 2009, 2012). The objectives of this study were to assess genetic diversity of 30 Ruthenia Medic accessions collected from Inner Mongolia using ISSR and SSR molecular markers.

Materials and methods

Plant materials and DNA extraction

A total of 30 accessions Ruthenia Medic germplasm were analyzed in this study (Table 1). All accessions were obtained from National Medium-term Gene Bank of Forage Germplasm in The Institute of Grassland Research of Chinese Academy of Agricultural Science (CAAS), Inner Mongolia, Hohhot, China.

Table 1 Accessions of *Medicago ruthenica* used in the study

Accession no.	Origin	Accession no.	Origin
MR-1	Xilingol League	MR-16	Xing an League
MR-2	Xilingol League	MR-17	Xing an League
MR-3	Xilingol League	MR-18	Hulunbeier City
MR-4	Xilingol League	MR-19	Hulunbeier City
MR-5	Xilingol League	MR-20	Hulunbeier City
MR-6	Xilingol League	MR-21	Wulanchabu City
MR-7	Xilingol League	MR-22	Wulanchabu City
MR-8	Xilingol League	MR-23	Wulanchabu City
MR-9	Chifeng City	MR-24	Wulanchabu City
MR-10	Chifeng City	MR-25	Baotou City
MR-11	Chifeng City	MR-26	Baotou City
MR-12	Tongliao City	MR-27	Hohhot City
MR-13	Tongliao City	MR-28	Hohhot City
MR-14	Tongliao City	MR-29	Hohhot City
MR-15	Xing an League	MR-30	Hohhot City

Total genomic DNA was extracted from 10 young leaves of each accession following the CTAB method described by Doyle and Doyle (1987). DNA quality and quantity were checked in 1 % agarose gels and UN4802 Spectrophotometer (Unico, USA), respectively. Genomic DNA was stored at −20 °C for further analysis.

ISSR-PCR

ISSR primers used in this study were synthesized by Shanghai Sangon Biological Engineering Technology & Service Co., Ltd. (China), according to the primer set published by the University of British Columbia (UBC). Eighty ISSR primers were initially screened, and ten of them, which yielded bright and discernible bands, were used for the analysis (Table 2). ISSR amplification reactions were carried out in a 25 μL reaction volume containing 10× reaction buffer, 2.0 mM Mg^{2+}, 0.6 mM of each dNTP, 0.9 μM for

primer, 1.5 U of *Taq* DNA polymerase and 0.5 ng of genomic DNA template. Amplification was performed in an Eppendorf Master cycler Gradient PCR (Eppendorf, Germany) under the following conditions: 3 min at 94 °C, followed by 30 s at 94 °C, 45 s at 52 °C, and 1.5 min at 72 °C for 35 cycles, and 10 min at 72 °C. PCR products were separated by electrophoresis using 1.5 % agarose gel and stained using EB and photographed by Nikon D200 (Japan).

SSR-PCR

The SSR primer pairs mainly originated from 89 Truncatula (*Medicago truncatula* Gaertn.) (Bernadette et al. 2003) and alfalfa (*M. sativa* L.) (Diwan et al. 1997). Ten of them, which yielded bright and discernible bands, were used for the analysis (Table 2). SSR amplification reactions were carried out in 25 μL reaction volume containing 10× reaction buffer, 5.5 μL Mg^{2+}, 0.75 μL of each dNTP, 0.75 μL for forward and reverse primer, respectively, 5.0 U of *Taq* DNA polymerase and 408 ng of genomic DNA template. Amplification was performed in an Eppendorf Master cycler Gradient PCR under the following conditions: 3 min at 94 °C, followed by 25 s at 94 °C, 30 s at 55–66 °C, and 30 s at 72 °C, and 35 cycles, and a final extension of 10 min at 72 °C, the stored at 4 °C. The amplification products by 6 % denaturing polyacrylamide gel electrophoresis, constant power 80 W, 30 min. And then silver staining, decolorization and fixed, washed, and finally stained and developing.

Data scoring and statistical analysis

The ISSR and SSR bands were scored as present (1) or absent (0) across 30 accessions for each primer or primer combination. POPGENE v1.32 (Yeh et al. 1997) was used to calculate various genetic diversity parameters, including the percentage of polymorphic bands (PPB), Shannon's information index (I) and Nei's gene diversity (He). A dendrogram was constructed based on Jaccard's similarity coefficients (GS) using the un-weighted pair group method with arithmetic average (UPGMA) with the SAHN module of NTSYS-pc 2.20 (Rohlf 2000). The COPH and MXCOMP programs were used to calculate goodness-of-fit between the cluster analysis and original matrix. A principal coordinate analysis to construct a two-dimensional array of eigenvectors was performed using the DCENTER module of the NTSYS-pc2.20 program. Finally, a Mantel test was made with 1,000 permutations to determine the significance level between ISSR and SSR, ISSR and ISSR + SSR, and SSR and ISSR + SSR based on the Jaccard's coefficient using NTSYS-pc 2.20 software.

Results and analysis

ISSR analysis

A total of 94 scored bands were generated using the 10 primer previously screened across 30 Ruthenia Medic accessions (Table 2). The number of amplified bands per primer ranged from 8 (UBC809, UBC834,

Table 2 Primer sequence used for ISSR and SSR analysis in this study

ISSR primer		SSR primer	Forward primer (5′–3′)	Reverse primer (5′–3′)
UBC809	$(AG)_9G$	MTIC233	GCG TAA CGT AAC AAC ATT CA	AAG GAA CAA TCC CAG TTT TT
UBC834	$(AG)_8YT$	MTIC347	TCG GTG TAT TTC CGT GTT TG	GGT TGA AAT TGA AAG AAG AAT CG
UBC836	$(AG)_8YA$	MTIC258	CAC CAC CTT CAC CTA AGA AA	TGA AAT TCA CAT CAA CTG GA
UBC813	$(CT)_8T$	BI4BO3	GCT TGT TCT TCT TCA AGC TCA C	CTG ACT TGT GTT TTA TGC
UBC821	$(GT)_8T$	MTIC183	TTC TCT TCA AGT GGG AGG TA	AAA TGG AAG AAA GTG TCA CG
UBC843	$(CT)_8RA$	MTIC249	TAG GTC ATG GCT ATT GCT TC	GTG GGT GAG GAT GTG TGT AT
UBC823	$(TC)_8C$	MTIC27	CCC CGT TTT TCT TCT CTC CT	CGA TCG GAA CGA GGA CTT TA
UBC845	$(CT)_8RG$	MAL369471	AAA CCC TTA GCA CCG ACA	ATT CAC ACA AAC CCA TCT TC
UBC866	$(CTC)_6$	MTIC451	GGA CAA AAT TGG AAG AAA AA	AAT TAC GTT TGT TTG GAT GC
UBC822	$(TC)_8A$	MTIC432	TGG AAT TTG GGA TAT AGG AAG	GCC ATA AGA ACT TCC ACT T

UBC836) to 13 (UBC822) with an average of ten bands per primer (Table 3). Of the 94 amplified bands, 83 were polymorphic (88.5 %), ranging from 6 (UBC809, UBC836) to 11(UBC822), with a mean of 8.3 polymorphic bands per primer. The percentage of polymorphic bands across the primers ranged from 75 % (UBC809, UBC836) to 100 % (UBC813, UBC845), with an average of 88.0 % polymorphism. The Shannon's information index (I) and gene diversity (He) were conducted to further understand the genetic diversity of Ruthenia Medic from different origins and from the complete set of accessions. The Shannon's information index (I) and gene diversity (He) of Ruthenia Medic were 0.487 and 0.329, respectively. Genetic similarity coefficients were obtained with UPGMA algorithm using Jaccard's coefficient. The GS ranged from 0.514 (MR-8 and MR-24) to 0.885 (MR-2 and MR-5).

The dendrogram grouped the 30 accessions into four main clusters (with a GS of 0.64) (Fig. 1a). Accessions within Cluster I were further divided into two subcluster. The subcluster I-1 comprised 12 accessions including eight accessions from Xilingol League, MR-15, MR-16 and MR-17 from Xing an League, MR-18 from Hulunbeier City. Subcluster I-2 contained seven accessions, MR-21, MR-22 and MR-23 from Wulanchabu City, MR-25 and MR-26 from Baotou City, MR-27 and MR-28 from Hohhot City. Cluster II comprised four accessions including MR-13 and MR-14 from Tongliao City, MR-19 and MR-20 from Hulunbeier City. Cluster III contained six accessions including MR-9, MR-10 and MR-11 from

Table 3 Total no. of bands (TNB), no. of polymorphic bands (NPB), percentage polymorphic bands (PPB), Shannon's Information Index (I), and expected heterozygosity (He) revealed by ISSR and SSR marker in *M. ruthenica*

Primer	TNB	NPB	PPB	I	He
ISSR					
UBC809	8	6	75.00	0.464	0.323
UBC834	8	7	87.50	0.554	0.388
UBC836	8	6	75.00	0.479	0.336
UBC813	9	9	100.00	0.535	0.360
UBC821	9	8	88.89	0.493	0.330
UBC843	9	8	88.89	0.486	0.323
UBC823	10	9	90.00	0.531	0.364
UBC845	10	10	100.0	0.380	0.230
UBC866	10	9	90	0.508	0.345
UBC822	13	11	84.62	0.459	0.308
Total	94	83	88.5		
Mean	9.4	8.3	88.5	0.487 ± 0.0024	0.329 ± 0.0018
SSR					
MTIC233	4	3	75.0	0.438	0.285
MTIC347	4	3	75.0	0.168	0.074
MTIC258	5	3	60.0	0.292	0.177
BI4BO3	6	5	83.3	0.419	0.264
MTIC183	8	5	62.5	0.442	0.283
MTIC249	6	5	83.3	0.404	0.266
MTIC27	7	6	85.7	0.429	0.273
MAL369471	9	8	88.9	0.400	0.250
MTIC451	10	9	90.0	0.403	0.248
MTIC432	10	10	100.0	0.279	0.164
Total	69	57	80.4		
Mean	6.9	5.7	80.4	0.372 ± 0.0022	0.231 ± 0.0017

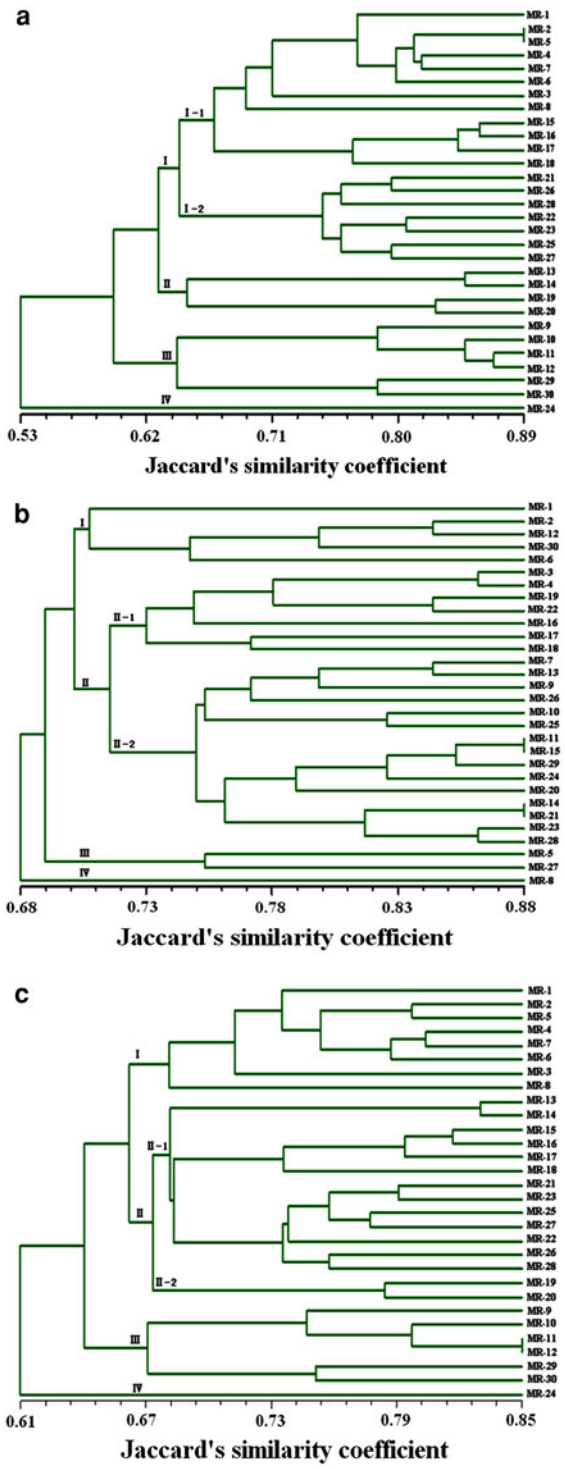


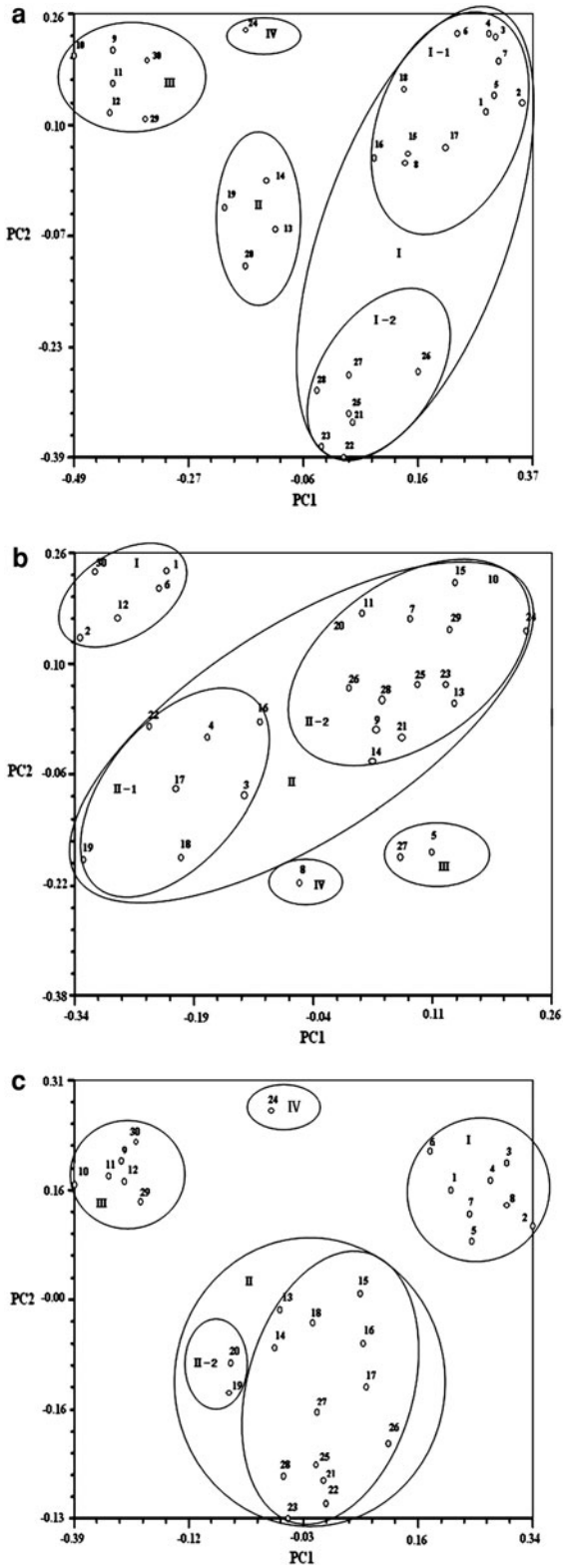
Fig. 1 Unweighted Pair Group Method with Arithmetic average (UPGMA) dendrogram using ISSR (**a**), SSR (**b**) and combined ISSR + SSR (**c**) of 30 Ruthenia Medic accessions

Chifeng City, MR-12 from Tongliao City, MR-29 and MR-30 from Hohhot City. Accession MR-24 from Wulanchabu City formed cluster IV. The results of the principal components analysis (PCA) were comparable to the cluster analysis (Fig. 2a). The first three components explain 35.24 % of the total variation.

SSR analysis

A total of 89 Truncatula and alfalfa different primer combinations were employed. Only ten pairs of them could produce clear and reproducible bands and were chosen in the following study (Table 2). A total of 69 bands were observed,of which 57 were polymorphic (80.4 %), ranging from 3 (MTIC233, MTIC347, MTIC258) to 10 (MTIC432) (Table 3). Average number of bands and polymorphic bands per primer combinations were 6.9 and 5.7, respectively. Percentage of polymorphic bands (PPB) ranged from 60 % (MTIC258) to 100 % (MTIC432) with an average polymorphism of 80.4 % across all the accessions.

The Shannon's information index (I) and gene diversity (He) of Ruthenia Medic were 0.372 and 0.231, respectively. The dendrogram using UPGMA analysis based on Jaccard's GS of the SSR markers of 30 accessions is showed in Fig. 1b. The GS varied from 0.7257 (MR-8 and MR-14) to 0.9571 (MR-11 and MR-12). The 30 Ruthenia Medic accessions were grouped into four clusters (with a GS of 0.705). Cluster I comprised five accessions, three accessions from Xilingol League, MR-12 from Tongliao City, MR-30 from Hohhot City. Cluster II comprised 22 accessions and was further divided into two subcluster. The subcluster II-1 contained seven accessions, MR-3 and MR-4 accessions from Xilingol League, MR-16 and MR-17 accessions from Xing an League, MR-18 and MR-19 accessions from Hulunbeier City, MR-22 accession from Wulanchabu City. Subcluster II-2 contained MR-7 from Xilingol League, three accessions from Chifeng City, two accessions from Tongliao City, one from Xing an League, one from Hulunbeier City, three accessions from Wulanchabu City, two from Baotou City, two from Hohhot City. Cluster III comprised MR-5 from Xilingol League, MR-27 from Hohhot City. Cluster IV comprised MR-8 from Xilingol League. The results of the principal components analysis (PCA) were basically consisting with the cluster analysis (Fig. 2b). The first three components explain 30.53 % of the total variation.



◀**Fig. 2** Two-dimensional plot (with vectors) of principal coordinate analysis of 30 Ruthenia Medic accessions using ISSR (**a**), SSR (**b**) and combined ISSR + SSR (**c**). The *numbers* correspond to those listed on Table 1

Combined (ISSR and SSR) data

In order to obtain more accurate genetic estimates, combined analysis was carried out using all the ISSR and SSR data together. The Jaccard's GS ranged from 0.5952 (MR-8 and MR-24) to 0.9030 (MR-11 and MR-12). The Jaccard's GS matrix based on combined data was used to generated a dendrogram showing genetic relationships among the accessions. The dendrogram grouped the 30 accessions into four main clusters (Fig. 1c). Eight accessions from Xilingol League formed cluster I. Cluster II is a biggest cluster consisting of 15 accessions and was further divided into two subcluster. Cluster II-1 included two from Tongliao City, three from Xing an League, MR-18 from Hulunbeier City, three accessions from Wulanchabu City, two accessions from Baotou City, two accessions from Hohhot City. Cluster II-2 included MR-19 and MR-20 which were from Hulunbeier City, respectively. The cluster III comprised three accessions from Chifeng City, MR-12 from Tongliao City, MR-29 and MR-30 from Hohhot City. Cluster IV comprised one accession from Wulanchabu City. The results of the principal components analysis (PCA) were comparable to the cluster analysis (Fig. 2c). The first three components explain 28.19 % of the total variation.

Discussion

Assessment of the genetic variability within a wild forage has important consequences in plant breeding and the conservation of genetic resources, and molecular markers offer an approach to unveil the genetic diversity among accessions based on nucleic acid polymorphisms. In the present study, two marker systems, ISSR and SSR, were applied for the first time to assess the level and pattern of genetic diversity of 30 Ruthenia Medic accessions from Inner Mongolia. Wide genetic variability among different accessions of Ruthenia Medic was observed in the study (Table 3). This high level of polymorphism in Ruthenia Medic has been reported before for its morphology level in

previous studies (Wang et al. 2008; Li et al.2011). In comparison to other legume forage species, the genetic diversity of Ruthenia Medic was higher than that of alfalfa (*M. sativa*) (ISSR,PPB = 60 %) (Touil et al. 2008) and red clover (*Trifolium pratense*) (RAPD, PPB = 74.2 %) (Ulloa et al. 2003). It was also lower than that found for other legume forage species such as *Astragals rhizanthus* (ISSR, PPB = 92.3 %) (Anand et al. 2010), and goat's rue (*Caragana korshinskii*) (AFLP, PPB = 93.9 %) (Wang et al. 2007). The applicability of ISSR and SSR as genetic markers to characterize the genetic diversity of Ruthenia Medic was compared. ISSR markers showed higher PPB (88.5 %) than the SSR markers (80.4 %). The average Nei gene diversity is also more higher for ISSR than that for SSR markers (Table 3) based on The Shannon's information index, Similar results were also observed in *Elymus* species (Li et al. 2005), Tobacco (Nie and Liu 2011). The present study showed clustering of accessions based on ISSR and SSR markers was not similar. The correlation between two Jaccard's similarity coefficient value resulted in low correlation between SSR and ISSR + SSR (r = 0.0191) and high for ISSR and ISSR + SSR (r = 0.956) and ISSR and SSR data (r = 0.0970). This shows that ISSR data is slightly more close to ISSR + SSR combined data. A possible explanation for the difference in resolution of two marker systems is that the two marker techniques targeted different portions of the genome. The ISSR markers scattered throughout the genome which revealed the diversity of the entire genome, while SSR markers only amplified target region of open reading frame (ORF), the functional regions (Yang et al. 2010b; Gimenes et al. 2007). These differences may also be attributed to marker sampling errors and/or the level of polymorphism detected, reinforcing the importance of the number of loci and their coverage of the overall genome in obtaining reliable estimates of genetic relationships among cultivars (Loarce et al. 1996). Although a part of accessions (e.g. from Xilingol League) are consistently grouped together in all four cluster analysis, the dendrograms did not show clear clustering pattern of geographically closer accessions, which indicated that the association between genetic similarity and geographical distance was less significant. Similar results were reported in Azuki bean (Yee et al. 1999). Our results indicate the presence of great genetic variability among Ruthenia Medic accessions. Genetic variation among Ruthenia Medic accessions based on ISSR and SSR analysis could be useful to select parents to be crossed for generating appropriate populations intended for both genome mapping and breeding purposes. The variation of Ruthenia Medic is poorly understood and basic systematic analyses of the different variants that have been recognized need to be carried out. Although there has been recent acquisition of germplasm,there is still a need for additional germplasm collection of the apparently wide range of ecotypes of this species and evaluation of their agronomic potential. There also needs to be an evaluation of the possibility of gene transfer to alfalfa, particularly those characteristics favoring drought and cold resistance (Small 2011). Ruthenia Medic occurs in remote areas of Asia that are subject to genetic erosion, and so conservation evaluation is desirable. The very recent collections acquired by the National Medium-term Gene Bank of Forage Germplasm will ensure that much of the genetic variation will be available in the future (Small 2011).

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References

Anand KK, Srivastava RK, Chaudhary LB (2010) Analysis of genetic diversity in *Astragalus rhizanthus* Benth. ssp. *rhizanthus* var. *rhizanthus* (Fabaceae) using molecular markers from India. J Bot 2010:1–9

Ariss JJ, Vandemark GJ (2007) Assessment of genetic diversity among nondormant and semidormant alfalfa populations using sequence related amplified polymorphisms. Crop Sci 47:2274–2284

Bernadette J, Sandrine F, Philippe B, Gaelle C, Sylvain S, Thierry H, Christian H (2003) Construction of two genetic linkage maps in cultivated tetraploid alfalfa (*Medicago sativa*) using microsatellite and AFLP markers. BMC Plant Biol 3:9

Beyene Y, Botha AM, Myburg AA (2006) Genetic diversity among traditional Ethiopian highland maize accessions assessed by simple sequence repeat (SSR) markers. Genet Resour Crop Evol 53(8):1579–1588

Campbell TA, Bao G, Xia ZL (1997) Agronomic evaluation of *Medicago ruthenica* collected in Inner Mongolia. Crop Sci 37:599–604

Campbell TA, Bao G, Xia ZL (1999) Completion of the agronomic evaluations of *Medicago ruthenica* [(L.) Ledebour]

germplasm collected in Inner Mongolia. Genet Resour Crop Evol 46:477–484

Deshwall RPS, Singh R, Malik K, Randhawa GJ (2005) Assessment of genetic diversity and genetic relationships among 29 populations of *Azadirachta indica* A. Juss. using RAPD markers. Genet Resour Crop Evol 52:285–292

Diwan N, Bhagwat AA, Bauchan GR, Cregan PB (1997) Simple sequence repeat (SSR) DNA markers in alfalfa and perennial and annual *Medicago* species. Genome 40:887–895

Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull 19:11–15

Gen HZ, Wu YF, Cao ZZ (1995) Chinese alfalfa. China Agriculture Press, Beijing 14

Gimenes MA, Hosino AA, Barbosa AVG, Palmieri DA, Lopes CR (2007) Characterization and transferability of microsatellite markers of cultivated peanut (*Arachis hypogaea*). BMC Plant Biol 7:9

Hao JH, Shi FL (2006) Study on drought resistance of *Medicago ruthenica* accessions. Chine J Grassland 28:39–42

Iwata H, Imon K, Tsumura Y, Ohsawa R (2005) Genetic diversity among Japanese indigenous common buckwheat (*Fagopyrum esculentum*) cultivars as determined from amplified fragment length polymorphism and simple sequence repeat markers and quantitative agronomic traits. Genome 48:367–377

Laurentin HE, Karlovsky P (2006) Genetic relationship and diversity in sesame (*Sesamum indicum* L.) germplasm collection using amplified fragment length polymorphism (AFLP). BMC Genet 7:1–10

Li YX, Li SS, Li LH (2005) Comparison of genetic diversity of twelve *Elymus* species using ISSR and SSR markers. Sci Agric Sin 38(8):1522–1527

Li HY, Li ZY, Mi FG, Lu XX, Shi WG (2008a) Appraisal of *Medicago ruthenica* Germplasms by SSR markers. Acta Agriculturae Boreali-sinica 23(3):67–71

Li HY, Mi FG, Ning HM, Shi WG, Zhao CG (2008b) Genetic diversity of *Medicago ruthenica* based on SSR markers. Chin J Grassland 30(2):34–38

Li ZY, Shi WG, Li HY, Li XY, Liu L (2009) Genetic diversity and relationship of three *Medicago ruthenica* ecotypes from Inner Mongolia. Acta Bot Boreal Occident Sin 29(12):2421–2428

Li HY, Li ZY, Mi FG, Shi WG, Cai LY, Wang CH, Xing JJ (2011) The genetic diversity of *Medicago ruthenica* were analyzed by phenotype and SSR marker in China. J Northwest A&F Univ 39(8):1–9

Li ZY, Li HY, Shi FL, Wang XL, Zhao CG, Shi WG, Cai LY (2012) Genetic Diversity Analysis of *Medicago ruthenica* by ISSR in China. J Plant Genet Resour 13(1):48–51, 56

Loarce Y, Gallego R, Ferrer E (1996) A comparative analysis of genetic relationships between *rye* cultivars using RFLP and RAPD markers. Euphytica 88:107–115

Nie Q, Liu R (2011) Genetic diversity of 23 tobacco germplasm resources by SSR and ISSR analysis. Southwest China J Agric Sci 24(1):15–19

Ofori A, Becker HC, Kopisch-ObuchF J (2008) Effect of crop improvement on genetic diversity in oilseed *Brassica rapa* (turnip–rape) cultivars, detected by SSR markers. J Appl Genet 49:207–212

Panguluri SK (2007) RAPD and ISSR fingerprinting in cultivated chickpea (*Cicer arietinum* L.) and its wild progenitor Cicer reticulatum Ladizinsky. Genet Resour Crop Evol 54:1235–1244

Qi XH, Yang JH, Zhang MF (2008) AFLP-based genetic diversity assessment among Chinese vegetable mustards (*Brassica juncea* (L.) Czern.). Genet Resour Crop Evol 55:705–711

Rohlf FJ (2000) NTSYS-PC version 2.10 s. Numerical taxonomy and multivariate analysis system. Exeter Publications, Setauket

Small E (2011) Alfalfa and relatives. Evolution and classification of *Medicago*. NRC Research Press, Ottawa, pp 567–570

Small E, Jomphe M (1989) A synopsis of the genus *Medicago* (Leguminosae). Can J Bot 67:3260–3294

Teklewold A, Becker HC (2006) Geographic pattern of genetic diversity among 43 Ethiopian mustard (*Brassica carinata* A. Braun) accessions as revealed by RAPD analysis. Genet Resour Crop Evol 53:1173–1185

Touil L, Guesmi F, Fares K, Ferchichi A (2008) Genetic diversity of some mediterranean populations of the cultivated alfalfa (*Medicago sativa* L.) using ISSR markers. Biotechnology 7(4):808–812

Ulloa O, Ortega F, Campos H (2003) Analysis of genetic diversity in red clover (*Trifolium pratense* L.) breeding populations as revealed by RAPD genetic markers. Genome 46(4):529–535

Uysal H, Fu YB, Kurt O, Peterson GW, Diederichsen A, Kusters P (2010) Genetic diversity of cultivated flax (*Linum usitatissimum* L.) and its wild progenitor pale flax (*Linum bienne* Mill.) as revealed by ISSR markers. Genet Resour Crop Evol 57(7):1109–1119

Wang Z, Gao HW, Wu YQ, Han JG (2007) Genetic diversity and population structure of *Caragana korshinskii* revealed by AFLP. Crop Sci 47:1737–1743

Wang JE, Wang Z, Wang YQ, Gao HW, Chapurin VF (2008) Morphological diversity of *Galega officinalis* L. germplasm resources. J Plant Genet Resour 9(2):201–205

Wang Z, Kenworthy KE, Wu YQ (2010a) Genetic diversity of common carpetgrass revealed by amplified fragment length polymorphism markers. Crop Sci 50:1366–1374

Wang Z, Wu YQ, Martin DL, Gao HW, Samuels T, Tan CC (2010b) Identification of vegetatively propagated turf bermudagrass cultivars using simple sequence repeat markers. Crop Sci 50:2103–2111

Yang GS, Wang Y, Sun ChY, Jlang SC, Zhang MP (2010a) SSR analysis of genetic diversity of *Panax ginseng*. Med Plant 1(7):50–53

Yang Y, Pan YZ, Gong X, Fan M (2010b) Genetic variation in the endangered Rutaceae species *Citrus hongheensis* based on ISSR fingerprinting. Genet Resour Crop Evol 57(8):1239–1248

Yee E, Kidwell KK, Sills GR, Lumpkin TA (1999) Diversity among selected *Vigna angularis* (Azuki) accessions on the basis of RAPD and AFLP markers. Crop Sci 39:268–275

Yeh FC, Yang R-C, Boyle TBJ, Ye Z-H, Mao JX (1997) POPGENE, the user-friendly shareware for population genetic analysis. Molecular Biology and Biotechnology Centre. University of Alberta, Canada