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# Transferability of Expressed Sequence Tag of Single Sequence Repeats Markers of Tomato Fruit in Different Vegetable Varieties

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Authors NC and HF designed the study. Authors JY, YS and TZ performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors SP and YY managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.*

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***Short Communication***

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## ABSTRACT

**Aims:** The expressed sequence tag of single sequence repeats (EST-SSR) primers designed from tomato fruit were studied for their polymorphism and transferability to other vegetable varieties.
**Study Design:** Primers were designed from ESTs in which the repeat bases of SSR were more than 18 bp. Twenty primer pairs chosen randomly were tested for their ability to amplify among 14 tomato varieties. The primer pairs were further used for 8 Chinese cabbage varieties, 8 muskmelon varieties and 8 eggplant varieties.
**Place and Duration of Study:** College of Biological Science and Technology, Shenyang Agricultural University, China, between February 2015 and March 2016.

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**Methodology:** Twenty primer pairs selected randomly were used for the amplification of genomic DNA in 14 tomato varieties. Genomic DNA of tomato was isolated and amplified, and tested the transferability of primers.
**Results:** Eighteen of primer pairs showed the amplification, and 2 primer pairs showed the non-pre-amplification. Seventeen of 18 primer pairs revealed polymorphism. Eighteen primer pairs were further used for PCR to genomic DNA from 8 Chinese cabbage varieties, 8 muskmelon varieties and 8 eggplant varieties. Twelve of 18 primer pairs showed amplification in all of materials provided, and the individual ratios of amplification were 77.8%, 83.3% and 83.3%, the ratios of polymorphism of them were 65.0%, 46.7% and 80%, respectively.
**Conclusion:** Eighteen primer pairs showed the amplification. Seventeen of 18 primer pairs revealed polymorphism. Twelve of 18 primer pairs showed amplification in 8 Chinese cabbage varieties, 8 muskmelon varieties and 8 eggplant varieties. Tomato EST-SSR markers had highly transferability to other plant species.

*Keywords: Tomato; EST-SSR; transferability.*

## 1. INTRODUCTION

Tomato (*Solanum lycopersicum* L.) has been studied as a model plant of Solanaceae [1]. The total number of tomato EST sequences in NCBI (National Center for Biotechnology Information) is 298289 (http://www.ncbi.nlm.nih.gov/nucest), provides an effective resource for the development of tomato EST-SSR markers [2].

The EST from the gene encoding region, may be directly identified so as to isolate the alleles which determine some important characters, and can also be prepared for microarray gene expression [3]. Because the flanking sequences of SSR have high conservation and universality, they can be used for the study of comparative genomics [4,5].

The number of EST sequence in tomato ranks the first in Solanaceae crops, and others are less than 100 thousand (http://www.ncbi.nlm.nih.gov/nucest?term=est%20tomato). Given the developing complexity of EST-SSR markers and the limited EST sequences in some species, it is very necessary that the universality from EST-SSR primers of tomato is tested in sibling species and sobrinus species. Polymorphism is the effective criterion that determines the usability of genetic marker [6,7].

In this research, we designed the EST-SSR primers from tomato fruit, and then identified their polymorphism and transferability to 8 Chinese cabbage varieties, 8 muskmelon varieties and 8 eggplant varieties.

## 2. MATERIALS AND METHODS

### 2.1 Plant Materials

All plant materials were provided by Institute of Vegetable Research, Shandong Academy of Agricultural Sciences (Table 1).

### 2.2 Primer Design

To calculate the preprocessed sequence aminimum of 18 repeat bases of its SSR was used for primer design. The primers were designed from unique sequences flanking regions of the SSR using the Primer Premier 5.0 program with length of 17-24 bp, annealing

**Table 1. Materials of transferability testing**

| Plants' materials | Name |
|---|---|
| 14 tomato varieties | Aihuangzhenzhu $F_2$, Hongfentianshi $F_2$, Bawang $F_{11}$ youqingjian, Qifen $F_8$, Shanghai 908$F_7$, Huafen $F_6$, Lv-2010-150lv, Lv-2010-152lv, Lv-2010-153lv, Tianzheng 110-822, Tianzheng110-83, Tianzheng110-820, Tianzheng110-620, Tianzheng 10-1320 |
| 8 cabbage varieties | 08-162 (Hua xin), 07-253 (He bao), 08-216 (Zhi tong), Guan291 (Die bao), Jinhuanghou (Hua xin), Qiuza3hao (Zhitong), Jingdichun (Hebao), Lubai1hao (Diebao) |
| 8 muskmelon varieties | Jinyanyilisanbai, Luhoutian1, Luhoutian2, Jinyu, $L_2$ male parent, $L_2$ female parent, 135 male parent, 135 female parent |
| 8 eggplant varieties | 21×22, 25×24, 1×7, 25×29, 24, 34, 17, 36 |

temperature of 50-60℃, and product sizes ranging from 100 to 400 bp.

### 2.3 Isolation and Amplification of Genomic DNA

Twenty primer pairs selected randomly were used for the amplification of genomic DNA in 14 tomato varieties (Table 2). Then, they were used for the amplification of the genomic DNA in 8 cabbage varieties, 8 muskmelon varieties and 8 eggplant varieties, respectively.

Polymerase chain reactions (PCR) were performed in a total volume of 20 μL, including 45 ng of tomato DNA, 10 μM of each primer, 10 μL reaction mix and 7 μL $ddH_2O$.

PCR reactions were performed on MyCycler Thermal Cycler (Bio-Rad, Laboratories, USA) with an initial 5 min of denaturation at 94℃, followed by 35 cycles of 94℃ for 30s, appropriate annealing temperature for 45s, 72℃ for 1 min, and a final extension at 72℃ for 10 min.

PCR products were separated on 8% polyacrylamide gel with TBE buffer according to the standard protocol.

**Table 2. Primers for isolation and amplification of genomic DNA of tomato varieties and to test the transferability**

| Primer code | GenBank accession(s) | Tm/℃ | The length of amplification /bp | The sequences of primers (5'-3') |
|---|---|---|---|---|
| HML2 | gi\|117720747\| | 52.74/50.47 | 381 | S 5' TGTCCCAAAGTCCAAGAT 3';<br>A 5' TTAAGCACTAGCCTCAAT 3' |
| HML3 | gi\|117720536\| | 55.02/51.65 | 128 | S 5' GAACCATGCCAAATAGCC 3',<br>A 5' GATGATGGGTTGAAATAAAG 3' |
| HML4 | gi\|117719773\| | 55.02/52.74 | 225 | S 5' GCGAAGGGAAAGCACAAA 3',<br>A 5' AAACCCGCTCCAAACATA 3' |
| HML5 | gi\|117719554\| | 52.74/50.47 | 269 | S 5' ATTGTCTTCTTCCGTCAC 3',<br>A 5' AATGGGTATTTGTGGATC 3' |
| HML6 | gi\|117717696\| | 51.09/50.47 | 193 | S 5' TTTACGGCATTGTTTGTTG 3',<br>A 5' ATCCCGCATAATACTTCA 3' |
| HML10 | gi\|117712661\| | 52.74/50.47 | 291 | S 5' GTTTCATCATCGTCGTCA 3',<br>A 5' CCATACAAACAAGGGATT 3' |
| HML11 | gi\|117712614\| | 55.02/53.70 | 193 | S 5' GGGGTTTGTTCTGTCTGT 3',<br>A 5' AAGCCAATGATCTACTCTTC 3' |
| HML12 | gi\|117711890\| | 55.02/52.74 | 293 | S 5' AAAAGGGTAGTCGCTGAG 3',<br>A 5' TGCCGTGAAGTCCATTAA 3' |
| HML13 | gi\|117710343\| | 52.74/52.74 | 175 | S 5' GGTCTTGTTGAGGCTTAT 3',<br>A 5' TGACGGGTTGTACTTTCT 3' |
| HML14 | gi\|117707059\| | 57.30/55.02 | 185 | S 5' CTCCGAACCCTACACCTA 3',<br>A 5' GGCATTACATCCTCCACT 3' |
| HML15 | gi\|117706032\| | 57.30/53.07 | 300 | S 5' CTTGCCTGATTCTGACCC 3',<br>A 5'CTAAGAGGAGGAAGTGTTTT 3' |
| HML16 | gi\|117704602\| | 52.74/55.02 | 193 | S 5' AAGCGAAGTTGGATAAGG 3',<br>A5' GAAGCATCTGAACCCTGA 3' |
| HML17 | gi\|117704414\| | 51.65/55.02 | 107 | S 5' TAATGAGTTTCTTGACCCTA 3',<br>A 5' GATCGTCGTCTTCTTGTG 3' |
| HML18 | gi\|117704334\| | 54.48/50.48 | 311 | S 5'ATTGGGAATTTGCTAATGAAGG 3',<br>A 5' TTTGATTTCTGTCCCTTG 3' |
| HML19 | gi\|117701576\| | 55.02/50.47 | 100 | S 5' ACTCCTCCTCCTTATCCT 3',<br>A 5' AAGGTGGTGGTTGTAAAT 3' |
| HML20 | gi\|117701243\| | 52.74/51.09 | 298 | S 5' GCGACTATGGTAAGATTG 3',<br>A 5' CTTTAGCCTGTATTTCACT 3' |
| HML21 | gi\|117696769\| | 52.74/55.02 | 258 | S 5' GATGGAAGAAACCGAAAG 3',<br>A 5' ATGTCCCAACTGACCTGA 3' |
| HML22 | gi\|117696148\| | 52.74/55.02 | 170 | S 5' TTGCTTTAGCTGCTTCAC 3',<br>A 5' CCCAGATGCCAAATAGAC 3' |
| HML25 | gi\|117692348\| | 55.02/52.74 | 217 | S 5' TAATCGAAGGCGAAGGAG 3',<br>A 5' CACTGGGATAAAGGAACA 3' |
| HML26 | gi\|117692049\| | 52.74/52.74 | 178 | S 5' TGTGGTGGTGATGTTGAT 3',<br>A 5' ACAGGAAATCGGGAATAG 3' |

## 3. RESULTS

### 3.1 The Amplification of Genomic DNA in Tomato

Twenty primer pairs selected randomly were used for the amplification of genomic DNA in 14 tomato varieties (Fig. 1). Eighteen of 20 primer pairs except HML6 and HML15 showed the amplification in all genomic DNA of 14 tomato varieties, and the ratio of amplification was 90%. Six primer pairs (HML5, 10, 12, 13, 26 and 22) only showed the predicted amplification, and 8 primer pairs (HML2, 3, 4, 11, 17, 18, 19 and 25) showed predicted amplification and non-predicted amplification, and 4 primer pairs (HML14, 16, 20 and 21) only showed the non-predicted amplification. Sixteen primer pairs except HML18 and HML15 revealed polymorphisms and the ratio of polymorphisms was 88.9%.

### 3.2 The Amplification of Genomic DNA in Other Materials

Eighteen primer pairs which showed the amplification to all genomic DNA in 14 tomato varieties were further used for the amplification to genomic DNA in 8 Chinese cabbages varieties, 8 muskmelon varieties and 8 eggplant varieties.

Fourteen primer pairs showed the amplification to all genomic DNA in 8 Chinese cabbage varieties, and fifteen primer pairs showed the amplification to all genomic DNA in 8 muskmelon varieties as well as 8 eggplant varieties. The ratios of amplification were 77.8%, 83.3%, 83.3%, respectively. Twelve primer pairs showed the amplification to all genomic DNA in the three materials. Two primer pairs did not show the amplification to all genomic DNA in the three materials, however, some primers including HML2, HML10, HML22 and HML25 showed the amplification in genomic DNA in the two materials. Some of them revealed polymorphisms, the ratios of them that showed polymorphisms were 65.0%, 46.7%, 80% (Table 3 and Fig. 2).

## 4. DISCUSSION

The present results show that the expressed sequence tag (EST) is from encoding region and is conservative, so EST markers are more universal because of the conservation [8]. The establishment of EST-SSR markers among different species will greatly improve the screening and utilizing of specific function genes. EST-SSR production is due to the instantaneous abscission and mismatch of polymerase during replication of DNA, according with stepwise mutation model, so it can be used to explain the history process for the origin of species, evolution junction and artificial selection. The stable EST-SSR markers can be used for the reconstruction of more distant evolutionary events [9,10], so EST-SSR marker may also be applied to the research on species origin and genetic difference of vegetable crops.

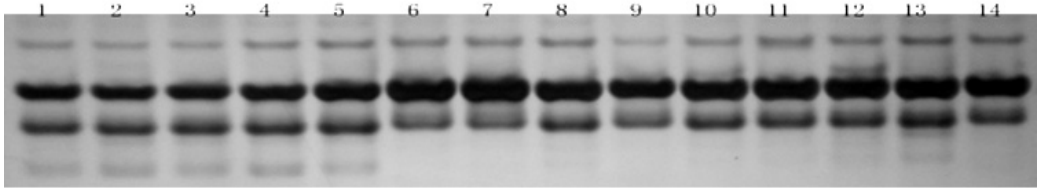


**Fig. 1. HML19 PCR amplification to genomic DNA in 14 tomato varieties**

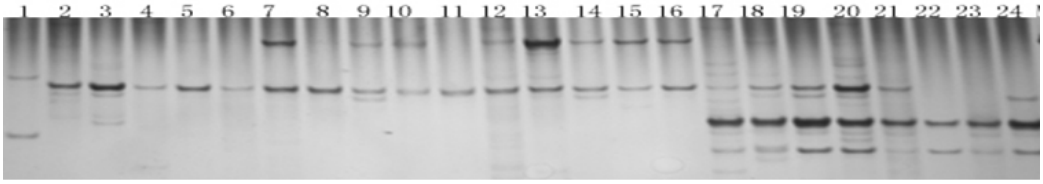


**Fig. 2. HML3 PCR amplification to genomic DNA in 8 Chinese cabbage varieties, 8 muskmelon varieties and 8 eggplant varieties (1-8: 8 Chinese cabbages varieties; 9-16: 8 muskmelon varieties; 17-24: 8 eggplant varieties)**

**Table 3. Results of 18 primer pairs amplification**

| The code of primers | Chinese cabbages varieties | | Muskmelon varieties | | Eggplant varieties | |
|---|---|---|---|---|---|---|
| | The length of amplification | Polymorphisms | The length of amplification | Polymorphisms | The length of amplification | Polymorphisms |
| HML2 | B | NP | AB | NP | ------- | -------- |
| HML3 | B | P | B | P | AB | P |
| HML4 | B | P | B | P | B | P |
| HML5 | B | P | B | NP | AB | P |
| HML10 | AB | NP | ------- | -------- | AB | P |
| HML11 | A | P | B | NP | A | NP |
| HML12 | AB | P | AB | P | A | P |
| HML13 | AB | P | AB | P | AB | NP |
| HML14 | AB | NP | AB | NP | AB | P |
| HML16 | AB | P | AB | NP | AB | P |
| HML17 | B | P | B | P | A | P |
| HML18 | AB | NP | B | NP | A | P |
| HML19 | AB | P | B | P | B | P |
| HML20 | ------ | ------- | ------- | ------- | -------- | ------- |
| HML21 | ------ | ------- | ------- | ------- | -------- | ------- |
| HML22 | ------ | ------- | A | NP | A | P |
| HML25 | ------ | ------- | B | P | B | P |
| HML26 | A | NP | A | NP | AB | NP |

*Note: A predict amplification; B: No predict amplification; P: Polymorphism; NP: No polymorphism*

EST-SSR marker is an effective functional molecular marker and has been used in study of many plants, such as wheat [11], soybean [12], common bean [13], cabbage [14]. Those sequences, in which the total number of repeat bases of SSR was at least 18bp, selected from 2039 ESTs for primer design. As found from the present study, we got 526 ESTs coming up to the requirements, occupying 25.8% in total ESTs containing EST-SSRs. We found that some ESTs could not be used for primer design, because SSRs were in the either ends of the ESTs. In this research, some ESTs could not be used for primer design because they were located in head or tail.

Eighteen of 20 primer pairs selected randomly showed amplification in all of tomato varieties, and the ratio of amplifying was 90%, the other 2 primer pairs did not show amplification, because the sequence of primer crosses the two exons or there was an intron between two primers. Seventeen of 18 primer pairs showed polymorphism. SSR polymorphism results from simple sequence length polymorphism and random amplifying satellite polymorphism produced by the number of repeated motif and the different bases. It was generally acknowledged that mutation in frequency of SSR positively correlated to the number of repeated motifs, that is, the more large possibility of SSR mutation, the more the number of repeated motifs [15,16]. It indicated that tomato markers had highly transferability among tomato varieties.

Eighteen primer pairs were tested for their transferability in 8 cabbage varieties belonging to the cruciferae, 8 muskmelon varieties belonging to the cucurbitaceae and 8 eggplant varieties belonging to the solanaceae. The results revealed that the amplification rates were larger than 75%. It showed polymorphism in three different plants, and the ratio of polymorphism in 8 eggplant varieties was larger than others. Whether or not amplification led to polymorphism was the effective criteria and that could be used as a genetic marker [6]. The results showed that EST-SSR markers of tomato have high transferability in other plants.

## 5. CONCLUSION

Eighteen of the primer pairs tested were amplified. Seventeen of 18 pairs exhibited polymorphism. Twelve of 18 primer pairs showed amplification in 8 Chinese cabbage varieties, 8 muskmelon varieties and 8 eggplant varieties. Tomato EST-SSR markers had highly transferability to other plant species.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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