SRAP Markers and Morphological Traits Could Be Used in Test of Distinctiveness, Uniformity, and Stability (DUS) of Lettuce (*Lactuca sativa*) Varieties

Lijuan Liu, Zaochang Liu (Corresponding author), Hairong Chen, Liguo Zhou

Yunhua Liu & Lijun Luo (Corresponding author)

Shanghai Agrobiological Gene Center, Shanghai 201106, China

Tel: 86-21-6220-2914　　　E-mail: lzc@sagc.org.cn; lijun@sagc.org.cn

Abstract

The test of distinctiveness, uniformity, and stability (DUS) is a necessary step for variety identification and new variety application. The objective of this study is to provide molecular marker-assisted approach combined with morphological trait-based testing for more convenient and fast DUS test and identification of varieties. Eighteen pairs of SRAP markers and 40 morphological traits for DUS test were applied for genetic diversity analysis of 50 lettuce (*Lactuca sativa*) varieties. Average polymorphism information content (PIC) of the SRAP markers was 0.80, ranging from 0.39 to 0.97. Cluster analysis using UPGMA of the band patterns amplified by SRAP marker and morphological trait-based clustering separated the varieties into three groups. The correlation coefficient of SRAP marker and morphological traits was 0.5455 reflecting that the two clustering results shared some similarity and consistence. It revealed that the combination of both SRAP marker and morphological trait analysis is more conducive to proper identification and classification of plant varieties, which will undoubtedly bring an alternative choice to DUS testing of plant new varieties and conservation of plant germplasm.

Keywords: DUS test, Genetic diversity, Lettuce (*Lactuca sativa*), Morphological traits, SRAP

1. Introduction

Lettuce (*Lactuca sativa*) is an annual or biennial herb belonging to the genus *Lactuca* Linn, of the family Asteraceae. It is comparatively rich in minerals and vitamins, with high nutritional value (Jiang, 2007). In recent years, the utilization of lettuce including those imported from abroad, and the progress of lettuce breeding have made germplasm resources in China becoming much more diversified, which have laid a solid foundation for innovation of lettuce varieties. But at the same time, it also poses new challenges for new variety application and variety identification and protection for lettuce.

Founded in 1961, the Union for Protection of New Varieties of Plants (UPOV) provides a lot of guidelines and standards for dealing with matters of economically useful plant species. The test for distinctiveness, uniformity, and stability (DUS) is one major part of the guidelines and standard provided by UPOV which laid the foundation for the testing and protection of new varieties. China in 1999 became the organization's 39th member states, making the regulations on the protection of new varieties of plants an official practice in China (Li and Li, 2003; Kwon et al., 2005).The traditional system of DUS testing technique basically tests morphological traits, including the choice of standard unified morphological characteristics or agronomic traits and the order of testing technology, as well as the database based on these traits. The morphological trait-based testing is a time-consuming process, requiring large areas of land for planting and easily subjecting to environmental impact (Cooke, 1995; Van Beuningen and Busch,1997), while the testing based on genome DNA fingerprinting technique is relatively simple and accurate, which could identify the difference of species that are difficult to be indentified by phenotypes (Wiel, 1999**).**

There are a variety of DNA marker techniques, such as random amplified polymorphic sequence (RAPD) (Juchum et al., 2007; Ro et al., 2007), amplified restriction fragment polymorphism (AFLP) (Percifield, 2007; Yuan et al., 2007) and microsatellite markers (SSR) (Legesse et al., 2007; Tommasini et al., 2003). They are

widely applied in species or varieties identification, evaluation of genetic diversity, but few reports are available on systematically comparative analysis of two kinds of testing approaches, such as morphological method and molecular marker method. Comprehensive analysis with morphological and DNA molecular markers helps to overcome the shortcomings of morphological markers, improve DUS testing system, and to ensure the authorized objectiveness and impartiality and accuracy of plant new variety test , which plays an important role in the protection of intellectual property rights of new varieties of plants (Zhang et al., 2000).

In this study, based on morphological characteristics of DUS testing techniques in lettuce, combined with sequence-related amplified polymorphism (SRAP) markers (Li and Quiros, 2001), comparative analysis of genetic relationship on 50 different types of lettuce varieties was carried out which aimed at identification and innovation of lettuce germplasm resources, and at providing a scientific basis for molecular marker-assisted breeding in lettuce as well as providing the necessary reference for further improvement of the DUS testing technology.

2. Materials and methods

2.1 Materials and chemical reagents

Fifty varieties of lettuce used in this study were provided by Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences (Table 1). Geographical distribution of these materials almost covers whole mainland of China.

SRAP markers were synthesized by Shanghai Sangon Biological Engineering Technology Company. Tag- DNA polymerase, dNTPs, $MgCl_2$, 10×Reaction PCR Buffer were purchased from Shanghai Shenergy Bioscience & Technology Co., Ltd, China.

2.2 Methods

2.2.1 SRAP marker testing

The seeds were sterilized, sowed in Petri dishes and kept in refrigerator at 4 ℃ for 2-3 days, and then transferred to 25 ℃ culture incubator until plants grow to the three-leaf stage. Genome DNA was extracted from lyophilized leaf tissues by 2% CTAB and precipitated by 1.5 volume of 1% CTAB according to the method by Murry and Thompson with modification (1980).

Primers were designed as described by Li and Quiros (2001). The primers applied in this study were listed in Table 2. The PCR mixture consists of 50 ng of DNA template, 0.75 mmol of each primer, 1×Reaction Buffer, 2.0 mmol Mg^{2+}, 0.2 mmol dNTPs, 1U *Tag* polymerase.in a total volume of 20 μl. The amplification was carried out in four steps: pre-denature at 94℃ for 5 min, followed by 5 cycles of 1 min denature at 94℃,1min annealing at 35℃ and 1 min extension at 72℃, then 30 cycles of 1 min at 94℃, 1min at 51℃and 1 min at 72℃. And finally, a final step of extension at 72 °C for 7 min completed the SRAP-PCR amplification. The PCR products (2.5 μl) were separated by electrophoresis on a 6% denaturing acrylamide gel at 60 W constant power for 1h. Then remove the gel for silver staining (Calvert et al., 1995).

2.2.2 Morphological trait-based testing

The 50 varieties of lettuce were planted in the experimental fields by randomized block design with three replications. Lettuce plants were planted by 25 cm spacing in rows with 40 cm row spacing. In accordance with the DUS testing requirements for lettuce by UPOV, 40 traits had been tested (Table 3).

2.2.3 Data collection and analysis

The PCR amplified products were scored as 1 or 0 respectively for the presence or absence of bands across the genotypes to generate a binary matrix. The binary matrix was analyzed using the NTSYS-PC version 2.10 software to calculate the similarity values and to generate the phylogram. At the same time, polymorphism information content (PIC) was calculated from the formula (Smith et al., 1997).

$$PIC_i = 1 - \sum_j P^2_{ij}$$

While Pij is the frequency of the jth SRAP allele in clones or varieties (i).

In order to compare phenotypic data with the molecular marker analysis by SRAP, phenotypic data were classified into qualitative traits and quantitative traits. For qualitative traits, the presence and absence of the

characteristic traits were scored as 1 and 0, respectively. For quantitatively traits, the data were transformed to binary form matrix followed the recommendation by UPOV and the methods by Giancola et al. (2002).

Similarity coefficient was calculated using the software NTSYS-PC version 2.10 (Nei, 1973; Nei and Li, 1979). Cluster analysis was conducted on similarity using the unweighted pair group method on arithmetic averages (UPGMA). The correspondence between the morphology and SRAP-based similarity coefficient matrixes was tested on the basis of correlation analysis.

3. Results and analysis

3.1 Amplified polymorphism using SRAP maker

In the preliminary experiments, all 255 SRAP primer pairs (combinations of 17 forward primers and 15 reverse primers) were used to screen a set of 6 lettuce varieties differing in morphological traits, namely 'Xiang', 'Wanlvye', 'Wanziye', 'Boli', 'Cuiye', and 'Yuanyeqin'. Of the 255 primer pairs, 18 (7.06%) revealed best polymorphism in varieties of the different morphological lettuce types (data not shown). As a result, all the 50 testing varieties were examined for genetic polymorphism using by using these 18 primer pairs.

A total of 549 bands were amplified from the 18 primer pairs for the 50 lettuce varieties, with an average number of amplified bands over 30 for each primer pair. Of the 549 bands, 464 were polymorphic bands, with an average of 25 polymorphic bands per primer pair (Table 4). Polymorphism rate generated by different primer pairs varies from 60-92% with an average of 83.7%. The No.9 primer pair produced the most bands, reaching to 59 bands in which 53 were polymorphic bands (90%). These findings indicated that SRAP marker could generate higher polymorphism, suitable for analysis of genetic diversity.

PIC value (Polymorphic information content), is a correlation function of allele frequency and change in allele number, reflecting the degree of gene variation among different varieties (Smith et al., 1997). Based on Bostein's (1980) point of view, when PIC value of a loci is higher than 0.5, this loci is of high diversity. While PIC value falls between 0.25 and 0.5, the loci belongs to middle diversity loci. If PIC value of a loci is lower than 0.25, it is a low diversity loci. Thereafter, markers with higher PIC value possess higher power to identify varieties. In this study, the PIC values of selected SRAP markers were estimated at 0.393-0.975 with an average of 0.798, indicating that SRAP markers have high capacity to identify lettuce species and the testing varieties possess relatively high genetic diversity.

3.2 Analysis of genetic relationships between lettuce varieties using SRAP markers

The genetic similarity coefficient shows the genetic relationship between two varieties. The 18 polymorphic SRAP markers used in this study were able to distinguish 46 lettuce varieties tested. Only variety No. 29/31 and No. 38/39 did not show diversity by all these markers and could not be distinguished from each other. Similarity coefficient of the SRAP markers ranged from 0.39 to 0.97.

The cluster analysis on the basis of genetic correlation coefficient generated by SRAP markers classified the tested varieties into three main clusters (Fig. 1). All varieties in the first cluster（No.1, 2, 3, 4） and the third cluster（No.5, 9, 10, 49, 50）were leaf lettuce. Three varieties in the first cluster (No.2 Wanlvye, No.3 Wanziye, No4 Ziye) were from the same Longyan city in Fujian Province, while three varieties No. 9 Cuiye, No. 10 Biyu and No.50 Jieqiu were from the same Shanghai city. The 2nd cluster contained the most varieties of lettuce, including mainly 41 stem lettuce varieties

3.3 Cluster analysis based on phenotypic traits

Investigation of forty morphologic traits in the fifty lettuce varieties revealed significant genetic differences among the varieties. Similarity coefficient ranged from 0.44 to 0.93 (Fig.2), reflecting wide genetic variation. Except variety No.43 (Baipijitui) and No.48 (Liuye), all other varieties could be distinguished from each other. The lettuce varieties tested in this study were clustered into three main groups, which was basically the same as the clustering result by SRAP markers as to the numbers of main group. But there were also some differences between the SRAP markers and morphologic traits clustering results as to the specific varieties in each group if we compare the clustering results generated by these two methods in this study. Cluster one and cluster three by SRAP correspond to cluster one and cluster two by phenotypic traits (Fig. 1 and Fig. 2). Many varieties are common in the clusters by the two methods, but cluster one and two by phenotypic traits contain much more varieties than cluster one and three by SRAP.

In the first group, three of all the six varieties（No1, 5, 9, 10, 49, 50）were from Shanghai local region. Six of the thirteen varieties in the second group were leaf lettuce, clustered into sub-group Ⅱ-1, mainly from cities of

Fujian and Guangdong provinces, South-Eastern China. Another sub-groupⅡ-2 was stem lettuce, mainly from Anhui and Henan province, Eastern and Central China. The third group contained the most varieties, twenty one of the tested fifty varieties, all belonging to stem lettuce varieties. As a result, clustering analysis by morphological traits classified the tested varieties into stem and leaf lettuces according to the edible habits. Furthermore, varieties with near geography origin were clustered into a relatively minor range, reflecting the geographical differences in the ecological types of lettuces.

3.4 Correlation and comparison between SRAP markers and morphological traits

The comparison between SRAP marker and morphological trait-based clustering results indicated that the two kind of markers resulted in difference to some extend in genetic correlation coefficient between varieties (the similarity coefficient ranged from 0.44 to 0.93 for morphological traits compared with that for SRAP markers ranging from 0.89 to 0.99). But the result of clustering analysis as a whole was consistent between the phenotypic and SRAP methods. For those varieties with similar morphological traits, they were also clustered into the same group by SRAP markers. The correspondence between the morphological traits and the SRAP-based similarity coefficient matrices was tested in a correlation analysis (Fig.3). The correlation coefficient was 0.5255. The SRAP marker-based clustering of lettuce varieties showed similarity to the dendrogram topoloies of the morphological trait-based clustering, although there were some differences in the positioning of lettuce varieties in the sub-groups.

4. Discussion

There are many examples of successful application of molecular markers in genetic diversity analysis and cultivar identification. SRAP marker with the advantages of RAPD markers and AFLP markers is a relatively new type of molecular marker and is more suitable for application in practice because of its features such as simple, low-cost test, security and rich in polymorphism (Li and Quiros, 2001; Li and Zhang, 2005). Rich polymorphism of these features is the most important characteristics. Ferriol et al. (2004) found in their study that 11 pairs of SRAP primers in amplifying DNAs of 47 pumpkin materials produced 148 bands, of which 98 were polymorphic bands, with polymorphism rate of 66.2%. While in the study by Guo and Luo (2006), the polymorphism rate was even higher, being 80.88%. The result of polymorphism rate in our experiment was 83.7%, basically in the same trend with the previous studies.

SRAP markers with high polymorphism made its application in DUS testing and analysis of genetic diversity become possible. To different varieties with similar agronomic traits, traditional morphological markers were difficult to identify clearly, but SRAP markers made the discrimination among these varieties simple. In the present study, tested cultivars No. 43 (Baipijitui) and No. 48 (Liuye) could not be separated by morphological traits, but SRAP markers discriminated them well.   However, there were also varieties (Longtai and Woshun; Xwqing and Baipiyuan) that could not be distinguished from each other by the SRAP marker in this study. It was the same as in the study by Ferriol et al. (2003), which maybe caused by the small genetic differences between commercial cultivars.   Therefore, a more viable solution is to increase the number of SRAP markers.

The morphological traits are strongly affected by environmental condition which is often questionable in distinction and identification of the varieties. Furthermore now popularized hybrid varieties showed low variation at the genetic level which brings tremendous inconvenience for DUS test. The SRAP markers directly amplify genetic material-DNA and the results of amplification reflect the differences in genome level without anything to do with the external environmental conditions, greatly increasing the reliability and stability of the test results (Li and Quiros, 2001; Pan et al., 2005). These methods meet the basic requirements of DUS test.

In addition, the clustering results from SRAP molecular markers and morphological traits in this study were not exactly the same, indicating different marker methods revealed differences in studying genetic diversity. It may be due to different molecular markers reflect the genetic variation in different regions of genome. Each marker method analyzed different loci numbers, causing relatively some variation in genetic distance and thus possibly leading to minor differences in clustering results by different markers. Several researchers have reported the difference to some extend in calculating genetic distance between morphological traits and molecular marker analysis, or even between different molecular markers in studying genetic diversity of rape, pepper and other crops (Fufa et al., 2005; Tang et al., 2008). Despite these minor differences between varieties in the specific positions of cluster tree in the current study, the main cluster trend was the same, and the correlation coefficient of 0.5255 clearly reflected this trend.

To make a summary, the various features of SRAP markers are very beneficial to DUS testing of new plant varieties and analysis of genetic relationship. With the increasing number of new lettuce varieties and reference

collections, the application of molecular markers such as SRAP marker would be a nice option. It could effectively avoid the defects of traditional morphological markers. The result in the present study revealed that the combination of both SRAP marker and morphological trait analysis is more conducive to proper identification and classification of plant varieties, which will undoubtedly bring a new breakthrough to DUS testing of plant new varieties.

Acknowledgements

References

Botstein, D., White, R.L., Skolnic, Davis R.W. (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *American Journal of Human Genetics*, 32:314-331. PMCID: PMC1686077

Calvert, R.J., Weghorst, C.M., Buzard, G.S. (1995). PCR amplification of silver-stained SSCP bands from cold SSCP gels. *Biotechniques*, 18: 782-786.

Cooke, R.J. (1995). Varietal identification of crop plants. In: Skerritt JH, Appels R(eds.) New Diagnostics in Crop Sciences, CAB, Wallingford, Int, pp. 33-63.

Ferriol, M., Picó, B., Nuez, F. (2003). Genetic diversity of a germplasm collection of *Cucurbi*ta *pepo* using SRAP and AFLP markers. *Theor Appl Genet*, 107: 271-82.

Ferriol, M., Picó, B., de Córdova, P.F., Nuez, F. (2004). Molecular diversity of a germplasm collection of squash(*Cucurbita moschata*) determined by SRAP and AFLP markers. *Crop Sci.*, 44: 653-664. http://dx.doi.org/10.2135/cropsci2004.6530

Fufa, H., Baenziger, P.S., Beecher, B.S., Dweikat, I., Graybosch, R.A., Eskridge, K.M. (2005). Comparison of phenotypic and molecular marker-based classifications of hard red winter wheat cultivars. *Euphytica*, 145: 133-146. http://dx.doi.org/10.1007/s10681-005-0626-3

Giancola, S., Marcucci, Poltri P.S., Lacaze, P., Hopp, H.E. (2002). Feasibility of integration of molecular markers and morphological descriptors in a real case study of a plant variety protection system for soybean. *Euphytica*, 127:95-113. http://dx.doi.org/10.1023/A:1019923923805

Guo, D-L., Luo, Z-R. (2006). Genetic relationship of some PCNA persimmons (*Diospyros kaki Thunb.*)from China and Japan revealed by SRAP analysis. *Genetic Resources and Crop Evolution*, 53:1597-1603. http://dx.doi.org/10.1007/s10722-005-8717-5

Jiang, Y. (2007). Study on extraction and characters of red pigment from purple acute leaves of *Lactuca Sativa*. *Acta Agriculturae Shanghai*, 23:39-43.

Juchum, F.S., Leal, J.B., Santos, L.M., Almeida, M.P., Ahnert, D., Corrêa, R.X. (2007). Evaluation of genetic diversity in a natural rosewood population using RAPD markers. *Genetics and Molecular Research*, 6:543-553.

Kwon, Y.S., Lee, J.M., Yi, G.B., Yi, S.I., Kim, K.M., Soh, E.H., Bae, K.M., Park, E.K., Song, I.H., Kim, B.D. (2005). Use of SSR markers to complement tests of distinctiveness, uniformity, and stability (DUS) of pepper (*Capsicum annuum L.*) varieties. *Molecules and Cells*, 19:428- 435.

Legesse, B.W., Myburg, A.A., Pixley, K.V.，Botha, A.M. (2007). Genetic diversity of African maize inbred lines revealed by SSR markers. *Hereditas*, 144: 10-17. http://dx.doi.org/10.1111/j.2006.0018-0661.01921.x

Li, G., Quiros, C.R. (2001). Sequence-related amplified polymorphism(SRAP) a neap marker system based on a simple PCR reaction: its application to mapping and gene tagging in *Brassica*. *Theor Appl Genet.*, 103:455-461. http://dx.doi.org/10.1007/s001220100570

Li, X., Li, X. (2003). New plant variety protection and DUS testing technique system. *Scientia Agriculture Sinica*, 36:1419-1422.

Li, Y., Zhang, C. (2005). Studies on genetic diversity with a molecular markers SRAP of watermelon hybrids. *Acta Horticulturac Sinica*, 32: 643-647.

Murry, H.G.,Thompson, W.F. (1980). Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Research*, 8:4321-4325.

Nei, M. (1973). Analysis of gene diversity in subdivided population. *PNAS*, 70:3321-3233.

Nei, M., Li, W.H. (1979). Mathematical Model for Studying Genetic Variation in Terms of Restriction Endonucleases. *PNAS*, 76:5269-5273.

Pan, J., Wang, G., Li, X., He, H., Wu, A., Cai, R. (2005). Construction of a genetic map with SRAP markers and localization of the gene responsible for first-flower-node trait in cucumber (*Cucumis sativus* L.). *Progress in Natural Science*, 15:407-413. http://dx.doi.org/10.1080/10020070512331342310

Percifield, R.J., Hawkins, J.S., McCoy, J., Widrlechner, M. P., Wendel, J.F.(2007).Genetic diversity in Hypericum and AFLP Markers for species-specific identification of *H. perforatum* L. *Planta Medica*, 73:1614-1621. http://dx.doi.org/10.1055/s-2007-993749

Ro, H.S., Kim, S.S., Ryu, J.S., Jeon, C., Lee, T.S., Lee, H. (2007). Comparative studies on the diversity of the edible mushroom Pleurotus eryngii: ITS sequence analysis, RAPD fingerprinting, and physiological characteristics. *Mycological Research*, 111:710-715. http://dx.doi.org/10.1016/j.mycres.2007.03.016

Smith, J.S.C., Chine, C.L., Shu, H., Smith, O.S., Wall, S.J., Senior, M.L., Mitchell, S.E., Kresovich, S., Ziegle, J. (1997). An evaluation of the utility of SSR loci as molecular markers in maize (*Zea mays* L.): comparisons with data from RFLPS and pedigree. *Theor Appl Genet.*, 29:163-173.

Tang, S., Dai, W., Li, M., Zhang, Y., Geng, Y., Wang, Li., Zhong, Y. (2008). Genetic diversity of relictual and endangered plant Abies ziyuanensis (*Pinaceae*) revealed by AFLP and SSR markers. *Genetica*, 133: 21-30. http://dx.doi.org/10.1007/s10709-007-9178-x

Tommasini, L., Batley, J., Arnold, G.M., Cooke, R.J., Donini, P., Lee, D., Law, J.R., Lowe, C., Moule, C., Trick, M., Edwards, K.J. (2003). The development of multiplex simple sequence repeat (SSR) markers to complement distinctness, uniformity and stability testing of rape (*Brassica napus* L.) varieties. *Theor Appl Genet.*, 106:1091-1101. http://dx.doi.org/10.1007/s00122-002-1125-8

Van Beuningen, L.T., Busch, R.H. (1997). Genetic diversity among North American spring wheat cultivars: III. Cluster analysisbased on quantitative morphological traits. *Crop Sci.*, 37 :981-988.

Wiel, C.V.D., Arens, P., Vosman, B., Clemens, V.D.W., Paul, A., Ben, V. (1999). Mireosatellite retieval in lettuce(*Lactuca sativa* L.). *Genome*, 42:139-149.

Yuan, Z., Yin, Y., Qu, J., Zhu, L., Li,Y. (2007). Population genetic diversity in Chinese pomegranate (*Punica granatum* L.) cultivars revealed by fluorescent-AFLP markers. *Journal of Genetics and Genomics*, 34: 1061-1071. http://dx.doi.org/10.1016/S1673-8527(07)60121-0

Zhang, S.H., Li, X.H., Tian, Z.G. (2000). Effects of biotechnology on maize breeding and variety protection. *Crops*, 1:20-23.

Table 1. Materials and their origins in China

No.	Varieties (type)	Origin	No.	Varieties (type)	Origin
1	Xiang (leaf lettuce)	Yibin	26	Nchong (stem lettuce)	Nanchong
2	Wanlvye (leaf lettuce)	Longyan	27	Smhong (stem lettuce)	Shimian
3	Wanziye (leaf lettuce)	Longyan	28	Bianganhng (stem lettuce)	Shimian
4	Ziye (leaf lettuce)	Longyan	29	Qingyuanye (stem lettuce)	Changde
5	Boli (leaf lettuce)	Guangdong	30	Jianyezi (stem lettuce)	Linxia
6	Yanzhi (leaf lettuce)	Nanchang	31	Xwqing (stem lettuce)	Xiuwu
7	Hongyeyou (leaf lettuce)	Conghua	32	Lnqing (stem lettuce)	Luoyang
8	Yuanye (leaf lettuce)	Xiamen	33	Xxhong (stem lettuce)	Xianxian
9	Cuiye (leaf lettuce)	Shanghai	34	Xxyuan (stem lettuce)	Xixian
10	Biyu (leaf lettuce)	Shanghai	35	Huiye (stem lettuce)	Shennong
11	Jianye (stem lettuce)	Jiashan	36	Ziye (stem lettuce)	Xuanen
12	Yuanyeqin (stem lettuce)	Jiashan	37	Lilou (stem lettuce)	Wufeng
13	Yaunyezi (stem lettuce)	Jiashan	38	Longtai (stem lettuce)	Wuxi
14	Lvjianye (stem lettuce)	Tongling	39	Woshun (stem lettuce)	Xianfeng
15	Lvyuanye (stem lettuce)	Tongling	40	Hong (stem lettuce)	Baoding
16	Baipi (stem lettuce)	Pengshan	41	Guashihong (stem lettuce)	Zhuzhou
17	Yang (stem lettuce)	Hefei	42	Yzyuan (stem lettuce)	Yongzhou
18	Qingpi (stem lettuce)	Jinzhai	43	Baipijitui (stem lettuce)	Yinchuan
19	Zipixiang (stem lettuce)	Wuhu	44	Grwoshun (stem lettuce)	Guangrao
20	Dajianye (stem lettuce)	Maanshan	45	Jituishun (stem lettuce)	Dingxi
21	Ailao (stem lettuce)	Wuhu	46	Bendituanye (stem lettuce)	Chenggong
22	Cxziye (stem lettuce)	Chaoxian	47	Biganzhong (stem lettuce)	Hangzhou
23	Wwjianye (stem lettuce)	Wuwei	48	Liuye (stem lettuce)	Beijing
24	Baipijian (stem lettuce)	Jintang	49	Gaohua (leaf lettuce)	Xichang
25	Baipiyuan (stem lettuce)	Nanchong	50	Jieqiu (leaf lettuce)	Shanghai

Table 2. Primer sequences used for SRAP analysis

No.	Forward primers	Reverse primers
1	5'-TGAGTCCAAACCGGAAT-3'	5'-GACTGCGTACGAATTTGC-3'
2	5'-TGAGTCCAAACCGGAAT-3'	5'-GACTGCGTACGAATTATG-3'
3	5'-TGAGTCCAAACCGGACC-3'	5'-GACTGCGTACGAATTTGC-3'
4	5'-TGAGTCCAAACCGGAAG-3'	5'-GACTGCGTACGAATTATT-3'
5	5-'TGAGTCCAAACCGGATG-3'	5'-GACTGCGTACGAATTAAT-3'
6	5'-TTCAGGGTGGCCGGATG-3'	5'-GACTGCGTACGAATTATT-3'
7	5-'GGTGAACGCTCCGGAAG-3'	5'-GACTGCGTACGAATTATT-3'
8	5'-TGAGTCCAAACCGGTAA-3'	5'-GACTGCGTACGAATTAAT-3'
9	5'-TGAGTCCAAACCGGTCC-3'	5'-GACTGCGTACGAATTCGA-3'
10	5'-TGAGTCCAAACCGGTCC-3'	5'-GACTGCGTACGAATTATT-3'
11	5'-TGAGTCCAAACCGGTGC-3'	5'-GACTGCGTACGAATTTGC-3'
12	5'-TGGGGACAACCCGGCTT-3'	5'-TGTGGTCCGCAAATTTAG-3'
13	5'-TGAGTCCAAACCGGATA-3'	5'-TGTGGTCCGCAAATTTAG-3'
14	5'-TGAGTCCAAACCGGAGC-3'	5'-GACTGCGTACGAATTATT-3'
15	5'-TGAGTCCAAACCGGATG-3'	5'-GACTGCGTACGAATTAAT-3'
16	5'-GGTGAACGCTCCGGAAG-3'	5'-GACTGCGTACGAATTCAA-3'
17	5'-TGAGTCCAAACCGGGCT-3'	5'-GACTGCGTACGAATTCGA-3'
18	5'-TGGGGACAACCCGGCTT-3'	5'-GACTGCGTACGAATTCGA-3'

Table 3. Morphological traits in DUS testing of lettuce

Trait No.	characteristics	Trait No.	characteristics
1	Type	21	Leaf:bitter
2	Seed:colour	22	Leaf:texture
3	Cotyledon:Anthocyanin coloration	23	Leaf:shape of tip
4	Cotyledon:shape	24	Leaf:hue of green colour of outer leaves
5	Cotyledon:length	25	Leaf:intensity of colour of outer leaves
6	Cotyledon:width	26	Leaf:anthocyanin coloration
7	Cotyledon:colour	27	Leaf:glossiness of upper side
8	Hypocotyl:anthocyanin	28	Leaf:dipcoat
9	Leaf:attitude	29	Leaf:blistering
10	Leaf:lobes of margin	30	Leaf:size of blisters
11	Plant:Width	31	Leaf blade:Type of undulation of margin
12	Plant:height (flowering plant)	32	Leaf blade:degree of undulation of margin
13	Plant:head formation	33	Leaf blade: incisions of margin on apical
14	Leaf:thickness	34	Leaf blade:depth of incisions on margin on apical part
15	Leaf:attitude(harvest maturity)	35	Leaf blade:density of incisions on margin on apical part
16	Leaf:shape	36	Leaf blade:type of incisions on apical part
17	Leaf:length	37	Leaf blade:venation
18	Leaf:breadth	38	Axillary sprouting
19	Leaf:costate size	39	Time of harvest maturity
20	Leaf:costate colour	40	Time of beginning of bolting under long day conditions

Table 4. Description for SRAP markers examined in the 50 lettuce varieties

| No.of primer pair	No.of amplified bands	No. of polymorphic bands	Rate of polymorphism	No. of allels	PIC (%)
1	33	27	0.82	22	94.4
2	32	29	0.91	10	79.1
3	37	24	0.65	17	78.9
4	24	20	0.83	10	64.3
5	25	22	0.88	21	89
6	12	10	0.83	9	56.8
7	32	27	0.84	20	90.5
8	39	34	0.87	14	71.6
9	59	53	0.9	48	97.5
10	43	39	0.91	22	92.4
11	24	22	0.92	27	89.5
12	22	16	0.73	8	39.3
13	20	12	0.6	40	96.8
14	31	27	0.87	13	90
15	30	27	0.9	11	55.6
16	29	27	0.93	43	96.7
17	25	20	0.8	10	80.4
18	32	28	0.88	8	73.1
total	549	464		353	
average	30.5	25.8	0.84	19.6	79.8
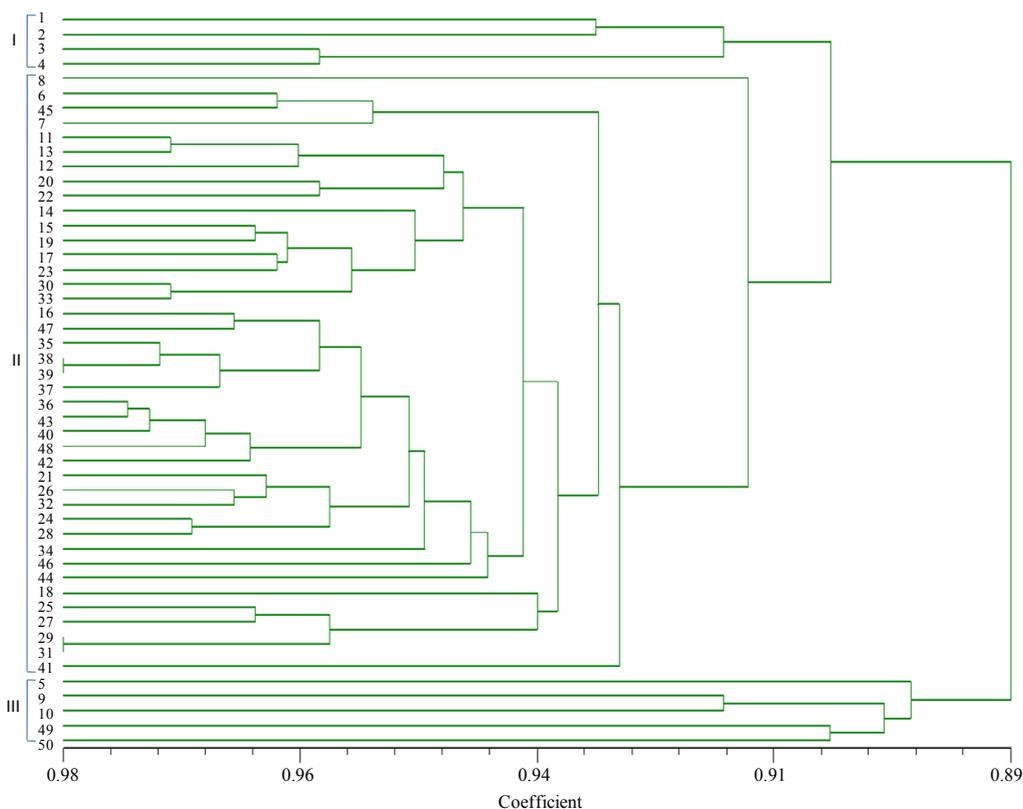
Figure 1. Dendrogram depicting the classification of the 50 lettuce varieties constructed using UPGMA and based on SRAP markers. The tested varieties were grouped into three major groups marked on the left side of the dendrogram. The scale at the bottom is the similarity coefficient
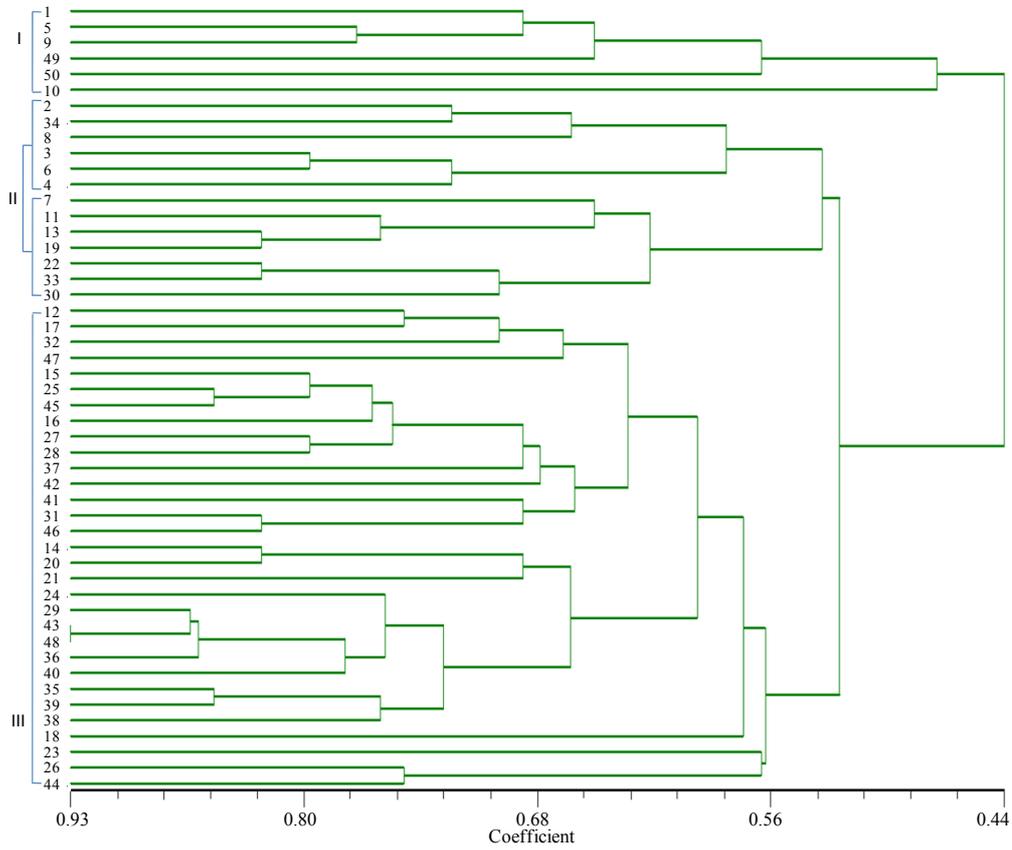
Figure 2. Dendrogram of the 50 lettuce varieties based on 40 morphological traits. The tested varieties were grouped into three major groups marked on the left side of the dendrogram. The scale at the bottom is the similarity coefficient
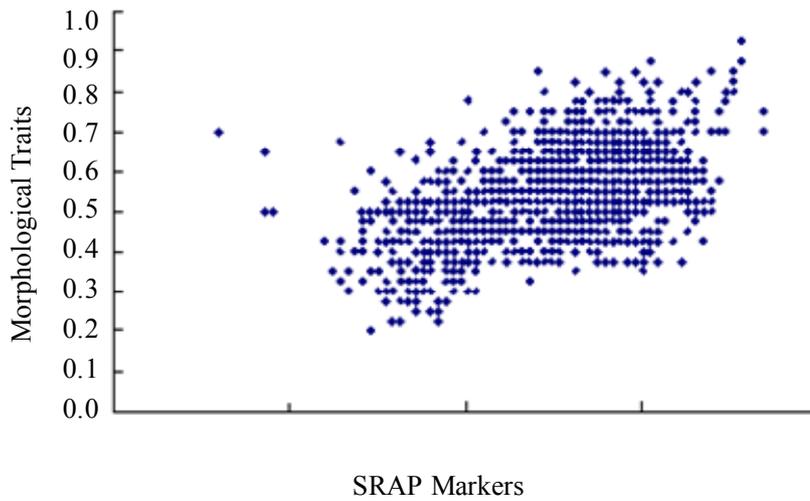

Figure 3. Comparison between morphological and molecular distance. Correlation coefficient is 0.5255