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Analysis of genetic diversity of salt-tolerant alfalfa germplasms

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ABSTRACT. Random amplified polymorphic DNA technology was used to analyze the genetic diversity of 25 salt-tolerant alfalfa varieties using 30 different primers. Results showed that the percentage of polymorphic loci between single-plant DNA was 81.52%, and that between mixed DNA of various varieties was 61.65%. Compared to the mixed DNA samples, single-plant DNA samples can better reveal the level of genetic variation among and between alfalfa varieties. The gene differentiation coefficients of 18 Chinese salt-tolerant alfalfa varieties and 7 American salt-tolerant alfalfa varieties were 0.271 and 0.152, respectively, showing that the exchange of genes between Chinese salt-tolerant alfalfa germplasms was more frequent than that of American germplasms. As a topical cross-pollinated plant, the genetic structure of biological populations of alfalfa was directly linked to its breeding system. According to the analysis of genetic distance (GD), 25 varieties can be divided into 9 groups, among which, the GD of Tumu No. 1 and Tumu No. 2 was the shortest (0.148), and the GD of Jieda No. 1 and Tumu was the longest (0.786). The analysis of genetic diversity

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of salt-tolerant alfalfa germplasms provided a theoretical basis for the creation of an alfalfa salt-tolerant core germplasm repository and for the selection and breeding of new salt-tolerant varieties.

Key words: Alfalfa; Salt-tolerant germplasms; Genetic diversity; Random amplified polymorphic DNA

INTRODUCTION

The worldwide extensive land salinization seriously affects the development of agriculture globally. To improve the salt tolerance of crops, cultivate new varieties with salt-tolerant traits, and increase crop production under salt stress have been the focus for scientists for a long time. The screening of salt-tolerant germplasms and obtaining salt-tolerant genes are the basis for crop introduction and breeding programs. As an important forage crop with high-quality proteins, alfalfa (*Medicago sativa* L.) is widely planted mainly on land with slight salinization (the salt concentration of the soil is less than 0.1%). The planting of alfalfa on land with medium or severe salinization is greatly restricted (Safarnejad, 2008; Khorshidi et al., 2009) because the lack of salt-tolerant germplasms and narrow genetic basis (Jin et al., 2010) of alfalfa have seriously limited the research and utilization of salt tolerance. Therefore, analysis of the genetic diversity of salt-tolerant alfalfa germplasms and the screening of salt-tolerant germplasms are vital to guide the selective breeding of new salt-tolerant alfalfa varieties and to explore the genetic mechanism of alfalfa salt tolerance. Liu and Zhang (1993) identified the salt tolerance of 65 alfalfa varieties by measuring 6 biological parameters including survival rate, relative plant height, and relative dry weight and 2 physiological indexes including membrane permeability of leaf blade and proline content of leaves under 0.3 or 0.4% salt stress. Afterwards, Han et al. (2003) also used various biological parameters or physiological indexes to screen alfalfa salt-tolerant germplasms. Recently, DNA molecular marker technology has been applied to screen and identify salt-tolerant alfalfa germplasms and analyze genetic diversity, and domestic and foreign scholars have successfully applied simple sequence repeat (Diwan et al., 1997; Mengoni et al., 2000; Liu et al., 2006) and amplified fragment length polymorphism (Julier et al., 2003) marker techniques to analyze the genetic diversity of different salt-tolerant varieties of alfalfa. Random amplified polymorphic DNA (RAPD) technology is frequently used in germplasm research, variety identification, polymorphism analysis, and breeding assistance. This study uses the RAPD technique to analyze the genetic diversity of salt-tolerant germplasms of alfalfa, which will facilitate the screening of salt-tolerant parents and the stacking of salt-tolerant genes, thereby providing a basis for alfalfa salt-tolerant breeding and salt-tolerance research.

MATERIAL AND METHODS

Experimental materials

In this study, 25 salt-tolerant alfalfa varieties (Table 1) were collected and identified by the Ethnic Region Biological Resources and Environment Institute of the College of Life Science in Dalian Nationalities University. Among them, 18 originated from the Grassland

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Institute, Branch of Animal Science, Jilin Academy of Agricultural Sciences, and 7 varieties were from the Alfalfa Institute, University of California at San Francisco, USA. These varieties were confirmed to display strong salt tolerance under the stress of 0.8% salt. For each variety, 10 different clones were randomly selected. Young leaves of these varieties were used as the experimental materials, and 3 replicates were prepared. After being rinsed and dried, the leaves were stored at -70°C for later use.

Table 1. Salt-tolerant alfalfa varieties and its source.

No.	Code	Variety	Scientific name	Source	Introduction time
1	AF	Aifeinite	*Medicago sativa* L. ssp *varia*	Jilin Province Academy of Agricultural Sciences	2003
2	AH1	Aohan No. 1	*Medicago sativa* L. ssp *varia*	Jilin Province Academy of Agricultural Sciences	2003
3	AH2	Aohan No. 2	*Medicago sativa* L. ssp *varia*	Jilin Province Academy of Agricultural Sciences	2003
4	CY1	Caoyuan No. 1	*Medicago sativa* L. ssp *varia*	Jilin Province Academy of Agricultural Sciences	2003
5	CY2	Caoyuan No. 2	*Medicago sativa* L. ssp *varia*	Jilin Province Academy of Agricultural Sciences	2003
6	GN1	Gongnong No. 1	*Medicago sativa* L. ssp *varia*	Jilin Province Academy of Agricultural Sciences	2003
7	GN2	Gongnong No. 2	*Medicago sativa* L. ssp *varia*	Jilin Province Academy of Agricultural Sciences	2003
8	GA3	Gannong No. 3	*Medicago sativa* L. ssp *varia*	Jilin Province Academy of Agricultural Sciences	2003
9	JD	Jieda	*Medicago sativa* L. ssp *varia*	Jilin Province Academy of Agricultural Sciences	2003
10	LM1	Longmu No. 801	*Medicago sativa* L. ssp *varia*	Jilin Province Academy of Agricultural Sciences	2003
11	LM3	Longmu No. 803	*Medicago sativa* L. ssp *varia*	Jilin Province Academy of Agricultural Sciences	2003
12	LM5	Liangmu No. 5	*Medicago sativa* L. ssp *varia*	Jilin Province Academy of Agricultural Sciences	2003
13	QX	Qianxian	*Medicago sativa* L. ssp *varia*	Jilin Province Academy of Agricultural Sciences	2003
14	TM1	Tumu No. 1	*Medicago sativa* L. ssp *varia*	Jilin Province Academy of Agricultural Sciences	2003
15	TM2	Tumu No. 2	*Medicago sativa* L. ssp *varia*	Jilin Province Academy of Agricultural Sciences	2003
16	XJ	Xinjiangdaye	*Medicago sativa* L. ssp *varia*	Jilin Province Academy of Agricultural Sciences	2003
17	ZD	Zhaodong	*Medicago sativa* L. ssp *varia*	Jilin Province Academy of Agricultural Sciences	2003
18	ZM	Zhongmu No. 1	*Medicago sativa* L. ssp *varia*	Jilin Province Academy of Agricultural Sciences	2003
19	AG	Az-Germsalt	*Medicago sativa* L. ssp *varia*	University of California, San Francisco	2004
20	AL	Alfaking	*Medicago sativa* L. ssp *varia*	University of California, San Francisco	2004
21	AS	Az90ncc-St	*Medicago sativa* L. ssp *varia*	University of California, San Francisco	2004
22	CH	Chilean	*Medicago sativa* L. ssp *varia*	University of California, San Francisco	2004
23	PE	Peruvian	*Medicago sativa* L. ssp *varia*	University of California, San Francisco	2004
24	WL	WL3-23	*Medicago sativa* L. ssp *varia*	University of California, San Francisco	2004
25	SU	Super 13R	*Medicago sativa* L. ssp *varia*	University of California, San Francisco	2004

Total DNA extraction and construction of DNA pool

The cetyltrimethylammonium bromide (CTAB) method was used to extract genomic DNA (Tucak et al., 2008). The DNA integrity was confirmed by agarose gel electrophoresis. The DNA purity and concentration were measured by an ultraviolet spectrophotometer. The samples were diluted to 100 ng/μL in 0.1X Tris-ethylenediaminetetraacetic acid. Single-plant DNA was extracted with the CTAB method, and single-plant DNA of each material was stored one by one. Meanwhile, 3 replicates of single-plant DNA samples of each variety were mixed in equal amounts, and a DNA pool of each variety was established and placed at -70°C for further use.

Polymerase chain reaction (PCR) amplification and detection

The total amplification system is 30 μL, including 50 ng template DNA, 5 mM $MgCl_2$, 40 pmol primer, 2X buffer, 0.5 mM dNTP, and 1.5 U Taq enzyme. The hot-cycle parameters are as follows: denaturation at 94°C for 3 min, followed by denaturation (94°C for 15 s), annealing (36°C for 15 s), and extension (72°C for 1 min). The PCR amplification was carried out for 45 cycles. A final extension step is at 72°C for 10 min, and the products were stored at 4°C. The amplified products of DNA were separated on 1.5% agarose gels containing ethid-

ium bromide at 100 V, and λ DNA/*Eco*RI+*Hin*dIII was used as a marker of standard molecular weight. After 2-3 h of electrophoresis, the PCR products were detected through screening by the automatic gel.

Analysis of genetic diversity

Eighty-five RAPD primers were designed and ordered from the TaKaRa Biotechnology Co., Ltd., Dalian, China. The primers were screened using an optimized RAPD reaction system with one alfalfa DNA pool as the template. The selected primers were used to amplify DNA pools of all the materials to further screen target primers, which could amplify specific bands of the tested varieties. The PCR results are recorded in types of having bands (1) and having no bands (0).

The percentage of polymorphic loci is ppl = i / j x 100%, where *i* and *j* represent the number of polymorphic loci and total locus number, respectively. The percentage of polymorphic loci is an important indicator that measures the level of genetic variation in a species group. A high percentage of polymorphic loci of a species group shows that its adaptive capacity to the environment is strong; otherwise, its adaptive capacity to the environment is weak, and it will be more likely to be eliminated in the long course of evolution.

The following genetic parameters were calculated according to the occurrence frequency of band spectra at various loci. The intravarietal genetic consistency was given by $j = \Sigma Xi^2$, where *Xi* is the frequency of locus *i*; the intravarietal genetic diversity was h = 1 - j; the mean value of the intravarietal genetic consistency was $J_s = \Sigma j / n$, where *n* is the variety type; the mean value of the intravarietal genetic diversity was $H_s = 1 - J_s$; the total genetic consistency was $J_T = \Sigma(\Sigma Xi / n)^2$; the total genetic diversity was $H_T = 1 - J_T$; and the coefficient of gene differentiation was $G_{ST} = (H_T - H_s) / H_T$.

Genetic distance (GD) and cluster analysis

The GD was calculated as GD = 1 - 2Mxy / (Mx + My) based on the method of Nei (Nei, 1975; Nei and Li, 1979). In the formula, *Mx* and *My* are total segments of *x* and *y* materials, respectively, and *Mxy* is the common segments of the 2 materials; the average genetic distance between varieties of the group is given by $GD = \Sigma(GD)_i / [n(n - 1) / 2]$, where it is the variety type in the group. The SAS8.2 software was used to cluster the GD by using unweighted pair group method with arithmetic average (UPGMA).

RESULTS

Polymorphism

We screened primers based on the intravarietal single-plant DNA amplification of 25 alfalfa germplasms. Criteria for screening primers are as follows: amplified bands are clear and consistent with good repeatability and abundant amplification. Thirty RAPD primers were selected from 75 primers, and the primer sequences and amplified results are shown in Table 2. Partial single-plant DNA and mixed DNA amplified maps are shown in Figure 1.

Table 2. Amplification results of 25 salt-tolerant alfalfa varieties with 30 random amplified polymorphic DNA primers.

Primer	Sequence	Number of bands		Number of polymorphic bands		Polymorphic percentage	
		Single plant	Blend	Single plant	Blend	Single plant	Blend
OPA-10	GTGATGGCAG	6	4	4	2	66.67	50.00
OPA-12	TCGGCGATAG	9	6	7	3	77.78	50.00
OPA-13	CACCACCCAC	15	11	11	5	73.33	45.45
OPA-17	GACCGCTTGT	23	16	19	9	82.61	56.25
OPB-01	GTTTCGCTCC	8	5	5	3	62.50	60.00
OPB-05	TGCGCCCTTC	13	7	11	4	84.62	57.14
OPD-05	TGAGCGGATA	18	9	17	6	94.44	66.67
OPD-07	TTGGCACGGG	20	11	18	7	90.00	63.64
OPD-12	CACCGTATCC	24	15	21	10	87.50	66.67
OPE-01	CCCAAGGTCC	11	8	10	6	90.91	75.00
OPE-04	GTCCACACGG	17	13	15	9	88.24	69.23
OPE-06	AAGACCCCTC	23	16	21	12	91.30	75.00
OPE-08	ACATCGCCCA	15	9	14	5	93.33	55.56
OPE-09	TCCACTCCTG	20	13	17	8	85.00	61.54
OPE-10	TTCCCCGCGA	16	13	12	7	75.00	53.85
OPE-12	TTATCGGCCC	18	10	15	5	83.33	50.00
OPE-16	GGTGACTGTG	26	17	21	10	80.77	58.82
OPF-03	CCTGATCACC	5	3	3	2	60.00	33.33
OPF-06	GGAGTACTGG	8	5	6	3	75.00	60.00
OPF-07	CCGATATCCC	16	9	14	8	87.50	88.89
OPF-13	GGCTGCAGAA	20	13	17	8	85.00	61.54
OPG-09	CTGACGTCAC	4	2	2	1	50.00	50.00
OPG-13	CTCTCCGCCA	8	6	5	3	62.50	50.00
OPG-14	GGATGAGACC	11	8	8	5	72.73	62.50
OPH-12	ACGCGCATGT	14	8	10	5	71.43	62.50
OPH-15	AATGGCGCAG	17	13	13	9	76.47	69.23
OPN-10	ACAACTGGGG	13	9	10	6	76.92	66.67
OPQ-13	GGAGTGGACA	5	3	4	2	80.00	66.67
OPQ-14	GGACGCTTCA	12	7	10	4	83.33	57.14
OPQ-15	GGGTAACGTG	18	10	13	6	72.22	60.00
Total		433	279	353	172		
Average		14.43	9.30	11.77	5.73	81.52	61.65

A total of 433 bands were obtained from 25 salt-tolerant alfalfa germplasms using 30 primers, among which, 353 bands were pleomorphic. The percentage of polymorphic loci reached 81.52%, indicating that there is intravarietal genetic differentiation, with a high level of genetic variation and strong adaptive capacity to the environment. Two hundred seventy-nine bands were amplified using 30 primers from mixed DNA of various varieties, including 172 polymorphic bands, and the percentage of polymorphic loci reached 61.65%. As shown in Table 2 and Figure 1, the polymorphism of mixed DNA of various varieties was obviously lower than that of single-plant DNA from various varieties, indicating that mixed DNA concealed the intravarietal DNA polymorphism and lowered the intravarietal polymorphism. Thus, for alfalfa, which is a cross-pollinated plant species, single-plant DNA samples can reveal intravarietal and intervarietal genetic variations and quantify intervarietal differences better than mixed DNA samples.

Among all the tested materials, the differences in the number of amplified DNA bands between primers were large. The number of amplified bands of single-plant DNA ranged from 4 to 26, with an average of 14.43, and that of mixed DNA ranged from 2 to 17, with an average 9.3, showing that there are large differences in DNA sequences between primers and the tested materials. For 2 DNA samples, AF and ZM, the primers OPA-17, OPD-05, OPD-12, OPE-06, OPE-08, OPE-09, OPE-16, and OPF-13 amplified many bands with a high percentage of polymorphic loci; these primers can be used to show characteristics of certain alfalfa varieties and act as the charac-

teristic bands of these varieties. In summary, as shown in Figures 1 and 2, certain differences were found in DNA sequences between the tested materials using 30 primers, indicating that RAPD technology is extremely applicable to the analysis of genetic diversity of alfalfa.

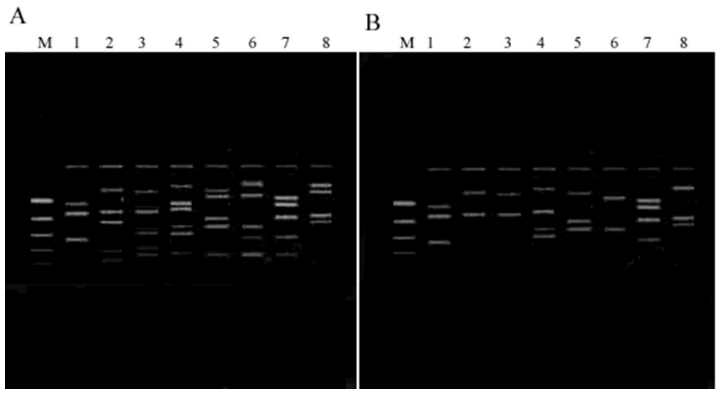

Figure 1. Patterns of random amplified polymorphic DNA by primer OPE-16 with single-plant DNA samples (**A**) and mixed-plant DNA samples (**B**). *Lanes 1-8* = amplification results derived from 8 varieties: AF, CY1, GN1, JD, QX, AG, WL, and PE (abbreviations are those given in Table 1). *Lane M* = molecular marker.

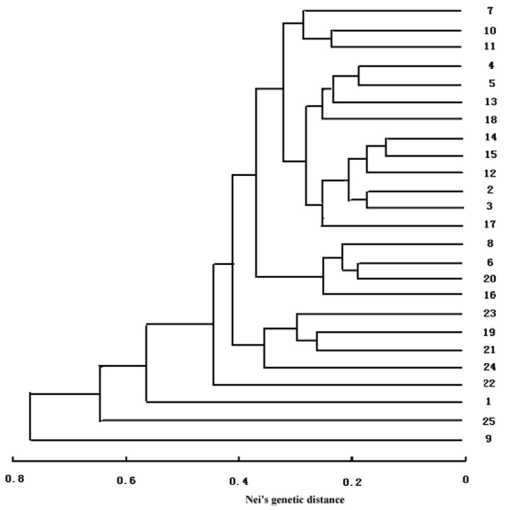

Figure 2. Clustering dendrogram of the 25 salt-tolerant alfalfa varieties based on random amplified polymorphic DNA markers. Numbers correspond to those given in Table 1.

Genetic variation

According to the genetic variation analysis of single-plant DNA samples of 18 salt-tolerant alfalfa varieties in Jilin, China, and 7 salt-tolerant alfalfa varieties in California, USA, the percentage of polymorphic loci of Tumu No. 2 is the highest (3.80%), and the lowest is Zhaodong alfalfa (48.36%). In terms of genetic diversity, the highest is Tumu No. 2 (h = 0.352), and

the lowest is Gongnong No. 1 (h = 0.225). Among 7 salt-tolerant alfalfa varieties in the USA, the percentage of polymorphic loci of the Peruvian variety is the highest (71.24%), and the lowest is the Chilean variety (50.26%). In terms of genetic diversity, Az90ncc-St is the highest (h = 0.357), and the lowest is Alfaking (h = 0.265).

As shown in Tables 3 and 4, the total genetic diversity of 18 salt-tolerant alfalfa varieties in Jilin, China (H_T = 0.398), is slightly larger than that of 7 salt-tolerant alfalfa varieties in California, USA (H_T = 0.355), showing that the Chinese salt-tolerant alfalfa resources are more diversified than the American resources. The coefficient of genetic differentiation mainly reflects the ratio of intervarietal variation to total variation. The coefficients of the 2 groups are 0.271 and 0.152, respectively. The above results showed that the genetic differentiation of alfalfa germplasms is mainly intravarietal and not intervarietal; further, the exchange of intravarietal genes of germplasm resources of Chinese salt-tolerant alfalfa is more frequent than that of American varieties. In summary, as a topical cross-pollinated plant species, the genetic structure of biological populations of alfalfa is directly related to its breeding system.

Table 3. Genetic differences of single-plant DNA samples from 18 salt-tolerant alfalfa varieties from Jilin, China.

Code	Polymorphic percentage	Gene identity	Gene diversity
AF	57.35	0.683	0.317
AH1	63.50	0.735	0.265
AH2	63.64	0.726	0.274
CY1	56.67	0.706	0.294
CY2	56.85	0.704	0.296
GN1	56.25	0.775	0.225
GN2	57.14	0.764	0.236
GA3	63.55	0.772	0.228
JD	68.55	0.664	0.336
LM1	62.10	0.704	0.296
LM3	62.25	0.697	0.303
LM5	62.64	0.708	0.292
QX	69.28	0.674	0.326
TM1	73.25	0.648	0.352
TM2	73.80	0.657	0.343
XJ	60.00	0.676	0.324
ZD	48.36	0.735	0.265
ZM	61.54	0.751	0.249
	J_S = 0.710; H_S = 0.290; J_T = 0.602; H_T = 0.398; G_{ST} = 0.271		

J_S = intravarietal genetic consistency; H_S = intravarietal genetic diversity; J_T = total genetic consistency; H_T = total genetic diversity; G_{ST} = coefficient of gene differentiation.

Table 4. Genetic differences of single-plant DNA samples from 7 salt-tolerant alfalfa varieties from California, USA.

Code	Polymorphic percentage	Gene identity	Gene diversity
AG	64.52	0.687	0.313
AL	56.85	0.735	0.265
AS	63.67	0.643	0.357
CH	50.26	0.713	0.287
PE	71.24	0.658	0.342
WL	61.57	0.673	0.327
SU	53.64	0.717	0.283
	J_S = 0.689; H_S = 0.311; J_T = 0.645; H_T = 0.355; G_{ST} = 0.152		

J_S = intravarietal genetic consistency; H_S = intravarietal genetic diversity; J_T = total genetic consistency; H_T = total genetic diversity; G_{ST} = coefficient of gene differentiation.

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Cluster analysis

According to the Nei genetic distance, 25 varieties were clustered by UPGMA. Under the threshold value of 0.8, all the tested materials were categorized into 9 groups. LM1, LM3, and GN2 were categorized into group I; CY1, CY2, QX, and ZM were categorized into group II; TM1, TM2, LM5, AH1, AH2, and ZD were categorized into group III; GA3, GN1, AL, and XJ were categorized into group IV; AG, AS, PE, and WL were categorized into group V; and CH, AF, SU, and JD were categorized into groups VI, VII, VIII, and IX, respectively. Among the 9 groups, the intervarietal genetic distance of the third group was the shortest, and the intervarietal genetic relationship is the closest, showing that the genetic relationship between alfalfa varieties that are distributed in the same area is relatively closer. The genetic distance between Tumu No.1 and No.2 in 25 varieties is the shortest (GD = 0.148), followed by Aohan No. 1 and No. 2 (GD = 0.182); the genetic distance between Jieda and Tumu No. 1 is the longest (GD = 0.786).

DISCUSSION

The RAPD technique is frequently used in germplasm research, variety identification, analysis of polymorphism and resistance, and breeding assistance because it is simple and rapid, has high detection efficiency of DNA polymorphism and broad coverage of genomes, and does not require the DNA sequences of research objects. However, because of the sensitivity to changes in reactant concentration, the stability of its results is poor. Therefore, few researchers have employed this technology to determine genetic information about pasture grass. Etch (1992) conducted RAPD analysis of backcrossed populations of diploid alfalfa and proposed that we can use RAPD markers to obtain some genetic information about alfalfa, which has limited genetic information. This is undoubtedly important for breeding programs and the evaluation of germplasm resources. Yu and Pauls (1993) used the RAPD technique to analyze the genetic relationship and heterozygosity of alfalfa varieties, confirming that RAPD markers can provide substantial genetic information to analyze the genetic relationship between backcrossed populations, obtain the maximum hybrid vigor, and choose proper parents. In this study, we used 30 primers to reveal the level of intravarietal and intervarietal genetic variations of 25 salt-tolerant alfalfa varieties as well as the genetic structure and distance of domestic and international salt-tolerant alfalfa varieties. Obviously, RAPD technology is very valuable to rapidly obtain the genetic information of alfalfa varieties.

Yu and Pauls (1993) used 10 RAPD-specific primers to differentiate 18-72 varieties (groups) using single plants from each variety (group). Yang et al. (2008) used intravarietal mixed DNA and 10 primers to analyze the genetic diversity of alfalfa germplasms in Gansu Province and did not find specific bands that were categorized into a single group. In this study, RAPD analysis of single-plant DNA samples and mixed DNA samples showed that polymorphism exists between and among different varieties. The analysis of mixed DNA samples concealed intravarietal polymorphism, while the intervarietal polymorphism was obviously reduced. For alfalfa, which is a cross-pollinated plant species, single-plant DNA samples can better reveal intravarietal and intervarietal genetic variations than the mixed DNA samples, and single-plant DNA samples can be used to analyze intervarietal differences quantitatively.

In recent years, studies on alfalfa salt tolerance mainly focused on screening salt-tolerant varieties and selection of salt tolerance. Studies that were conducted by Liu et al. (2008) showed that there were significant differences in salt tolerance among alfalfa varieties, and salt-tolerant varieties could be screened from varieties with extensive genetic specificity in different regions by selecting the genetic potential enhancing the salt tolerance. As shown in the clustering in Figure 2, among 25 alfalfa germplasms, most varieties (strains) from the same area were categorized into the same group. This result is consistent with that reported by Mao et al. (2007) and Wang et al. (2007). The reason could be that experimental materials from the same area are under directive breeding based on local breeding objectives, and the genetic basis is increasingly narrowing. Thus, the degree of genetic diversity is certainly low. However, in our group IV, the genetic distance between Gongnong No. 1 and Alfaking was the closest. Gongnong No. 1 was cultivated after 4 rounds of consecutive and selective breeding of the Alfking variety that was imported from the USA in 1922, and then it experienced 26 years of extensive selection and cultivation in Gongzhuling, Jilin. This observation indicated that the genetic diversity of salt-tolerant alfalfa germplasms is not only related to geographic positions but also closely associated with the origin of the parents. In terms of genetic distance, Eiffelnett and Jieda from Jilin, China, are closer to 7 American varieties than to other domestic varieties, suggesting that these 2 varieties would probably be new varieties that originated from the cultivation and selection of varieties that were imported from America or from hybridization with imported varieties.

Salt-tolerant alfalfa germplasms have a certain genetic diversity, and salt tolerance is a quantitative trait that is controlled by multiple genes with different expression patterns. Thus, we can improve the utilization rate and breeding efficiency of germplasm resources and promote salt-tolerant alfalfa breeding and production by analyzing the genetic diversity of salt-tolerant alfalfa genes, pertinently selecting alfalfa varieties with non-allelic salt-tolerant genes as the parent materials, collocating hybridized combinations, and obtaining varieties with pyramiding of salt-tolerant genes.

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