Quality Assurance and Safety of Crops & Foods, 2014; 6 (4): 445-452

Wageningen Academic Publishers

The use of principal component analyses and hierarchical cluster analyses in the quality evaluation of *Salvia miltiorrhiza* Bunge

Y.Y. Jiang[1], G.C. Zhong[1], L. Wang[2], T. Wang[1], M. Wang[1], L. Zhang[1*], Y.H. Zhou[2], C.B. Ding[1], R.W. Yang[1] and X.L. Wang[1]

[1]*Sichuan Agricultural University, College of Life and Basic Science, 46 Xinkang Road, Yaan, Sichuan 625014, China P.R.;* [2]*Sichuan Agricultural University, Triticeae Research Institute, 211 Huimin Road, Wenjiang, Sichuan 611130, China P.R.; zhang8434@sina.com*

Received: 11 April 2013 / Accepted: 26 July 2013

RESEARCH ARTICLE

Abstract

Principal component analysis (PCA) and hierarchical cluster analysis (HCA) are quite useful chemometrics tools for the data analysis in many fields. In China P.R., *Salvia miltiorrhiza* Bunge is a traditional medicinal plant. Due to the unique action of its biologically active compounds in treating cardiovascular disease, *S. miltiorrhiza* was planted in many regions as the local economic crop. The roots, which are also called 'Danshen', were the original economical products. In this study, PCA and HCA were used to determine the quality of Danshen from two aspects, namely, agronomic traits and active compounds. PCA revealed that the first principal component cumulatively accounted for 50.1% of the total variance in the data set, while PC2 and PC3 explained 20.0 and 13.1%, respectively. The quality descending order of all samples obtained by PCA was as follows: tissue culture lobular Danshen, tissue culture broad leaf Danshen, short stalk and lobular leaf Danshen, broad leaf Danshen, lobular Danshen, and white leaf Danshen. Samples were classified into three main groups by HCA. Therefore, based on the provisions in the Chinese pharmacopoeia about the content of tanshinone IIA, ZD was considered the best line for the farmers. The research showed that PCA and HCA also could be used in the quality evaluation of medicinal crops.

Keywords: hierarchical cluster analyses, principal component analyses, *Salvia miltiorrhiza*

1. Introduction

With the development of modern computer techniques, more and more chemometric tools are being used for characterisation, and determination of geographic origin, and quality control has recently become a very active research area (Patras *et al.*, 2011). Some authors have attempted to use these tools to monitor or evaluate their experiments (Aouidi *et al.*, 2012; Kunal and Gopinath 2009). Principal component analysis (PCA) is a multivariate statistical analysis technique which is often used to approximate data vectors that have many elements with a new set of data vectors that has fewer elements, while retaining most of the variability and information of the original data (Kara *et al.*, 2007). Hierarchical cluster analysis (HCA) refers to a set of analytical procedures that reduce complex multivariate data into smaller subsets or groups. Namely, it is a process to classify all the data into different groups and the data in one group can be considered similar (Leonard and Droege, 2008). PCA and HCA are among the most popular multivariate exploratory methods allowing for a relatively simple representation of similarities between samples on the basis of more-or-less complex analytical data. Moreover, some authors have used them to try and obtain a comprehensive understanding of research (Lima *et al.*, 2010; Patras *et al.*, 2011).

Cardiovascular diseases and their thrombotic complications are the leading cause of morbidity and mortality in developed countries and some developing countries. The World Health Organization indicated that the socio-economic burden would escalate in the future because of an ageing population and increased survival which produces more chronic cardiovascular patients. *Salvia miltiorrhiza* Bunge, a medicinal crop in China P.R., belongs to *Salvia* Linn and its roots are called 'Danshen' in Chinese pharmacopoeia and are used as a traditional Chinese herbal medicine. Its curative functions include invigorating and cooling the blood, removing stagnation, clearing heat in the heart, reducing carbuncles, and soothing irritability (China Pharmacopoeia Committee, 2010). The major active compounds are quinoid diterpenes called tanshinones and several phenolic acids (Matkowski *et al.*, 2008). According to modern investigations, the most important and frequent clinical application of the herb is the treatment of cardiovascular diseases, including angina pectoris, coronary artery spasm, myocardial infarction, and others (Cheng 2006). It has been used in numerous finished products, such as the Danshen dripping pill, either in mono or combined preparations. Due to its pharmacological importance, it has also been used in Western medicine (Li *et al.*, 2009; Liu *et al.*, 2007) and more importantly, the compound Danshen dropping pill has been through the American Food and Drug Administration II clinical trial.

In view of current market developments, the demand for them has increased steadily in recent years (He *et al.*, 2010; Liu *et al.*, 2011), and the increasingly stringent quality requirements of *S. miltiorrhiza* must be adhered to. However, sometimes only the amounts of effective ingredients in medicine plants were taken as the targets to make a quality evaluation (Li *et al.*, 2003, 2008; Shi *et al.*, 2006). Researchers ignored the fact that the characteristics of shape, colour, smell, and taste should also be considered, because they are closely related to the quality of a medicine crop (Wan, 2007). Wang *et al.* (2007) indicated that in the quality evaluation of a medicinal crop, the exclusive use of active compounds or target compounds contents as referenced is not reliable. Thus, in the present study, PCA and HCA were used to gain insights into the quality of *S. miltiorrhiza* based on agronomic traits and active compounds for the better development of the medicinal crop.

2. Materials and methods

Plant materials, instrumentation, and reagents

Materials that were included in the study, long stalk and broad leaf Danshen (Gaogan Daye; GD), short stalk and lobular leaf Danshen (Aigan Daye; AD), lobular Danshen (Xiaoye; XY), tissue culture broad leaf Danshen (Zupei Daye; ZD), tissue culture lobular Danshen (Zupei Xiaoye; ZX) and white leaf Danshen (Baiye; BY), were authenticated by Professor Yonghong Zhou of Triticeae Research Institute, Sichuan Agricultural University. All materials were planted in Zhongjiang County, Sichuan Province in China P.R. which is regarded as a genuine production area for *S. miltiorrhiza* and has been a planting site for centuries for this local economical crop. Meanwhile, tissue culture broad leaf Danshen (ZD) and tissue culture lobular Danshen (ZX) were propagated by tissue culture of short stalk and broad leaf Danshen (AD) and lobular Danshen (XY), respectively, and then also planted in Zhongjiang County. They were planted in April 2011, and harvested in January 2012. The cultivation management of materials was conducted according to the standardisation technical regulation for *S. miltiorrhiza* in Zhongjiang County.

A Shimadzu LC-10AVP high-performance liquid chromatography (HPLC) system (Shimadzu Technologies, Kyoto, Japan) equipped with online vacuum degasser, binary pump, autosampler, thermostated column compartment, and an ultraviolet detector, and Shimadzu Technologies Chemstation software for LC (1.24 SP1) were used. HPLC separation was performed on a Phenomenex Shim-pack-C18 column (5 μm, 250 mm × 4.60 mm i.d.; Phenomenex, Torrance, CA, USA).

Authentic standards of tanshinone IIA (TS-IIA) and salvianolic acid B (SA-B) were purchased from the National Institute for Control of Pharmaceuticals and Biological Products (Beijing, China P.R.). HPLC-grade acetonitrile and methanol were purchased from Fisher Scientific (Pittsburgh, NJ, USA). Deionised water was purified by Milli-Q system (Millipore, Bedford, MA, USA). All the other chemicals, reagents, and solvents used were of analytical grade.

Investigation of agronomic traits

After harvest, 20 plants of each line were selected randomly; their roots were dried in the shade and the skin colour (SKC), section colour (SC), and lignification level (LF) were described according to the standards presented in Table 1.

At the same time, 20 plants of each line were selected randomly and weighed respectively. The fresh roots were classified by diameter (<1 cm and >1 cm) (Shu *et al.*, 2007;

Table 1. Assignment standards of skin colour (SKC), section colour (SC) and lignification level (LF).

Variables	Characteristic	Value
SKC	Bright red	15
SKC	Dark red or brown red	10
SKC	Others	5
SC	Purple-brown	15
SC	Incanus or yellowish-white	10
SC	Others	5
LF	No	15
LF	Sporadic	10
LF	Yes	5

Zhang *et al*., 2002). The same class of each line was mixed, dried in the shade, and then the dry weight was taken. The average fresh weight per plant (FW), average dry weight per plant (DW), average drying rate per plant (DR), and average superior rate per plant (SR) of each line were calculated based on Equations 1-4.

$$FW = \text{total yield of fresh roots}/20 \quad (1)$$

$$DW = \text{total yield of dried roots}/20 \quad (2)$$

$$DR = \text{total yield of dried roots}/\text{total yield of fresh roots} \quad (3)$$

$$SR = \text{the yield of dried roots (diameter >1 cm)}/\text{total yield of dried roots} \quad (4)$$

Quantitative analysis of active compounds

The extracted method was used that is described in China Pharmacopoeia Committee (2010). The dried roots of the six lines of *S. miltiorrhiza* were comminuted and passed through 60-mesh sieves. After accurately weighing 0.3 g of the sample powder, samples for detection of TS-IIA were refluxed for 1 h with methanol as solvent, respectively. Methanol contributed to the final volume. Meanwhile, samples for detection of SA-B were extracted for 4 h with deionised water as solvent using the method of reflux extraction, respectively. Deionised water was added to the final volume. The final sample solutions were all filtered through a membrane (0.45 μm), and then 10 μl of each sample was injected into the HPLC system.

The chromatographic condition of TS-IIA: the mobile phase included a mixture of methanol and deionised water (75:25). The chromatographic separation was carried out using an isocratic elution. The flow rate was kept at 1.0 ml/min. Column temperature was maintained at 40 °C. The sample injection volume was 10 μl and the detection wavelength was set at 270 nm.

The chromatographic condition of SA-B: the mobile phase was a mixture of methanol, acetonitrile, formic acid and deionised water (30:10:1:59). Chromatographic separation was carried out using an isocratic elution. The flow rate was kept at 1.0 ml/min. Column temperature was maintained at 25 °C. The sample injection volume was 10 μl and the detection wavelength was set at 288 nm.

Data analysis

PCA is a data visualisation method that is useful for observing groupings within multivariate data (Dharmara *et al*., 2006) by reducing the dimensionality of the multivariate problem. The first process is to transform the original variables into a number of new and uncorrelated variables called principal components (PCs) (Mebatsion *et al*., 2012). Each principal component describes a percentage of the total variance of a data set and elaborates loadings or weights that each variable contributes to this variance. The coefficients of the principal components quantify the loading or weight of each variable to that amount of variance (Kara *et al*., 2007). In this paper, the use of PCA for the quality of six lines of *S. miltiorrhiza* was explained.

HCA calculates the distances (or correlation) between all samples using a defined metric such as Euclidean distance or Manhattan distance (Berrueta *et al*., 2007). All data were analysed by PCA and HCA using the software IBM SPSS Statistics version 19 (New York, NY, USA).

3. Results and discussion

Values of agronomic traits and active compounds

Agronomic traits and active compounds were considered to evaluate the quality of the six lines of *S. miltiorrhiza*. The values for them are shown in Table 2 and 3.

However, it is generally acknowledged that samples have different characteristics that make them suitable for various applications. The one with bright red skin colour, purple-brown section colour, and no lignifications is fit for traditional Chinese crude medicine. It is considered to have the best quality (China Pharmacopoeia Committee, 2010). The results (Table 2) indicated that ZX, ZD, GD, and AD were the best in skin colour, section colour and lignification level, whereas BY, which was greyish-white in colour and full of lignifications, was the worst. In addition, XY was also full of lignifications.

In Table 3, we found that ZX had the highest fresh weight (485.0 g/plant), dry weight (110.8 g/plant), and superior rate (42.0%), but the drying rate (22.8%) was lower than that of ZD, which had the highest drying rate (27.7%). Meanwhile, BY had the lowest fresh weight (237.5 g/plant) and superior rate (16.0%). The dry weight (49.00 g/plant) and drying rate (14.9%) of GD were the lowest. The fresh weight of XY, GD,

Table 2. Observed results of skin colour (SKC), section colour (SC) and lignification level (LF) of different *Salvia miltiorrhiza* lines.

Lines	SKC	SC	LF
Zupei Xiaoye	Bright red	Purple-brown	No
Xiaoye	Bright red	Purple-brown	Yes
Zupei Daye	Bright red	Purple-brown	No
Baiye	Yellowish-white	Yellowish-white	Yes
Gaogan Daye	Bright red	Purple-brown	No
Aigan Daye	Bright red	Purple-brown	No

Table 3. Values of agronomic traits and active compounds of different *Salvia miltiorrhiza* lines.[1]

Lines[2]	SKC	SC	LF	FW (g/plant)	DW (g/plant)	DR (%)	SR (%)	TS-IIA (mg/g)	SA-B (mg/g)
ZX	15	15	15	485.01±1.12	110.81±0.21	22.80±0.32	42.12±0.67	1.732±0.021	77.93±0.076
XY	15	15	5	311.50±0.54	53.80±0.07	17.31±0.12	40.01±0.98	3.035±0.097	119.7±0.121
ZD	15	15	15	372.51±1.20	103.01±0.11	27.72±0.08	36.04±0.12	2.233±0.076	116.0±0.094
BY	5	10	5	237.52±0.91	55.10±0.97	23.21±0.32	16.01±0.07	1.405±0.089	89.69±0.007
GD	15	15	15	329.02±1.63	49.00±0.23	14.92±0.16	33.02±0.51	2.735±0.044	106.4±0.011
AD	15	15	15	308.53±0.72	74.00±0.87	24.01±0.06	38.03±0.19	3.896±0.056	125.0±0.024

[1] DR = drying rate; DW = dry weight; FW = fresh weight; LF = lignification level; SA-B = salvianolic acid B; SC = section colour; SKC = skin colour; SR = superior rate; TS-IIA = tanshinone IIA.
[2] AD = Aigan Daye; BY = Baiye; GD = Gaogan Daye; XY = Xiaoye; ZD = Zupei Daye; ZX = Zupei Xiaoye.

and AD were 311.5, 329.0, and 308.5 g/plant, respectively, which showed an insignificant difference, but they were all lower than that of ZD (372.5 g/plant) and ZX (485.0 g/plant).

At the same time, the data indicated that the fresh weight and drying weight of ZX were 55.7 and 105.9%, respectively, higher than those of XY. The drying rate of ZX was 31.8% higher than that of XY. The difference between ZX and XY in terms of superior rate was not obvious compared with the differences in fresh weight, drying weight, and drying rate. The fresh weight of ZD was 13.2 and 20.7% higher than that of GD and AD, respectively. The drying weight of ZD was 110.2 and 39.2% higher than that of GD and AD, respectively. The drying rates of ZD were 85.9 and 15.4% higher than those of GD and AD, respectively. The difference among ZD, GD, and AD in superior rate was not obvious compared with the differences in fresh weight, drying weight, and drying rate. The results showed that the lines obtained by tissue cultures were successful in terms of the agronomic traits that have significant economic values for farmers.

Moreover, the current Chinese pharmacopoeia requires the amount of SA-B and TS-IIA in Danshen to exceed 30 and 2 mg/g, respectively (China Pharmacopoeia Committee, 2010). The results (Table 3) indicated that all lines except ZX and BY have the required amount of TS-IIA, values ranging from 1.405 to 3.896 mg/g. TS-IIA content decreased in the following order: AD>XY>GD>ZD>ZX>BY. The amount of SA-B of all samples were according the provisions of China Pharmacopoeia Committee (2010)and the values ranged from 77.93 to 119.7 mg/g. SA-B content decreased in the following order: AD>XY>ZD>GD>BY>ZX. But, the higher levels of effective component do not necessarily equate to a better quality of sample. So, PCA and HCA were performed to gain an overview of the similarities and differences among the six lines *S. miltiorrhiza* and to investigate the quality of them.

Principal component analysis

PCA was applied to the raw adsorption data given in Table 3 to view the relationship between the studied samples and variables, and the influence on quality of samples taken by variables. Before analysis, the data were normalised as mentioned earlier. PCA for variables indicated that the first principal component (PC1) explained 50.1% of the total variance in the data set while PC2 and PC3 explained 20.0 and 13.1%, respectively. PC1 had a loading of 0.966 to SKC and SC, and a loading of 0.945 to SR. PC2 had a loading of 0.776 to DW, and PC3 had a loading of 0.805 to DR. The loading plots for sample and variables are shown in Figure 1A, B and C and a number of observations may be made.

Firstly, Figure 1A revealed that ZX and ZD were loaded in the upper part of the plot, i.e. the two lines had a positive score on PC2; while GD, BY, AD and XY were loaded in the lower part of the PC2 zero part, i.e. they had a negative score on PC2. The locations of ZX and ZD may be explained by their high FW and DW values (Table 3). Interestingly, ZX was located some distance away from the others, indicating that its composition differs from the other samples, at least in terms of some of the analytes measured. The reason may be its lower values of TS-IIA and SA-B (Table 3) which were co-located in this region of the PC space (Figure 1B). The close distance between XY and AD may be caused by the high values of TS-IIA and SA-B (Table 3). Figures 1B and 1C illustrate the relationships between the parameters studied in the present work. SC and SKC were overlapped on the plots, which indicated they were highly correlated as shown in Table 4. Moreover, DR, DW, FW and LF were located on the upper right-hand quadrant of Figure 1B, with the exception of SR, SC, SKC, TS-IIA and SA-B. FW, DW and DR were found in opposition to TS-IIA and SA-B as evidenced by their Pearson correlation coefficients (Table 4). In Figure 1C, DR, TS-IIA, SA-B, DW and LF were located on the upper right-hand quadrant, but, SR, SC, SKC and FW were situated on the lower right-hand quadrant. More

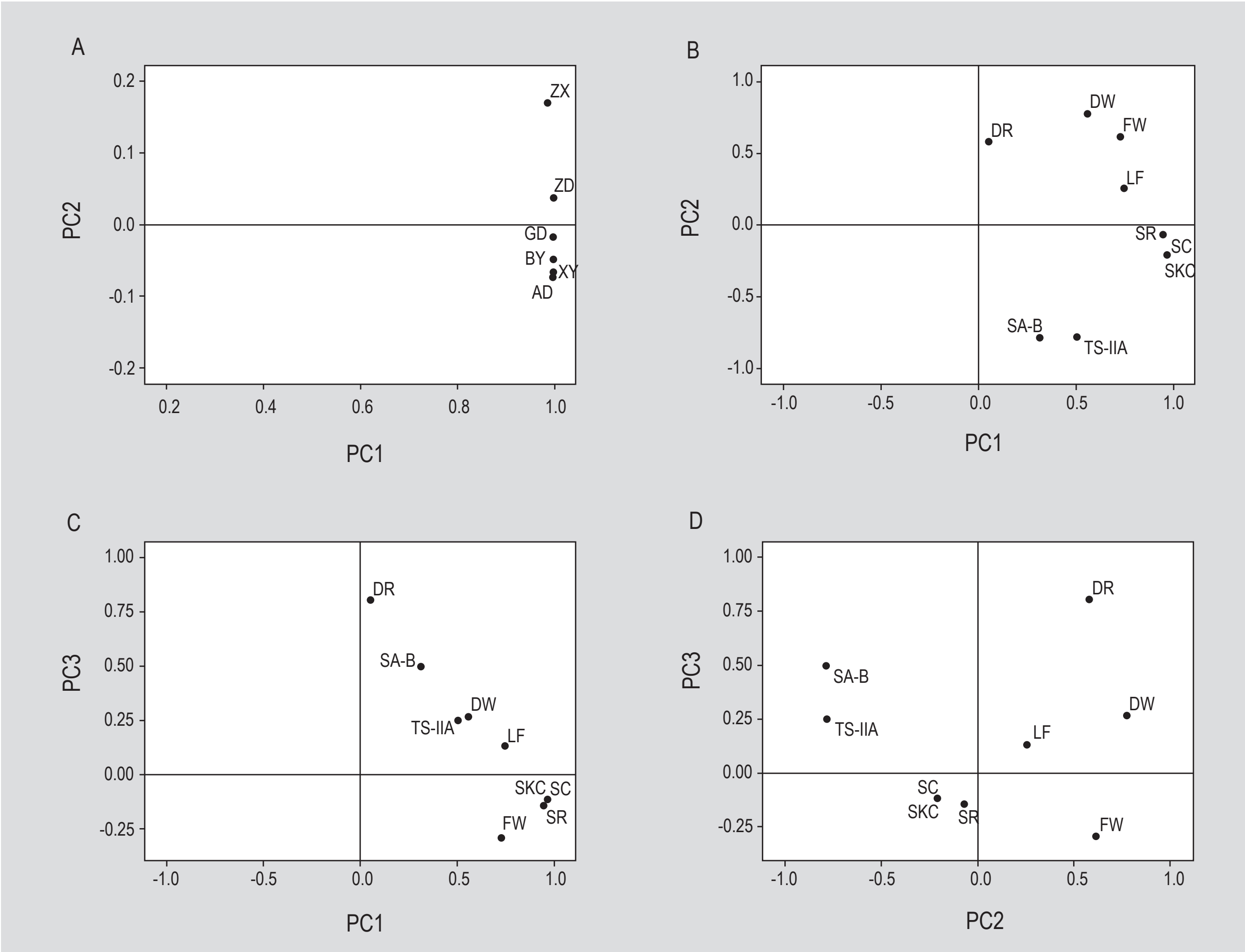

Figure 1. Principal component analysis (PCA) plots. (A) Loading plot for samples. (B) PCA loading plot for different variables on PC1 and PC2. (C) Loading plots for different variables on PC1 and PC3. (D) Loading plots for different variables on PC2 and PC3.

observations were quite apparent from Figures 1B and 1C. SCK, SC and SR are clustered together on the bottom right-hand side of the plots, as SCK, SC and SR were significantly correlated (Table 4). In addition, DR was removed from the others, which may be explained by the result in Table 4 showing that DR has a negative correlation with SKC, SC and SR.

To understand more about the relationship between the different variables, the loading plot about PC2 and PC3 was illustrated (Figure 1D). The locations of indexes which represent agronomic traits were not clustered together. The phenomenon may indicate that the investigation indexes were independent and influence each other, and importantly, they all influenced the quality of samples. The quality descending order of all samples obtained by PCA was as follows: ZX, ZD, AD, GD, XY, and BY.

Hierarchical cluster analysis

In hierarchical cluster analysis, samples were grouped based on similarities, without considering the information on class membership. It was interesting to observe what kind of classifications can be made solely on the basis of distances (Patras *et al.*, 2011). Following HCA, the results obtained were shown in the form of a dendrogram (Figure 2) in which three defined clusters were visible at 10 distances. The first cluster (A) was composed of ZX and ZD. The second cluster (B) included GD, AD and XY. It is important to note that, in cluster B, GD and AD was clustered first, and then clustered with XY; this may be explained by XY's lower value of LF. And the result was in accordance with the PCA showing that the distance between XY and AD is small. The third cluster (C) consisted of BY alone, which may be the reason for the low values in SKC, SC, LF, FW, SR, TS-IIA and SA-B.

Moreover, cluster A and cluster B were well separated due to variations in the values of FW, DW, and TS-IIA. And FW and DW were at a close distance in Figure 1C.

Table 4. Inter-correlation of analysed parameters of *Salvia miltiorrhiza*.

Variable[1]	SKC	SC	LF	FW	DW	DR	SR	TS-IIA	SA-B
SKC	1.000								
SC	1.000**	1.000							
LF	0.632	0.632	1.000						
FW	0.608	0.61	0.617	1.000					
DW	0.351	0.351	0.574	0.82*	1.000				
DR	-0.162	-0.162	0.231	0.168	0.708	1.000			
SR	0.944**	0.944**	0.506	0.710	0.472	-0.075	1.000		
TS-IIA	0.592	0.592	0.243	-0.184	-0.263	-0.226	0.502	1.000	
SA-B	0.427	0.427	0.046	-0.400	-0.292	-0.034	0.284	0.841*	1.000

* Significant at P<0.05; ** significant at P<0.01.

[1] DR = drying rate; DW = dry weight; FW = fresh weight; LF = lignification level; SA-B = salvianolic acid B; SC = section colour; SKC = skin colour; SR = superior rate; TS-IIA = tanshinone IIA.

Moreover, it was interesting to compare the dendrogram with the loading plot in Figure 1A. In both of these figures, ZD and ZX were in one cluster. And in HCA, the other four lines were also separated which differed from PCA. But, in other words, the HCA and the PCA visualisations could not produce opposite conclusions. Maybe, the HCA could capture chemical similarities more efficiently from a visual perspective than the PCA (Patras *et al*., 2011), but PCA supplied more information about the interaction of the considered variables.

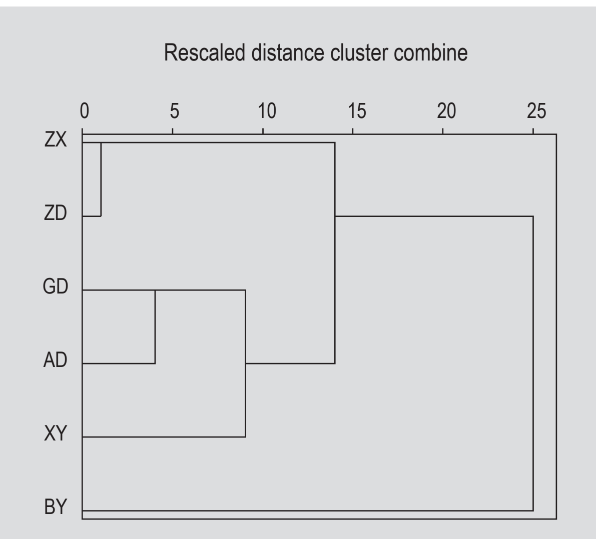

Figure 2. Dendrogram of hierarchical cluster analysis for samples. AD = Aigan Daye; BY = Baiye; GD = Gaogan Daye; XY = Xiaoye; ZD = Zupei Daye; ZX = Zupei Xiaoye.

4. Conclusions

Considerable variations were observed among the six lines in terms of agronomic traits and active compounds. The quality ranking orders obtained by PCA for the samples were: ZX, ZD, AD, GD, XY, and BY. HCA clustered the samples into three groups. In detail, ZX and ZD which were clustered into one group were at relatively higher levels of SKC, SC, FW and DW. The second cluster GD, AD and XY had high contents of TS-IIA and SA-B. In addition, BY which was in one group only had lower levels of SKC, SC, FW and TS-IIA. So, based on the provisions in CPA about the content of TS-IIA, ZD was considered the best line for the farmers.

Moreover, the application of PCA and HCA has accomplished a careful evaluation of the identified variables in which their strong and weak points were detected. This is the first report documenting the use of PCA and HCA to conduct the quality evaluation of Danshen (the root of *S. miltiorrhiza*) based on its agronomic traits and active compounds. The information contributed by this study would be useful for the further quality evaluation of other medicinal crops using PCA and HCA.

Acknowledgements

This work was financially supported by the science and technology department of Sichuan province, China P.R. (2011ZO0006, 2012ZR0050, 2012NZ0020), the funding programme of technological innovation for small and medium-sized sci-tech enterprises, Chengdu (11CXYB958JH-003), the programme of innovation fund for small technology-based firms, China P.R. (12C26215105863) and agriculture science technology achievement transformation fund of Sichuan Agriculture University.

References

Aouidi, F., Dupuy, N., Artaud, J., Roussos, S., Msallem, M., Perraud-Gaime, I. and Hamdi, M., 2012. Discrimination of five Tunisian cultivars by mid infrared spectroscopy combined with chemometric analyses of olive *Olea europaea* leaves. Food Chemistry 131: 360-366.

Berrueta, L., Alonso-salces, R. and Héberger, K., 2007. Supervised pattern recognition in food analysis. Journal of Chromatography A 1158: 196-214.

Cheng, T.O., 2006. Danshen: a versatile Chinese herbal drug for the treatment of coronary heart disease. International Journal of Cardiology 113: 437-438.

China Pharmacopoeia Committee, 2010. Pharmacopoeia of the People's Republic of China. Peoples Medicinal Publishing House, Beijing, China P.R., pp. 70.

Dharmara, S., Hossain, M., Zhari, S., Harn, G.L. and Ismail, Z., 2006. The use of principal component analysis and self-organizing map to monitor inhibition of calcium oxalate crystal growth by orthosiphon stamineus extract. Chemometrics and Intelligent Laboratory Systems 81: 21-28.

He, C.E., Wei, J.H., Jin, Y. and Chen, S.L., 2010. Bioactive components of the roots of *Salvia miltiorrhizae*: changes related to harvest time and germplasm line. Industrial Crops and Products 32: 313-317.

Kara, S., Güven, S. and Üven, S., 2007. Classification of macular and optic nerve disease by principal component analysis. Computers in Biology and Medicine 37: 836-841.

Kunal, R. and Gopinath, G., 2009. QSTR with extended topochemical atom (ETA) indices. 12. QSAR for the toxicity of diverse aromatic compounds to *Tetrahymena pyriformis* using chemometric tools. Chemosphere 77: 999-1009.

Leonard, S.T. and Droege, M., 2008. The uses and benefits of cluster analysis in pharmacy research. Research in Social and Administrative Pharmacy 4: 1-11.

Li, M.H., Chen, J.M., Peng, Y., Wu, Q.L. and Xiao, P.G., 2008. Investigation of Danshen and related medicinal plants in China. Journal of Ethnopharmacology 120: 419-426.

Li, L., Li, X.C., Wang, X.R. and Wang, S.W., 2003. Development of HPLC finger print barcode technique for authentication and quality assessment of radix *Salvia miltiorrhiza*. Chinese Traditional and Herbal Drugs 34: 649-653.

Li, Y.G., Song, L., Liu,M., Hu, Z.B. and Wang, Z.T., 2009. Advancement in analysis of *Salviae miltiorrhizae* Radix et Rhizoma (Danshen). Journal of Chromatography A 1216: 1941-1953.

Lima, D., Santos, A., Araujo, R., Scarminio, I., Bruns, R. and Ferreiraa, S., 2010. Principal component analysis and hierarchical cluster analysis for homogeneity evaluation during the preparation of a wheat flour laboratory reference material for inorganic analysis. Microchemical Journal 95: 222-226.

Liu, Y.R., Qu, S.X., Maitz, M.F., Tan, R. and Weng, J., 2007. The effect of the major components of *Salvia miltiorrhiza* Bunge on bone marrow cells. Journal of Ethnopharmacology 111: 573-583.

Liu, F.Y., Wang, X.D., Wang, S.H., Zou, Z.R. and Liang, Z.X., 2011. Effect of drought stress on growth and accumulation of active constituents in *Salvia miltiorrhiza* Bunge. Industrial Crops and Products 33: 84-88.

Matkowski, A., Zielinńska, S., Oszmianński, J. and Lamer-zarawska, E., 2008. Activity of extracts from leaves and roots of *Salvia miltiorrhiza* Bunge, *S. przewalskii* Maxim., and *S. verticillata* L. Bioresource Technology 99: 7892-7896.

Mebatsion, H.K., Paliwal, J. and Jayas, D.S., 2012. Evaluation of variations in the shape of grain types using principal components analysis of the elliptic Fourier descriptors. Computers and Electronics in Agriculture 80: 63-70.

Patras, A., Brunton, N., Downe, G., Rawson, A., Warriner, K. and Gernigon, G., 2011. Application of principal component and hierarchical cluster analysis to classify fruits and vegetables commonly consumed in Ireland based on *in vitro* antioxidant activity. Journal of Food Composition and Analysis 24: 250-256.

Shi, S.H., Wang, Q., Yuan, L.C. and Xu, H., 2006. Constituent determination and quality evaluation on resource plant species of *Salvia* L. in Yunnan. Journal of Plant Resources and Environment 15: 73-74.

Shu, Z.M., Liang, Z.S., Sun, Q., Ye, Z.L., Zhang, X.W. and Fu, L.L., 2007. Comparison and evaluation of biological traits in different *Salvia miltiorrhiza* germplasm. Journal of Xi'an University of Arts and Science 10: 24-29.

Wan, D.G., 2007. The varieties quality and potency of traditional Chinese herb. Shanghai Scientific and Technical Publishers, Shanghai, China P.R., pp 59.

Wang, W.Q., Peng, J., Wan, J., Fang, J.G., Tang, J. and Liu, Y.H., 2007. Chemical pattern recognition of quality assessment of *Folium isatidis* from various habitats. Chinese Traditional and Herbal Drugs 38: 921-925.

Zhang, X.G., Wang, Y.M., Luo, G.A. and Cheng, F.S., 2002. Studies on resource characteristics of *Salvia miltiorrhiza* varieties. Chinese Traditional and Herbal Drugs 33: 742-747.