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BIOLOGY

# Analysis on genetic diversity of 51 Grape germplasm resources

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**ABSTRACT**: *The objective of this study is to research the genetic diversity of the ' Zuijinxiang ' grape and its mutant breeding F1 plants, we screened the excellent mutant plants with potential breeding value. 50 mutated single plants obtained from $^{137}$Cs-γ irradiated 'Zuijinxiang' grape seeds were used as research objects, and SCoT molecular marker technology was used for genetic diversity and variation analysis, and clustering research was carried out. The results showed that: (1) 36 SCoT primers produced abundant polymorphisms, and the amplification results showed obvious bright bands, and the amplification efficiency and polymorphism rate were 100%. (2) A total of 221 bands were amplified by 36 primers, of which 175 were rich in polymorphism, the average polymorphic percentage was 80.3%, and the average genetic similarity coefficient was 0.916. (3) The number of observed alleles (Na) ranged from 4 to 8, with an average of 6.1389; the number of effective alleles (Ne) ranged from 1.2772 to 5.6322 with an average of 3.5968; the desired heterozygosity (He) The range is from 0.2192 to 0.8344, the average is 0.6965; the observed heterozygosity (Ho) ranges from 0.1656 to 0.7808 with an average of 0.3035; the Nei's gene diversity index (H) ranges from 0.2170 to 0.8224 with an average of 0.6863; Shannon-Wiener The index (I) ranges from 0.5186 to 1.8597 with an average of 1.4517. (4) UPGMA clustering of 51 materials showed that the test materials could be divided into three groups when the genetic distance was 0.856. The experiment shows that the genetic diversity of the 'Zuijinxiang' radiation variation germplasm resources is rich. In addition, SCoT molecular marker technology can distinguish the materials with close genetic distance, and can be used for early identification techniques of grape mutant materials. This study provides a theoretical basis for the development of excellent mutant germplasm of 'Zuijinxiang' grapes.*
**Key words**: *grape, SCoT markers, genetic diversity, cluster analysis.*

## Análise da diversidade genética da videira 51

**RESUMO**: *O objetivo deste estudo é investigar a diversidade genética da uva 'Zuijinxiang' e de suas plantas F1 reprodutoras mutantes. Foram selecionadas as melhores plantas mutantes com potencial e valor genético. Utilizaram-se como objeto de pesquisa 50 plantas individuais mutantes obtidas de sementes de uva irradiadas com 137Cs-γ 'Zuijinxiang', e a tecnologia de marcadores moleculares SCoT para análise de diversidade genética e variação, e foi realizada uma pesquisa de agrupamento. Os resultados mostraram que: (1) 36 iniciadores de SCoT produziram polimorfismos abundantes, e os resultados de amplificação mostraram bandas claras óbvias, e a eficiência de amplificação e taxa de polimorfismo foram de 100%. (2) Um total de 221 bandas foi amplificado por 36 iniciadores, dos quais 175 eram ricos em polimorfismo, a porcentagem polimórfica média foi de 80,3% e o coeficiente médio de similaridade genética foi de 0,916. (3) O número de alelos observados (Na) variou de 4 a 8, com uma média de 6,1389; o número de alelos efetivos (Ne) variou de 1,2772 a 5,6322 com uma média de 3,5968; a heterozigosidade desejada (He), o intervalo é de 0,2192 a 0,8344, a média é de 0,6965; a heterozigosidade observada (Ho) varia de 0,1656 a 0,7808 com uma média de 0,3035; o índice de diversidade genética (H) de Nei varia de 0,2170 a 0,8224 com uma média de 0,6863; Shannon-Wiener o índice (I) varia de 0,5186 a 1,8597 com uma média de 1,4517. (4) O agrupamento de 51 materiais da UPGMA mostrou que os materiais de teste poderiam ser divididos em três grupos quando a distância genética era de 0,856. O experimento mostra que a diversidade genética dos recursos de germoplasma de variação de radiação "Zuijinxiang" é rica. Além disso, a tecnologia de marcadores moleculares da SCoT pode distinguir os materiais com uma distância genética próxima, e pode ser usada para técnicas de identificação precoce de materiais mutantes da uva. Este estudo fornece uma base teórica para o desenvolvimento de germoplasma mutante excelente de uvas "Zuijinxiang".*
**Palavras-chave**: *videiras, marcadores SCoT, diversidade genética.*

## INTRODUCTION

*Vitis vinifera* is a perennial vine deciduous fruit tree of the genus *Victaceae* (*Vitis* L.) (FANG et al., 2014). At the same time, it is also one of the earliest and most widely distributed fruit trees in the world. The global grape industry output ranks fifth in the world's agricultural products (TAO et al., 2012). Because of it's sweet taste and high nutritional value, it is deeply loved and cultivated in both north and south of China. After long-term production practice, our breeding workers have developed cultivation techniques adapted to grape

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cultivation in China and cultivated many new varieties with good quality (WAN et al., 2015). However, in the southern planting areas, there are still problems such as single structure, lack of good resistance, and high quality varieties that mature earlier than the typhoon season. Therefore, it is particularly important to breed new varieties of grapes that are high quality and resistant.

‘Zuijinxiang’ is a hybrid variety of European and American hybrids selected from the horticultural research of Liaoning Academy of Agricultural Sciences, so that “Shenyang Rose (7601)” is the female parent and “Jiufeng” is a male parent. It has high yield and high quality. It has grown in all grape producing areas of the country (ZHOU et al., 2015). In order to adapt to the southern planting areas to further improve grape germplasm, mutation breeding, cross breeding and biotechnology breeding are usually used. Radiation mutagenesis can not only achieve genetic recombination, shorten the breeding cycle, more importantly, the mutagenized offspring have many advantages such as high mutation rate, excellent maternal traits and improved unfavorable traits, and thus are widely used in breeding improvement (SHANG et al., 2017).

The Start Codon Targeted Polymorphism (SCoT) is a novel molecular marker method based on single-primer amplification reaction developed by Collard and Mackill in rice in 2009 (COLLARD & MACKILL, 2009). SCoT markers produce different polymorphism differences due to their different regions amplified in the genome. Because of their simple operation, low cost and rich genetic information, SCoT markers can effectively produce a series of markers linked to traits and universality of primers. It is widely used in plant genetic diversity research (GUAN et al., 2019). In recent years, SCoT markers have been applied to kiwifruit (CHEN et al., 2018), pear (HE et al., 2016), watermelon (ZHANG et al., 2015), rapeseed (YANG et al., 2015), Junqianzi (WU et al., 2016) and other horticultural plants for identification and pedigree analysis of germplasm resources, genetic diversity, construction of genetic linkage maps and plant gene mapping studies (CHEN et al., 2018). The laboratory irradiated ‘Zuijinxiang’ grape seeds with 137Cs-γ-rays, and 50 mutated plants were obtained after cultivation and cultivation. 51 grape germplasm samples were used as experimental samples, and genetic diversity analysis was carried out based on SCoT molecular technology to clear the difference between the ‘Zuijinxiang’ grape and other new strains is to further analyze the genetic information of the mutant germplasm resources and lay the foundation for future breeding and gene mapping research of new varieties.

## MATERIALS AND METHODS

### *Lexperiment material*

The test materials were taken from the ‘Zuijinxiang’ grape test base in Cixi Xinpu Grape Theme Park, Ningbo City, Zhejiang Province. On September 26, 2012, the mutant young plants obtained from 137Cs-γ-ray irradiation of ‘Zuijinxiang’ grape seeds were planted in Daejeon. After cultivating from 2012 to 2015, there were 50 strains of individual plants. From June 2017 to July 2018, a number of disease-free annual fresh young leaves were collected and labeled. All samples were placed in an ice box and immediately returned to the laboratory at -80 °C for storage. Table 1 shows the test material code and name.

Table 1 - Experiment materials used for SCoT analysis of grape.

| No. | Name | No. | Name | No. | Name | No. | Name |
|---|---|---|---|---|---|---|---|
| 1 | C1-1 | 14 | C2-3 | 27 | C4-4 | 40 | C6-4 |
| 2 | C1-3 | 15 | C2-5 | 28 | C4-5 | 41 | C6-5 |
| 3 | C1-5 | 16 | C2-7 | 29 | C4-6 | 42 | C6-6 |
| 4 | C1-6 | 17 | C2-9 | 30 | C4-9 | 43 | C6-7 |
| 5 | C1-9 | 18 | C2-12 | 31 | C4-10 | 44 | C6-8 |
| 6 | C1-10 | 19 | C2-13 | 32 | C5-2 | 45 | C6-9 |
| 7 | C1-11 | 20 | C2-15 | 33 | C5-3 | 46 | C6-11 |
| 8 | C1-12 | 21 | C2-17 | 34 | C5-6 | 47 | E2 |
| 9 | C1-13 | 22 | C3-1 | 35 | C5-7 | 48 | 1 |
| 10 | C1-14 | 23 | C3-4 | 36 | C5-9 | 49 | 2 |
| 11 | C1-15 | 24 | C3-11 | 37 | C5-10 | 50 | 4 |
| 12 | C1-16 | 25 | C3-12 | 38 | C6-1 | 51 | Zuijinxiang |
| 13 | C2-1 | 26 | C4-3 | 39 | C6-3 | | |

*Main reagents and equipment*

Plant DNA Extraction Kit (OMEGA); 36 SCoT primers (Shenzhen Huada Biological Company); 2×EasyTaq PCR SuperMix (Full Form Golden Biotechnology Co., Ltd.); Trichloromethane, isoamyl alcohol, ethanol, β-mercaptoethanol (National Pharmaceutical Group Chemical Reagent Co., Ltd.); RNaseA, 10×Buffer, DL2000 Marker, agarose, TE buffer, l×TAE running buffer (Shanghai Shenggong Bioengineering Co., Ltd.).

Acid detector (Eppendorf BioPhotometer plus), spectrophotometer NanoDropTM2000 (Thermo Fisher Scientific), gel imaging system (Gel Doc XR+, Bio-RAD), PCR instrument (Eppendorf Mastercycler Nexus), temperature gradient PCR instrument (Eppendorf Mastercycler Pros), refrigerated centrifuge (Eppendorf Centrifuge 5430R), electrophoresis apparatus and power supply (Beijing Liuyi Instrument Factory DYY-6C), palm type centrifuge (Eppendorf TGL 16G), sterilization pot (SANYO MLS-3750), micro pipette , constant temperature oscillator, ultra-clean workbench, constant temperature metal bath (G506106, Shanghai Bioengineering Co., Ltd.).

*Evaluation of grappe genomic DNA quality*

Referring to OMEGA's plant DNA kit extraction method, 1 μL of DNA solution was mixed with 5 μL of DNA loading Buffer, and 5 μL of the mixed product was taken for 1% agarose gel electrophoresis. Taking 1.5 μL of DNA sample the OD260/OD280 value and concentration value of the DNA sample were determined by NanoDropTM2000 to determine the purity and concentration of the DNA template, and then the DNA sample was stored in a refrigerator at -20 °C for use.

*SCoT primer screening and SCoT-PCR amplification*

36 SCoT primers were referenced by Collard and Mackill (Collard and Mackill, 2009) and synthesized by Shenzhen Huada Biotech Co., Ltd. By consulting the relevant literature and conducting experimental optimization screening, the PCR reaction procedure was determined as follows: pre-denaturation at 94 °C for 5 min, denaturation at 94 °C for 5 s, annealing at 50 °C for 1 min, extension at 72 °C for 1 min, a total of 35 cycles, and finally, 72 °C. Extend for 8 min and store at 4 °C. Reaction system:
2×EasyTaq PCR SuperMix - 10 μL
Primer - 1 μL
RNase-Free water - 8 μL
DNA Template - 1 μL
Total - 20 μL

To detect the availability of SCoT primers, a genomic DNA template of five sample materials was randomly selected and mixed to construct a mixed gene pool. Therefore, SCoT-PCR amplification was performed as a single template. After the amplification, 8 μL of the amplified product was subjected to constant pressure electrophoresis on a 2% agarose gel in l×TAE running buffer, voltage 120 V, current 120 mA, electrophoresis for 30 mins. The electrophoresis is terminated by the indicator running through the gel for about 2/3, and then the image is acquired using a gel imaging system. A clear and polymorphic SCoT primer was screened for subsequent experiments.

*Data statistics and analysis*

According to the presence or absence of the same electrophoretic mobility (same molecular weight fragment) of each molecular marker, binary data of all the sites were statistically obtained, and the amplified band was recorded as 1, and the band-free was recorded as 0. Jaccard similarity analysis was performed by using software NTSYSpc 2.10e, and then cluster analysis was performed by SHAN program unweighted arithmetic and arithmetic average method (UPGMA) to establish a genetic relationship clustering map. And use the molecular tag data format conversion software Data Formater to convert the strip data into the POPGENE software input file. The average number of alleles (Na), effective alleles (Ne), observed heterozygosity (Ho), expected heterozygosity (He), Nei's gene diversity index (H), and Shannon diversity were calculated using POPGENE 1.32 software. (Shannon-Wiener) index (I) and so on.

## RESULTS

*DNA extraction quality testing*

In this study, the material 'Zuijinxiang' grape contains a large amount of polysaccharides, polyphenols and tannins and other secondary metabolites, which are easy to combine with nucleic acids to produce a complex. In particular, polyphenols tend to oxidize DNA to produce browning. In addition, polysaccharides are easily combined with DNA to produce sticky gelatinous substances, all of which affect the quality of DNA extraction. Therefore, this experiment obtained a higher quality DNA template by means of an improved kit extraction method, using TE buffer to dissolve DNA, and optimizing extraction conditions.

Figure 1 is a genomic DNA electrophoresis map of 'Zuijinxiang' and some of its mutant materials.

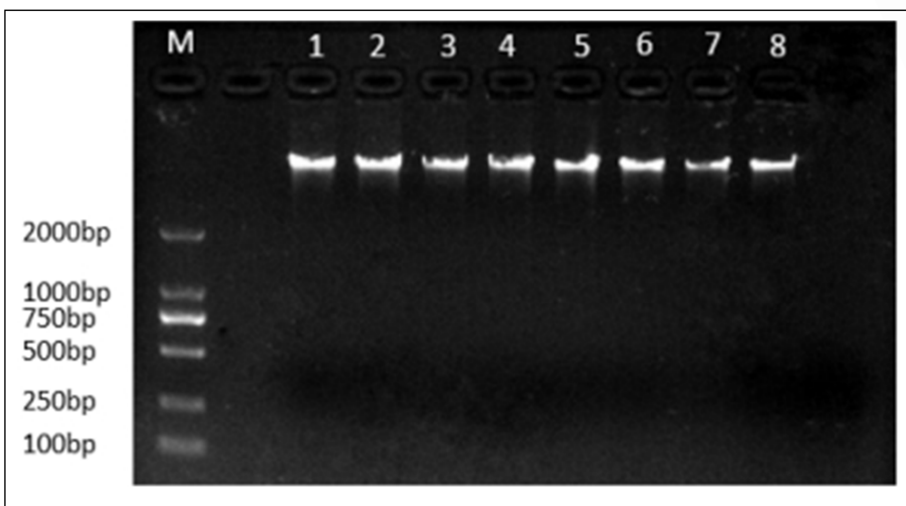


Figure 1 - DNA electrophoresis results figure of ‘Zuijinxiang’ and its Mutants of *Vitis vinifera* L.

According to the results, there was no obvious degradation and tailing. In addition, the detection by the nucleic acid detector indicates that OD260/OD280 values are between 1.7-2.0. As the result, the protein, saccharide and phenolic substances of the material have been substantially removed, and the DNA extracted by the test has high purity, which meets the requirements of further SCoT amplification analysis.

*SCoT orimer screening*

To detect the availability of SCoT primers, genomic DNAs of C1-1, C1-6, C3-11, C6-11, and E2 were randomly selected as sample templates for mixed gene pools, and polymorphisms were performed on 36 SCoT primers as a single template. According to figure 2, all the 36 SCoT primers produced rich polymorphisms by gel imaging, and the amplification results showed obvious bright bands, and the amplification efficiency and polymorphism rate were 100%, so 36 SCoT primers were obtained. Subsequent experimental studies are available.

*Polymorphism analysis of SCoT-PCR amplification products*

The 36 primers obtained by screening were used for SCoT-PCR reaction, and the amplification results were clear and rich in polymorphism. Figure 3 (a-d) shows the amplification results of 51 grape germplasms by primers SCoT7, SCoT20, SCoT25

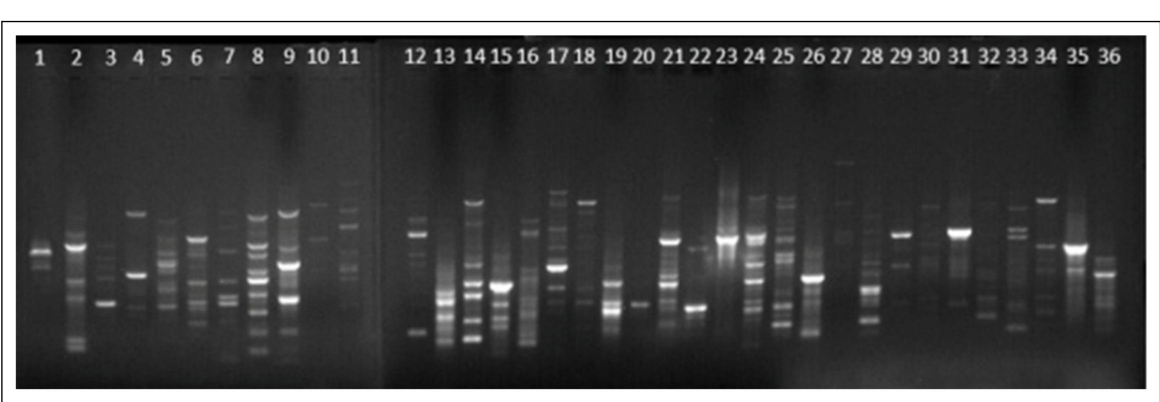


Figure 2 - Amplification electrophoresis map of 36 SCoT primer pairs mixed gene pool DNA template.

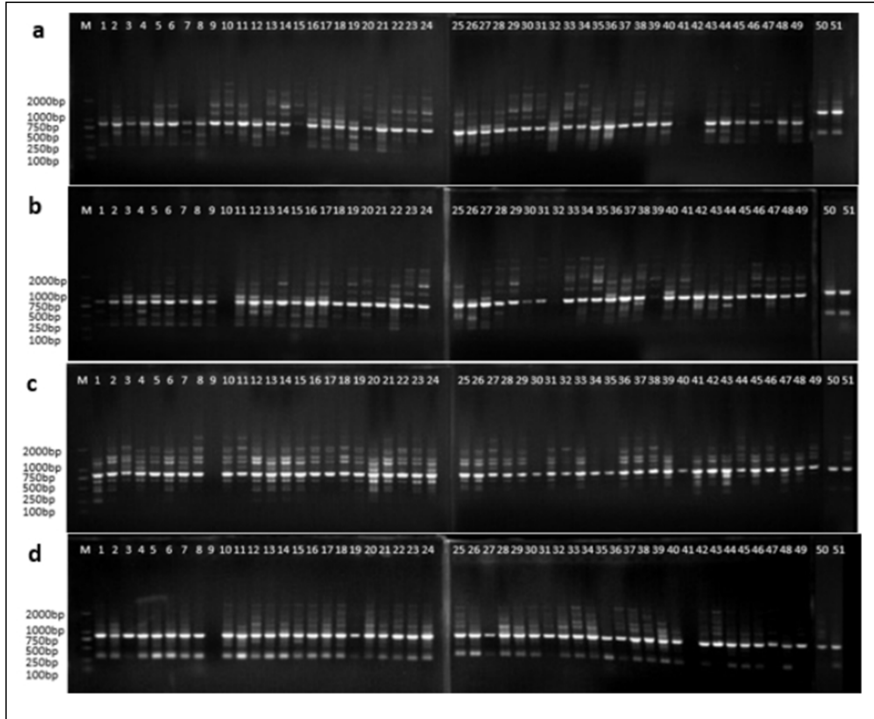


Figure 3 - Electrophoresis of genomic DNA amplification of 51 test materials by primers SCoT7, SCoT20, SCoT25 and SCoT35.

and SCoT35. According to the statistical analysis in table 2, a total of 221 bands were amplified by 36 primers, of which 175 were rich in polymorphism, the average polymorphic percentage was 80.3%, and the amplified fragments were concentrated between 250 bp and 2000 bp. From the results of the amplification, The number of bands amplified by different primers is not the same, and the number of polymorphic sites is also different. The number of amplified bands is between 4 and 8, and the average number of amplified bands is 6.1. In addition, the primers with the largest number of amplified bands were SCoT6, SCoT11, SCoT19, SCoT22, SCoT28, SCoT30 and SCoT35, all of which amplified 8 bands; The primers with the smallest number of amplified bands were SCoT5, SCoT18, SCoT21, SCoT26 and SCoT33, all of which amplified 4 bands. In addition, the percentage of primer polymorphism bands is between 57.1% and 100%.There were 10 primers in the 36 primers with a polymorphic band rate of 100%, namely SCoT1, SCoT6, SCoT7, SCoT10, SCoT13, SCoT17, SCoT18, SCoT21, SCoT26 and SCoT33;In addition, the primer SCoT32 amplified the least number of polymorphic sites, and the percentage of polymorphism was only 57.1%.According to the amplification results, the genetic diversity of the grapes is extremely rich. The SCoT molecular marker can analyze the polymorphism of the 'Zuijinxiang' grape under radiation mutagenesis treatment. At the same time, it was found that the SCoT-PCR amplified bands were rich in polymorphism, and the efficiency of detecting grape genetic diversity was also significant.

*Analysis of genetic diversity revealed by SCoT markers*

According to the SCoT amplification results, the Jaccard similarity coefficient analysis was performed using NTSYSpc 2.10e, and the genetic similarity coefficient between the samples was obtained by calculation. Statistics are available, ranging from 0.840 to 1.00 with an average genetic similarity coefficient of 0.916.The greater the genetic similarity coefficient between the samples, the closer the genetic relationship between the samples is. At the same time, according to the test results, it can be concluded that the genetic similarity coefficient between the tested materials is highly variable, and thus has high genetic diversity.

According to the POPGENE1.32 software, the SCoT-PCR amplified band data can be calculated

Table 2 - Sequence and amplification efficiency of 36 pairs of primers.

| Primer | Sequence | Total band number | Polymorphic band number | Percentage of polymorphic bands(%) |
|---|---|---|---|---|
| SCoT1 | CAACAATGGCTACCACCA | 5 | 5 | 100 |
| SCoT2 | CAACAATGGCTACCACCC | 6 | 4 | 66.7 |
| SCoT3 | CAACAATGGCTACCACCG | 5 | 4 | 80 |
| SCoT4 | CAACAATGGCTACCACCT | 6 | 5 | 83.3 |
| SCoT5 | CAACAATGGCTACCACGA | 4 | 3 | 75 |
| SCoT6 | CAACAATGGCTACCACGC | 8 | 8 | 100 |
| SCoT7 | CAACAATGGCTACCACGG | 5 | 5 | 100 |
| SCoT8 | CAACAATGGCTACCACGT | 6 | 4 | 66.7 |
| SCoT9 | CAACAATGGCTACCAGCA | 5 | 3 | 60 |
| SCoT10 | CAACAATGGCTACCAGCC | 6 | 6 | 100 |
| SCoT11 | AAGCAATGGCTACCACCA | 8 | 5 | 62.5 |
| SCoT12 | ACGACATGGCGACCAACG | 7 | 5 | 71.4 |
| SCoT13 | ACGACATGGCGACCATCG | 6 | 6 | 100 |
| SCoT14 | ACGACATGGCGACCACGC | 5 | 3 | 60 |
| SCoT15 | ACGACATGGCGACCGCGA | 6 | 4 | 66.7 |
| SCoT16 | ACCATGGCTACCACCGAC | 7 | 5 | 71.4 |
| SCoT17 | ACCATGGCTACCACCGAG | 6 | 6 | 100 |
| SCoT18 | ACCATGGCTACCACCGCC | 4 | 4 | 100 |
| SCoT19 | ACCATGGCTACCACCGGC | 8 | 7 | 87.5 |
| SCoT20 | ACCATGGCTACCACCGCG | 6 | 5 | 83.3 |
| SCoT21 | ACGACATGGCGACCCACA | 4 | 4 | 100 |
| SCoT22 | AACCATGGCTACCACCAC | 8 | 6 | 75 |
| SCoT23 | CACCATGGCTACCACCAG | 6 | 4 | 66.7 |
| SCoT24 | CACCATGGCTACCACCAT | 7 | 6 | 85.7 |
| SCoT25 | ACCATGGCTACCACCGGG | 7 | 5 | 71.4 |
| SCoT26 | ACCATGGCTACCACCGTC | 4 | 4 | 100 |
| SCoT27 | ACCATGGCTACCACCGTG | 6 | 5 | 83.3 |
| SCoT28 | CCATGGCTACCACCGCCA | 8 | 5 | 62.5 |
| SCoT29 | CCATGGCTACCACCGGCC | 6 | 5 | 83.3 |
| SCoT30 | CCATGGCTACCACCGGCG | 8 | 6 | 75 |
| SCoT31 | CCATGGCTACCACCGCCT | 7 | 5 | 71.4 |
| SCoT32 | CCATGGCTACCACCGCAC | 7 | 4 | 57.1 |
| SCoT33 | CCATGGCTACCACCGCAG | 4 | 4 | 100 |
| SCoT34 | ACCATGGCTACCACCGCA | 6 | 5 | 83.3 |
| SCoT35 | CATGGCTACCACCGGCCC | 8 | 6 | 75 |
| SCoT36 | GCAACAATGGCTACCACC | 6 | 4 | 66.7 |
| Total | | 221 | 175 | - |
| Mean | | 6.1 | 4.9 | 80.3 |

and analyzed. The results of the genetic diversity index are shown in table 3. The number of observed alleles (Na) ranges from 4 to 8, with an average of 6.1389. In general, the higher the number of alleles per locus, the more polymorphic the population is; the number of effective alleles (Ne) reflects the size of the population variation. When the number of effective alleles is closer to the absolute value of the detected alleles, it can be concluded that the alleles are evenly distributed in the population. Conversely, the alleles are unevenly distributed. The statistics range from 1.2772 to 5.6322 with an average of 3.5968; the expected heterozygosity (He) ranges from 0.2192 to 0.8344 with an average of 0.6965.Generally, its value is used to measure the genetic diversity of a population. Overall the higher the value of (He), the lower the genetic consistency of the revealed population, and the richer the genetic diversity of the population is; the observed heterozygosity (Ho) ranged from 0.1656 to 0.7808 with an average of

Table 3 - Polymorphism of 36 SCoT primers in‘Zuijinxiang’ and its Mutants of *Vitis Vinifera* L.

| Primer | Na | Ne | He | Ho | H | I |
|---|---|---|---|---|---|---|
| SCoT1 | 5 | 2.5525 | 0.6142 | 0.3858 | 0.6082 | 1.2308 |
| SCoT2 | 6 | 3.9186 | 0.7547 | 0.2453 | 0.7448 | 1.5299 |
| SCoT3 | 5 | 3.4792 | 0.7235 | 0.2765 | 0.7126 | 1.3790 |
| SCoT4 | 6 | 3.1038 | 0.6845 | 0.3155 | 0.6778 | 1.4357 |
| SCoT5 | 4 | 3.9512 | 0.7683 | 0.2317 | 0.7469 | 1.3801 |
| SCoT6 | 8 | 3.4473 | 0.7169 | 0.2831 | 0.7099 | 1.6352 |
| SCoT7 | 5 | 1.2781 | 0.2198 | 0.7802 | 0.2176 | 0.5186 |
| SCoT8 | 6 | 1.2772 | 0.2192 | 0.7808 | 0.2170 | 0.5220 |
| SCoT9 | 5 | 3.1494 | 0.6916 | 0.3084 | 0.6825 | 1.3002 |
| SCoT10 | 6 | 2.3656 | 0.5830 | 0.4170 | 0.5773 | 1.2511 |
| SCoT11 | 8 | 3.2717 | 0.7012 | 0.2988 | 0.6943 | 1.5551 |
| SCoT12 | 7 | 4.4454 | 0.7923 | 0.2077 | 0.7750 | 1.6909 |
| SCoT13 | 6 | 4.9388 | 0.8161 | 0.1839 | 0.7975 | 1.6687 |
| SCoT14 | 5 | 4.6667 | 0.7971 | 0.2029 | 0.7857 | 1.5716 |
| SCoT15 | 6 | 3.0475 | 0.6785 | 0.3215 | 0.6719 | 1.4300 |
| SCoT16 | 7 | 5.1914 | 0.8215 | 0.1785 | 0.8074 | 1.8081 |
| SCoT17 | 6 | 3.0403 | 0.6777 | 0.3223 | 0.6711 | 1.4032 |
| SCoT18 | 4 | 3.9670 | 0.7681 | 0.2319 | 0.7479 | 1.3820 |
| SCoT19 | 8 | 3.0781 | 0.6818 | 0.3182 | 0.6751 | 1.5571 |
| SCoT20 | 6 | 2.4006 | 0.5892 | 0.4108 | 0.5834 | 1.2443 |
| SCoT21 | 4 | 3.3543 | 0.7108 | 0.2892 | 0.7019 | 1.2767 |
| SCoT22 | 8 | 5.3473 | 0.8259 | 0.1741 | 0.8130 | 1.8597 |
| SCoT23 | 6 | 2.9624 | 0.6690 | 0.3310 | 0.6624 | 1.4083 |
| SCoT24 | 7 | 5.6322 | 0.8344 | 0.1656 | 0.8224 | 1.8255 |
| SCoT25 | 7 | 5.1914 | 0.8215 | 0.1785 | 0.8074 | 1.8081 |
| SCoT26 | 4 | 3.9512 | 0.7683 | 0.2317 | 0.7469 | 1.3801 |
| SCoT27 | 6 | 3.9186 | 0.7547 | 0.2453 | 0.7448 | 1.5299 |
| SCoT28 | 8 | 5.3473 | 0.8259 | 0.1741 | 0.8130 | 1.8597 |
| SCoT29 | 6 | 3.1038 | 0.6845 | 0.3155 | 0.6778 | 1.4357 |
| SCoT30 | 8 | 3.2717 | 0.7012 | 0.2988 | 0.6943 | 1.5551 |
| SCoT31 | 7 | 4.4454 | 0.7923 | 0.2077 | 0.7750 | 1.6909 |
| SCoT32 | 7 | 5.1914 | 0.8215 | 0.1785 | 0.8074 | 1.8081 |
| SCoT33 | 4 | 3.3543 | 0.7108 | 0.2892 | 0.7019 | 1.2767 |
| SCoT34 | 6 | 2.3656 | 0.5830 | 0.4170 | 0.5773 | 1.2511 |
| SCoT35 | 8 | 3.0781 | 0.6818 | 0.3182 | 0.6751 | 1.5571 |
| SCoT36 | 6 | 2.4006 | 0.5892 | 0.4108 | 0.5834 | 1.2443 |
| Mean | 6.1389 | 3.5968 | 0.6965 | 0.3035 | 0.6863 | 1.4517 |

0.3035, which represents the percentage of observed heterozygous individuals as a percentage of the total number of samples; Nei’s gene diversity index (H) ranges from 0.2170 to 0.8224, with an average of 0.6863. Its value indicates the genetic distance to analyze the richness of genetic diversity, and the genetic diversity is evaluated according to the proportion of different genes in the population; The Shannon-Wiener index (I) indicates the genetic polymorphism of the population, ranging from 0.5186 to 1.8597 with an average of 1.4517.

*Cluster analysis based on SCoT marker*

Jaccard similarity analysis was performed by software NTSYSpc 2.10e, and then the genetic similarity coefficient (UPGMA) clustering analysis was carried out by genetic similarity coefficient and SHAN program non-weighted allocation to establish a genetic relationship clustering map. The results are shown in figure 4. When the genetic distance is 0.856, 51 test materials can be divided into three groups: A, B, and C. It can be seen from the genetic relationship clustering map of ‘Zuijinxiang’ and mutant lines

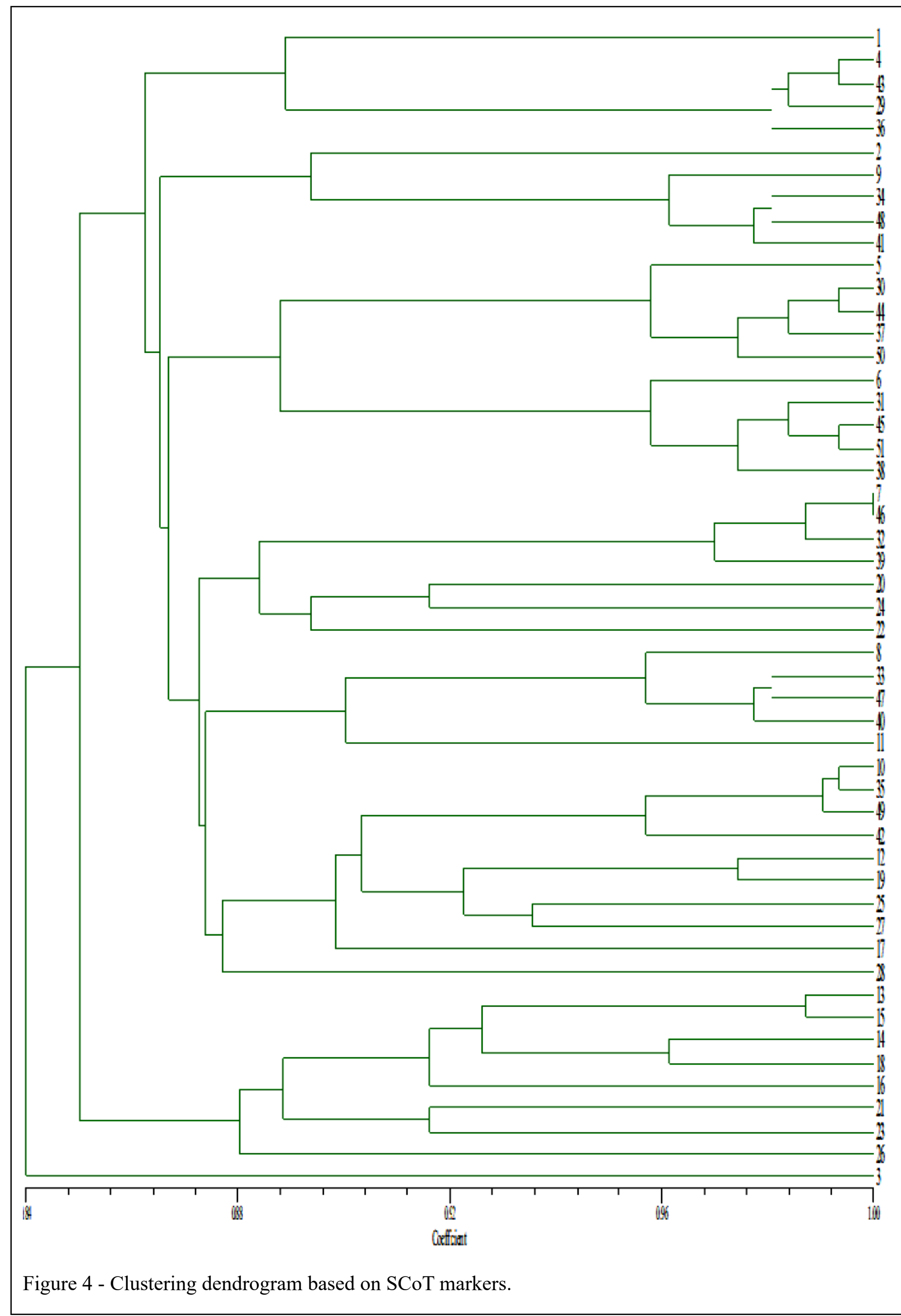

Figure 4 - Clustering dendrogram based on SCoT markers.

that the genetic similarity distance is the smallest (C1-11) and 46 (C6-11), the similarity coefficient is 1.00, genetic similarity The largest distance is the No. 3 (C1-5) sample and No. 1 (C1-1) germplasm with a similarity coefficient of 0.84.According to the clustering chart, it can be concluded that the C group contains at least only 3 samples; In addition, in group B, 26 (C4-3), 23 (C3-4), 21 (C2-17), 16 (C2-7), 18 (C2-12), 14 (C2) -3), 15 (C2-5), 13 (C2-1) are grouped together; The genetic similarity coefficient is about 0.884, and the 26th (C4-3) is separated. The genetic similarity coefficients are approximately 0.912, 21 (C2-17) and 23 (C3-4) are separated, then No. 16 (C2-7), No. 18 (C2-12), No. 14 (C2-3), No. 15 (C2-5), No. 13 (C2-1) is a sub-category. The genetic similarity coefficient is about 0.920, and the 16th (C2-7) is separated. The genetic similarity coefficients are about 0.928, 18 (C2-12), 14 (C2-3) are grouped together, and 15 (C2-5) and

13 (C2-1) are grouped together. In addition, the group A group is the most complex, including 'Zuijinxiang', No. 28 (C4-5), No. 17 (C2-9), No. 27 (C4-4), No. 25 (C3-12), No. 19 (C2 -13), 12 (C1-16), 42 (C6-6), 49 (2), 35 (C5-7), 10 (C1-14), 11 (C1-15), No. 40 (C6-4), No. 47 (E2), No. 33 (C5-3), No. 8 (C1-12), No. 22 (C3-1), No. 24 (C3-11), No. 20 (C2 -15), 39 (C6-3), 32 (C5-2), 46 (C6-11), 7 (C1-11), 38 (C6-1), 45 (C6-9) ), 31 (C4-10), 6 (C1-10), 50 (4), 37 (C5-10), 44 (C6-8), 30 (C4-9), 5 (C1-9), 41 (C6-5), 48 (1), 34 (C5-6), 9 (C1-13), 2 (C1-3), 36 (C5-9) ), No. 29 (C4-6), No. 43 (C6-7), No. 4 (C1-6), No. 1 (C1-1) and other materials. As the result of analysis, most of the mutant strains have less genetic similarity with 'Zuijinxiang', and their genetic distance is relatively close. In addition, SCoT markers can be used to classify sample materials with close genetic distances. Therefore, SCoT markers can be used to reveal the phylogenetic relationships between similar lines in the early stage of radiation mutagenesis breeding for screening and genetic identification.

## DISCUSSION

The use of radiation mutagenesis breeding to screen new varieties has a long history and significant effects. Usually, various types of radiation are used as inducing conditions to cause genetic variation of materials, which can effectively shorten the breeding period and obtain more mutant types of mutant materials. Screen for new materials with excellent traits. According to the official FAO/IAEA data, until the 2016, deciduous fruit tree breeding workers in various countries have used mutation breeding techniques to screen and cultivate 55 new varieties of deciduous fruit trees, including 1 new grape variety and 1 apricot variety. , 1 plum tree variety, 2 jujube varieties, 2 new pomegranate varieties, 6 peach tree varieties, 8 pear tree varieties, 13 new apple varieties and 21 new cherry germplasms (WU et al., 2016). In this experiment, 137Cs-γ-rays were used to irradiate the seeds of 'Zuijinxiang' grapes, and then the mutant plants were obtained, in order to screen new germplasm with excellent variation. In order to carry out comprehensive and in-depth research on the new germplasm resources obtained, molecular genetic analysis was used to analyze the genetic diversity. Generalized genetic diversity is usually the genetic difference between species or interspecific, including all genetic information, and narrowly refers to the integration of genetic variation between different individuals within a species or between different groups, that is, intraspecific inheritance. Diversity (LI et al., 2016). The basic conditions of biological evolution are often rich in genetic diversity and also constitute rich biodiversity. Therefore, the study of genetic diversity of populations is conducive to the genetic improvement of plant populations and the provision of rich germplasm resources for the breeding of new varieties. The results of the test were calculated. The Shannon-Wiener diversity index of 'Zuijinxiang' and its mutant lines was 1.4517, and its Nei's gene diversity index (H) was 0.6863, and the average polymorphic percentage was 80.3%. Through UPGMA cluster analysis, it is found that when the genetic distance is 0.856, the test materials can be divided into three groups. Overall, the test materials are rich in genetic diversity.

SCoT molecular marker is a novel dominant molecular marker based on PCR technology in 2009. It is widely used because of its rich polymorphism and simple operation. Chen Bolun et all used SCoT technology to analyze the variation and genetic diversity of 17 kiwifruit mutant materials (CHEN et al., 2018). The study found that SCoT molecular technology can effectively distinguish the mutant material and can be used as an early identification technique for kiwifruit. He Shiyu et all used 24 parts of the excellent nutrient variation system of Prunus to test the genetic distance by SCoT-PCR amplification in 2016 (HE et al., 2016). Jiang Qiaoqiao et all carried out SCoT molecular marker study on 24 sweet orange bud strains, and the results showed that 12 variant materials could be well identified and differentiated, and other materials need to be combined with other markers (JIANG et al., 2011). Compared with the main field crops such as wheat and rice, the research on grape molecular marker-assisted breeding is relatively backward. Although the application of SCoT molecular markers in grape research is limited to the stage of system optimization and diversity analysis, it is found by this experiment that SCoT markers can classify grape varieties with close genetic distance and can be used for early identification of grape mutant materials. It will provide theoretical support for grape variety improvement and new variety selection. SCoT molecular markers have been proved to have good application potential gene differential expression analysis in plant, germplasm identification, genetic linkage mapping and functional gene cloning. It is foreseeable that SCoT molecular markers have broad prospects in grape research. It is significance to promote grape molecular marker-assisted breeding and genetic research.

Using molecular marker technology to identify germplasm resources and genetic diversity analysis, compared with traditional morphological and cytological studies, the germplasm identification technology has the advantages of high reliability, stable and efficient, and easy operation. However,

SCoT molecular marker technology still has certain limitations. Because its amplification bands are often dominant markers, it is impossible to effectively identify homozygotes and heterozygotes. Therefore, SCoT molecular marker technology cannot be used for germplasm identification in cross breeding. Mutation breeding usually results in a variety of mutations due to mutations in randomness and uncertainty, and often has less favorable variation. In order to ensure the acquisition of excellent mutant germplasm, the number of mutant populations should be increased to a certain extent, thus increasing the chance of germplasm screening. When identifying mutant germplasm resources, research should be carried out by combining traditional morphological or cytological research methods with molecular marker techniques.

## CONCLUSION

In summary, the genetic diversity of 50 'Zuijinxiang' mutant germplasm resources is abundant. All 36 SCoT primers produced abundant polymorphisms, and the amplification results showed obvious bright bands. SCoT molecular marker technology can distinguish between materials with close genetic distance and can be used for early identification of grape mutant materials. This study provides a theoretical and practical basis for the study of genetic diversity of Mutant Breeding and the development and utilization of high quality germplasm resources.

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## AUTHORS' CONTRIBUTIONS

Qingchun Yue and Yueyan Wu conceived and designed experiments. Qingchun Yue , Chenfei Zhang , Qinghao Wang , Wenjing Wang , Jinyang Wang performed the experiments and statistical analyses of experimental data. Qingchun Yue prepared the draft of the manuscript. All authors critically revised the manuscript and approved of the final version.

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