# Molecular Characterization of Genetic Diversity of Underutilized Crops: Buckwheat as an Example

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**Abstract**

**China has many kinds of underutilized crops which are widely distributed and used for various purposes such as healthy food, industrial materials, cash crops and medicines. Buckwheat is one such crop and considered as a healthy food for the people. China has collected and conserved more than 3000 accessions of buckwheat, composed of landraces, cultivars and wild species. Characterization for agro-economic traits of buckwheat accessions was carried out in China and phenotypic diversity was documented. Recognizing the importance of molecular tools in characterizing crop genetic diversity and identifying useful traits from germplasm collections, Bioversity International in cooperation with the national partners in China aim to characterize genetic diversity of buckwheat with molecular tools such as AFLP, ISSR and SSR, in order to understand the nature of genetic diversity of buckwheat and relationships between the groups with different origins. A total of 79 accessions of Tartary buckwheat (*F. tartaricum*) were assessed with 15 pairs of AFLP primers and 19 ISSR primers. The results showed that AFLP and ISSR markers have produced high polymorphic information content (PIC) values on buckwheat accessions and could be used for investigating the genetic diversity of buckwheat. Both AFLP and ISSR markers showed that the highest genetic diversity was presented in accessions from Yunnan which is considered the centre of origin of buckwheat. Molecular markers could play an important role in assessing genetic diversity, constructing linkage maps and locating useful genes in underutilized crops.**

## INTRODUCTION

Buckwheat (*Fagopyrum* spp.) is an underutilized crop and considered a healthy food for the people in China. Tartary buckwheat (*F. tartaricum*) is one of the two cultivated species of the genus *Fagopyrum*. It is cultivated under low input conditions and is adapted to marginal lands with harsh environments. China is the centre of origin of Tartary buckwheat and has rich genetic diversity with a wide distribution in the southwest and west of China (Jiang and Jia, 1990). Tartary buckwheat seeds and leaves are mainly used for human consumption and animal fodder. Tartary buckwheat flour has a higher protein content and lower fat content than other grain crops such as maize. Tartary buckwheat is rich in flavonoid, Fagopyritol, D-chiro inositol, peptides and other high activity functional components (Lin et al., 2002). It is reportedly very effective in controlling and treating tumours, heart disease and is an anti-oxidant, purging free radicals, improving pancreatic function, regulating blood-glucose, blood-fat and blood-pressure. Therefore, Tartary buckwheat is used as a nutritional food, or healthy food in China. It has been considered as the best healthy food in the 21st century (Xu et al., 2000). However, buckwheat research has been neglected in terms of genetic resources and improvement of its production and quality.

In recent years, genetic diversity of buckwheat has been studied using molecular tools, such as isozymes (Ohnishi et al., 1985), Random Amplified Polymorphic DNA (RAPD) markers (Wang et al., 2004) and amplified fragment length polymorphism

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(AFLP) (Iwata et al., 2005). Inter-simple sequence repeat markers (ISSR) are a novel PCR technique that uses repeat-anchored or non-anchored primers to amplify DNA sequences between two inverted SSR (Zietkiewicz et al., 1994). ISSR markers are highly reproducible due to their primer length and to the high stringency achieved by the annealing temperature, and provide highly polymorphic fingerprints. ISSR markers have been successfully used for the investigation of genetic resources (Godwin et al., 1997; Moreno et al., 1998), genetic mapping (Cekic et al., 2001; Sankar and Moore, 2001), gene localization (Ammiraju et al., 2001), genetic diversity analysis (Esselman et al., 1999), evolution studies (Fang et al., 1998) and phylogenetic development (Huang and Sun, 2000). The repeatability of ISSR-PCR is better than RAPD-PCR because ISSR primers are longer and hence have higher annealing temperatures (Blair et al., 1999; Gilbert et al., 1999). The objectives of this study were to develop and identify suitable ISSR and AFLP markers that could be used for genetic studies of Tartary buckwheat, assess the genetic diversity of Tartary buckwheat accessions in China, and explore the relationships among accessions from different origins.

## MATERIALS AND METHODS

### Plant Materials

Seventy-nine accessions of Tartary buckwheat were used in this study (Table 1).

### Methods

**1. DNA Extraction.** The total DNA was extracted from the leaf tissue of 20 young plants of each accession using the modified CTAB method (Saghai-Maroof et al., 1984). Each sample was ground to a fine powder in liquid nitrogen and mixed with 22.5 ml of the CTAB extraction buffer. The sample was then shaken gently at 65°C for 20 min. After adding 22.5 ml of 24:1 chloroform/isoamyl alcohol, the sample was centrifuged at 4000 rpm for 20 min. The aqueous phase was transferred into a 2/3 volume of isopropanol to precipitate the DNA. The DNA precipitate was recovered with 70% ethanol, then dried for more than 1 h and finally dissolved in 1.5 ml of the 1×TE buffer and stored at -20°C.

**2. AFLP Analysis.** Genomic DNA digested fragments were ligated to EcoRI and *Mse*I adapters by using an AFLP Core Reagent Kit (Invitrogen). Pre-amplification was conducted by using the appropriate primer combinations with no additional selective nucleotides: E00/M00. Selective amplification was conducted by using five primer combinations with three and two additional selective nucleotides (Table 2).

*Pre-Amplification.* PCR was carried out in 20 µl reaction volumes containing of sterile distilled water, 0.6 units of *Taq* polymerase, 2.0 µl of 10× PCR buffer (supplied with the enzyme), 0.4 µl of dNTP, 0.6 µl (50 ng/µl) of primer and 2.0 µl DNA. The reaction was over-laid with sterile mineral oil prior to PCR. The PCR amplification was performed on a PTC-100 (MJ Research Inc.) thermocycler under the following conditions: 94°C for 2 min and 94°C for 35 s, 56°C for 35 s, at 72°C for 1 min and 30 cycles of 35 s at 94°C, after 30 cycles, there was a final extension of 5 min at 72°C followed by soaking at 4°C.

*Selective Amplifications.* PCR amplifications were carried out in 20 µl reaction volumes containing sterile distilled water, 0.7 units of *Taq* polymerase, 2.0 µl of 10× PCR buffer (supplied with the enzyme), 0.45 µl of dNTP, 1 µl (50 ng/µl ) of primer and 2.0 µl pre-amplification DNA. The reaction was over-laid with sterile mineral oil prior to PCR. The PCR amplification was performed on a PTC-100 (MJ Research Inc.) thermocycler under the following conditions: 94°C for 2 min, 94°C for 35 s, and 65°C for 35 s with each cycle minus 0.7°C, at 72°C for 1 min and 12 cycles of 35 s at 94°C, after 12 cycles, 94°C for 2 min, 56°C for 35 s, at 72°C for 1 min 24 cycles of 35 s at 94°C after that there was a final extension of 5 min at 72°C followed by soaking at 4°C.

*Electrophoresis of PCR Products.* Amplification products were electrophoresed in 5% polyacrylamide gel. Electrophoresed PCR products were visualized by silver staining (Bassam et al., 1991).

**3. ISSR Analysis.** Primers. A total of 108 ISSR primers (UBC primer set # 9, John Hobbs, NAPS Unit, University of British Columbia, Vancouver, V6T 1Z3 Canada) were tested for DNA amplification; 19 of them produced sharp and clear banding profile with polymorphism and these were used for genotyping of 79 Tartary buckwheat accessions (Table 3).

*PCR Procedure.* PCR was carried out in 25 µl reaction volumes containing sterile distilled water, 1 unit of *Taq* polymerase, 2.5 µl of 10× PCR buffer (supplied with the enzyme), 0.5 µl of dNTP (made from an equimolar solution of 10 mM, each of dATP, dCTP, dGTP and dTTP), 1.0 µl (5 µmol/L) of primer and 60 ng of template DNA. The reaction was over-laid with sterile mineral oil prior to PCR. The PCR amplification was performed on a PTC-100 (MJ Research Inc.) thermocycler programmed for 45 cycles with an initial strand separation at 94°C for 5 min and 94°C for 30 s, followed by an annealing temperature of 52°C for 1 min and extension at 72°C for 2 min. After 45 cycles, there was a final extension of 10 min at 72°C followed by soaking at 4°C.

*Electrophoresis of PCR Products.* Amplification products were electrophoresed in 5% metamorphic polyacrylamide gel. 4.5 µl loading buffer (0.25% (w/v) bromophenol blue, 0.25% (w/v) xylene cyanol FF, 30% (w/v) glycerol) was added to each PCR product and 5 µl mix was loaded in each of slot of the gel. Electrode strips were soaked with 25 ml of electrode buffer (0.2 M Tris pH 8.0, 0.2 M Tricine and 0.55% SDS) and placed in contact with the gel at the top and bottom or cathodal and anodal ends of the gel. Electrophoresed PCR products were visualized by silver staining (Bassam et al., 1991).

**4. Data Analysis.** The individual ISSR and AFLP bands were considered as loci and scored as 1 or 0 (presence or absence) for each sample. The genetic similarity matrix was calculated using NTSYS pc version 2.1 software (Rohlf et al., 1992). An unweighted pair group of arithmetic means (UPGMA) dendrogram was constructed based on Nei's coefficient.

## RESULTS

### Genetic Diversity of Tartary Buckwheat Revealed by AFLP Markers

Fifteen pairs of AFLP primers were used for the analysis of the 79 accessions. A total of 621 reproducible bands were amplified, with an average of 41.4 bands per primer. Of these, 537 were polymorphic, with an average of 35.8 bands per primer. The average PIC (Polymorphic information content) of the 15 primers was 0.946 and primer pair E18/M58 showed the highest PIC (0.973). The proportion of polymorphic bands reached 86.5% (Table 4), which suggested that AFLP primers amplified the fragments in bulked DNA samples from 79 Tartary buckwheat accessions have high variation. On the basis of bulked analysis of 79 accessions of Tartary buckwheat, the coefficient among all accessions ranged from 0.32 to 0.97. The genetic differentiation among all accessions was significant. The data of polymorphic AFLP fragments were used to construct an UPGMA dendrogram (Fig. 1). Accessions on the tree were clearly divided into four major groups.

In the first group, 22 accessions were mainly from Qinghai, Guizhou, Hunan, Yunnan and Hubei province. This group could be further divided into two subgroups. The first subgroup included 12 accessions from Qinghai, Guizhou, Ningxia, and Hunan and one cultivar from Yunnan province. The second subgroup included 10 accessions mainly from Yunnan and Hubei. One cultivar accession from Gansu and one landrace from Yunnan were placed the second subgroup. In the second group, 14 accessions were mainly from Guizhou, Hubei and Yunnan province. Two cultivar accessions from Yunnan were placed this group. In the third group, 23 accessions were mainly from Qinghai, Yunnan, Guizhou. In the fourth group, 11 accessions were mainly cultivars. Interestingly, accessions TB111, TB141, TB137 and TB142 did not cluster with any of above groups and formed their own outgroups.

Results show that generally the clustered groups were associated with the origins of accessions. The landraces of Tartary buckwheat obviously showed 'district marker' characteristics. The cultivars were mainly clustered into one subgroup and about 60% of

cultivars had a coefficient of 0.84.

There are differences in the variation of accessions from different origins (Table 5). The accessions from Yunnan had the highest genetic variation with 54.9% of polymorphic bands and 0.9526 for Simpson's index. The accessions from Shanxi had the lowest genetic variation compared to the other accessions. The order of variation was Yunnan>Guizhou>Qinghai>Hubei>Hunan>Shaanxi>Ningxia>Gansu>Shanxi. It was noted that some provinces such as Shaanxi, Shanxi, Gansu and Ningxia only have 2 accessions each, which might affect the results of diversity analysis for accessions from these provinces.

**Genetic Diversity of Tartary Buckwheat Revealed by ISSR**

Nineteen ISSR primers were used for the analysis of the 79 accessions. Most of primers produced a high level of visible bands. A total of 546 reproducible bands were amplified, with an average of 28.7 bands per primer in bulked DNA samples. Among these bands, 506 were polymorphic, with an average of 26.6 per primer. The proportion of polymorphic bands achieved 100% in most of the primers, with an average of 92.6% in all primers (Table 6). Two primers, M04 and U852, amplified the highest and lowest levels of polymorphic bands, i.e., 58 and 11. It was suggested that ISSR primers amplified the fragments in bulked DNA samples from 79 Tartary buckwheat accessions were high polymorphic.

The analysis showed that the coefficient among all accessions ranged from 0.39 to 0.93. The data of polymorphic ISSR fragments were used to construct an UPGMA dendrogram (Fig. 2). Accessions on the tree were clearly divided into two major groups.

In the first group, 37 accessions were mainly from Qinghai, Guizhou and Hubei provinces. This group could be further divided into five subgroups. The accessions formed sub-clusters that corresponded well with their geographic origin. In the second group, there were 38 accessions from Hunan, Yunnan, and Guizhou. This group could be further divided into three subgroups. In the first subgroup, there were two cultivars ('TB129' and 'TB131') from Hunan. In the second subgroup 17 accessions were all cultivars from Hunan, Shanxi, Guizhou and Yunnan. In the third subgroup, 19 accessions were mainly from Yunnan province. Accessions TB137, TB095, TB127, and TB142 were quite distinct from all others.

The results indicated that landraces of Tartary buckwheat presented a higher genetic diversity than cultivars. Most of cultivars showed a close relationship with the landraces from Yunnan. In general, the cultivars tended to be homogenous as over 60% of accessions had a similarity coefficient of 0.77.

The analysis also showed the accessions from Yunnan had the highest genetic variation as the percentage of polymorphic bands (67.0%) and Simpson's index (0.9531) of Yunnan accessions was the highest, while the accessions from Shaanxi had the lowest genetic variation (Table 7). The order of variation of accessions was Yunnan>Guizhou> Hubei>Qinghai>Hunan>Ningxia>Shanxi>Gansu>Shaanxi. The result was very similar to that of the AFLP markers.

**DISCUSSION**

Tartary buckwheat originated in China and is distributed mainly in the west and southwest of China. It has been used for food and medicine for a long time, particularly in the ethnic groups in southwest of China. The diversity of buckwheat was less understood than other crops such as rice, maize or wheat. Tsuji and Ohnishi (1998) had constructed a NJ tree on buckwheat materials based on RAPD markers by employing 40 primers.

**Genetic Diversity Revealed by AFLP**

The average PIC for these fifteen pairs of primers was 0.946. Compared with the other markers, AFLP markers revealed a higher level of polymorphism and are an effective tool in analyzing the genetic diversity of buckwheat germplasm.

The percentage (86.5%) of polymorphic fragments amplified is similar to that

reported in rice, maize and grape (Moreno et al., 1998; Blair et al., 1999; Kantety et al., 1995). However, Wang et al. (2004) used RAPD to analyse wild buckwheat germplasm in Yunnan, with an average of 8.5 bands per primer in bulked DNA samples, and the percentage of polymorphic fragments reached 94.8%. The variation revealed by AFLP on Tartary buckwheat accessions is higher than on common buckwheat obtained by Iwata (2005).

Tsuji and Ohnishi (2001) used AFLP markers and revealed the relationship between wild buckwheat and Tartary buckwheat, and compared two dendrograms based on AFLP and RAPD. They indicated that Tartary buckwheat perhaps originated from the east of Tibet or the northwest of Yunnan in China. The current study also indicated that Yunnan is a centre of diversity of Tartary buckwheat.

It was found that the accessions from the same geographic origin were not grouped together in the cluster analysis. This phenomenon might suggest that considerable gene flow existed among different regions through germplasm exchange.

**The Analysis of Genetic Diversity by ISSR**

ISSR markers for Tartary buckwheat had not previously been available. The 19 markers that we developed showed high levels of polymorphism, indicating that they can be powerful tools for the genetic study of Tartary buckwheat. In our study, the percentage (92.6%) of polymorphic fragments amplified in bulked DNA analysis of 79 Tartary buckwheat populations was actually similar to that (78, 95 and 94%) of rice, maize and grape (Moreno et al., 1998; Blair et al., 1999; Kantety et al., 1995).

The genetic diversity of Tartary buckwheat has evolved over time through natural and artificial selection. This study showed that landraces of Tartary buckwheat from different origins exhibit considerable genetic variation. The landraces of Tartary buckwheat from Yunnan were different from those from Guizhou and Hubei. This differentiation shows the diversity of ecotypes from geographically isolated populations. However, few of them might have been derived from crosses between landraces. For example, Baikuqiao from Hubei was highly related to the landraces of Tartary buckwheat from Guizhou and might be derived from a cross with landrace from there. The close relations between accessions from Guizhou and those from Hebei may also indicate that the active germplasm exchange for Tartary buckwheat had happened between the growers of south and north of China.

## CONCLUSION

Both AFLP and ISSR produced highly polymorphic bands and are suitable for genetic diversity analysis on Tartary buckwheat germplasm. With AFLP markers, all the accessions were distinct and revealed enough genetic diversity for identification of individual accessions, but little geographical information was revealed. With ISSR marker, all the accessions were distinct and revealed enough genetic diversity for identification and showed a strong relationship with the origins of accessions. For example, Tartary buckwheat accessions from Yunnan, Hubei and Qinghai clustered separately in different groups. Yunnan Tartary buckwheat accessions are the most polymorphic and important for varietal improvement in the future.

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## Tables

Table 1. List of Tartary buckwheat accessions used for AFLP and ISSR analysis.

| No. | Accession name | Types | Origin |
|---|---|---|---|
| TB91 | MAZIKU | Landrace | Minhe, Qinghai |
| TB92 | BAIKUQIAO | Landrace | Minhe, Qinghai |
| TB93 | KUQIAO | Landrace | Xunhua, Qinghai |
| TB94 | MAKUQIAO | Landrace | Minhe, Qinghai |
| TB95 | MAKUQIAO | Landrace | Minhe, Qinghai |
| TB96 | KUQIAO | Landrace | Xinghai, Qinghai |
| TB97 | QIAOMAI | Landrace | Minhe, Qinghai |
| TB98 | KUQIAO | Landrace | Minhe, Qinghai |
| TB99 | KUQIAO | Landrace | Minhe, Qinghai |
| TB100 | KUQIAO | Landrace | Yuedu, Qinghai |
| TB101 | ERBAIKUQIAO | Landrace | Weining, Guizhou |
| TB102 | DABAIKUQIAO | Landrace | Weining, Guizhou |
| TB103 | BAIKUQIAO | Landrace | Shuicheng, Guizhou |
| TB104 | DAKUQIAO | Landrace | Weining, Guizhou |
| TB105 | ERBAIKUQIAO | Landrace | Weining, Guizhou |
| TB106 | KUQIAO | Landrace | Haozhang, Guizhou |
| TB107 | DAJIANZUKUQIAO | Landrace | Weining, Guizhou |
| TB108 | CHANGJIANZUKUQIAO | Landrace | Haozhang, Guizhou |
| TB109 | SILENGQIAO | Landrace | Weining, Guizhou |
| TB110 | HEIKUQIAO | Landrace | Haozhang, Guizhou |
| TB111 | KUQIAO | Landrace | Panshui, Shennongjia, Hubei |
| TB112 | KUQIAO | Landrace | Jiuhu, Shennongjia, Hubei |
| TB113 | KUQIAO | Landrace | Yangri, Shennongjia, Hubei |
| TB114 | KUQIAO | Landrace | Muyu, Shennongjia, Hubei |
| TB115 | KUQIAO | Landrace | Qingtian, Shennongjia,Hubei |
| TB116 | KUQIAO | Landrace | Hongping, SHennongjia, Hubei |
| TB117 | KUQIAO | Landrace | Tianjiashan, Shennongjia, Hubei |
| TB118 | GAOSHANKUQIAO | Landrace | Tianjiashan, Shennongjia, Hubei |
| TB119 | GAOSHANKUQIAO | Landrace | Tianjiashan, Shennongjia, Hubei |
| TB120 | BAIKUQIAO | Landrace | Zhaoyang, Shennongjia, Hubei |
| TB121 | GUYUANKUQIAO | Cultivar | Guyuan, Ningxia |

Table 1. Continued.

| No. | Accession name | Types | Origin |
|---|---|---|---|
| TB122 | HAIYUANKUQIAO | Cultivar | Haiyuan, Ningxia |
| TB123 | WEININGKUQIAO | Cultivar | Weining, Guizhou |
| TB124 | ZHENBAKUQIAO II | Cultivar | Zhenba, Shaanxi |
| TB125 | JIUJINAGKUQIAO | Cultivar | Jiujiang, Jiangxi |
| TB126 | LIUQIAONO.1 | Cultivar | Liupaishui,guizhou |
| TB127 | LIUQIAONO.2 | Cultivar | Liupaishui,guizhou |
| TB128 | LIUQIAONO.3 | Cultivar | Liupaishui,guizhou |
| TB129 | HUNAN3-1 | Cultivar | Fenghuang, Hunan |
| TB130 | HUNAN1-2 | Cultivar | Fenghuang, Hunan |
| TB131 | HUNAN2-2 | Cultivar | Fenghuang, Hunan |
| TB132 | HUNAN3-2 | Cultivar | Fenghuang, Hunan |
| TB133 | HUNAN4-2 | Cultivar | Fenghuang, Hunan |
| TB134 | HUNAN5-2 | Cultivar | Fenghuang, Hunan |
| TB135 | HUNAN6-2 | Cultivar | Fenghuang, Hunan |
| TB136 | HUNAN7-2 | Cultivar | Fenghuang, Hunan |
| TB137 | FENGHUANGKUQIAO | Cultivar | Fenghuang, Hunan |
| TB138 | YT-37 | Cultivar | Kunming, Yunnan |
| TB139 | YT-5 | Cultivar | Kunming, Yunnan |
| TB140 | YUNNANHUIKUQIAO | Cultivar | Kunming, Yunnan |
| TB141 | HEIFENEG NO.1 | Cultivar | Shouyang, Shanxi |
| TB142 | KUNMINGHUIKUQIAO | Cultivar | Kunming, Yunnan |
| TB143 | DIANNING NO.1 | Cultivar | Kunming, Yunnan |
| TB144 | JINQIAOMAI NO.2 | Cultivar | Shouyang, Shanxi |
| TB145 | WEININGNO.3 | Cultivar | Weining, Guizhou |
| TB146 | ZHAOKU NO.1 | Cultivar | Zhaojue, Sichuan |
| TB147 | XINONG9909 | Cultivar | Yulin, Shaanxi |
| TB148 | DING98-1 | Cultivar | Dingxi,Gansu |
| TB149 | WEI93-8 | Cultivar | Weining,Guizhou |
| TB150 | LIJIANGKUQIAO | Landrace | Lijiang, Yunnan |
| TB151 | WENSHANKUQIAO | Landrace | Wenshan, Yunnan |
| TB152 | WENSHANKUQIAO | Landrace | Wenshan, Yunnan |
| TB153 | DALIKUQIAO | Landrace | Dali, Yunnan |
| TB154 | DALIKUQIAO | Landrace | Dali, Yunnan |
| TB155 | QIUBEIKUQIAO | Landrace | Qiubei, Yunnan |
| TB156 | QIUBEIKUQIAO | Landrace | Qiubei, Yunnan |
| TB157 | SHIZONGKUQIAO | Landrace | Shizong, Yunnan |
| TB158 | XUANWEIKUQIAO | Landrace | Xuanwei, Yunnan |
| TB159 | XUANWEIKUQIAO | Landrace | Xuanwei, Yunnan |
| TB160 | QIANWEINO.3 | cultivated | Weining,Guizhou |
| TB161 | ZHAOTONGKUQIAO | Landrace | Zhaotong, Yunnan |
| TB162 | DINGXIKUQIAO | cultivated | Dingxi,Gansu |
| TB163 | NINGLANGKUQIAO | Landrace | Ninglang, Yunnan |
| TB164 | HUIZEKUQIAO | Landrace | Huize, Yunnan |
| TB165 | HUIZEKUQIAO | Landrace | Huize, Yunnan |
| TB166 | HUIZEKUQIAO | Landrace | Huize, Yunnan |
| TB168 | MALONGKUQIAO | Landrace | Malong, Yunnan |
| TB169 | MALONGKUQIAO | Landrace | Malong, Yunnan |

Table 2. The sequences of adapters and primers of AFLP.

| Adapter and primers | Nucleotide sequences | Application |
|---|---|---|
| *Eco*R I Adaptor | 5'-CTCGTAGACTGCGTACC-3'<br>3'-CTGACGCATGGTTAA-5' | Adapter ligation |
| *Mse* I Adaptor | 5'-GACGATGAGTCCTGAG-3'<br>3'-TACTCAGGACTCAT-5' | Adapter ligation |
| *Eco*R I Primer | | |
| E00 | 5'-GACTGCGTACCAATTC | Pre-selective amplification |
| E15 | 5'-GACTGCGTACCAATTC CA-3' | Selective amplification |
| E18 | 5'-GACTGCGTACCAATTC CT-3' | |
| E19 | 5'-GACTGCGTACCAATTC GA-3' | |
| E20 | 5'-GACTGCGTACCAATTC GC-3' | |
| E21 | 5'-GACTGCGTACCAATTC GG-3' | |
| E25 | 5'-GACTGCGTACCAATTC TG-3' | |
| E45 | 5'-GACTGCGTACCAATTC ATG-3' | |
| E48 | 5'-GACTGCGTACCAATTC CAC-3' | |
| E61 | 5'-GACTGCGTACCAATTC CTG-3' | |
| E64 | 5'-GACTGCGTACCAATTC GAC-3' | |
| E70 | 5'-GACTGCGTACCAATTC GCT-3' | |
| *Mse* I Primer | | |
| M00 | 5'-GATGAGTCCTGAGTAA-3' | Pre-selective amplification |
| M12 | 5'-GATGAGTCCTGAGTAA AC-3' | Selective amplification |
| M16 | 5'-GATGAGTCCTGAGTAA CC-3' | |
| M17 | 5'-GATGAGTCCTGAGTAA CG-3' | |
| M18 | 5'-GATGAGTCCTGAGTAA CT-3' | |
| M22 | 5'-GATGAGTCCTGAGTAA GT-3' | |
| M26 | 5'-GATGAGTCCTGAGTAA TT-3' | |
| M37 | 5'-GATGAGTCCTGAGTAA ACG-3' | |
| M58 | 5'-GATGAGTCCTGAGTAA CGT-3' | |
| M66 | 5'-GATGAGTCCTGAGTAA GAT-3' | |
| M67 | 5'-GATGAGTCCTGAGTAA GCA-3' | |

Table 3. ISSR primers used in this study.

| Primers | Nucleotide sequences | Primers | Nucleotide sequences |
|---|---|---|---|
| U808 | AGA GAG AGA GAG AGA GC | U888 | BDB CAC ACA CAC ACA CA |
| U815 | CTC TCT CTC TCT CTC TG | M01 | CACACACACACAR |
| U818 | VDV CTC TCT CTC TCT CT | M02 | CACACACACACARY |
| U834 | AGA GAG AGA GAG AGA GYT | M03 | CACACACACACARG |
| U840 | GAG AGA GAG AGA GAG AYT | M04 | GTGTGTGTGTGTYR |
| U842 | GAG AGA GAG AGA GAG AYG | M05 | GCT GCT GCT GCT Y |
| U845 | CTC TCT CTC TCT CTC TRG | M06 | AGC AGC AGC AGC Y |
| U852 | TCT CTC TCT CTC TCT CRA | M07 | AGC AGC AGC AGC GY |
| U857 | ACA CAC ACA CAC ACA CYG | M08 | AGC AGC AGC AGC AY |
| U886 | VDV CTC TCT CTC TCT CT | | |

Note: R=(A,G); Y=(C,T); B=(C,G,T) (i.e., not A); D=(A,G,T) (i.e., not C); V=(A,C,G) (i.e., not T).

Table 4. Polymorphic detected by AFLP marker in Tartary buckwheat.

| Primer | No. of polymorphic | No. of bands | Percentage of polymorphic bands (PPB) (%) | Polymorphic information content (PIC) |
|---|---|---|---|---|
| E21/M66 | 38 | 48 | 79.2 | 0.953 |
| E61/M17 | 49 | 52 | 94.2 | 0.960 |
| E15/M67 | 40 | 46 | 87.0 | 0.958 |
| E19/M58 | 39 | 41 | 95.1 | 0.957 |
| E70/M12 | 40 | 48 | 83.3 | 0.963 |
| E70/M18 | 33 | 41 | 80.5 | 0.956 |
| E61/M22 | 42 | 42 | 100 | 0.967 |
| E18/M58 | 47 | 47 | 100 | 0.973 |
| E20/M37 | 27 | 33 | 81.8 | 0.895 |
| E25/M37 | 37 | 51 | 72.5 | 0.955 |
| E48/M26 | 45 | 45 | 100 | 0.967 |
| E64/M17 | 27 | 29 | 93.1 | 0.943 |
| E61/M16 | 10 | 18 | 55.6 | 0.835 |
| E70/M16 | 38 | 49 | 77.5 | 0.957 |
| E45/M22 | 25 | 31 | 80.6 | 0.945 |
| Sum | 537 | 621 | 86.5 | |
| Average | 35.8 | 41.4 | | 0.946 |

Table 5. Genetic variation within Tartary buckwheat detected by AFLP markers.

| Origin | No. of accessions | No. of polymorphic bands | PPB (%) | Simpson's index |
|---|---|---|---|---|
| Qinghai | 10 | 194 | 36.1 | 0.8998 |
| Guizhou | 18 | 276 | 51.4 | 0.9440 |
| Hubei | 10 | 258 | 48.0 | 0.8997 |
| Shanxi | 2 | 119 | 22.2 | 0.4939 |
| Shaanxi | 2 | 78 | 14.5 | 0.5000 |
| Hunan | 9 | 259 | 48.2 | 0.8877 |
| Yunnan | 22 | 295 | 54.9 | 0.9526 |
| Gansu | 2 | 88 | 16.4 | 0.4996 |
| Ningxia | 2 | 42 | 7.8 | 0.4999 |
| All accessions | 77 | 537 | | |

Table 6. Polymorphism in Tartary buckwheat detected by ISSR markers.

| Primers | No. of bands | No. of polymorphic bands | PPB (%) | PIC |
|---|---|---|---|---|
| U808 | 26 | 23 | 88.4 | 0.918 |
| U815 | 24 | 24 | 100 | 0.940 |
| U818 | 23 | 20 | 86.9 | 0.869 |
| U834 | 29 | 27 | 93.1 | 0.942 |
| U840 | 22 | 20 | 90.9 | 0.941 |
| U842 | 33 | 30 | 90.9 | 0.944 |
| U845 | 35 | 34 | 97.1 | 0.945 |
| U852 | 11 | 11 | 100 | 0.866 |
| U857 | 35 | 30 | 85.7 | 0.938 |
| U886 | 32 | 31 | 96.9 | 0.947 |
| U888 | 18 | 18 | 100 | 0.933 |
| M01 | 31 | 27 | 87.1 | 0.945 |
| M02 | 34 | 34 | 100 | 0.928 |
| M03 | 37 | 32 | 86.4 | 0.944 |
| M04ma | 58 | 58 | 100 | 0.963 |
| M05 | 25 | 20 | 80.0 | 0.937 |
| M06 | 32 | 30 | 93.7 | 0.947 |
| M07 | 27 | 25 | 92.6 | 0.913 |
| M08 | 14 | 12 | 85.7 | 0.824 |
| Total | 546 | 506 | 92.6 | |
| Average | 28.7 | 26.6 | | 0.919 |

Table 7. Genetic variation within Tartary buckwheat detected by ISSR markers.

| Origin | No. of accessions | No. of polymorphic bands | PPB (%) | Simpson's index |
|---|---|---|---|---|
| Qinghai | 10 | 92 | 18.2 | 0.8971 |
| Guizhou | 18 | 364 | 71.9 | 0.9436 |
| Hubei | 10 | 265 | 52.4 | 0.8991 |
| Shanxi | 2 | 80 | 15.8 | 0.4991 |
| Shaanxi | 2 | 107 | 21.1 | 0.4975 |
| Hunan | 9 | 284 | 56.1 | 0.8882 |
| Yunnan | 22 | 339 | 67.0 | 0.9531 |
| Gansu | 2 | 92 | 18.2 | 0.4985 |
| Ningxia | 2 | 62 | 12.3 | 0.4997 |
| All accessions | 77 | 506 | | |

**Figures**

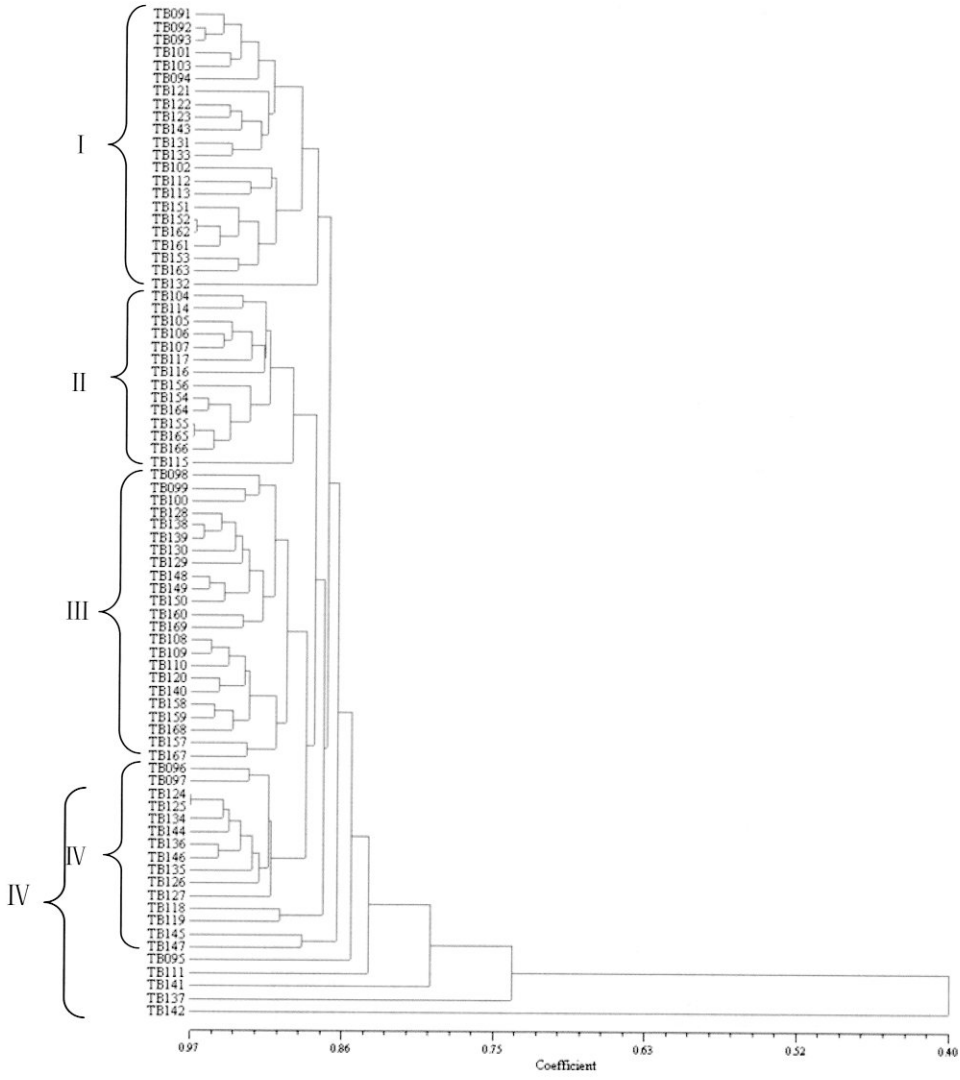


Fig. 1. Dendrogram of 79 Tartary buckwheat accessions based on AFLP data.

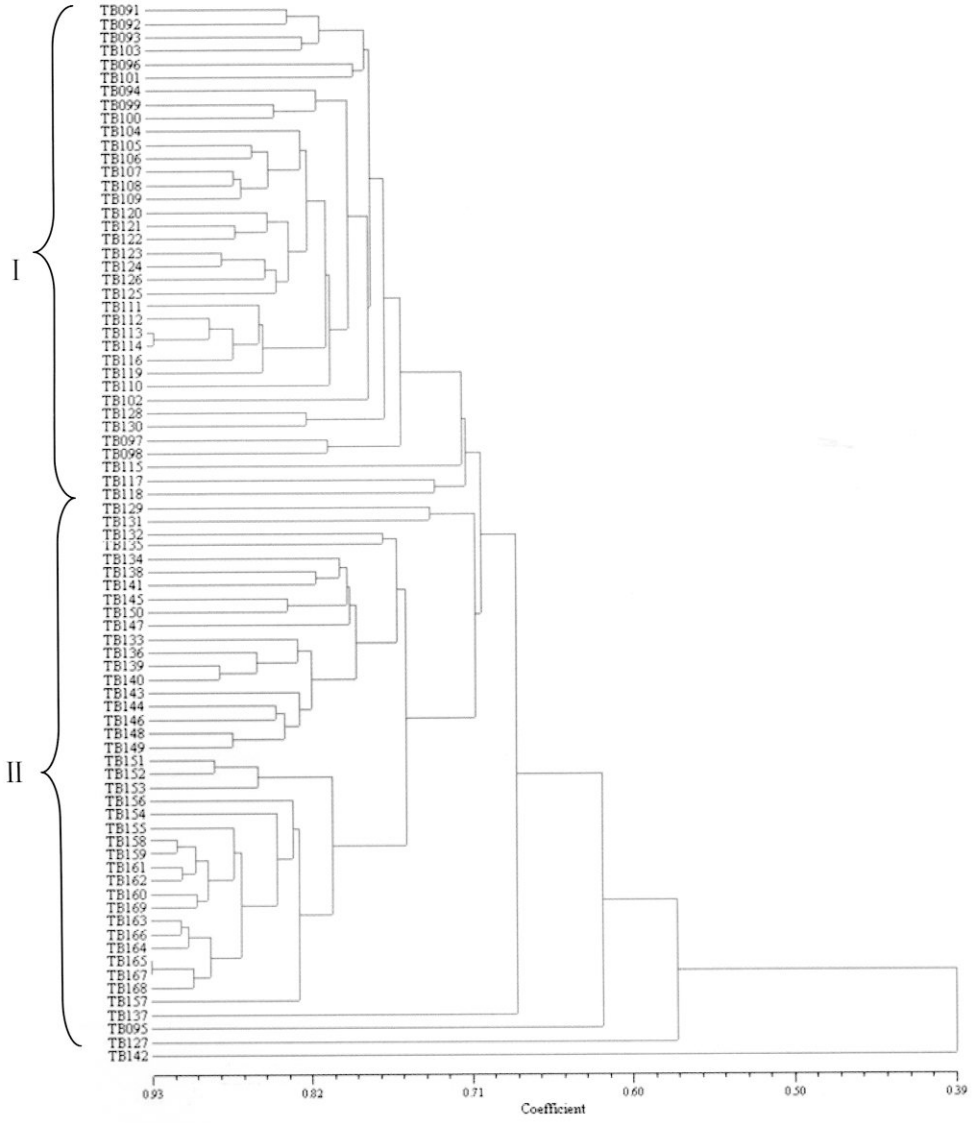


Fig. 2. Dendrogram of 79 Tartary buckwheat accessions based on ISSR data.