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Full Length Research Paper

Genetic diversity of taraxacum germplasm revealed by sequence-related amplified polymorphism (SRAP) analysis

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Sequence-related amplified polymorphism (SRAP) and morphological markers were employed to determine the genetic diversity and relations among 11 population of taraxacum in northeast of China. Data on 34 morphological traits were collected and analyzed. A total of 795 polymorphic SRAP's bands were scored with 20 combinations of primers. The genetic relationships analyzed with un-weighed pair-group method with arithmetic mean (UPMGA) showed that 11 population of taraxacum were grouped into three clusters. The results reveal by SRAP molecular markers were consistent with those based on the agronomic traits, suggesting that SRAP markers could be used in the taxonomic analysis of taraxacum germplasm.

Key words: Taraxacum, sequence-related amplified polymorphism (SRAP), genetic diversity, un-weighed pair-group method with arithmetic mean (UPMGA).

INTRODUCTION

Taraxacum is one of the important categories in Compositae and currently, there are more than 2000 species of taraxacum naturalized worldwide (Ge, 1998). China has 70 species and 1 variant. Among them, there are 19 species, 1 variant and 3 forms in northeast of China (Li, 2004). Taraxacum in China could be used as the traditional Chinese medicine, having sound anti-infec-tion effect, according to the Chinese Pharmacopoeia of 2010. Obviously, the species of taraxacum from different regions may have diverse components account-ing for the effectiveness and stability as medicine. Therefore, it is urgent and crucial that scientific systems of sorting, identifying and evaluating the germplasm resource of taraxacum plants should be established.

Molecular markers are powerful tool to assess the genetic diversity and population structure, because they are plentiful, independent of tissue or environmental effects (Englbrecht et al., 2000; Whitehead et al., 2003; Liu et al., 2006). As one of the DNA-based markers, sequence-related amplified polymorphism (SRAP) is designed to amplify open reading frames (ORFs) (Li and Quiros, 2001) based on specially designed primer pairs. The forward primers preferentially amplify exonic regions and the reverse primers preferentially amplify intronic regions and regions with promoters. Compared with other marker systems, SRAP has been demonstrated to be a useful tool for population genetic studies because of its simplicity, reproducibility and disclosure of multiple markers from a single two-primer reaction, when compared with other marker systems (Li and Quiros, 2001; Ferriol et al., 2003; Esposito et al., 2007).

Currently, SRAP markers have been successfully used to study genetic diversity and relationships in many

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Abbreviations: SRAP, Sequence-related amplified polymorphism.

Table 1. Taraxacum materials and origin of the entire collection.

Number	Population name	Latin	Origin
1	Dandong Dandelion	*Taraxacum antungense*	Shenyang Liaoning
2	Liaodong Dandelion	*Taraxacum liaotungense*	Chaoyang Liaoning
3	Jipian Dandelion	*Taraxacum asiaticum*	Chaoyang Liaoning
4	Xing'an Dandelion	*Taraxacum falcilobum*	Chaoyang Liaoning
5	Chaoxian Dandelion	*Taraxacum coreanum*	Shenyang Liaoning
6	Guangbao Dandelion	*Taraxacum lamprolepis*	Chaoyang Liaoning
7	Tujian Dandelion	*Taraxacum sinomongolicum*	Chifeng Neimeng
8	Yaoyong Dandelion	*Taraxacum officinale*	Panshi Jilin
9	Menggu Dandelion	*Taraxacum mongolicum*	Chaoyang Liaoning
10	Juanbao Dandelion	*Taraxacum urbanum*	Benxi Liaoning
11	Dongbei Dandelion	*Taraxacum ohwianum*	Dandong Liaoning

Table 2. Primers sequence used in this study.

Code	Forward primers sequence	Code	Reverse primers sequence
ME1	5'-TGAGTCCAAACCGGATA-3'	EM1	5'-GACTGCGTACGAATTAAT-3'
ME2	5'-TGAGTCCAAACCGGAGC-3'	EM2	5'-GACTGCGTACGAATTTGC-3'
ME3	5'-TGAGTCCAAACCGGA AT-3'	EM3	5'-GACTGCGTACGAATTGAC-3'
ME4	5'-TGAGTCCAAACCGGACC-3'	EM4	5'-GACTGCGTACGAATTTGA-3'
		EM5	5'-GACTGCGTACGAATTAAC-3'

crops, such as cotton (Lin et al., 2003), corn (Jiang et al., 2007) and some vegetable crops including hot pepper (Ren et al., 2004), *Brassica napus* (Riaz, 2001), pea (Espostio, 2007), potato (He et al., 2007), eggplant (Mutlu, 2008) and *Cucurbita pepo* (Ferriol et al., 2003). In addition, SRAP markers were also used on some Chinese herbal medicine including *Codonopsis tangshen* (Chen et al., 2009), *Astragalus* root (Qian et al., 2009) and *Houttuynia cordata* (Zhong et al., 2010). Yet, not much is known about whether SARP could be used on taraxacum. Thus, the objective of this study was to investigate genetic diversity and genetic distance for 11 populations of taraxacum from the northeast of China in order to classify the taraxacum populations on the molecular level and provide precision genetic information for the future breeding program.

MATERIALS AND METHODS

Plant materials

A total 11 populations of taraxacum were collected from the northeast of China. The locations for the populations are listed in Table 1.

Morphological traits and data analysis

Several traits were analyzed in order to characterize the plant morphological diversity. The roots of taraxacum collected were cultivated in June, 2009. Flower, plant, leaf and achene were divided into 34 qualitative characters, which with reference to Flora Northeast, Flora of China and Atlas of higher plants in China. Data were collected from September of 2009 for 36 phenotypic traits. Genetic distances were calculated according to Dice genetic similarity (GS). Phenograms were constructed based on un-weighted pair-group method analysis (UPGMA) (Sokal and Michener, 1958) using the NTSYS-pc 2.1 software (Rohlf, 2000).

DNA extraction and SRAP analysis

Young leaves of taraxacum were harvested in April, 2010 and the genomic DNA was extracted by CTAB method (Wu, 1999). Working stock solutions were made by diluting the samples to 25 mg l^{-1} and stored at 4°C for analysis.

SRAP is a PCR-based marker system with two primes, a forward primer of 17 bases and a reverse primer of 18 bases. The primers were composed by Invitrogen Co. Ltd (Table 2). Each 15 ul PCR reaction mixture consisted of 1.5 µl of 10× PCR buffer (25 mM of $MgCl_2$), 1.2 µl dNTPs, 1 µl forward and reverse primer (10 mM), 0.5 µl Taq DNA polymerase (2.5 U/µl) and 1 µl genomic DNA (50 ng/µl). The reaction procedure was as follows: an initial denaturing at 95°C for 5 min, followed by 5 cycles of three steps: 30 s denaturing at 95°C, 30 s annealing at 37°C and 1 min elongation at 72°C. In the following 35 cycles, the annealing temperature was increased to 50°C, with a final elongation step at 72°C for 8 min. Amplification products were separated by electrophoresis in 12% polyacrylamide gel and silver stained (Brant, 1991). Each reaction was repeated at least two times to test for reproductively of the selected markers.

Data scoring and statistical analysis

DNA fragments were scored as "1" and "0", where "1" stands for the presence and "0" stands for the absence of each SRAP

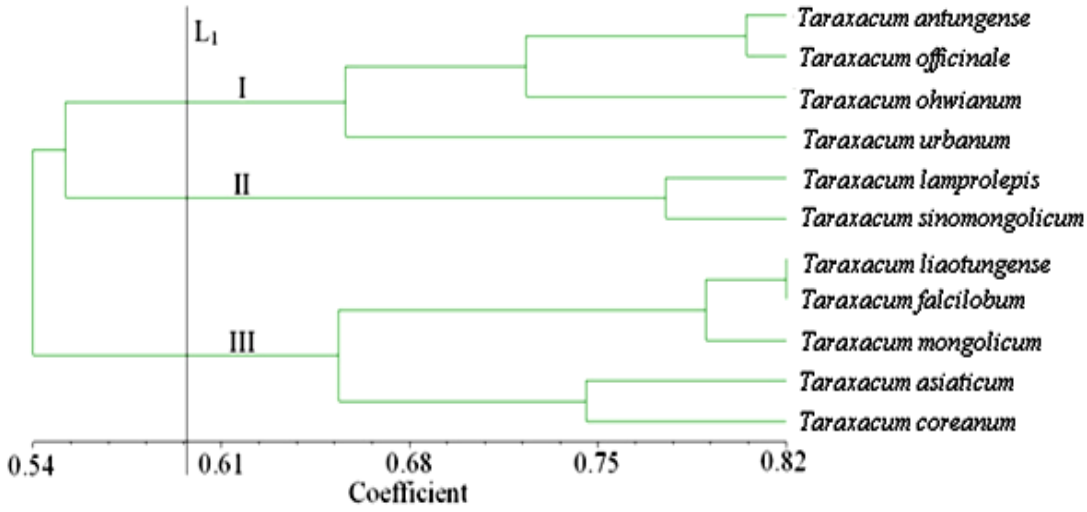

Figure 1. Dendrogram of 11 populations based on morphological data.

fragment. The fragment data were transformed into a binary (1/0) data matrix. Genetic similarity was analyzed using Nei and Li's index of similarity with the formula:

Similarity= N_{ab}/N_a+N_b

Where, N_{ab} represents the number of fragments shared by fragment a and b; N_a represents amplified fragments in sample a; N_b represents amplified fragments in sample b (Nei and Li, 1979). The inbreds were grouped using the un-weighted pair group method (UPGMA) (Sokal and Michener, 1958). Cluster analysis was performed on the similarity matrix in order to generate phenograms using the NTSYS-pc 2.1 software (Rohlf, 2000).

RESULTS

Morphological analysis

Relationships between the 34 morphological parameters revealed by cluster analyses are presented in Figure 1. Three main clusters can be observed. The first cluster includes the *Taraxacum antungense*, *Taraxacum officinale*, *Taraxacum ohwianum* and *Taraxacum urbanum*. The second cluster comprises *Taraxacum lamprolepis* and *Taraxacum sinomongolicum*. The third one includes *Taraxacum liaotungense*, *Taraxacum falcilobum*, *Taraxacum mongolicum, Taraxacum asiaticum* and *Taraxacum coreanum. T. liaotungense* had the most closely genetic relationship with *T. falcilobum* (GS = 0.82). In addition, *T. antungense* and *T. officinale* also showed close relationship (GS = 0.80). The results illustrate that the first cluster had closer relationship with the third cluster.

Polymorphism detected by SRAP

A total of 110 primer combinations were assayed on three representative populations of taraxacum. Primers banding patterns that were difficult to score and those that failed to amplify consistently were excluded. Consequently, 20 primer combinations, which consistently produced well defined bands, were selected to show polymorphisms (Table 3). A total of 831 bands were observed, among which 795 were polymorphic (95.7%), ranging between 22 and 55 per primer combination, with an average of 39.75 polymorphic bands per pair of primers. The combination of primers showing most polymorphism was the combination of EM3-ME4, EM4-ME3 and EM4-ME4 (100%), whereas the primer combination of EM5-ME3 had the lowest polymorphism (81%).

The bands that were unique could be used as the special marker to distinguish the genotypes of different taraxacum. For instance, in combinations of primer P6, material 7 and material 9 had two special amplified bands, respectively. In addition material 3 and material 11 showed one special amplified band, respectively (Figure 2, the special band was showed as red arrow); in the primer combination of P8, material 2 had one special band and material 6 showed two special bands (Figure 2). In the combinations of primer P10, material 9 and material 10 had two special bands, respectively (Figure 2). The special bands observed might be used to identify the genotypes of taraxacums.

Genetic relationships

A dendrogram based on the similarity coefficient of the 11 populations was constructed (Figure 3). Three major clusters can be identified at different similarity level. The first cluster (I) includes 5 populations of taraxacum: *T. mongolicum*, *T. liaotungense*, *T. asiaticum*, *T. falcilobum* and *T. coreanum*. *T. mongolicum* was closely related with

Table 3. Polymorphism based on 20 primer combinations.

Primer code	Primer combination	Number of amplified band	Number of polymorphism band	Percentage of polymorphic band (%)	Polymorphism information content
P1	EM1-ME1	42	41	0.97619	0.96412
P2	EM1-ME2	51	50	0.980392	0.971445
P3	EM1-ME3	36	33	0.916667	0.958293
P4	EM1-ME4	44	43	0.977273	0.969298
P5	EM2-ME1	35	32	0.914286	0.955748
P6	EM2-ME2	50	48	0.960000	0.969944
P7	EM2-ME3	45	42	0.933333	0.966598
P8	EM2-ME4	55	50	0.909091	0.975985
P9	EM3-ME1	22	21	0.954545	0.929031
P10	EM3-ME2	49	47	0.959184	0.969325
P11	EM3-ME3	46	45	0.978261	0.969941
P12	EM3-ME4	48	48	1.00000	0.968352
P13	EM4-ME1	47	46	0.978723	0.965883
P14	EM4-ME2	40	39	0.97500	0.965873
P15	EM4-ME3	38	38	1.00000	0.959897
P16	EM4-ME4	44	44	1.00000	0.964419
P17	EM5-ME1	35	34	0.971429	0.960081
P18	EM5-ME2	35	32	0.914286	0.96131
P19	EM5-ME3	32	26	0.812500	0.957403
P20	EM5-ME4	37	36	0.972973	0.964736
	Total	831	795		
	Mean	41.55	39.75	0.956679	0.963384

T. liaotungense at the similarity level of 0.74. The second cluster (II) consists of *T. lamprolepis* and *T. sinomongolicum* at the similarity level of 0.73, which were distinctly separated from other clusters. The third cluster (III) includes 4 taraxacum populations: *T. antungense* (Shenyang, Liaoning), *T. officinale* (Panshi, Jilin) *T. urbanum* (Benxi, Liaoning) and *T. ohwianum* (Dandong, Liaoning). The similarity coefficient between *T. officinale* and *T. urbanum* was 0.72.

DISCUSSION

SRAP marker may have various applications for genetic studies and practical breeding programs in taraxacum. The dominant SRAP markers could provide more accurate information on population genetic diversity than traditional methods. The results provide evidence that SRAP is an efficient approach suitable for taxonomic analysis of different populations of taraxacum.

Previous studies have demonstrated that in the genetic diversity analysis, the information derived from SRAP marker was more concordant to the morphological variability and to the evolutionary history of the morphotypes than that of other molecular markers (Ferriol et al., 2003). The results of SRAP in our study show good amplification, stability and productivity with easily found polymorphism in the populations of taraxacum plants. Similar clusters of taraxacum were identified with both the morphological and SRAP markers, indicating that it is possible to evaluate the genetic diversity with SRAP molecular marker.

Both of the two methods divided the 11 populations of taraxacum into three clusters, nonetheless, the results of cluster analyses from the morphological and SRAP markers showed some differences, which probably due to the two detecting system based on different criteria. It is likely that some of the polymorphic site detected by SRAP marker and shown as the morphological traits could hardly be observed by naked eyes or might be neglected. It remains unclear whether the polymorphic sites detected by SRAP makers reflected the morphologic traits in our study. We suggested that some specific combinations of primer be explored to unify the classification from the two markers.

In this study, the DNA-based SRAP marker was successfully used; which distinguished the genetic diversity of taraxacum from the molecular level. The SRAP marker was suitable for the molecular investigation of phylogenic relationships between different populations of taraxacum. This study revealed the utility of SRAP markers in detecting genetics diversity and relations among populations of taraxacum, which could be useful to identify, select and breed varieties of taraxacum.

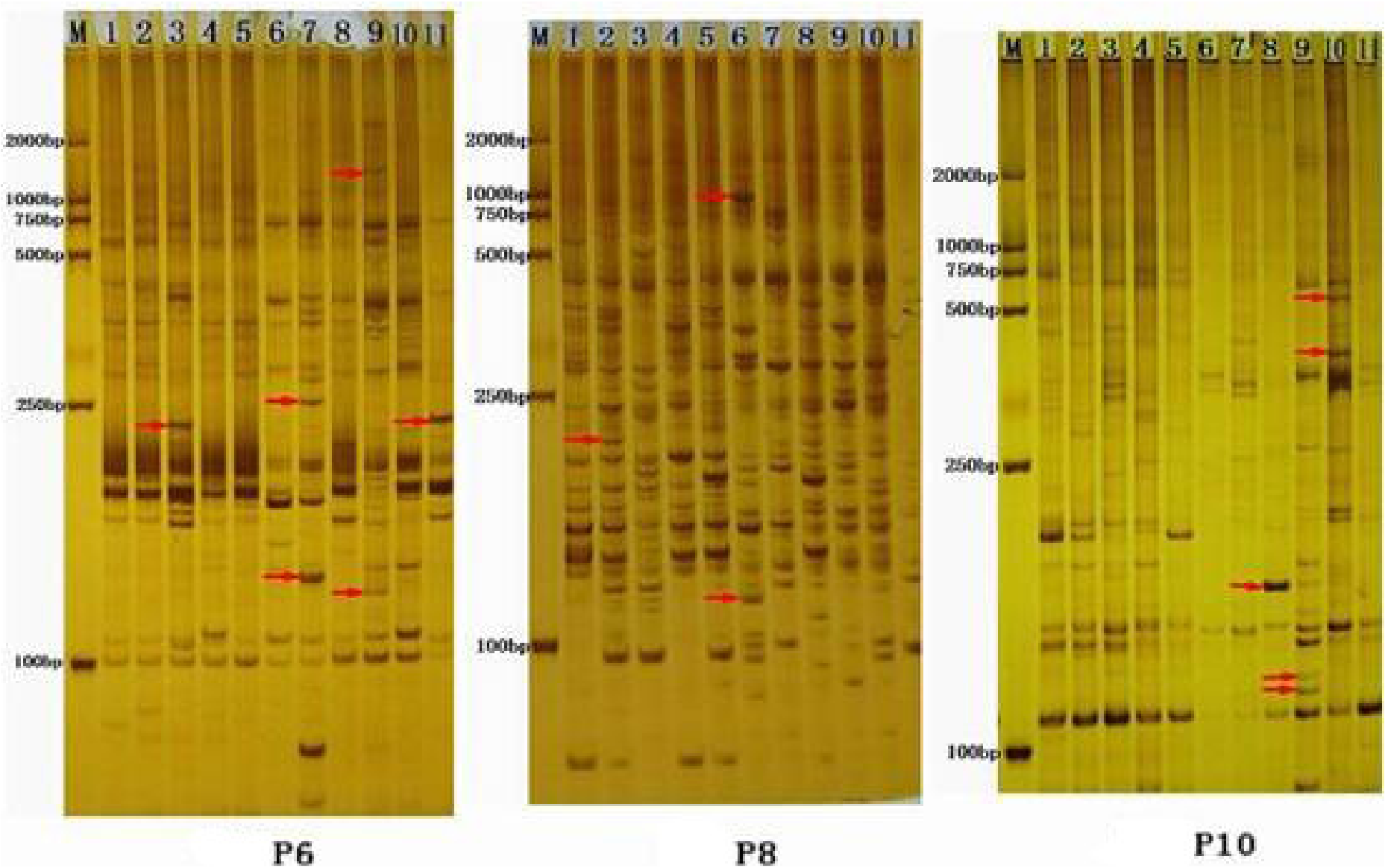

Figure 2. Parts of the SRAP amplification profile of prime combination. The arrows are polymorphic bands, M is molecular markers; the number were the same as those in Table 1.

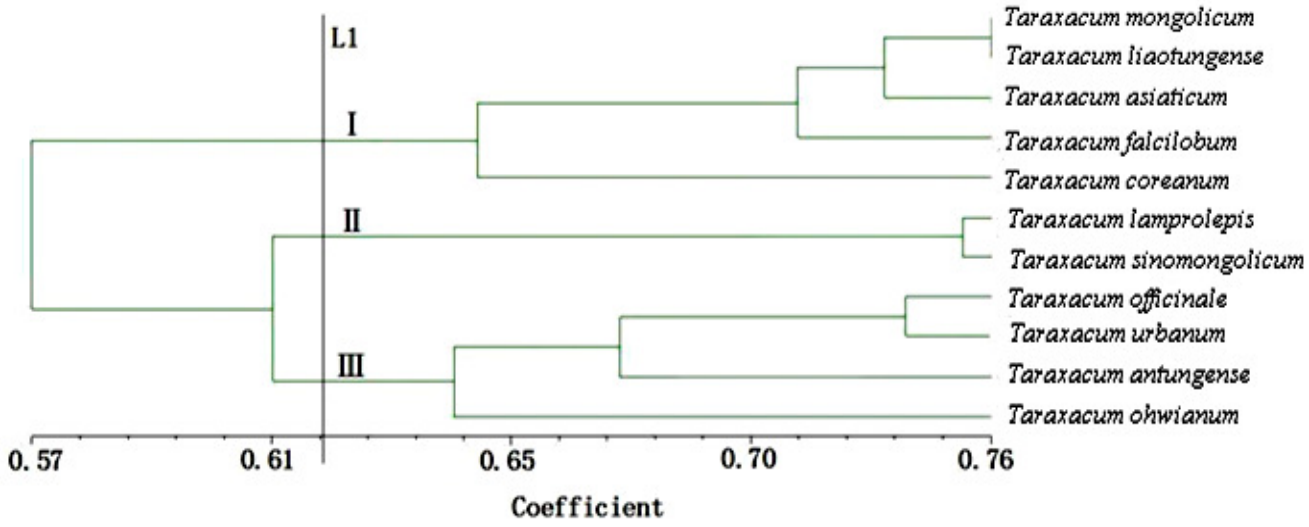

Figure 3. An un-weighted pair-group method with arithmetic averages (UPGMA) dendrogram of phylogenetic relationships among 11 populations based on similarity coefficients from the 20 SARP primer combinations. The resulting three clusters are labeled I, II and III.

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