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# Genetic inter-relationships among Chinese wild grapes based on SRAP marker analyses

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## Summary

**Sequence-Related Amplified Polymorphism (SRAP) markers were used to assess genetic inter-relationships among 39 grape genotypes. These included 22 indigenous Chinese grape species/varieties, the north American *V. riparia* and the European *V. vinifera* L. 'Thompson seedless' and 'Pinot noir'. Of the 72 SRAP primer combinations tested, 25 primers generated 135 reliable bands, with an average of 5.52 bands per primer pair. Further analysis shows that 106 of 135 bands were generated by 25 polymorphic primer pairs, with a polymorphism efficiency of 79 %. The similarity coefficients of SRAP polymorphism varied from 0.463 to 0.981 among the genotypes analysed. A dendrogram analysis divided the 39 *Vitis* accessions into 21 groups with similarity coefficients of 0.83. It reveals broadly similar genetic relationships among the genotypes examined to those previously determined using classical taxonomic methods. Our results define *V. heyneana* subsp. *ficifolia* and *V. baihensis* as subspecies of *V. heyneana* and *V. bashanica*, respectively. We question the placement of *V. davidii* var. *cyanocarpa* and *V. davidii* var. *ningqiangensis* as varieties in *V. davidii*.**

Key words: *Vitis*; species; genotypes; polymorphism; genetic diversity.

## Introduction

The genus *Vitis* includes genotypes that have been cultivated since antiquity in many places around the world. The genus contains over 70 named species and most are native to Eurasia, north America or eastern Asia. The Chinese wild grape species have their centres of origin mainly in eastern Asia, and these comprise some 54 accessions which include 40 distinct species, one subspecies and 13 varieties. Together the Chinese wild grapes account for approximately 60 % of the total number of *Vitis* species known (Wan *et al.* 2008). At some stage in their evolutions, a good number of the Chinese wild grapes have acquired superior resistance traits to numerous agriculturally important pathogens and have also adapted to quite severe environmental conditions, including to extremes of heat, or cold or drought (He and Chao 1982, Yang *et al.* 2007, Lin *et al.* 2009, Wang *et al.* 2011). While this wild germplasm is known to be derived from many geolocations and to possess many superior resistance traits, just how these have evolved and how they may be interrelated genetically, is somewhat obscure. Numerous taxonomic studies have been conducted using traditional botanical methods including morphology, anatomy (Niu and He 1996), palynology (Niu and Zhang 2000) and isozyme technology (Liu *et al.* 1998). Nevertheless, these studies have been largely unable to clearly define their genetic and evolutionary inter-relationships. This is primarily due to their very variable morphological characteristics. Furthermore, the taxonomic status of many of the indigenous Chinese species and varieties remains obscure. Examples are: *V. amurensis var. Yanshanensis*, *V. davidii* (Roman) Foëx var.*ningqiangensis, V. bryoniaefolia* Bge., *V. qinlingensis* He P.C., *V. flexuosa* var. *parvifolia* (Roxb.) Gagnep, *V. baihensis* Niu L.X. and *V. tiubaensis* Niu L.X. The classical methods are particularly challenged here, because of the frequent occurrence of interspecies crosses. Also, there are complex interactions among a range of ecological, environmental and genetic factors which along with the genus's diverse and complex morphologies render unambiguous definition and classification almost impossible (Liu and Kong 1995, Luo *et al.* 2001). Fortunately, molecular and genomic marker technologies are able to resolve genotypic differences at nucleotide level and so provide powerful tools for addressing genetic inter-relationships and for resolving various classification and taxonomical ambiguities (Chen *et al.* 2011, Guo *et al.* 2012, Jiang and Liu 2011, Zhang *et al.* 2011).

One of the marker techniques recently employed in numerous studies for defining genetic inter-relationships among plants is Sequence-Related Amplified Polymorphism (SRAP). This was first developed in *Brassica* (Li and Quiros 2001), with the aim of amplifying open reading frames (ORFs) using primers on the basis of conservation in exons and variation in introns. SRAP has already been used successfully to study genetic relationships in a number of plants, including radish (Liu *et al.* 2008), celery (Wang

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*et al.* 2011), orchid (CAI *et al.* 2011), cherry (ABEDIAN *et al.* 2012) and citrus (AMAR *et al.* 2011).

Recently, GUO *et al.* used SRAP to analyse genetic relationships among eight species of Chinese wild grapes (GUO *et al.* 2012). In this study, we extend their work to include up to 22 species or varieties. Our comprehensive analyses reveal complex genetic inter-relationships among these species and germplasms. We discuss possible genetic and environmental interactions among these genotypes.

## Material and Methods

Plant material and DNA extraction: A total of 39 grape accessions were obtained from the grape germplasm repository of the Horticulture Institute at Northwest A&F University in Yangling. These included one *V. riparia* L. accession, two cultivars of *Vitis Vinifera* and 22 species of Chinese indigenous grapes. More detailed information on these is presented in Tab. 1. Genomic DNA was isolated from 0.5-1.0 g samples of frozen fresh leaves of each accession using the CTAB protocol (LODHI *et al.* 1994). The quality and concentration of the DNA extracted was determined using a 0.8 % agarose gel and a spectrophotometer (NanoDrop America). The DNA samples isolated were diluted to 20 $ng \cdot \mu L^{-1}$ for the SRAP analysis. Both the stock and diluted portions were stored at -40 °C in a freezer.

SRAP amplification: A total of 72 SRAP primer combinations were used (Tab. 2). These comprised eight forward primers and nine reverse primers as previously

Table 1

The diverse plant materials of genus *Vitis* used in this study

| Species | No. | Accession | Origin |
|---|---|---|---|
| *V. amurensis* var. *yanshanensis* D.Z.Lu et H.P.Liang | 1 | Yanshan-1 | Hebei Prov. China |
| *V. bashanica* He P.C. | 2 | Baihe-41 | Shannxi Prov. China |
| | 12 | Baihe-42 | Shannxi Prov. China |
| *V. heyneana* Roem.&Schult subsp. *ficifolia* (Bge.) C.L.Li | 6 | Weinan-3 | Shannxi Prov. China |
| | 7 | Sangyeshandong | Shandong Prov. China |
| *V. davidii* var.*cyanocarpa* (Gagnep.) Gagnep | 8 | Zhen-3 | Shannxi Prov. China |
| *V. davidii* (Roman) Foëx var.*ningqiangensis* Niu L.X | 35 | Ningqiang-6 | Shannxi Prov. China |
| *V. davidii* (Roman. Du Caill.) Foëx | 32 | Tangweiputao | Jiangxi Prov. China |
| | 33 | Ji'nan-1 | Shandong Prov. China |
| | 34 | Ji'nan-2 | Shandong Prov. China |
| *V. bryoniaefolia* Bge. | 10 | Taishan-1 | Shandong Prov. China |
| | 13 | Taishan-2 | Shandong Prov. China |
| *V. sinocinerea* W.T. Wang | 11 | Lan-2 | Shannxi Prov. China |
| *V. wilsonae* Veitch | 14 | Zhengzhouwangmai | Henan Prov. China |
| *V. qinlingensis* He P.C. | 20 | Ping-5 | Shannxi Prov. China |
| *V. amurensis* Rupr. | 21 | Taishan-11 | Shandong Prov. China |
| | 22 | Heilongjiangshisheng | Heilongjiang Prov. China |
| *V. heyneana* Roem.&Schult. | 23 | Taishan-24 | Shandong Prov. China |
| | 24 | Shang24 | Shannxi Prov. China |
| *V. romaneti* Roman. Du Caill. ex Planch. | 25 | Jiangxi-1 (♀) | Jiangxi Prov. China |
| | 26 | Jiangxi-2 (♂) | Jiangxi Prov. China |
| *V. bryonifolic* | 27 | Anlin-2 | Zhejiang Prov. China |
| | 28 | Anlin-3 | Zhejiang Prov. China |
| *V. pseudoreticulata* W.T. Wang | 29 | Baihe-35-1 | Shannxi Prov. China |
| | 30 | Baihe -35-2 | Shannxi Prov. China |
| *V. flexuosa* var. *parvifolia* (Roxb.) Gagnep. | 31 | Shangnan-2 | Shannxi Prov. China |
| *V. hancockii* Hance | 36 | Lingyeshandong (♀) | Shandong Prov. China |
| | 37 | Jiangxi-3 | Jiangxi Prov. China |
| *V. baihensis* Niu L.X. | 5 | Baihe-40 | Shannxi Prov. China |
| *V. tiubaensis* Niu L.X. | 9 | Liuba-10 | Shannxi Prov. China |
| *V. piasezkii* Maxim.var.(no Latin variety name yet) | 4 | Mei-6 | Shannxi Prov. China |
| | 19 | Nanzheng-2 | Shannxi Prov. China |
| *V. piasezkii* Maxim.var.*pagnucii* (Planch.) Rehd. | 15 | Baishui-40 | Shannxi Prov. China |
| | 17 | Gansu-91 | Gansu Prov. China |
| *V. piasezkii* Maxim | 16 | Liuba-9 | Shannxi Prov. China |
| | 18 | Liuba-6 | Shannxi Prov. China |
| *V. vinifera* L. | 38 | Thompson seedless | Mid Asia |
| | 39 | Pinot noir | West Europe |
| *V. riparia* L. | 3 | Hean-3 | North America |

Table 2

Forward and reverse primers used for the SRAP analysis in this study

| Forward primers | Reverse primers |
|---|---|
| Me1, 5'-TGAGTCCAAACCGGATA-3' | Em1, 5'-GACTGCGTACGAATTAAT-3' |
| Me2, 5'-TGAGTCCAAACCGGAGC-3' | Em2, 5'-GACTGCGTACGAATTTGC-3' |
| Me3, 5'-TGAGTCCAAACCGGAAT-3' | Em3, 5'-GACTGCGTACGAATTGAC-3' |
| Me4, 5'-TGAGTCCAAACCGGACC-3' | Em4, 5'-GACTGCGTACGAATTTGA-3' |
| Me5, 5'-TGAGTCCAAACCGGAAG-3' | Em5, 5'-GACTGCGTACGAATTAAC-3' |
| Me6, 5'-TGAGTCCAAACCGGTAA-3' | Em6, 5'-GACTGCGTACGAATTGCA-3' |
| Me7, 5'-TGAGTCCAAACCGGTCC-3' | Em7, 5'-GACTGCGTACGAATTGAG-3' |
| Me8, 5'-TGAGTCCAAACCGGTGC-3' | Em8, 5'-GACTGCGTACGAATTGCC-3' |
| | Em9, 5'-GACTGCGTACGAATTTCA-3' |

reported (Li and Quiros 2001, Ahmad *et al.* 2004). Of the 72 primer combinations, 25 gave clear, reproducible and polymorphic bands in an initial screening of three representative accessions ('Baihe-40', 'Weinan-3', 'Sangyeshandong'). Accordingly, these 25 primer pairs were used to study band polymorphism among the 39 accessions. The SRAP-PCR reaction was carried out in 25 μL reaction mixtures which consisted of 2.5 μL of 10 × reaction buffer, 2.0 μL of 25 mM $Mg^{2+}$, 2.0 μL of 2.5 mM dNTPs, 0.5 μL of each 10 μM primer, 0.2 μL of 5 U Taq DNA polymerase and 2.0 μL of 20 ng·$\mu L^{-2}$ genomic DNA. All PCR reactions were carried out using a BIO-RAD thermal cycler (Mexico) with the following parameters: denaturing at 94 °C for 5 min, followed by the first five cycles of 94 °C for 1 min, 36 °C for 1 min, 72 °C for 1 min and then 35 cycles of 94 °C for 1 min, 56 °C for 1 min, 72 °C for 1 min and a final extension at 72 °C for 10 min. The PCR reaction of each primer pair was carried out twice. The amplified products were separated on 1.5 % agarose gels in 1 × TAE buffer at 120 V constant voltages for 50 min. The gels were stained with ethidium bromide (EB) solution and photographed in the Gene Genius Bioimaging System (SYNGENE, England). Clear, reproducible bands were scored and their molecular weights calculated using DNA marker 5000 (Transgene, China).

Data analyses: All clear, repeatable fragments were scored as either 1 for presence or 0 for absence to generate a binary data matrix which was then used to calculate the SM similarity coefficient and to construct a dendrogram for all 39 accessions based on the unweighted pair group method with arithmetic averaging (UPGMA) by SHAN module of NTSYS-pc 2.10e.

## Results

SRAP polymorphism: A total of 135 reproducible bands were amplified using the 25 selected SRAP primer combinations. These ranged in size from 100 bp to 3000 bp (Fig. 1, Tab. 3). Of the 135 bands identified, 106 were polymorphic with an average of 5.4 fragments per primer set. The primer combination Me3/Em8 gave up to nine polymorphic bands while primers Me6/Em9 and Me8/Em1 gave only three bands (Tab. 3). The percentage of polymorphic bands varied among primer combinations, ranging from 60 % (Me4/Em7, Me5/Em6, Me5/Em7 and Me7/Em3) to 100 % (Me1/Em2, Me2/Em9, Me6/Em4 and Me6/Em8) with an average percentage of 78.5 %. These results show that the SRAP markers developed were able to reveal genetic diversity in *Vitis* and that high polymorphism existing among the 39 grape accessions examined.

Detection of genetic diversity among 39 grape species: The pairwise similarity coefficient

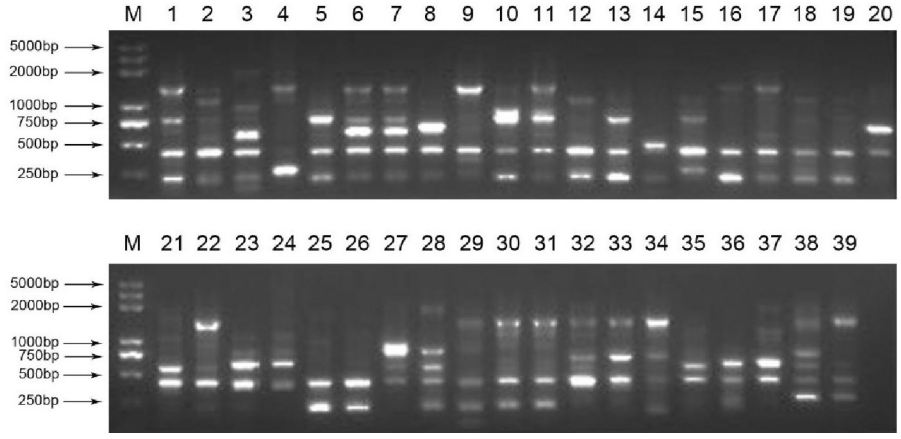


Fig. 1: SRAP application profiles by primer combination Me8/Em8 from grape germplasm. M: DL5000 Marker; numbers in this Figure are the same as those in Tab. 1.

Table 3

Numbers of total and polymorphic fragments per SRAP primer combination

| Primer combination | No. of fragments | No. of polymorphic fragments | Percentage of polymorphic fragments % |
|---|---|---|---|
| Me1/Em1 | 7 | 5 | 71.4 |
| Me1/Em2 | 5 | 5 | 100 |
| Me1/Em4 | 4 | 3 | 75 |
| Me2/Em1 | 5 | 4 | 80 |
| Me2/Em4 | 5 | 4 | 80 |
| Me2/Em8 | 8 | 6 | 75 |
| Me2/Em9 | 4 | 4 | 100 |
| Me3/Em2 | 4 | 3 | 75 |
| Me3/Em8 | 9 | 7 | 77.8 |
| Me4/Em7 | 5 | 3 | 60 |
| Me4/Em9 | 6 | 5 | 83.3 |
| Me5/Em6 | 5 | 3 | 60 |
| Me5/Em7 | 5 | 3 | 60 |
| Me6/Em4 | 6 | 6 | 100 |
| Me6/Em8 | 4 | 4 | 100 |
| Me6/Em9 | 3 | 2 | 66.7 |
| Me7/Em3 | 5 | 3 | 60 |
| Me7/Em5 | 5 | 4 | 80 |
| Me7/Em6 | 4 | 3 | 75 |
| Me7/Em7 | 5 | 4 | 80 |
| Me7/Em8 | 7 | 6 | 85.7 |
| Me8/Em1 | 3 | 2 | 66.7 |
| Me8/Em3 | 7 | 6 | 85.7 |
| Me8/Em7 | 6 | 5 | 83 |
| Me8/Em8 | 8 | 6 | 75 |
| Total | 135 | 106 | 78.5 |
| Mean | 5.40 | 4.24 | 78.5 |

for the SRAP markers ranged from 0.453 to 0.991, with an average of 0.72. This reflected a high level of genetic diversity among the 39 accessions of the genus *Vitis*. The highest genetic similarity was found between 'Taishan-24' and 'Shang 24' from *V. heyneana*, followed by the value of 0.98 between 'Weinan-3' and 'Sangyeshandong' from *V. heyneana* subsp. *Ficifolia*. The lowest similarity was between 'Hean-3' (one *Vitis Riparia* accession) and 'Baihe-42', suggesting a relatively distant evolutionary relationship between these two accessions. This may result from their different geographic origins and species.

The dendrogram generated by UPGMA based on the SRAP data shows that among the Chinese wild grapes species examined (Fig. 2), *V. piasezkii* Maxim and *V. piasezkii* Maxim. var. ('Mei-6' and 'Nanzheng-2') share similar morphologies and were assembled together with a similarity coefficient of 0.824. We located Line 1 at this point and define it as the "species line" used for distinguishing different species. Accessions of each taxonomic unit were clustered in one group (Fig. 2).

Except for *V. flexuosa* var. *parvifolia* which is grouped with *V. pseudoreticulata* and the division of accessions from *V. piasezkii var.* ('Mei-6' and 'Nanzheng-2') into two different clades, the remaining 20 taxonomic units are clearly discriminated by Line 1. These include *V. amurensis* var. *yanshanensis*, *V. davidii*, *V. sinocinerea*, *V. bryonifolic*, *V. piasezkii*, *V. piasezkii* var. *pagnucii*, *V. davidii* var.*cyanocarpa*, *V. romaneti*, *V. bryoniaefolia*, *V. amurensis*, *V. bashanica*, *V. baihensis*, *V. heyneana* subsp. *ficifolia*, *V. heyneana*, *V. qinlingensis*, *V. tiubaensis*, *V. davidii* var.*ningqiangensis*, *V. hancockii*, *V. wilsonae* and *V. vinifera*.

According to Fig. 2, all 39 grape accessions examined are grouped by Line 2 into three main clusters. This is generally in line with their taxonomic placements (north American grapes, wild species and European grapes of *V. vinifera*).

Cluster I is the largest. It includes 28 accessions and includes 16 wild species native to China. We define six subgroups within cluster I. Subgroup 1 includes *V. amurensis* var. *yanshanensis*, *V. davidii*, *V. sinocinerea* and *V. bryonifolic*. Subgroup 2 includes three species having compound leaflets (*V. piasezkii* var. ('Mei-6' and 'Nanzheng-2'), *V. piasezkii* and *V. piasezkii* var.*pagnucii*), along with *V. davidii* var.*cyanocarpa*, *V. romaneti* and *V. bryoniaefolia*. In subgroup 2, two accessions of *V. piasezkii* var. ('Mei-6' and 'Nanzheng-2') were found not to be classified closely in one branch, though the three species with compound leaflets were grouped together in a larger branch. *Vitis piasezkii* first clustered with *V. piasezkii* var. ('Mei-6' and 'Nanzheng-2') and then with *V. piasezkii* var. *pagnucii*. We infer from this a probable evolutionary relationship between *V. piasezkii* var. *pagnucii* and *V. piasezkii var.* ('Mei-6' and 'Nanzheng-2'). Subgroup 3 contains only *V. amurensis*. Subgroup 4 includes *V. bashanica* and *V. baihensis*, which are closely related. Subgroup 5 includes *V. heyneana* subsp. *ficifolia* and *V. heyneana*. Subgroup 6 contains only 'Ping-5' of *V. qinlingensis* as an outgroup. This was furthest from the other wild species in this cluster.

Cluster II consists of six Chinese wild species (containing eight lines) and two cultivars of *V. vinifera*, which fall into two subgroups. Subgroup 1 includes three wild species *V. tiubaensis*, *V. davidii* var.*ningqiangensis* and *V. hancockii* and subgroup 2 includes *V. vinifera*. *V. wilsonae*, *V. pseudoreticulata* and *V. flexuosa* var. *Parvifolia*.

Cluster III contains only the north American *V. riparia*, suggesting a relationship distant from both the Chinese indigenous species and also from the European *V. vinifera*.

## Discussion

SRAP markers: SRAP technology has been used successfully for evaluation of genetic diversity in many plant species, including in fruits (AI *et al.* 2011, ABEDIAN *et al.* 2012), vegetables (JING *et al*. 2011), flowers (FU *et al.* 2008, SHAO *et al.* 2010, CAI *et al.* 2011), medicinal plants (CHEN *et al.* 2011) and grasses (BUDAK *et al.* 2004). Here, we identify 25 SRAP markers that gave a high level of polymorphism (78.5 %) useful for the analysis of genetic variation among our 39 accessions of *Vitis*. This polymorphism level is higher than in some earlier studies with grape (LUO *et al.* 2001) (RAPD, 68.7 %) and (ERGÜL *et al.* 2004) (AFLP, 33.7 %). Our work shows there is a high level of diversity among the *Vitis* germplasm resources we examined. This is reflected in our observation that 104 polymorphic SRAP

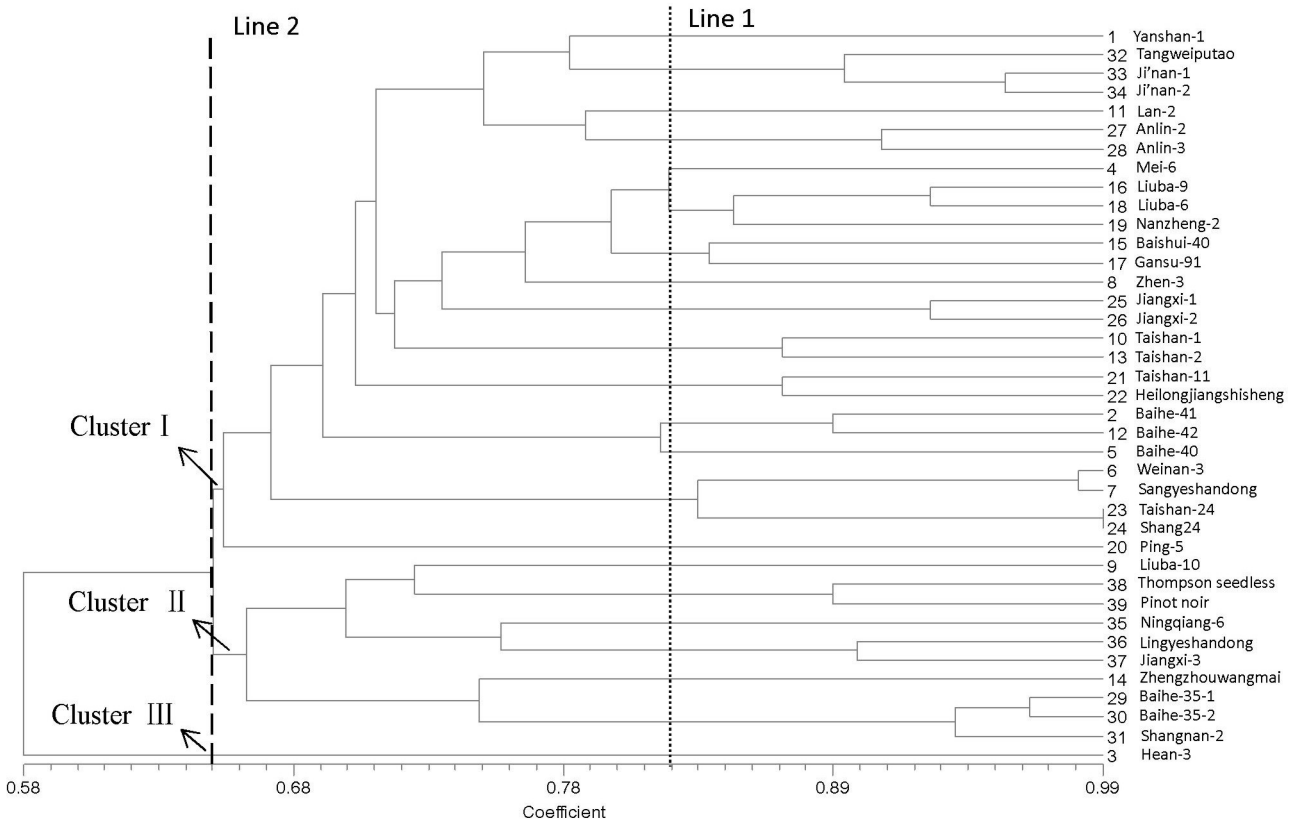


Fig. 2: UPGMA dendrogram based on SRAP markers among 39 grape accessions. Numbers in this Figure are the same as those in Tab. 1.

bands were unambiguously identified among 39 assessed genotypes. The majority of the species we examined can be clearly discriminated and are well supported. Moreover, this classification result is in agreement with that of classical taxonomy (Liu and Kong 1995, Liu *et al.* 1998, Niu and Zhang 2000). All these results indicate that the SRAP technique is effective and efficient and that it is useful for analysing genetic diversity and inter-relationships in the *Vitis* genus.

Cluster analysis of Chinese wild grapes: Using average similarity values > 0.65 as the cut-off point, the SRAP markers were used to define clusters (Fig. 2). All 39 accessions can be divided into 21 clades based on the value of Line1 (0.824). This clearly distinguishes all species except *V. piasezkii* var. ('Mei-6' and 'Nanzheng-2') and *V. flexuosa* var. *parvifolia*. We identify *V. riparia* L. as an outgroup of the Chinese wild grape species and showing obvious geographic isolation. *Vitis riparia* is alone in a cluster originating from north America, while all the Chinese wild grapes examined are grouped into two other clusters. This result is consistent with earlier reports (Luo *et al.* 2001, Hou *et al.* 2010). Intriguingly, 'Thompson seedless' and 'Pinot noir' (both *V. vinifera* L.) are grouped with four species and two varieties of Chinese wild grapes in Cluster II, while the other Chinese wild grapes are in Cluster I. This indicates these Chinese wild grapes appear to be more closely related genetically to European grapes than to north American grapes. This result is not in line with earlier studies (Luo *et al.* 2001, Tang *et al.* 2004, Fang *et al.* 2010). It raises the question of how this has happened - both genetically and geographically. One hypothesis proposes that hybridisation could have contributed to integration of the European grape genome into the Chinese germplasm or *vice versa* (This *et al.* 2006, Tröndle *et al.* 2010). This is based on the observation that these Chinese wild species come from regions where viticulture (*V. vinifera*) has been common since antiquity. This will have increased the possibility of dispersal (anthropogenic and/or natural) of these accessions among populations of cultivated grapes (*V. vinifera*). This hypothesis is further supported by the observation that the genomes of cultivated grapes are very often integrated into wild populations. We also conjecture that these divergent results could be ascribed to differences in sample sizes and compositions in earlier research.

It is recognised that clear boundaries between species and subspecies are not always evident. In line with earlier classifications based on isozyme analysis (Chao 1988), on morphological features (Niu and He 1996) and on RAPD marker identification (Luo *et al.* 2001) our finding that *V. davidii* var. *cyanocarpa* and *V. davidii* var. *ningqiangensis* are both genetically distant from *V. davidii* challenges the accepted view that the former (two) should be treated as subspecies of the latter (one). Meanwhile, the placement of *V. bryonifolic* in *Vitis* has been controversial with no study able to clarify whether V. *bryonifolic* should be combined with *V. bryoniaefolia*, or placed in the latter as a subspecies (Wang 1988) or treated as an independent species (Wang *et al.* 2000). Here, we suggest *V. bryonifolic* to be a subspecies of *V. bryoniaefolia* because these two species are grouped separately in the first and second subgroups in cluster I, so revealing a somewhat distant relationship.

All grapes having compound leaves are treated as a single species in traditional taxonomy. However, here

we find grapes with different compound leaf types show relatively distant genetic relationships. This suggests that a high level of diversity exists among these grapes having compound leaves. Here we find five species and varieties of compound-leafed *Vitis* are in different clades (by Line 1). *Vitis tiubaensis* Niu L.X is in cluster II, while the other four are in cluster I. This indicates these grapes are not a pure species but that some interspecies transitional types are probably present. This is also implied by the polymorphism of the compound-leafed grapes. From the evolutionary relations of *V. piasezkii* and *V. piasezkii* var. *pagnucii* in Fig. 2, we infer that *V. piasezkii* var. *pagnucii* may be derived from *V. piasezkii* var. ('Mei-6' and 'Nanzheng-2') which is in line with WANG *et al.* (2000) who came to the same view based on a stepwise clustering methodology. *Vitis baihensis* and *V. bashanica* are closely clustered in subgroup 4 of cluster I. A similar result was obtained by NIU and HE (1996) who showed a close relationship between these two species and placed them in one group. We suggest that the former species originated from the latter because compound-leafed grapes are more highly evolved than simple-leafed grapes (KONG 2004). The polymorphisms found in various compound-leafed grapes suggest a complex genetic context. Therefore, we come to much the same conclusion as ZHANG *et al.* (2011), namely that polyphyletic evolution has taken place in the compound-leafed grapes. Our interpretation conflicts with that of NIU and HE (1996) who consider the relationship between *V. tiubaensis* and other compound-leafed grapes to be close. Further and more comprehensive genetic analysis is needed to clarify this.

Similarly, the placement of *V. heyneana* subsp. *ficifolia* in the *Vitis* genus remains to be clarified. Among all the genotypes we examine here, we find the closest intra-species relationship between *V. heyneana* and *V. heyneana* subsp. *ficifolia*. These two species and subspecies are here clustered in one clade, with a similarity coefficient of 0.83. Based on this, we suggest it is probable that *V. heyneana* subsp. *ficifolia* is derived from *V. heyneana*. This supports the earlier conclusion of NIU and HE (1996) and WANG *et al.* (2008) that *V. heyneana* subsp. *ficifolia* is a subspecies of *V. heyneana*.

In conclusion, our study shows that the SRAP technique is a highly reproducible and efficient tool for assessing genetic relationships among the Chinese wild grapes. Our analyses suggest that the wild grape germplasm domesticated in China is highly variable and contains abundant genetic diversity.

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