

## Genetic Diversity and Genetic Structure in Natural Populations of *Prunus davidiana* Germplasm by SSR Markers

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### Abstract

*Prunus davidiana*, which is a wild species, can be used as rootstocks for cultivation of fruit trees, breeding germplasm for improving resistance to diseases and insects, pioneer trees for recovering vegetation in arid areas, decoration trees for parks and gardens, and a potential medicinal plant for human health. Despite the valuable characteristics of *P. davidiana*, the genetic variations of resources of *P. davidiana* remain unclear. In this study, we used seven natural populations (GE; GH; ST; SF; SJ; SY; NX short for Heshui, Gansu; Huating Gansu; Taibai, Shaanxi; Fuxian, Shaanxi; Jiaocheng, Shanxi; Yangquan, Shanxi; Xiji, Ningxia, respectively) of *P. davidiana* collected at distribution center in China to generate a fingerprint using 15 SSR markers by multifluorophore fragment analysis to assess transportability of the markers, genetic diversity and genetic structure within and among populations. Our data show that a 92% transportability rate was found from closely related species of *P. davidiana* based on SSR markers developed, and DNA polymorphisms were also detected among accessions by the selected SSR markers. The SSR markers amplified 137 alleles in total for all accessions with an average value of 9.13 alleles, ranging from the highest value of 15 alleles for UDP96-013 to the lowest value of 3 alleles for CPPCT017. Rare alleles, present at less than or equal to 5% of all accessions by marker,

showed the highest value in BPPCT 020. By analyzing all accessions, the majority of the 192 accessions from seven populations were found to cluster together according to the given populations, while the minority from the given populations was distributed within clusters. The SY population appeared to have the most diversity among populations, followed by the NX population according to comprehensive analysis of all indices. The seven populations analyzed by unweighted parsimony and principal coordinate analysis were divided into two groups: ST, SY and SF; NX, GE, SJ and GH. A stark difference was found in the genetic variation—with 16% among populations and 84% within populations. Our results from this study imply that most SSR markers developed from related species can be used for genetic analyses of *P. davidiana*; some markers have more relevant applications for genetic analyses such as UDP96-013 for diversity evaluation and BPPCT 020 for mining rare alleles in *P. davidiana*; SY and NX are preferred when considering conservation and agronomic utilization.

**Keywords:** *Prunus davidiana*, Natural population, Simple sequence repeat, Genetic characteristics

## 1. Introduction

*Prunus davidiana* (Carr.) Franch, belonging to subgenus *Amygdalus*, distributed in Northwestern and Northern parts of China, is a wild species related to *P. persica* (L.) Batsch (peach and nectarine) (Wang, 1988). The adult plant of *P. davidiana* usually grows like shrub with weeping shoots or tree with upright shoots, with a reddish-brown bark; the leaves are long, glabrous, ovate-lanceolate and broadest near the base; its flowers are in white or light pink color, blooming earlier and last longer than ones of any other related species; its fruit pit is small and pitted; its flesh with very thin layer is freestone and inedible (Bassi and Monet, 2008) (Fig1). Although *P. davidiana* is not cultivated for its fruits, it still represents an important value of natural resource, including the tolerance to coldness, dryness, alkali of soil and resistance to peach aphid (Wang and Zhuang, 2001). In cultivation of peach, nectarine, almond, apricot and plum, *P. davidiana* is often used as rootstock, especially in the Northwestern and Northern parts of China because of its strong adaptability and potential for selection of new rootstock by sport mutation. Furthermore, accessions of the species can be crossed with ones of peach and nectarine cultivars to improve the resistance to diseases including plum pox, powdery mildew and leaf curl, etc. (Moing et al., 2003), to nurture interspecific rootstocks adaptable to marginal soils or more resistant to replant problems (Edin and Garcin, 1994; Pisani and Roselli, 1983; Roselli et al., 1985) or to select for self-incompatibility genes (Foulongne et al., 2003). Besides its agronomic importance, *P. davidiana* can be directly used as gardening trees because of earlier flowering and bright color of bark, and solid timber due to hardness and no cracking traits of wood. In addition, *P. davidiana* has been widely used as an alternative prior species for ecologic restoration in dry and arid areas for the northwestern parts of China (Wang and Zhuang, 2001). Seeds of *P. davidiana* content of 45% oil that has the therapeutic application in Chinese medicine (Wang and Zhuang, 2001), and stems of *P. davidiana* plants contain some health-promoting components that show the inhibitory activity on both total reactive oxygen species (ROS) and hydroxyl radicals ( $\cdot\text{OH}$ ) generation (Choi, 1991; Jung et al., 2003).

In order to use *P. davidiana* germplasm efficiently and extensively, basic information on its molecular biological characteristics is needed. Many methods from botany, to agronomy and to molecular biology have been developed to screen plant genetic diversity. The molecular biology approach has been preferentially applied in resolving limitations of enzyme analyses and macro-observations with morphology and agronomy (Beyene et al., 2005; Mondini et al., 2009; Zeinalabedini et al., 2008). SSR (Simple Sequence Repeat or Microsatellite DNA) technology is the most commonly used as compared to other molecular biology methods due to its advantages of displaying co-dominant inheritance, hypervariability and having high cross-species transferability (Bell and Ecker, 1994; Guilford et al., 1997; Tauraz, 1989; Sosinski et al., 2000; Wünsch, 2009). More than 300 SSR markers have been isolated and characterized in the subgenus *Amygdalus* (Genome Database for Rosaceae <http://www.bioinfo.wsu.edu/gdr/>). The markers provide a very reliable and convenient tool for analyzing genetic diversity of *P. davidiana*. Recent literatures from many studies reported genetic variations on peach and related species in the subgenus *Amygdalus* using molecular biological analyses (Aranzana et al., 2002; Aranzana et al., 2003; Bouhadida et al., 2007; Cheng, 2007a; Cheng, 2007b; Cheng and Huang, 2009; Cipriani et al., 1999; Dirlwanger et al., 2002; Shiran, 2007; Sosinski et al., 2000; Testolin et al., 2000; Wang et al., 2002), however, the genetic diversity and genetic structure of natural populations of *P. davidiana* still remains unknown.

Given these important traits of *P. davidiana* in agronomy, ecology and medicine mentioned above, it is very important to study genetic characters of *P. davidiana* in order to preserve and use resource of *P. davidiana* from a lot of individuals and various distributions, which is really a challenge. As documented, *P. davidiana* evolved from *P. kansuensis* Rehd. (Wang and Zhuang, 2001) is native to Gansu and Shannxi provinces (Yu, 1979), so we had to focus the sample collection of *P. davidiana* populations in Gansu, Shannxi and their borders such as Shanxi, Ningxia. We investigated the geological distribution of *P. davidiana* in these areas above and other

places, and collected accessions of different populations over the past few years. Genetic variation and population structure of 192 accessions of *P. davidiana* representing seven natural populations were studied using selected 15 SSR markers. Our results provide valuable information on guidance for conservation and further exploration of the important wild germplasm in breeding cultivated peach and nectarine.

## 2. Materials and methods

### 2.1 Plant materials

Accessions of *P. davidiana* were collected from seven populations (GE, GH, ST, SF, SJ, SY and NX abbreviated for Heshui, Gansu; Huating, Gansu; Taibai, Shaanxi; Fuxian, Shaanxi; Jiaocheng, Shanxi; Yangquan, Shanxi; and Xiji, Ningxia, respectively) within four Chinese provinces and prefecture: Gansu, Shaanxi, Shanxi and Ningxia, during the past few years (Fig.2). Young leaves of more than 30 accessions per population were collected and stored in sealed bags with silica material for DNA extraction.

### 2.2 Molecular analysis

Genomic DNA of plants was extracted using the modified CTAB extraction protocol (Doyle and Doyle, 1987). To be effective for using plates of 96 wells, DNA samples of accessions from each population were 26-28 (less than 30 leaf samples of accessions from each population) to conform to 192 wells of two plates in total. PCR amplifications were run on two platforms with different conditions for each. For fragment separation on 3% high resolution MetaPhor® (Cambrex Charles City Inc, IA) agarose – 1X TBE gels, the amplifications were performed in a total volume of 20 µl with final concentrations of 50 ng of DNA, 0.2 µM of both primers, 200 µM of each dNTP (New England Biolabs, Ipswich, MA) and 0.5 U of New England Biolabs' *Taq* DNA polymerase in 10 mM Tris-HCl pH 8.3, 1.5 mM MgCl<sub>2</sub> and 50 mM KCl. The process was analyzed with 25 published SSR markers which were previously mapped in peach (Table 1) for screening transportability and polymorphic characters. For fragments detected using the ABI 3130 (Applied Biosystems, Foster City, CA) with 15 SSR markers selected from the above mentioned 25 SSRs, PCR conditions were the same as above with the exception of 0.02 µM of M-13–tagged forward primer, 0.2 µM of reverse primer and 0.2 µM of M-13–tagged dye (6'-FAM, VIC, NED or PET) (ABI). Thermo Scientific MBS Satellite Thermal Cyclers (Thermo Fisher Scientific, Waltham, MA) were used. The PCR program was performed for 3 min of initial denaturation at 94°C, followed by 35 cycles of 1 min at 94°C, 1 min at the annealing temperature ( $T_a$ ) and 1 min at 72°C, then a final extension step of 5 min at 72°C for each set of primer combinations.

When performing PCR for multifluorophore fragment analysis, the conditions above were followed except for primer pairs with  $T_a$  significantly lower than 58°C ( $T_a$  for M-13 forward primer). In such cases, 4 additional cycles were performed at the annealing temperature of the SSR marker followed by 35 cycles at the annealing temperature of the M-13, as described above. PCR amplicons, using 3% MetaPhor® - 1X TBE agarose gels along with New England Biolabs' low molecular weight DNA marker, were visualized either with ethidium bromide under UV light or by pooling together (4 different fluorophore). The samples were cleaned up with ExoSAP-IT (USA Scientific or USB) according to manufacturer protocol and run on ABI 3130 with GeneScan™ 600 LIZ® (Applied Biosystems) internal size standard. PCR products were separated on agarose gel and then scored visually or analyzed by GeneScan with the ABI 3130 and read by Gene Mapper V.4.0 (Applied Biosystem) for multifluorophore fragments (Fig.3).

### 2.3 Data analysis

The band patterns from automated sequences were scored and converted into digital (0/1) format to make a binary data file: 1 indicates the presence of a band; 0, the absence of a band. All genetic parameters including diversity of amplified DNAs of primers and populations, genetic structure and principal component analysis (PCA) were obtained by GenALEX6.2 software (Peakall and Smouse, 2006) according to relative rules except that clusters of accessions and populations were constructed based on the matrix of the genetic distances using the unweighted parsimony method by MEGA4.0 (Tamura et al., 2007).

## 3. Results

### 3.1 Transportability of cross species

Twenty-five SSR markers developed and mapped in peach from other species related to *P. davidiana* were tested, of which 23 gave good amplification and polymorphism in *P. davidiana* accessions (Fig.4). On the other hand, two markers, BPPCT014 and pchcms2, failed to produce any amplification product although three experimental repeats had been conducted using original protocols. High transportability, with a ratio of 92%, of all SSR markers and polymorphic characters indicates that most of the SSR primers developed from peach and other related species can be used for molecular genetic studies in *P. davidiana* (Table 1).

### 3.2 Polymorphism of microsatellite primers

DNAs of the accessions from seven populations were amplified with 23 transportable primer pairs, and amplified products were electrophoresed and visualized on a high resolution gel. Based on the number and diversity of bands produced from the selected accessions amplified with all markers, 15 SSR markers were selected for further multifluorophore PCR analysis. Products of multifluorophore amplification by automated capillary sequencers were used to display genetic variations of 192 accessions of the seven populations, inferring that alleles amplified by the markers for all accessions were from three alleles of the marker CPPCT017 to 15 alleles of the marker UDP96-013, with an average value of 9.13 alleles for all primers. Eight SSR markers, each with 10 alleles or more (higher than mean value of 9.13 as standard), shared 53.33% of all the markers, while only two markers, each with five alleles or less, shared 13.33 % (Table 2). Alleles comprising less than or equal to 5% of all experimental accessions for a marker can be used as an indication of rarity, which is determined by screening primers. BPPCT020 with seven rare alleles had the most among all markers, followed by UDP98-409 with five rare alleles and then five other markers with three rare alleles each, while CPPCT 022 showed no rare alleles at all (Table 2).

### 3.3 Genetic diversity of populations

Results from the 15 SSRs using DNAs from the seven natural populations mentioned indicate that SY has the highest value with 109 alleles, whereas GE has the lowest value with 99 alleles, and the populations were average 102.7 alleles (Table 3). Rare alleles (present at less than or equal to 5% of all accessions by marker) were distributed in SY and NX. From analyses of the populations with private alleles (exclusive to one population), SY had the most with five, followed by NX and GE with two each. Shannon's Information Index (Table 3) displays that SY has the highest index figure compared to the other populations. Expected heterozygosity of the populations was similar to the trends of unbiased expected heterozygosity which range from a low of 0.16 for GE to a high of 0.19 for SY. The expected heterozygosity ranged from the lowest value of 0.15 for GE to the highest value of 0.18 for SY, NX, SJ and GH, with an average value of 0.17 for all populations. The populations with the highest percentage of polymorphic bands are ordered as SY>NX>SJ>GH = SF>GE>ST (Table3). This pattern remains the same regardless of which indices mentioned above are used to evaluate diversity of the populations: SY has the highest values, followed by NX with the exception of their values of expected heterozygosity equaling those of SJ and GH.

### 3.4 Genetic relation and genetic structure

Based on clusters of all accessions from the seven populations with genetic distances, our results revealed that most accessions were allocated together according to the given populations. However, some of the clusters displayed mixtures of populations (Fig. 5). There were ten groups: the first and second groups were composed of two individuals from GE and NX separately; the third group, of individuals of NX; the fourth group, of individuals from SJ except one from GH; the fifth group, of ones from four populations (SJ, ST, SY and SF); the sixth group, of ones from SY besides one from ST; the seventh group, of ones from ST besides one from SF and one from NX; the eighth group, of ones from both GH and SJ besides two from GE and SY; the ninth group, of ones from NX and SF besides three from SY and two each from ST and GH; the tenth group, of ones mostly from GE and a few from the other six populations.

As all populations were clustered with units of populations from different geographic locations, two groups formed: one group contained ST, SY and SF which further divided into two subgroups; another group came from NX, GE, SJ and GH—the former two and the latter two distributed into two subgroups, respectively (Fig. 6). We used PCA to test harmony of the clusters. Percentage of variation is explained by the first 3 axes: Coord.1, coord.2 and coord.3 recorded as 33.56, 28.09 and 14.43, respectively. From coord.1, the plot was divided into two groups: one was ST, SY and SF; the other was NX, GE, SJ and GH (Fig. 7) as the same groups clustered by the unweighted parsimony method. Under coord.1, using coord.2 could identify two subgroups in each group as the same by clustering with unweighted parsimony. The result displayed that populations clustered without relation to geographic locations. For genetic structural analysis from recorded data, the percentage of molecular variance within populations was 84%—five times more than the variation between populations, 16%.

## 4. Discussions

There are no reports on the population genetic analysis of *P. davidiana* using SSR markers or other molecular methods; therefore, it makes sense that SSR markers from related species in the subgenus *Amygdalus* were used for DNA amplification of *P. davidiana*. The results also agree with previous reports about cross-species transportability of SSR markers of *P. persica* for *P. domestica* (European plum), *P. salicina* (Japanese plum), *P. armeniaca* (apricot), *P. dulcis* (almond), *P. avium* (sweet cherry) and *P. cerasus* (sour cherry) (Cipriani et al.,

1999); *P. avium* (Dirlewanger et al. 2002); *P. armeniaca* (Zhebentyayeva et al. 2003); *P. dulcis* (Wünsch, 2009). Our results also offer the future possibility of using SSR markers from related species to study the genetic diversity and structure of *P. davidiana*.

Alleles obtained from the fragment analysis of the 15 SSR markers display an average value of 9.1, which is almost equal to mean value of 9 alleles from the wild species of *P. avium*, *P. cerasus*, *P. mahaleb*, *P. angustifolia* and *P. laurocerasus* in *Prunus* (Turkoglu et al. 2010), much higher than those from other species in the subgenus *Amygdalus*, such as almond with mean value of 6.6 alleles per locus (Shiran et al., 2007), *P. persica* with an average of 3.0 alleles per locus observed in 28 cultivars (Sosinski et al., 2000), with 4.5 alleles per locus in 50 cultivars (Testolin et al., 2000) and with 7.3 alleles per locus observed in a set of 212 cultivars using 16 polymorphic SSR markers (Aranzana et al., 2003). Although the average number of alleles per SSR marker for the studies of *P. persica* mentioned above is associated with the number of experimental accessions, alleles of 192 accessions from seven populations in our study appeared higher than ones of 212 peach cultivars even though accession number of the former was less than that of the latter. A possible explanation for this case is that accessions of *P. davidiana* originated from wild species within different geographical and ecological areas and had multiple loci including more than three alleles amplified with most of the markers. Other studies also gave the evidence that order of allelic richness indicated wild relatives > landraces > cultivars (Ruter, 1999; Ram, et al. 2007; Wang, et. al, 2010). *P. davidiana* has not only rich alleles, but also special alleles. Some of the 15 SSR markers in this study revealed that particular alleles were represented in less than or equal to 5% of all accessions. We can use this informative data of markers like BPPCT020 and UDP98-409 to further discover rare alleles. For uncovering private alleles in the populations, SY has the most potential. These rare and private alleles may be useful markers for improving breeding and cultivation through enhanced rootstocks and peach production.

Although most accessions of *P. davidiana* were distributed into one or a few groups, the phylogeny relations of all accessions could not thoroughly cluster into seven groups based on their originating places, which implies that progenies in the same place came from different progenitors and had interbred among populations. The phenomena also were observed in *P. armeniaca* and *P. avium* in wild populations whose accessions from diverse origins clustered in a group (He et al., 2007; Guarino et al. 2009). We clustered seven populations using the unweighted parsimony method and principal component analysis, which consistently showed that the seven populations were divided into two groups. SY and NX from each of the two groups had more alleles, rare alleles and private alleles as well as a higher Shannon's Information Index and a higher percentage of polymorphic alleles than other populations in the groups.

Our field observations revealed that the two genetic populations represent two different kinds of botanical characteristics. For example, trees from SY population usually grow more vigorously and higher with slightly upright shoots of a light pink color, and white flower as compared with trees of NX which are a type of shrub with red brown weeping shoots and pink flowers. SY and NX populations represent two different types in terms of geographic and climatic factors as well. The SY population grows in warm, temperate zones and half-wet climate zones near Northern China, while that of NX is located in temperate and transition zones of half wetness and aridity in Northwestern China. Previous studies of other species suggest that agronomic characters were harmonized with molecular data on the basis of analysis of diversity for constructing core collections (Qiu et al., 2006; Wang et al., 2009; Zhang et al., 2009). From the combination of molecular, botanical, geographic and climatic information in this study and others' successful experiments, it seems that the two populations SY and NX will be conserved preferentially. The genetic structure, molecular variance of which within and between populations in this study was 84% and 16%, respectively, was found to be almost the same as related species: genetic variation of *P. armeniaca* mainly resides among individuals within populations (83.6-86.3%) (He, et al., 2007), and genetic variations between and within groups for *P. persica* are 11.9, 88.1%, respectively (Cheng, 2007a). The result indicates that we should pay attention to the amount of accessions from one population rather than separately from all populations in proportion to get the same amount when collecting accessions for conservation and utilization. How to collect accessions from one population for conservation rests on diversity indices of different individuals in this population. For conservation of accessions from different populations, if we select more than two populations, accessions from the population with higher indices of diversity should be collected more than from the less diverse population. For example, for two preferentially conserved populations in this study, more accessions should be collected from SY population than from NX population.

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Table 1. Amplified characteristics of the 25 SSR markers screened via Metaphor® agarose gel in *P. davidiana*.

Marker	Linkage group	Amplification of DNA	Polymorphism (Yes/No)	Reference
BPPCT 006	G8	Clear PCR product	Yes	Dirlewanger et al. 2003
BPPCT 007	G3	Clear PCR product	Yes	
BPPCT 008	G6	Clear PCR product	Yes	
BPPCT014	G5	No PCR product	NA	
BPPCT 015	G4	Clear PCR product	Yes	
BPPCT 017	G5	Clear PCR product	Yes	
BPPCT 020	G1	Clear PCR product	Yes	
BPPCT 025	G6	Clear PCR product	Yes	
BPPCT 028	G1	Clear PCR product	Yes	
CPPCT 002	G3	Clear PCR product	Yes	Aranzana et al. 2002
CPPCT 006	G8	Clear PCR product	Yes	
CPPCT 016	G1	Clear PCR product	Yes	
CPPCT 017	G7	Clear PCR product	Yes	
CPPCT 022	G7	Clear PCR product	Yes	
CPPCT 033	G7	Clear PCR product	Yes	
pchems2	G7	No PCR product	NA	Wang et al. 2002
UDP96-001	G6	Clear PCR product	Yes	Cipriani et al. 1999 Testolin et al. 2000
UDP96-003	G4	Clear PCR product	Yes	
UDP96-005	G1	Clear PCR product	Yes	
UDP96-013	G2	Clear PCR product	Yes	
UDP97-403	G3	Clear PCR product	Yes	
UDP98-024	G4	Clear PCR product	Yes	
UDP98-025	G2	Clear PCR product	Yes	
UDP 98-409	G8	Clear PCR product	Yes	
UDP98-412	G6	Clear PCR product	Yes	



Table 2. Characteristics of the 15 SSR markers studied via multifluorophore fragment analysis in *P. davidiana*

Marker name	No. Alleles	Allele size † (bp)	No. rare alleles* (allele size bp)
BPPCT 007	10	79, 101, 107, 122, 130, 149, 163, 173, 185, 366	1 (366)
BPPCT 008	8	74, 90, 96, 105, 111, 117, 123, 132	1 (90)
BPPCT 017	8	42, 66, 75, 84, 129, 150, 158, 164	3 (84, 158, 164)
BPPCT 020	13	146, 149, 158, 161, 169, 182, 187, 197, 202, 208, 240, 244, 248	7 (182, 187, 197, 202, 208, 240, 244)
BPPCT 025	9	144, 154, 162, 167, 182, 191, 199, 217, 223	3 (191, 199, 223)
CPPCT 016	6	174, 180, 184, 192, 208, 220	2 (192, 220)
CPPCT 017	3	75, 150, 170	1 (170)
CPPCT 022	5	217, 221, 231, 247, 291	0
UDP96-001	6	41, 84, 106, 163, 215, 255	2 (215, 255)
UDP96-003	10	54, 58, 73, 84, 90, 96, 102, 107, 112, 122	3 (54, 58, 73)
UDP96-005	10	84, 103, 113, 125, 133, 150, 161, 173, 185, 204	3 (173, 185, 204)
UDP96-013	15	129, 149, 157, 163, 170, 180, 194, 206, 214, 222, 237, 278, 320, 423, 483	3 (206, 278, 320)
UDP98-024	12	42, 47, 50, 56, 68, 77, 80, 86, 97, 107, 110, 118	1 (118)
UDP98-409	11	102, 118, 123, 129, 137, 147, 173, 180, 188, 202, 209	5 (102, 173, 180, 188, 202)
UDP98-412	11	43, 77, 93, 100, 104, 111, 118, 123, 132, 159, 179	2 (93, 159)

\*Alleles present in equal to or less than 5% of all accessions for a given marker.

†Allele sizes analyzed by GeneScan with the ABI 3130 machine and read by Gene Mapper V.4.0 software (Applied Biosystem) for multifluorophore fragments.

Table 3. Characteristics of seven *P. davidiana* populations amplified by 15 SSRs.

Population	ST	SY	SF	NX	SJ	GH	GE
No. alleles*	101	109	102	107	101	100	99
No. alleles freq. = <5%†	18	26	16	22	9	16	14
No. private alleles‡	1	5	1	2	0	0	2
Mean I §	0.26	0.29	0.27	0.28	0.28	0.28	0.25
SE of Mean I	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Mean He **	0.17	0.18	0.17	0.18	0.18	0.18	0.15
SE of Mean He	0.02	0.01	0.02	0.02	0.02	0.02	0.02
Mean UHe ***	0.17	0.19	0.17	0.18	0.18	0.18	0.16
SE of mean UHe	0.02	0.02	0.02	0.02	0.02	0.02	0.02
%P****	67.88	77.37	70.80	75.91	72.26	70.80	68.61

\*No. of different alleles

†No. of rare alleles with a frequency = <5%

‡No. of alleles unique to a single population

§Shannon's Information Index =  $-1 * (p * \ln(p) + q * \ln(q))$

\*\*Expected heterozygosity =  $2 * p * q$

\*\*\*Unbiased expected heterozygosity =  $(2N / (2N - 1)) * He$

\*\*\*\*Percentage of polymorphic alleles in each population

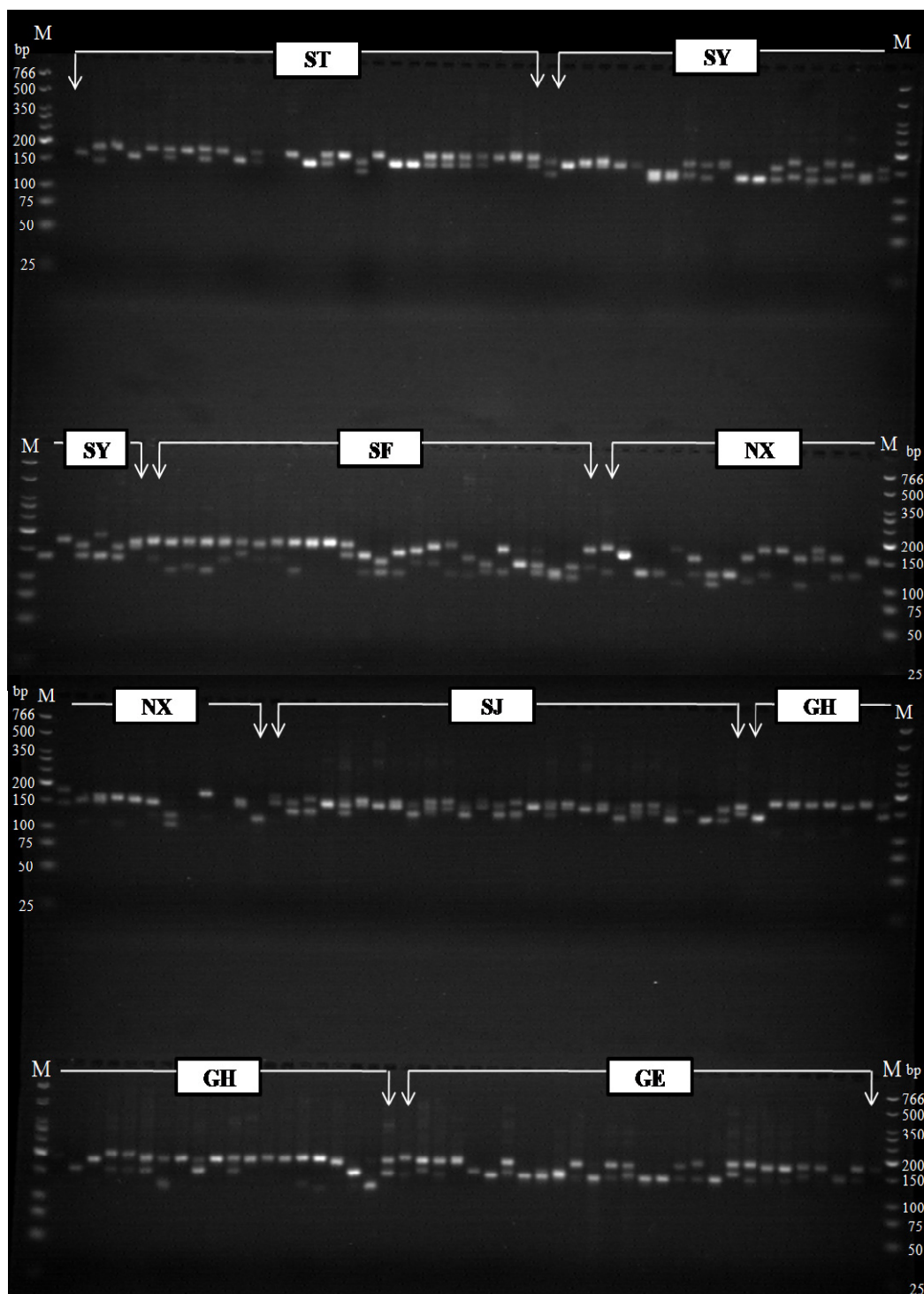
For diploid binary data, assuming Hardy-Weinberg equilibrium,  $q = (1 - \text{Band freq.})^{0.5}$  and  $p = 1 - q$ .



Figure 1. Photos of trees, leaves, flowers and fruits of *P. davidiana*



Figure 2. Seven populations (GE, GH, ST, SF, SJ, SY, NX with red mark abbreviated for Heshui, Gansu; Huating Gansu; Taibai, Shaanxi; Fuxian, Shaanxi; Jiaocheng, Shanxi; Yangquan, Shanxi; and Xiji, Ningxia, respectively, below as the same for the study) of *P. davidiana* collected from the areas



M: DNA ladder.

Figure 3. Photos of all accessions of seven populations of *P. davidiana* amplified with UDP96-005 primer pair

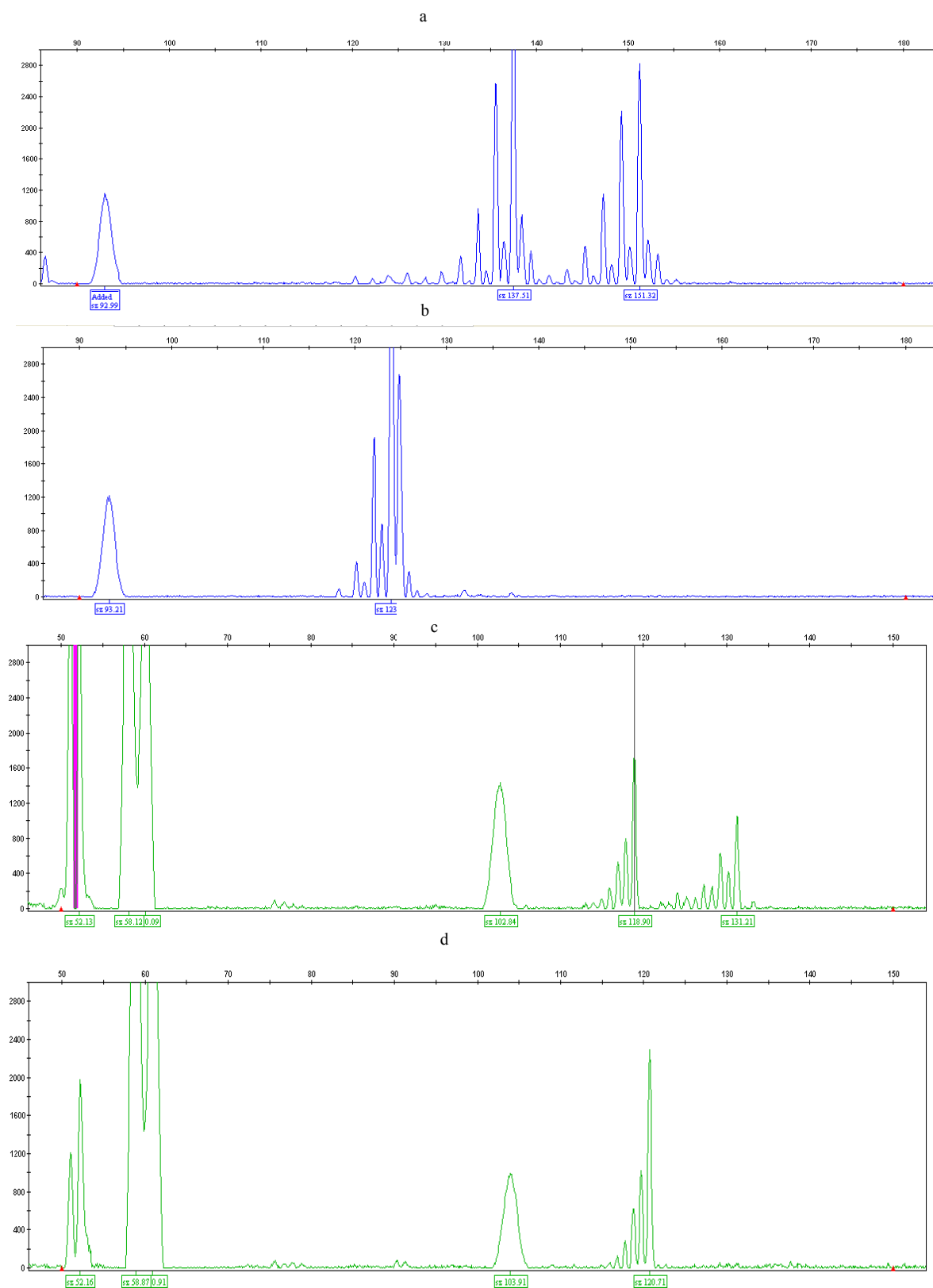


Figure 4. Multifluorophore fragment analysis. Plots display products of four accessions of *P. davidiana* from the ST population amplified by BPPCT008 for plots a and b and UDP96-003 for plots c and d.

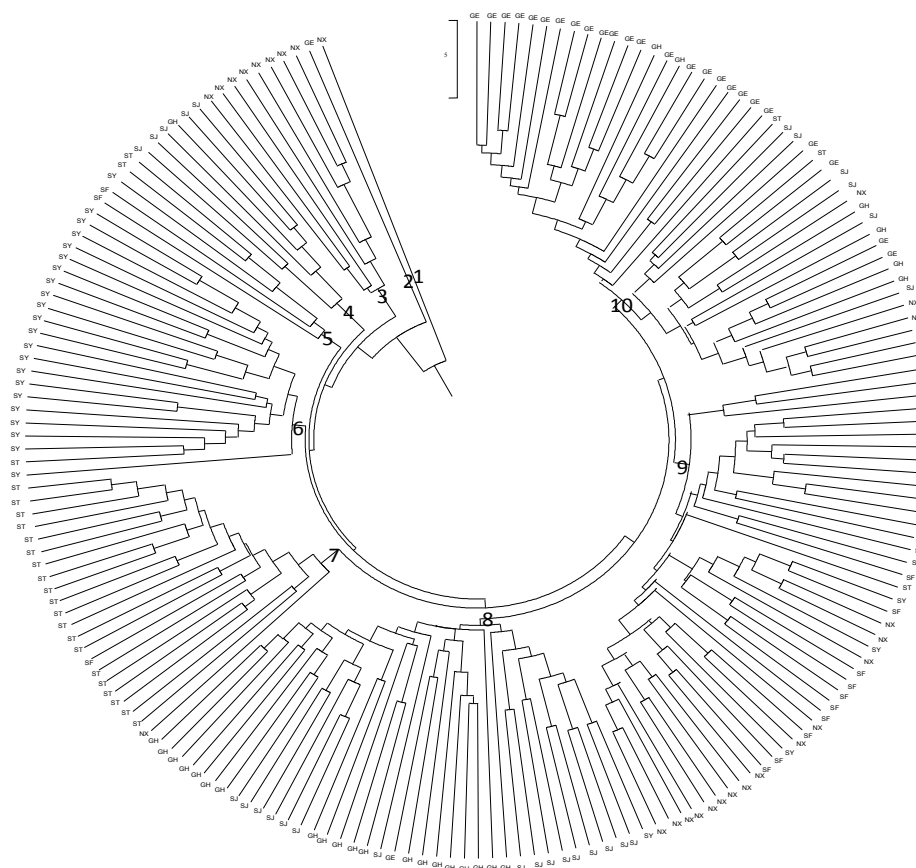


Figure 5. Cluster of all accessions from the seven populations of *P. davidiana*

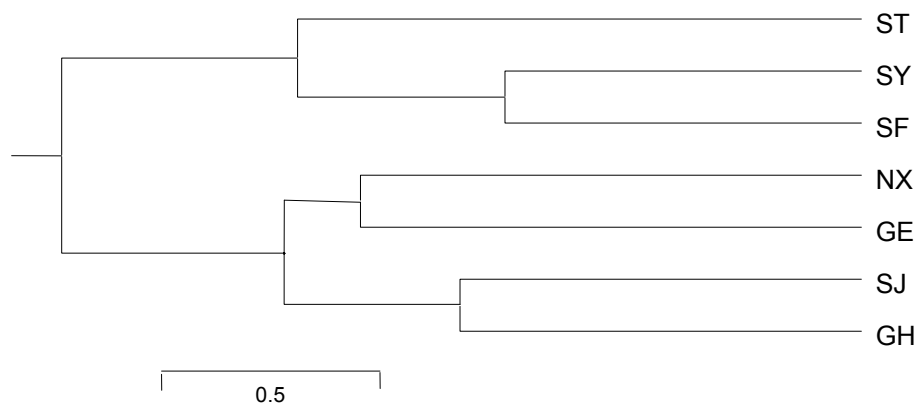


Figure 6. Cluster of the seven populations of *P. davidiana*

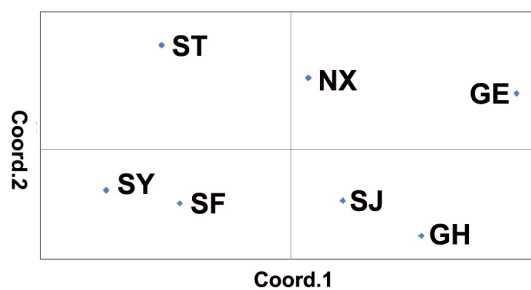


Figure 7. Rincipal Component Analysis (PCA) of the seven populations of *P. davidiana*